Mapping of the conserved antigenic domains shared between potato apyrase and parasite ATP diphosphohydrolases: potential application in human parasitic diseases

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SUMMARY

Evolutionary and closer structural relationships are demonstrated by phylogenetic analysis, peptide prediction and molecular modelling between Solanum tuberosum apyrase, Schistosoma mansoni SmATPase 2 and Leishmania braziliensis NTPDase. Specific protein domains are suggested to be potentially involved in the immune response, and also seem to be conserved during host and parasite co-evolution. Significant IgG antibody reactivity was observed in sera from patients with American cutaneous leishmaniasis (ACL) and schistosomiasis using potato apyrase as antigen in ELISA. S. mansoni adult worm or egg, L. braziliensis promastigote (Lb) and Trypanosoma cruzi epimastigote (EPI) have ATP diphosphohydrolases, and antigenic preparations of them were evaluated. In ACL patients, IgG seropositivity was about 43% and 90% for Lb and potato apyrase, respectively, while IgM was low (<19%) for both. In schistosomiasis patients IgM (>40%) or IgG (100%) seropositivity for both soluble egg (SEA) and adult worm (SWAP) antigens was higher than that found for potato apyrase (IgM = 10%; IgG = 39%). In Chagas disease, IgG seropositivity for EPI and potato apyrase was 97% and 17%, respectively, while the IgM was low (3%) for both antigens. The study of the conserved domains from both parasite proteins and potato apyrase could lead to the development of new drug targets or molecular markers.

Key words: ATP diphosphohydrolase, apyrase, NTPDase, Leishmania braziliensis, Schistosoma mansoni, Trypanosoma cruzi, Solanum tuberosum, antibody, molecular modelling, phylogenetic.

INTRODUCTION

ATP diphosphohydrolase (EC 3.6.1.5), also known as apyrase, or nucleoside triphosphate diphosphohydrolase (NTPDase), has been characterized in plants, mammals, bacteria, fungi and parasites (Handa and Guidotti, 1996; Vasconcelos et al., 1996; Gendron et al., 2002). These ubiquitous enzymes share several common features, such as ability to hydrolyse di- and triphosphate nucleosides upon bivalent metal ion activation, and are members of the ATP diphosphohydrolase family, which includes proteins that are related in sequence, sharing 5 apyrase-conserved regions (ACRs) (Handa and Guidotti, 1996; Vasconcelos et al. 1996; Gendron et al. 2002). The mammalian NTPDase family is the most extensively studied and includes 6 membrane-bound enzymes – NTPDases 1–4, 7 and 8, and 2 soluble species – NTPDases 5 and 6, all of them involved in several physiological processes that include modulation of signals mediated by cell-surface purinergic receptors (Gendron et al., 2002; Murphy-Piedmonte et al. 2005).

ATP diphosphohydrolase activity has been characterized in parasites such as Toxoplasma gondii, Schistosoma mansoni, Leishmania (L.) amazonensis, Trichomonas vaginalis and Trypanosoma cruzi (Bermudes et al. 1994; Vasconcelos et al. 1996; Coimbra et al. 2002; Tasca et al. 2004; Fietto et al. 2004). The first description of this protein family is recent (Handa and Guidotti, 1996; Vasconcelos et al.).

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S. mansoni or L. braziliensis (unpublished data) and L. (V.) braziliensis (Coimbra et al. 2008) and L. (L.) amazonensis (Coimbra et al. 2008) and L. (V.) braziliensis (unpublished data) promastigotes, suggesting that these proteins share epitopes. These data were confirmed by immunoprecipitation assays, since antibodies against different potato apyrase isoforms, isolated from Solanum tuberosum, immobilized on Sepharose-Protein A depleted the ATPase and ADPase activities from these detergent-solubilized samples (Vasconcelos et al. 1996; Coimbra et al. 2008). Furthermore, sera from experimentally S. mansoni or L. (L.) amazonensis infected-mice show cross-immunoreactivity with potato apyrase, suggesting the presence of conserved epitopes and the antigenicity of the parasite isoforms (Faria-Pinto et al. 2004; Coimbra et al. 2008).

Due to the previous evidence of cross-immunoreactivity, in this study we performed an analysis of the structural relationship between these enzymes, the putative 3-dimensional structures of soluble potato apyrase, S. mansoni SmATPDase 2 and Leishmania braziliensis NDPase using molecular modelling, putative epitope availability, and their evolutionary relations to other NTPDase family members. Moreover, we analysed the antibody reactivity against the conserved domains using potato apyrase or parasite preparations as antigen, and sera obtained from patients with American cutaneous leishmaniasis, schistosomiasis or Chagas disease.

**MATERIALS AND METHODS**

In silico analyses, molecular model construction and epitope prediction

Comparison of the S. tuberosum, S. mansoni and Leishmania apyrases in the Swiss-Prot database was carried out using the Fasta3. Sequence alignments were produced with T-Coffee (Notredame et al. 2000), and manipulated and hand-edited with Jalview. This alignment was utilized to determine the 4 maximally diverse representatives of each family. The best templates for potato apyrase and SmATPDase 2 or L. braziliensis NDPase model construction were determined by threading methods, using Bio-info meta server (http://bioinfo.pl/meta/), as the structures of (PDB file 1T6C), determined by X-ray diffraction that shared around 52% and 37% identity, respectively, in an un-gapped alignment with potato apyrase. Using this template, the models were constructed using Modeller 8.0 (Sali and Blundell, 1993). The predicted B cell epitopes were ranked according to the potential ability to bind with high affinity one or more (promiscuous sequences) out of 51 different HLA-DR molecules (Singh and Raghava, 2001).
2001). Patients confirmed with schistosomiasis (n = 31) were diagnosed by 3 stool samples examined for S. mansoni eggs according to the Kato-Katz method. Patients with indeterminate or cardiac clinical forms of Chagas disease (n = 30) were diagnosed by indirect immunofluorescence, ELISA and indirect haemagglutination assays for Trypanosoma cruzi (Gomes et al. 2005). As a control, 10 selected sera from healthy individuals from non-endemic areas for these diseases, and without any other parasitic disease, were also tested. The study protocols complied with the regulations of the Brazilian National Council of Research in Humans and were approved by the Ethical Committee for Human Research of Centro de Pesquisas Rene Rachou (CPqRR), Belo Horizonte, Minas Gerais, Brazil, under protocols CEPSH/CPqRR 06/2001 and 04/2005, for Chagas disease and schistosomiasis, respectively. For leishmaniasis, the project was approved by the Ethical Committee for Human Research of the Universidade Federal de Alfenas, Alfenas, MG, Brazil, under process no. 141/2006.

**Antibody analyses by enzyme-linked immunosorbent assays (ELISA)**

Potato apyrase was purified from a commercial strain of Solanum tuberosum, and used as coating antigen by ELISA as previously described (Kettlun et al. 1992; Faria-Pinto et al. 2004). Potato apyrase (0.5 μg/well in 0.1 M NaHCO₃, pH 9.6) was absorbed overnight onto flat-bottomed Immunolon microtitre plates. Following a blocking step (0.3% Tween-20, 5% non-fat dry milk, 0.15 M phosphate buffer solution, pH 7-2), sera diluted 1:200 from healthy individuals or from patients with schistosomiasis mansoni (n = 31), American cutaneous leishmaniasis (n = 21), and Chagas disease (n = 30) were tested in duplicate, in 3 different experiments. Leishmania (L.) braziliensis promastigotes (MHOM/BR/1975/M2903 strain) homogenized (Pedras et al. 2003), soluble egg antigen (SEA) or worm adult (SWAP) antigen from S. mansoni (Makarova et al. 2003) and epimastigote-derived antigens from Y strain T. cruzi (Gomes et al. 2005) were used as positive references, under the same experimental conditions. Antibodies bound to the antigen-plate were detected using peroxidase-conjugated antibodies to anti-IgG and anti-IgM human specific immunoglobulin (Sigma; St Louis, MO; PharMingen, San Diego, CA, USA), and OPD/H₂O₂ as substrate. The subsequent colour reaction was read at 492 nm on a microplate reader (Molecular Devices Corp., Menlo Park, CA, USA).

**Statistical analyses**

For comparative analysis of antibody reactivities, ELISA units were calculated as the mean of optical density (OD; 492 nm) values of each duplicate serum sample from each patient divided by the mean of the optical density of sera from healthy individuals (n = 10) plus 2 standard deviations [OD of each sample/(XOD control + 2 s.d.)]. The mean of the OD of serum samples from these selected healthy individuals plus 2 standard deviations correspond to an ELISA unit value of 1. Therefore, values greater than this cut-off level were considered to be seropositive. GraphPad Prism Software (version 4) was used for statistical analysis. The median and the 95% confidence interval of the ELISA units were calculated, and the data were analysed using the Mann-Whitney test to compare 2 groups, or Kruskal-Wallis test to compare 3 groups. P values <0.05 were considered significant.

**RESULTS**

In silico analyses

The alignment of 32 members of the ATP diphosphohydrolase family was performed, and included mammalian, helminth and protozoan parasites, mosquitoes and plant proteins, found in the National Center for Biotechnology Information (NCBI) database. Phylogenetic analysis (Fig. 1) shows 2 main branches, indicating 2 different evolutionary pathways. The first is clearly composed of membrane-associated human NTPDases 1–4, 7–8, mouse NTPDases 1–4 and 6–8, S. mansoni SmATPDase 1, P. falciparum NTPDase 1 and T. gondii NTPase 1. The second is composed of human and mouse NTPDases 5 and human NTPDase 6, plant apyrases, S. mansoni SmATPDase 2, S. japonicum NTPDase6-like protein and Leishmania NDPases, showing a closer structural relation between them. The proteins with higher homology with potato apyrase were the Leishmania NDPases (30–33% identity and 45–46% similarity over 401 to 408 amino acids), followed by S. mansoni SmATPDase 2 (28% identity and 43% similarity over 433 amino acids). Lower identity was found for T. cruzi NTPDase 1 (27% identity and 43% similarity over 433 amino acids).

The highest identity between potato apyrase and Leishmanias NDPases (50–82.4%) or SmATPDase 2 (41–70%) were found in the A, B, C, D, E, F and G regions (Table 1), that are shown in grey columns in Fig. 2. The Regions A, C, D and E include the characteristic conserved domains of the ATP diphosphohydrolase family, the ACR1, ACR2, ACR3 and ACR4, respectively (Fig. 2). No significant immunological identity was observed in the linear amino acid sequence of the region corresponding to ACR5 (Fig. 2). Peptides found in both Regions A and D from L. braziliensis NDPase, S. mansoni SmATPDase 2 and T. cruzi NTPDase 1 show a high score (>0.8) for antibody binding (Fig. 2 and Table 1). In Region C, only L. braziliensis and...
T. cruzi proteins have peptides with high score (>0.8) for reactivity by antibodies. Analysis of Region E suggests that it may be a domain containing epitopes in L. braziliensis NDPase or S. mansoni SmATPDase 2 (Fig. 2 and Table 1). In addition, with the exception of Region E, that includes the ACR 4, all the others were predicted as promiscuous, since they have nanomeric peptides predicted to bind 10 (20%) to 47 (92%) of 51 different human leukocyte antigen (HLA)-DR alleles used in this matrix-based algorithm and, therefore, theoretically have a high probability to induce a T cell immune response (Fig. 2 and Table 1).

The Region F, but not G, in L. braziliensis NDPase and T. cruzi NTPDase 1 has high score as potential antibody-binding region (>0.8; Fig. 2 and Table 1). These regions did not bind antibodies in SmATPDase 2 according to prediction (Fig. 2 and Table 1). For all the parasite proteins, Regions F and G have promiscuous peptides capable to bind HLA-DR molecules (Fig. 2 and Table 1). Regions F and G are not characteristics of the ATP diphosphohydrolase family and also share identity with soluble NTPDases 5 and 6, and/or human membrane associated NTPDase 1 (Fig. 2).

On the other hand, Region B from L. braziliensis NDPase (50% identity and 57% similarity over 38 amino acids) or SmATPDase 2 (48% identity and 67% similarity over 37 amino acids) shows high identity with potato apyrase in the absence of gaps (Fig. 2). Furthermore, it has a high score to bind antibodies (>0.8) and several promiscuous peptides capable of binding to HLA-DR molecules used in this matrix-based algorithm (Fig. 2 and Table 1). This Region B from potato apyrase is also shared with S. japonicum, L. infantum or L. major putative NDPases (Fig. 2), and with other plant apyrase isoforms found in GeneBank (data not shown). Interestingly, it shows lower identity with either soluble NTPDases 5 and 6 (32–40%) or membrane-associated NTPDase 1 (20%) counterparts, and no significant similarity was found with T. cruzi.

Fig. 1. Phylogenetic tree of several ATP diphosphohydrolases from different organisms. This tree was constructed using T-Coffee, excluding positions with gaps. GeneBank Accession numbers of the sequences are: Solanum tuberosum apyrase, P80595; Dolichos biflorus apyrase, AF156781; Glycine soja apyrase, AAG32959; Medicago truncatula apyrase, AAO23007; Pismum sativum apyrase, BAB85978; Schistosoma mansoni SmATPDase2, DQ868522; Schistosoma japonicum NTPDase6-like protein, AAW26231; NTPDase6-Homo sapiens, AAP92131; NTPDase5-Homo sapiens, NP_001240; NTPDase5-Mus musculus, NP_001021385; Aedes aegypti NDPase, EAT42846; Anopheles gambiae CD39-like protein, XP_320057; Trypanosoma cruzi NTPDase1, AA875599; Leishmania braziliensis NDPase, CAM42020; Leishmania major NDPase, CAJ03227; Leishmania infantum NDPase, CAM66723; Plasmodium falciparum NTPDase1, XP_00138471; Schistosoma mansoni SmATPDase1, AAP94734; NTPDase1-Homo sapiens, NP_001767; NTPDase1-Mus musculus, AAAH11278; NTPDase2-Homo sapiens, NP_982293; NTPDase2-Mus musculus, OS5026; NTPDase8-Homo sapiens, AAR04374; NTPDase8-Mus musculus, NP_082369; NTPDase3-Homo sapiens, NP_001239; NTPDase3-Mus musculus, NP_848791; NTPDase4-Homo sapiens, NP_004892; NTPDase4-Mus musculus, NP_080450; NTPDase7-Homo sapiens, NP_065087; NTPDase7-Mus musculus, NP_444333; Toxoplasma gondii NTPase1, Q27893; NTPDase6-Mus musculus, NP_742115.
Parasites and potato apyrases share antigenic domains

Table 1. Theoretical prediction of peptides that are either antibody epitopes or are capable of binding HLA-DR alleles, within the parasite NTPDase domains shared with potato apyrase

(The number of HLA-DR molecules that bind nanomeric peptide from the parasite protein is relative to the 51 alleles analysed, and the percentage is shown in parentheses.)

| Potato apyrase Region | L. braziliensis NTPDase Antibody (score)* | HLA-DR molecule | S. mansoni SmATPase 2 Antibody (score)* | HLA-DR molecule | T. cruzi NTPDase 1 Antibody (score)* | HLA-DR molecule |
|-----------------------|------------------------------------------|-----------------|-----------------------------------------|-----------------|-------------------------------------|-----------------|
| A (46–64)             | >0.8(+)                                  | 12/51 (24)      | >0.8(+)                                 | 25/51 (50)      | >0.8(+)                             | 10/51 (20)      |
| B (78–117)            | >0.8(+)                                  | 42/51 (83)      | >0.8(+)                                 | 22/51 (43)      | NS                                   | NS              |
| C (120–133)           | >0.8(+)                                  | 33/51 (65)      | <0.8                                    | 47/51 (92)      | >0.8(+)                             | 10/51 (20)      |
| D (164–185)           | >0.8(+)                                  | 19/51 (37)      | >0.8(+)                                 | 39/51 (76)      | >0.8(+)                             | 32/51 (63)      |
| E (194–204)           | >0.8(+)                                  | 02/51 (04)      | >0.8(+)                                 | 01/51 (02)      | <0.8                                | 01/51 (02)      |
| F (240–252)           | >0.8(+)                                  | 15/51 (29)      | <0.8                                    | 40/51 (78)      | >0.8(+)                             | 27/51 (53)      |
| G (392–410)           | <0.8                                    | 27/51 (53)      | <0.8                                    | 12/51 (24)      | <0.8                                | 27/51 (53)      |

* Peptides with score >0.8(+) within the domain have higher probability as epitopes. NS, no significant identity with potato apyrase.

NTPDase1, suggesting that this particular amino acid sequence is shared between the plant apyrases and those of Leishmania and Schistosoma isofoms.

The modelled structures of isolated either potato apyrase in different angles (Fig. S1A and Fig. S2A), L. braziliensis NTPDase (Fig. S1B) or S. mansoni SmATPase 2 (Fig. S2B), and in junction (Fig. S1C and Fig. S2C) are shown in the supplementary figures in the Online version only. The models consist of a mixed 5-stranded-sheet with the second strand in an anti-parallel position to the rest. The connections between strand 1 and 4 and between strand 4 and 5 contain helical segments, all on the same side of the sheet. The connections are significantly longer in the C-terminal domain than in the N-terminal domain. Accordingly, 5 R-helices are present in the C-terminal domain and only 2 in the N-terminal domain (Fig. S1A or Fig. S2A, Fig. S1B and Fig. S2B, Online only).

It is interesting to note the high level of homology that exists between the predicted 3-dimensional structures of potato apyrase and either L. braziliensis NTPase or SmATPase 2, and the coincident localization of the regions shown in Fig. 2. As observed in the models, these regions are exposed and possibly able to bind antibodies (Fig. S1A or Fig. S2A, Fig. S1B and Fig. S2B, Online only). For better clarification of the structural relationship between these proteins, the ACR regions in both L. braziliensis NTPase (Fig. S1B, Online only) and SmATPase 2 (Fig. S2B, Online only) were marked in red, and white arrows were added to indicate specific antigenic loops to both models. Furthermore, the 3 other regions indicated in this paper, B, F and G (Fig. 2 and Table 1), were detached by other colour tonalities in potato apyrase (blue, Fig. S1A and Fig. S2A, Online only), L. braziliensis NTPase (brown; Fig. S1B, Online only) or SmATPase 2 (green; Fig. S2B, Online only), and were adequately

denominated. Therefore, antigenic loops are shown as conserved functional regions, suggesting a clear association between structure and antigenicity.

**Antibody levels against potato apyrase**

IgG and IgM antibody levels were quantified in diluted serum samples 1:200 from patients with American cutaneous leishmaniasis (ACL), schistosomiasis or Chagas diseases, using potato apyrase as coating antigen in ELISA. The IgG antibody level against potato apyrase in serum samples from ACL (O.D. = 0.227 ± 0.082; P < 0.001) and schistosomiasis (O.D. = 0.161 ± 0.086; P < 0.05) patients, but not from Chagas disease patients (O.D. = 0.126 ± 0.038), was significantly higher than that found in healthy individuals (O.D. = 0.094 ± 0.020; control). The IgM antibody level against potato apyrase was similar between infected patient and healthy individual groups (control). IgG and IgM antibody levels of these serum samples diluted 1:200 were also evaluated against either the L. (V.) braziliensis promastigote preparation (Lb), derived antigens from T. cruzi epimastigote (Epi) or soluble egg (SEA) and adult worm (SWAP) antigens obtained from S. mansoni. In ACL patients, no significant difference was observed when their IgM or IgG antibody levels against Lb were compared to the respective control (healthy individuals). In schistosomiasis patients, the IgG or IgM antibody levels against SEA and SWAP showed values significantly higher (P < 0.001) than those found in controls. In serum samples from Chagas disease patients, IgG, but not IgM antibody, against the T. cruzi epimastigote preparation showed a value significantly (P < 0.001) higher than that found in controls (data not shown).

The results were then calculated as ELISA units. Medians, maximum and minimum values are shown in Figs 3 and 4. Significantly higher IgG antibody
Fig. 2. For legend see opposite page.
reactivity was found in sera from ACL patients (median 1·485), when compared to those from either schistosomiasis (median 0·879; *P*<0·05) or Chagas disease (median 0·637; *P*<0·001) patient groups. Sera from Chagas disease patients had low IgG antibody reactivity against potato apyrase, significantly (*P*<0·01) lower than that found in schistosomiasis patients (Fig. 3).

IgG and IgM antibody reactivities against potato apyrase found in serum diluted 1:200 from ACL patients were compared to that against the *L. (V.) braziliensis* promastigote preparation (Fig. 4 A and B). It was observed that the IgG reactivity against the potato apyrase (median 1·485) was significantly (*P*<0·001) higher than that observed for the parasite preparation (median 0·424). Curiously, the seropositivity analyses showed that 43% (*n*=9) and 90% (*n*=19) of the 21 ACL patients were reactive with the parasite preparation or potato apyrase respectively, suggesting that this shared epitope is highly effective in ACL patients (Fig. 4 A). On the other hand, IgM antibody reactivity was low with either the parasite preparation (median 0·020) or potato apyrase (B, median 0·678). Antibodies from only 1 (5%) patient reacted with the parasite preparation, while antibodies from 4 (19%) individuals reacted with potato apyrase (Fig. 4 B).

Soluble egg (SEA) and adult worm (SWAP) antigens obtained from *S. mansoni* were also used for comparative analyses of the IgG and IgM antibody reactivities from patients with schistosomiasis (Fig. 4 C and D). The sera diluted 1:200 from schistosomiasis patients showed a high and similar IgG antibody reactivity against both SWAP (median 1·942) and SEA (median 2·197), with 100% seropositivity for both preparations and was significantly higher (*P*<0·001) than that observed when potato apyrase was used as antigen (Fig. 4 C; median 0·879). In schistosomiasis, the IgG antibody reactivity against potato apyrase was observed in 39% (12/31) of the patients (Fig. 4 C). The IgM antibody reactivity against either SWAP (median 1·126; 68% seropositivity) or SEA (median 0·979; 42% seropositivity) showed similar results, and was significantly higher than that observed against potato apyrase (Fig. 4 D; median 0·610). In schistosomiasis, 10% (3/31) of the patients showed IgM antibody reactivity against potato apyrase (Fig. 4 D).

In addition, the IgG and IgM antibody reactivities against *T. cruzi* epimastigote derived antigens and potato apyrase were also evaluated (Fig. 4 E and F). The IgG seropositivity analyses showed that 97% (*n*=29) of the 30 Chagas disease patients were strongly reactive with the parasite preparation (median 3·224), while only 17% (*n*=5) of them showed a low and positive reactivity with potato apyrase, slightly above the threshold of 1 (Fig. 4 E; median 0·637). IgM antibody reactivity was low with either the parasite preparation (median 0·465) or potato apyrase (Fig. 4 F; median 0·323), and antibodies from only 1 (3%) patient reacted to these antigens (Fig. 4 F).

**DISCUSSION**

By evaluation of the cross-immunoreactivity with potato apyrase, we showed the possible occurrence of conserved domains as functional regions in parasite ATP diphosphohydrolases and the associated antigenicity of these proteins in human parasitic diseases. Two groups of ATP diphosphohydrolase isoforms were identified by phylogenetic analysis, which showed independent branches indicating 2 main different evolutionary pathways, possibly due to
ancient divergence. Our results reveal that there is a closer relationship between potato apyrase, *L. braziliensis* NDPase and *S. mansoni* SmATPDase 2 in the primary amino acid sequences, which show particular regions of high identity among them. Putative 3-dimensional models generated by us suggest that these regions may be exposed and available for antibody binding. In addition, prediction of

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**Fig. 4.** Comparative IgG or IgM antibody reactivity against different antigens. The IgG (A, C, E) or IgM (B, D, F) antibody reactivity was determined by ELISA, using potato apyrase (APY) as coating antigen and serum samples (diluted 1:200) from American cutaneous leishmaniasis (A, B), schistosomiasis (C, D) or Chagas disease (E, F) patients. The IgG or IgM antibody reactivity of serum samples was also determined using as coating the antigenic preparations of *Leishmania (V.) braziliensis* promastigote (LB), soluble adult worm (SWAP), egg (SEA) or *Trypanosoma cruzi* epimastigote (EPI). Antibody levels are expressed as ELISA units (U). The horizontal line represents the cut-off value. The statistical significance of group differences was determined using Mann-Whitney or Kruskal-Wallis test. *P* values are <0.01** and <0.001***.
Parasites and potato apyrases share antigenic domains

MHC Class-II binding peptides in *L. braziliensis* NDPase and *S. mansoni* SmATPase 2 showed regions, also shared with potato apyrase, with a high probability of eliciting a regulatory T cell immune response. Recently, we observed that potato apyrase stimulates *in vitro* the production of significant amounts of Th1 and Th2 cytokines by immune cells of *S. mansoni*-infected mice, suggesting that this hypothesis could be explored in humans (unpublished data). Hosts and parasites have co-evolved over thousands of years, and in mammals these parasites live for years using a wide range of mechanisms to evade and manipulate the host’s immune response (Requena et al. 2000; Dunne and Cooke, 2005). It is possible that specific ATP diphosphohydrolase regions were conserved between the different parasite species so far studied, and possibly in others. It may be related to the success of the parasitism, through host molecular mimicry and/or disease immunomodulation.

Our results suggest that these structural homologies occur, since ATP diphosphohydrolase epitopes of the parasite, shared with potato apyrase, are promptly recognized by IgGs from patients with different diseases, as observed in the ELISAs where this vegetable protein was utilized as antigen. Higher reactivity was found in the ACL patient group, followed by antibodies from individuals with schistosomiasis. These results concur with the sequence alignments, which showed higher identity between potato apyrase and *L. braziliensis* NDPase. Region B from these parasites is of particular interest, since it has lower homology with mammalian proteins and could be responsible for the antibody reactivity described here. In previous work we demonstrated by immunocytochemistry and confocal microscopy that rabbit polyclonal antibodies to potato apyrase recognize ATP diphosphohydrolase isoforms from *S. mansoni* egg, but not the mammalian NTPDases, suggesting the presence of unique motifs shared between the parasite and the vegetable protein, and that autoantibodies are not induced by potato apyrase immunization (Faria-Pinto et al. 2006).

Regions A, C, D and E, which include the characteristic conserved domains of the ATP diphosphohydrolase family (Handa and Guidotti, 1996; Vasconcelos et al. 1996) are potentially able to bind antibodies and/or HLA-DR molecules, which could induce an autoimmune response or elicit a regulatory T cell response. These regions, as well as Regions F and G, have high homology with human NTPDases. It is possible that self epitopes contained in these domains are unable to induce immune responses. On the other hand, it is interesting to note that 5 patients with Chagas disease have IgG seropositivity for potato apyrase. The Region B of the potato apyrase, shared with *L. (V.) braziliensis* NDPase or *S. mansoni* SmATPase 2, did not have significant identity with *T. cruzi* NTPDase. Since the antibody epitopes and HLA-DR predictions show regions in *T. cruzi* NTPDase shared with potato apyrase, potentially capable of inducing humoral and cellular immune responses, we believe that this low immune response should not be neglected. We believe that further investigations of larger populations will allow us to determine whether HLA type is correlated with the reactivity to apyrase and the development of the parasitic diseases here discussed. The highly distinct humoral immune response profiles of IgG antibodies from patients with ACL, schistosomiasis or Chagas disease, associated with parasite life-cycles, suggest also that these antigens are processed and presented to effector cells from the host immune system by different pathways. Since *T. cruzi* and *Leishmania* have antigens associated with autoimmune responses (Requena et al. 2000), the regions from parasite proteins identified in this work may be interesting targets for further investigation of their role on the immune response against the parasite infection.

Soluble egg (SEA) and adult worm (SWAP) antigens from *S. mansoni*, *L. (V.) braziliensis* promastigotes and *T. cruzi* epimastigote preparations are in some instances used in epidemiological studies (Makarova et al. 2003; Pedras et al. 2003; Gomes et al. 2005; Marques et al. 2006). Crude antigens are valuable for detection of general patterns in infected populations but, for comparative studies, use of serological surveys on single antigens permit a better definition not only of the humoral response but also of an analysis of the role of the antigen in inducing an effective cellular response (Mutapi, 2001). Furthermore, effective cross-immunity between different *Leishmania* species and the identification of shared and species-specific antigens could be useful for formulation of an anti-leishmanial vaccine (Kedzierski et al. 2006), and diagnostic methods among other investigational areas. Therefore, we consider that further studies of both parasite and potato apyrase domains indicated in this work, obtained by peptide synthesis or by cloning and heterologous expression, will allow comparison between the different infectious diseases and studies on the immunodominance of these epitopes. These domains could be relevant as molecular markers for the study of infected populations, or for the development of vaccines, and these experiments are being carried out in our laboratory.

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