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Brazilian studies on the genetics of *Schistosoma mansoni*

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Abstract

The parasite *Schistosoma* is known to exhibit variations among species, strains and genera, such as, the levels of infectivity, pathogenicity and immunogenicity. These factors may differ among parasite populations according to the local epidemiological conditions. Diversity observed in *S. mansoni* from different geographical regions or within individuals of the same region can be determined by differences in the genotype of each parasite strain. However, until recently, finding adequate genetic markers to investigate infectivity or other epidemiological characteristics of a transmission area proved difficult. Several studies have been conducted to evaluate the genetic variability of *S. mansoni*, using different techniques. Intraspecific variability was observed in morphological characters, isoenzyme studies, mtDNA, ribosomal gene probes, RAPD and microsatellites. The sequencing of the *S. mansoni* genome was the most important achievement concerning genetic approaches to the study of this parasite and may improve the development of drugs, vaccines and diagnostics of schistosomiasis. The knowledge of the genetic structure of schistosome populations in relation to epidemiological data and host variability is essential for the understanding of the epidemiology of the disease and the design of control strategies.

Geographical genetic variation of schistosomes is reflected in several biological characteristics of the species and is also relevant for the epidemiology of the disease (Le et al., 2000). There is evidence that *Schistosoma* exhibit variations not only among species, but also among strains and between males and females, other than the obvious sexual characteristics (McCutchan et al., 1984), associated with variations in the levels of infectivity, pathogenicity and immunogenicity (Rollinson et al., 1997). These factors may differ among parasite populations according to the local epidemiological conditions (Sire et al., 1999). Therefore, parasites variability can be influenced by spatial heterogeneity in host exposition and susceptibility to infection, host species present in a transmission area, population dynamics and behavior of the hosts. Geographic patterns in the pathology of schistosomiasis have been observed. However, they are frequently explained by the differences in the transmission intensity among foci, although some of the disease manifestations may be directly related to the parasite genetics (Curtis et al., 2000). Furthermore, genetic differences among geographically separated strains cannot be evaluated without taking into account differences in a local scale, such as, within an endemic locality or municipality.

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Diversity found in *S. mansoni* from different geographical regions or within individuals of the same region can be determined by differences in the genotype of each parasite strain (Fletcher et al., 1981b). However, until recently, finding adequate genetic markers to investigate infectivity or other epidemiological characteristics of a transmission area proved difficult. The parasite genome recently sequenced (Haas et al., 2007) contains 8 chromosome pairs, with one heterologous pair, WZ (Short and Menzel, 1979; Grossman et al., 1980) and contains a large number of polymorphic markers such as microsatellites and SNPs (Simões et al., 2007).

Several studies have been conducted to evaluate the genetic variability of *S. mansoni*, using different techniques. Intraspecific variability was observed in morphological characters of eggs, cercarial shedding pattern and adult worms (Kastner et al., 1975; Machado-Silva et al., 1995; Théron et al., 1995; Bogea et al., 1996; Machado-Silva et al., 1998; Neves et al., 1998). Isoenzymes were the first genotypic markers used to detect variability in *S. mansoni* strains (Fletcher et al., 1981a; Navarro et al., 1992). After the development of the Polymerase Chain Reaction (PCR) technique in the end of the 80's, several molecular markers were used to access the genetic variability of *S. mansoni*, such as mtDNA (Despres et al., 1991; Despres et al., 1993; Pena et al., 1995; Blair et al., 1999; Le et al., 2000; Morgan et al., 2005), ribosomal gene probes (Vieira et al., 1991), RAPD (Barral et al., 1993; Dias et al., 1993a; Barral et al., 1996; Sire et al., 1999) and microsatellites (Durand et al., 2000; Rodrigues et al., 2002a; Rodrigues et al., 2002b).

Here we describe the most important results obtained in the study of the genetics of *S. mansoni* carried out with Brazilian strains using different techniques.

Genotypic variations may result in morphological intraspecific polymorphisms observed among individuals or populations. Before the advent of genetic techniques, morphological criteria were commonly used to elucidate taxonomic questions and in phylogenetic studies. Morphometric studies of *S. mansoni* adult worms in Brazil compared different strains (Paraense et al., 1963; Magalhães et al., 1973; Machado-Silva et al., 1995) or worms recovered from several host species using bright field, electronic or confocal microscopy (Dias et al., 1980; Machado-Silva et al., 1998; Neves et al., 1998). The first study used morphometric characteristics to detect the existence of different *S. mansoni* strains in Brazil, each related to a different species of intermediary host (*B. glabrata* and *B. tenagophila*), was conducted by Paraense & Corrêa (1963). Later, a similar comparison was performed by the same authors confirming a host-parasite adjustment of *S. mansoni* to different hosts, showing intraspecific variations in the length of adult worms, size of eggs, the duration of the prepatency period and in the infection rates. The different strains also produced viable offspring able to infect different hosts (Paraense et al., 1981). Magalhães & Carvalho (1973), studied the same strains and observed differences in the length of the worms, in the distances between the oral and ventral suckers and in the number of testes. However, we must be careful in the interpretations using morphological criteria because morphologic variations can also be attributed to environment.

Considering the wild reservoirs of *S. mansoni*, a few studies compared the morphology between strains isolated from human infections and rodent infections. Dias e Piedrabuena (1980) studied the morphology of *S. mansoni* eggs and worms recovered from the rodent *Holochilus brasiliensis* and observed no differences when compared to the typical description of *S. mansoni*. Machado-Silva et al. (1994) compared adult worms recovered from experimentally infected mice to those recovered from the wild water-rat *Nectomys squamipes* naturally infected in the Sumidouro Municipality, Rio de Janeiro State. The worms obtained from wild infections had larger body length and smaller testes than the laboratory strain. Similar observations were made in relation to human isolates. The authors concluded that the morphology of adult worms could be used to characterize *S. mansoni* strains among different hosts (Machado-Silva et al., 1995). Human isolates were also observed to display differences

in the testicular lobes and in the morphology of the eggs when compared to worms recovered from the wild reservoir *Nectomys squamipes* (Neves et al., 1998).

Genotyping studies were first conducted with the use of polymorphic enzymes, isoenzymes (Parker et al., 1998). Isoenzymes are the multiple molecular forms of the same enzyme of a species, as a result of the presence of more than one allele of a gene coding for the protein. Isoenzymes present co-dominant expression, and it is one of the most accessible genetic techniques. The main limitations of the method are the number of loci that can be detected and the number of alleles per locus. Thus, they are not the markers of choice for investigating large genomes.

Enzyme studies of *S. mansoni* have been performed since the 60's (Conde del Pino et al., 1966; Conde del Pino et al., 1968; Coles, 1970; Coles, 1971). However, the first report on enzyme polymorphism in *S. mansoni* based on single worms was a study by Fletcher et al. (1981b), that used samples from Africa, Asia and America, including Brazilian strains from Bahia. The authors found seven polymorphic loci among 18 analyzed in several populations of *S. mansoni*, although presenting low genetic variability between African and American strains. Those results were the first genetic evaluations which supported the theory that *S. mansoni* was introduced in the Americas from Africa. Navarro et al., (1992) used isoenzymes to compare Brazilian and Venezuelan strains of *S. mansoni* and found no intrastrain variation, but observed variation in the isoenzyme patterns among the strains.

Before the availability of a large number of cDNA or genomic sequences, polymorphism in the genome of *S. mansoni* was explored by the random amplification of polymorphic DNA (RAPD), a method that permits the annealing of primers under lower stringency conditions to genomic DNA and the amplification of regions where primers annealed at opposite strands in close proximity (Dias et al., 1993a). The use of this methodology provided some insight into the genomic diversity of *S. mansoni*, but a very low level of polymorphism was observed (Dias et al., 1993b). Low Stringency Single Specific Primer PCR also yielded similar results (Simpson et al., 1995). One alternative approach to low stringency amplification of genomic DNA was the use of the more polymorphic mitochondrial DNA.

The exploration of the mitochondrial and nuclear genomes provided additional tools for the investigation of the genetic variability of schistosomes. The mitochondrial DNA (mtDNA) of *S. mansoni* has been sequenced (NC_002545) (Le et al., 2001). The mitochondrial genome contains 36 genes being 12 protein coding. Phylogeographic analysis of *S. mansoni* using mtDNA as a genetic marker from eight Brazilian localities showed very little variation among them. This observation may have resulted from a severe reduction in mitochondrial diversity at the time of establishment of the parasite in Brazil, and few mutations would be expected to be fixed in 500 years, since the introduction of the species (Galtier et al., 2000; Morgan et al., 2005). Although mitochondrial genetic markers have been widely used for genetic studies of populations, it has been demonstrated that in *Schistosoma* it is not the preferred marker due to the low level of polymorphism (Curtis et al., 2001). DNA markers of genomic origin with a higher level of polymorphisms were required.

The genome size of *S. mansoni* is estimated to be 270 Mbp (Simpson et al., 1982; Marx et al., 2000). The genome is 40% repetitive and contains typical telomeres (Simpson et al., 1982; Moyzis et al., 1988). The sequence of the genome has been completed and it is currently under the annotation process (Haas et al., 2007). In addition to the genome sequence, over 205,000 expressed sequence tags (ESTs, short sequence segments from cloned cDNAs) are available (Verjovski-Almeida et al., 2003; Oliveira et al., 2004; Oliveira et al., this issue).

One type of polymorphic markers that are widely used for genetic analysis is microsatellites. Microsatellites are short sequences (1-6 bp) repeated in tandem. Microsatellites are widely

distributed in eukaryotic genomes and are generally neutral and inherited in a co-dominant fashion (Tautz et al., 1984; Ashley et al., 1994). Microsatellite repeats are polymorphic due to the variation in the number of tandem repeats among individuals, making it one of the most useful markers for genotyping and population studies (Schlotterer, 2000). Microsatellites have been increasingly used to assess the genetic structure of *S. mansoni* populations. A large number of microsatellites have been developed for *S. mansoni* (Rodrigues et al., 2002a; Rodrigues et al., 2002b; Rodrigues et al., 2007). In Brazil, the use of this type of marker has demonstrated that field isolates are more diverse than laboratory strains (Rodrigues et al., 2002a). Interestingly, published and unpublished data frequently indicate a lower than expected level of heterozygosity (Rodrigues et al., 2002a; Rodrigues et al., 2002b; Rodrigues et al., 2007). One possible interpretation for this observation is that infected individuals sample only a fraction of the available parasite genotypes. It was also recently shown with microsatellite markers that upon drug pressure parasite diversity decreases (Oliveira, unpublished). Currently, the variation of population structure at transmission sites upon mass chemotherapy is under evaluation (Oliveira, unpublished). Other useful genomic markers have also been explored.

Recently, a survey of the EST dataset revealed a large number of single nucleotide polymorphisms (SNPs) (Simões et al., 2007). SNPs occur in the genome with a greater frequency than microsatellites. Several genes, including those coding for vaccine candidates were shown to be polymorphic. In addition, the frequency of polymorphism was higher in isolates from endemic sites in contrast to laboratory strains. The higher variability of wild populations, made with the use of microsatellites and SNPs, are relevant for the development of vaccines. As usually, only laboratory strains are investigated.

Although there are several studies on the genetic variability of *S. mansoni*, there are few quantitative evaluations of the genetic diversity of this parasite in naturally infected non-human host populations. *S. mansoni* geographic distribution comprises three continents and a wide range of host species beyond humans. For this reason, schistosomes are expected to present a large amount of genetic variability resulting in different strains among populations, adapted to a wide spectrum of environments and microhabitats according to the host species. As host species may present intraspecific differences in the susceptibility and maintenance of the parasite, infrapopulation variations of the parasite may also occur.

Some rodent species, especially the water-rats, constitute an important group of mammals which has an important role in the transmission of the schistosomiasis as wild reservoirs and are able to maintain the transmission without human presence (Picot, 1992; Theron et al., 1992; Maldonado et al., 1994; D'Andrea et al., 2000; Gentile et al., 2006; Edgardo Borda et al., 2006). Studies with molecular DNA markers are currently being conducted in parasites recovered from the water-rat. (Gentile, unpublished).

Considering the intermediary host, few studies evaluated the genetic diversity of the *S. mansoni* within molluscan populations. Minchella et al., (1995) observed a diverse array of *S. mansoni* genotypes obtained from naturally infected *B. glabrata* in Belo Horizonte using hybridization of southern blots to a polymorphic repetitive DNA element. They concluded that dispersion combined with high levels of genetic variability facilitates multi-genotype transmission and helps maintain the parasite genetic diversity. This result was contrasting to those observed in Guadeloupe (Sire et al., 1999; Sire et al., 2001).

Conclusion

The advances in molecular biology have made a dramatic impact on the understanding of parasitic organisms which are responsible for several human diseases. The history of host-

parasite relationship, radiation and adaptation to local conditions became clearer with studies on genetic variability of the parasites.

Several aspects of molecular parasitology have an impact on the control of schistosomiasis. Such studies have been providing the basic knowledge to support the development and improvement of drugs for treatment, vaccines and diagnostic techniques. The genome sequences are the major recent advances for molecular studies of schistosomes (Haas et al., 2007; McManus et al., 2004).

Different natural populations of *S. mansoni* adapt to local conditions of the environment concerning not only abiotic parameters, but also the host species, and the characteristics of the host. Populations of the parasite may develop particular host-parasite interactions characteristic, which may result in different clinical histories of the disease. Therefore, control actions must consider local characteristics in order to produce efficient results for the reduction of transmission. Non-human hosts must also be taken into account, as they may significantly contribute to the genetic variability of the parasite, resulting in different profiles of the disease in endemic areas and isolated foci. Gentile et al. (2006) observed that water-rats may present different levels of importance in the transmission dynamics of *S. mansoni* infection cycle at distinct endemic sites, and can be considered important wild-reservoirs of this human disease.

For all these reasons, the knowledge of the genetic structure of parasite populations, in the context of epidemiological, ecological and geographical data, associated with educational and public health political programs, is of major importance for understanding the epidemiology of the disease and for the design of control strategies.

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