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Commentary

Rationale for selection of developmentally regulated genes as vaccine candidates against *Leishmania infantum* infection

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The complex life cycles of parasites challenge elaboration of effective vaccines, as evidenced by the absence of a licensed vaccine against any human parasitic disease [1]. In the case of *Leishmania* spp., genome sequences are available, which makes possible the study of differential gene expression profiles in the main developmental processes of the parasite: growth and differentiation of promastigotes to a highly infective stage (metacyclogenesis) within the gut of the sand fly vector (*Phlebotomus* spp. or *Luztomyia* spp.) or in axenic culture, and development into the intraphagocytic amastigote stage within the mammalian host [2–6]. These data constitute a source of potential prophylactic molecules and drug targets. A set of developmentally regulated genes from *L. infantum* have been selected as vaccine candidates according to the rationale explained below.

Immunity against *Leishmania* spp. has been extensively described to be dependent of the T helper response triggered by MHC II-driven antigen presentation. Th1 has been associated to protection because the nitrosative response is activated for clearance of amastigotes, whereas Th2 is thought to lead to progression of the disease. The major cytokines involved in the immune response against the parasite are IL-12, IFN-γ, TNF-α (Th1) and IL10 (Th2). The dichotomy is clear in the *L. major* murine infection model [7]. However, a balanced Th1/Th2 response is observed in the canine and the human model [8,9]. In addition, the progression of the disease is also dependent on the individual response. Recent studies on visceral leishmaniasis have brought to light that T cell exhaustion and loss of spleen architecture are relevant factors for progression of chronic disease [10]. For these reasons, the complexity of the immune response against *Leishmania* spp. makes the selection of potential vaccine candidates difficult. In our opinion, considering *in silico* prediction of MHC II-binding epitopes and the intrinsic features of the parasitic proteins under study is important as explained next.

The rationale of vaccine candidate selection presented herein is based on these grounds: (i) the molecule must be visible to the immune system (through MHC II restriction in this case); (ii) sequence dissimilarity is essential to avoid immune tolerance [11]; and (iii) empirical association between certain biological functions and the protective ability of the molecule have been reported [12,13]. Hence, genes up-regulated in the infective stages of the parasite code potential vaccine candidates, which has been considered herein together with biological function, sequence dissimilarity and *in silico* MHC II binding predictions. TriTrypDB (www.tritrypdb.org) is a database that contains genome sequences of trypanosomatid parasites, their annotated genes, hyperlinks to

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Table 1
Vaccine candidates against *Leishmania infantum* infection. The TriTrypDB identifiers, names of protein products and differential gene expression profiles (DGEP) inferred with the Student's *t*-test ($p < 0.05$) are detailed. The references containing the exact fold-change and p-values and discussing these DGEP are cited in the right column (Ref.). Abbreviations: aa, amino acids; Amas, amastigotes; Pro-Pper, promastigotes isolated from the anterior midgut of the sand fly *P. perniciosus*; Pro-Log, cultured promastigotes isolated at the logarithmic phase; Pro-PNA$^+$, peanut lectin-agglutinating stationary phase promastigotes (procyclic); Pro-PNA$^-$, peanut lectin non-agglutinating stationary phase promastigotes; Pro-Stat, cultured promastigotes isolated at the stationary phase.

TriTrypDB Id.	Protein	DGEP ($p < 0.05$)	Ref.
LinJ.03.0540	Hypothetical protein (NCBI: surface antigen)	Amas/Pro-Stat	[2]
LinJ.05.0350	Trypanothione reductase	Amas/Pro-Stat	[2]
LinJ.05.1210	Surface antigen-like protein 2	Amas/Pro-Log	[2]
LinJ.07.0010$^{\#}$	Ubiquitin activating enzyme E1	Pro-Pper/Pro-Stat, Pro-Pper/Amas	[4,5]
LinJ.07.0150$^{\#}$	Mitochondrial acyl-CoA dehydrogenase	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.08.0680*	Amastin superfamily	Amas/Pro-Stat, Amas/Pro-Pper, Pro-Pper/Pro-Stat, Pro-PNA$^-$/Pro-PNA$^+$	[2–5]
LinJ.08.0830§	DNA polymerase beta	Pro-Stat/Pro-Log, Pro-Stat/Ama	[20]
LinJ.10.1370	Hypothetical protein, conserved	Amas/Pro-Pper	[4]
LinJ.12.0690$^{\#}$	Surface antigen protein 2 precursor	Pro-Pper/Pro-Stat	[5]
LinJ.14.1450	Myo-inositol 1-phosphate synthase	Amas/Pro-Stat	[2]
LinJ.15.0240$^{¥}$	Protein phosphatase 1 (PP1) N-terminal peptide (55 aa, 6.4 kDa)	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.16.0550$^{\#}$	Aspartate carbamoyl transferase	Amas/Pro-Log	[2]
LinJ.16.1070	Hypothetical protein, unknown function	Amas/Pro-Pper	[4]
LinJ.17.1520$^{\#}$	Otubain-like cysteine peptidase	Amas/Pro-Log	[2]
LinJ.20.1220	Calpain-like cysteine peptidase Clan CA, family C2	Pro-Pper/Pro-Stat	[5]
LinJ.22.1330	Hypothetical protein, conserved	Pro-Pper/Pro-Stat	[5]
LinJ.23.1170	Hypothetical protein, unknown function	Amas/Pro-Stat	[2]
LinJ.24.0770	DNA repair and recombination protein RAD54	Pro-Pper/Pro-Stat	[5]
LinJ.24.0910$^{□^{ca}}$	DNA polymerase theta, catalytic subunit	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.24.1460	Mismatch repair protein	Pro-Pper/Pro-Stat	[5]
LinJ.25.0080$^{\#}$	Poly(A)-binding protein 3 (PABP3)	Pro-Pper/Pro-Stat	[5]
LinJ.29.2420$^{\#}$	Enoyl-CoA hydratase/isomerase	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.31.0860	Triacylglycerol lipase	Pro-Pper/Pro-Stat	[5]
LinJ.31.1210	Hypothetical protein, conserved	Pro-Pper/Pro-Stat	[5]
LinJ.31.2210^{a}	Prostaglandin F2 alpha synthase	Pro-Pper/Pro-PNA$^-$	[6]
LinJ.31.2540	Lipase	Amas/Pro-Stat	[2]
LinJ.31.3070$^{\#}$	Repressor of differentiation kinase 2	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.31.3390$^{\#}$	Sodium stibogluconate resistance protein	Amas/Pro-Pper	[4]
LinJ.32.0470	Prostaglandin F synthase	Pro-Pper/Pro-PNA$^-$	[6]
LinJ.32.0500	Hypothetical protein, conserved	Pro-Pper/Pro-Stat	[5]
LinJ.32.1080	Protein kinase	Amas/Pro-Log	[2]
LinJ.32.1900$^{\#}$	Ser/Thr protein kinase A	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.32.3020	Hypothetical protein, unknown function	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.32.3820$^{\#}$	3-hydroxyisobutyryl-CoA hidrolase	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.32.4140	GIPL-Galf transferase (LPG1G)	Pro-Pper/Amas	[4]
LinJ.33.2430	UDP-glucose 4′-epimerase	Amas/Pro-Log	[2]
LinJ.33.2910$^{\#}$	Ubiquitin-conjugating enzyme	Pro-Pper/Pro-Stat, Pro-Pper/Amas	[4,5]
LinJ.34.0830$^{¥}$	Protein phosphatase 1 (PP1) N-terminal peptide (26 aa, 2.6 kDa)	Amas/Pro-Log, Amas/Pro-Stat	[2]
LinJ.34.0840$^{¥}$	Protein phosphatase 1 (PP1) N-terminal peptide (23 aa, 2.5 kDa)	Pro-Pper/Pro-Stat	[5]
LinJ.34.2680$^{\#}$	Protein kinase A regulatory subunit	Pro-Pper/Pro-Stat	[5]
LinJ.34.3740$^{\#}$	Expression site-associated glycoprotein (ESAG5)	Amas/Pro-Pper	[4]
LinJ.34.3770	Hypothetical protein, conserved	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.35.2580	Hypothetical protein, unknown function	Amas/Pro-Pper	[4]
LinJ.35.3080	Isoprenyl cysteine methyltransferase	Pro-Pper/Pro-Stat	[5]
LinJ.35.4200$^{\#}$	Poly(A)-binding protein 2 (PABP2)	Pro-Pper/Pro-Stat	[5]
LinJ.36.2050	DNA mismatch repair protein MSH6	Pro-Pper/Pro-Stat	[5]
LinJ.36.2090	Protein phosphatase 2B, catalytic subunit A2	Pro-Pper/Pro-Stat	[5]
LinJ.36.2490$^{□^{\times}}$	Tyrosine aminotransferase (TAT)	Pro-PNA$^-$/Pro-PNA$^+$	[3]
LinJ.36.4230$^{◆}$	Zinc carboxypeptidase, metalloexopeptidase	Pro-PNA$^-$/Pro-PNA$^+$, Pro-Pper/Pro-Stat	[3,5]
LinJ.36.6510$^{\#}$	Small G protein	Amas/Pro-Pper	[4]

Only one of the family members has been included in the immunoinformatic analysis as an example (*); genes already cloned in the expression plasmid vector pQE30 ($^{\#}$), pQE32 (§), pRSET-C ($^{□}$), pMAL-c2 ($^{○}$) and pAVA0421 ($^{◆}$) in *E. coli* for protein production and testing; peptides synthesized for testing ($^{¥}$); genes cloned in the pTEX (^{a}), pIR ($^{\times}$) trypanosomatid expression vectors.

functional databases (IUBMB EC, GO, PFAM, InterPro, etc.) and analysis tools. The immunoinformatic approach can be conducted with publicly available resources (IEDB, SYFPEITHI, etc.). TriTrypDB provides tools for prediction of transmembrane domains, signal peptides and epitopes. In this case, the IEDB resource was used (see below).

The set of 50 selected vaccine candidates up-regulated in the infective stages of the life cycle of the parasite is provided in Table 1. For example, the poly(A)-binding protein (PABP) LinJ.25.0080 (metacyclic promastigotes obtained from the sand fly anterior midgut) [5], the trypanothione reductase (TryR) and the aspartate carbamoyl transferase (ACT) (intraphagocytic amastigotes) [2]. Several candidates not included in Table 1 were filtered out according to dissimilarity to mammalian proteomes (sequence identity >30% and expect value <1e−10 cutoff in BLASTP alignments).

Functional criteria are more difficult to manage because there is no clear correlation between biological role and protective ability. In principle, priority may be assigned to proteins directly involved in differentiation, infectivity, virulence and resistance to oxidative and nitrosative stress because they are thought to protect the host against infection, as well as certain surface molecules. Empirical evidence also points to proteins involved in lipid metabolism and intracellular signaling as possible vaccine candidates [12,13]. However, none of these preferences guarantee correct election of the molecule. Surprisingly, non-membrane bound molecules like

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