Available online at www.sciencedirect.com
ScienceDirect
Molecular & Biochemical Parasitology 150 (2006) 236–248
MOLECULAR & BIOCHEMICAL PARASITOLOGY

Differential transcription profiles in *Trypanosoma cruzi* associated with clinical forms of Chagas disease: Maxicircle NADH dehydrogenase subunit 7 gene truncation in asymptomatic patient isolates☆

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Received 27 September 2005; received in revised form 18 August 2006; accepted 21 August 2006
Available online 15 September 2006

Abstract

The majority of individuals in the chronic phase of Chagas disease are asymptomatic (indeterminate form). Every year 2–3% of these individuals develop severe clinical manifestations (cardiac and digestive forms). In this study a *Trypanosoma cruzi* DNA microarray was used to compare the transcript profiles of six human isolates: three from asymptomatic and three from cardiac patients. Seven signals were expressed differentially between the two classes of isolates, including tryparedoxin, surface protease GP63, cyclophilin, some hypothetical proteins and the pre-edited maxicircle gene *NADH dehydrogenase subunit 7* (*ND7*). The approximately 30-fold greater signal in cardiac strains for *ND7* was the most pronounced of the group, and differential levels of pre-edited *ND7* transcript confirmed the microarray analysis. The *ND7* gene from asymptomatic isolates showed a deletion of 455 bp from nt 222 to nt 677 relative to *ND7* of the CL Brener reference strain. The *ND7* gene structure correlated with disease manifestation for 20 isolates from clinically characterised, chronic phase patients. The *ND7* lesion produces a truncated product that could impair the function of mitochondrial complex I. Possible links between the integrity of the electron transport chain and symptom presentation are discussed. We propose that *ND7* and other genes of the pathway constitute valuable targets for PCR assays in the differential diagnosis of the infective *T. cruzi* strain. While this hypothesis requires validation by the examination of additional recent parasite isolates from patients with defined pathologies, the identification of specific molecular markers represents a promising advance in the association between parasite genetics and disease pathology.

Keywords: DNA microarrays; Human strains; Diagnosis; NADH dehydrogenase subunit 7; Mitochondrial complex I; Tryparedoxin

1. Introduction

Chagas disease, caused by the protozoan *Trypanosoma cruzi*, affects 18 million people in Latin America. At present, a few hundred thousand seropositive individuals are estimated to live in the USA, Europe and Asia [1]. Chagas disease progresses through two successive stages: the acute phase and the chronic phase. The acute phase lasts 6–8 weeks and once it subsides most infected persons are asymptomatic. This presentation of the chronic phase is called the indeterminate form, and can persist indefinitely. In the majority of these individuals no abnormalities

Abbreviations: BER, Bayes error rate; COIII, cytochrome oxidase subunit III; ND7, NADH dehydrogenase subunit 7; EST, expression sequence tag; kDNA, kinetoplast DNA; LIT, liver infusion tryptose medium; TEUF and TENF, EST from a non-normalised (TEUF) and normalised (TENF) cDNA library of CL Brener epimastigotes

☆ *Note*: Nucleotide sequences reported in this paper have been submitted to the GenBank™ database with the accession numbers DQ663084–DQ663087.

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doi:10.1016/j.molbiopara.2006.08.008

are detected, while in others some functional alterations occur. The prognostic value of these alterations in the subsequent development of the chronic symptoms has not received appropriate attention. After several years or decades, 10–40% of the asymptomatic patients develop lesions in various organs, mainly the heart or digestive system. These presentations are called, respectively, the cardiac or digestive forms of Chagas disease [1].

The variability of symptoms and the geographical differences in the distribution of the chronic forms have been attributed to the diversity of *T. cruzi* strains. However the interplay with the immunogenetic background of the human host and the environment are also important for the outcome of Chagas disease. *T. cruzi* strains show substantial heterogeneity in biological and biochemical characteristics, which are resultant from a high genetic diversity (reviewed in [2]). Based on several DNA markers *T. cruzi* strains have been clustered into two major groups, which were named as *T. cruzi* I and *T. cruzi* II [3]. Further, *T. cruzi* II group has been divided into five subgroups IIa–IIe [4], several of which are the products of genetic fusions showing signs of recombination and heterozygosity [5]. Epidemiological studies suggest that *T. cruzi* I strains circulate in the silvatic cycle of the parasite transmission, whereas *T. cruzi* II strains are related to the domestic cycle in regions where Chagas disease is more severe [6].

In kinetoplastids the mitochondrial genome is represented by 20–50 maxicircles, which, together with thousands of minicircles, constitute a dense network, or kinetoplast (kDNA) that comprises approximately 15% of the total cellular DNA and thus represents an attractive diagnostic target. Genetic markers capable of differentiating *T. cruzi* II strains isolated from patients with cardiac and digestive forms were queried using a low-stringency single-primer PCR approach directed against the minicircle component of the kDNA, but it was not possible to identify a specific kDNA signature for strains isolated from a particular group of patients [7]. Minicircles carry the genes for a group of small transcripts referred to as guide RNAs that are involved in the Byzantine post-transcriptional process of RNA editing by uridine insertion and deletion [8]; due to the nature of their functional flexibility, these genes are largely transition tolerant, and may present hypervariability. The maxicircle genomes of the CL Brener and Esmeraldo strains have been assembled and annotated from data generated by the TIGR-SBRI-KI *T. cruzi* Sequencing Consortium [9]. Studies focusing on the phylogeny of the maxicircle relative to defined nuclear markers have defined three maxicircle clades [10,11] that are in agreement with an evolutionary schema proposed for *T. cruzi* including two major hybridisation events [5,9]. The functional constraints on maxicircle genes are also influenced by the RNA editing process, but these genes must maintain a higher degree of fidelity relative to the guide RNA genes.

One explanation for the distinct phenotypes and pathogenesis induced by *T. cruzi* strains may be the differential expression of particular genes. This hypothesis has been investigated in six strains classified into *T. cruzi* I and *T. cruzi* II groups by suppression subtractive hybridisation analysis [12]. Although diverse expression patterns were obtained for a few genes, no correlation between the gene expression and the classification of the strains was found. DNA microarray technology is a useful tool to discover new genes and to identify coordinated expression of genes. In a previous report, we demonstrated that despite the high genetic diversity of *T. cruzi* strains, DNA microarrays bearing predominantly expressed sequence tags (ESTs) of CL Brener are valid tools for comparative genomic studies and for the analysis of gene expression in this parasite [13]. In the present study a modified version of the microarray slide was constructed and used in the analysis of gene expression profiles in *T. cruzi* strains isolated from individuals presenting the indeterminate or the cardiac forms of Chagas disease. Among the DNA sequences differentially transcribed, the transcript from the mitochondrial maxicircle DNA-encoded gene for NADH dehydrogenase subunit 7 (*ND7*) was approximately 30-fold more abundant in the cardiac strains than in the asymptomatic strains. Detected differences were caused by a substantial deletion within *ND7*. The *ND7* locus was challenged by a set of *T. cruzi* isolates associated with characterised pathologies and maintained its validity. Thus the *ND7* gene may be a gateway for diagnostic and prognostic tests, and analysis of differentially expressed *T. cruzi* markers may advance the understanding of the basis of Chagas disease pathogenesis.

2. Materials and methods

2.1. Parasite stocks and cultivation

The characteristics of the *T. cruzi* strains employed in this study are summarised in Table 1. The strains were classified into *T. cruzi* I and *T. cruzi* II groups as previously described [14]. For the microarray experiments six Brazilian strains were analysed: the 115, B147, and B13-167 strains isolated from patients presenting severe electrocardiographic alterations [15], and the VL10, Famema, and Berenice 62 strains isolated from individuals with normal electrocardiograms and normal chest X-rays and considered to be asymptomatic (indeterminate form) (Table 1). The isolates were obtained by haemoculture in different years. In order to minimise parasite selection, positive blood cultures in liver infusion tryptose (LIT) medium with 10% fetal calf serum were maintained in individual tubes for a short period of time without passage. LIT medium was added every 10–15 days for a maximum of 8 weeks. Stocks were cryopreserved in liquid Nitrogen. For the validation of the PCR assay targeted at the *ND7* gene 25 isolates were analysed. The relevant information on reference strains was obtained from the literature (Table 1). These stocks were maintained in liquid Nitrogen (B.Z. laboratory) until use. Eleven human strains were isolated by haemoculture in the last 5 years from chronic patients from Minas Gerais (Brazil), who presented positive serology for Chagas disease (two tests). The clinical presentation of Chagas disease was meticulously assessed by one of us (E.D.G.) based on the results of electrocardiograms, echocardiograms, Holter monitoring, chest X-rays and radiology of the oesophagus and colon. This information will be published elsewhere. The patient characteristics are summarised in the lower section of Table 1. The same procedures described above were followed to minimise parasite selection. Pellets of 10^9 epimastigotes were frozen for DNA extraction.