Integrated Genetic Epidemiology of Infectious Diseases: The Chagas Model

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Genetic typing of pathogenic agents and of vectors has known impressive developments in the last 10 years, thanks to the progresses of molecular biology, and to the contribution of the concepts of evolutionary genetics. Moreover, we know more and more on the genetic susceptibility of man to infectious diseases. I propose here to settle a new, synthetic field of research, which I call 'integrated genetic epidemiology of infectious diseases' (IGEID). I aim at evaluating, by an evolutionary genetic approach, the respective impact, on the transmission and pathogenicity of infectious diseases, of the host's, the pathogen's and the vector's genetic diversity, and their possible interactions (co-evolution phenomena). Chagas' disease constitutes a fine model to develop the IGEID methodology, by both field and experimental studies.

Key words: co-evolution - genetic typing - evolutionary genetics - *Trypanosoma cruzi*

Genetic studies dealing with infectious agents, vectors and hosts (for example: genetic susceptibility of man to infectious diseases) have developed until now separately, in a compartmentalized manner. Nevertheless, in an evolutionary point of view, the three actors of infectious disease transmission (the pathogen, the host, and in the case of vector-borne diseases, the vector) have evolved together, and should be considered as the three linked components of a unique phenomenon of co-evolution. When the host evolves (for example, develops specific immune defenses to escape from the damage caused by the pathogen), it shapes in return the evolution and the genetic diversity of the pathogen. It is therefore distressing to analyze separately these three components. I have proposed (Tibayrenc 1998a, b) to settle a new, synthetic field of research, the 'integrated genetic epidemiology of infectious diseases' (IGEID), that will take into account simultaneously the impact, on the transmission and pathogenicity of infectious diseases, of the host's, the pathogen's and the vector's genetic diversity, as well as the interactions (phenomena of co-evolution) of these three parameters. I will advocate here that Chagas' disease constitutes a fine model for throwing the first bases of this ambitious approach.

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WHAT ABOUT THE GENETIC DIVERSITY OF *TRYPANOSOMA CRUZI*?

If we consider the putative impact of the host's, the pathogen's and the vectors' genetic diversity on the transmission and pathogenicity of Chagas' disease, there is little doubt that the best known element is *T. cruzi* genetic variability. Many studies have been published on this theme, and it is possible that *T. cruzi* is one of the pathogenic agents which evolutionary genetics is the best explored. Main results can be briefly summarized as follows: *T. cruzi* natural populations show considerable genetic polymorphism, as revealed by isoenzyme electrophoresis (Miles et al. 1978), kDNA RFLP analysis (Morel et al. 1980) and RAPD (Tibayrenc et al. 1993). The most parsimonious hypothesis to account for this huge genetic polymorphism is that it is the result of long-term clonal evolution with possible occasional bouts of genetic exchange (Tibayrenc et al. 1986, Tibayrenc & Ayala 1988). Recently, these suspected recombinant genotypes have been more precisely characterized as stable hybrid lines, that would propagate clonally after the hybridization event (Bogliolo et al. 1996, Carrasco et al. 1996, Brisse et al. 1998). Among the natural clones of *T. cruzi*, some are widespread and more frequently sampled. They have been given the name of 'major clones' (Tibayrenc & Ayala 1988), since it can be suspected that their epidemiological and pathogenic relevance is considerable. It is most probable that these 'clonal genotypes' identified by a limited set of genetic markers do not correspond to real clones, but rather, tho families of closely related clones. We have pro-

posed (Tibayrenc & Ayala 1991) the term of 'clonet' to refer to sets of stocks that appear identical for a given set of genetic markers in a clonal species. *T. cruzi* clonets are distributed into two main phylogenetic lineages within each of which genetic diversity remains considerable (Tibayrenc 1995, Souto et al. 1996). The second main phylogenetic lineage of *T. cruzi* appears as structured into five lesser subdivisions (Brisse et al. 1998), of which some correspond to either hybrid lines or to formerly identified 'major clones' (Tibayrenc & Ayala 1988) or both. By comparison with other pathogens, *T. cruzi* population structure can be defined as follows: it is a clonal species (Tibayrenc et al. 1986) that is structured into durable genetic subdivisions ('discrete typing units' or DTUs; Tibayrenc 1998a, b). The whole species *T. cruzi* is a DTU, as well as its main and lesser genetic subdivisions. All these DTUs can be characterized by specific genetic markers or 'tags' (Tibayrenc 1998a, b). To some extent, *T. cruzi* DTUs and tags can be equated respectively to monophyletic lineages (clades) and synapomorphic characters, although a strict cladistic approach is difficult here, due to the existence of occasional hybridization events. Still the fact remains that *T. cruzi* overall intraspecific phylogeny appears as robust, considering the strong agreement between the species phylogenies generated by independent sets of genetic markers: isoenzymes and RAPDs (Tibayrenc et al. 1993), and microsatellites (Macedo & Pena, pers. comm.). This striking concordance between three different kinds of genetic markers is clear evidence that the strong genetic distances recorded within *T. cruzi* are due to a real evolutionary divergence rather than to individual genetic diversity within a hypothetical, recent ancestral sexual species, as formerly envisaged (Tibayrenc et al. 1984). It is reasonable to expect that the evolutionary divergence accumulated between *T. cruzi* clonal lineages involves also those genes that govern relevant medical properties such as virulence or resistance to drugs. A possible link between *T. cruzi* genetic variability and Chagas'disease clinical diversity has been suspected by Miles et al. (1981). Montanat et al. (1996) have recently corroborated this hypothesis. Long-term experiments performed in our laboratory show a clear correlation between evolutionary divergence among *T. cruzi* clonal lineages and amount of differences for relevant biological properties such as pathogenicity in mice, *in vitro* drug sensitivity or culture growth speed (Laurent et al. 1997, Pinto et al. 1998, Revollo et al. 1998, De Lana et al. 1998). Certain experiments suggest an interaction between clonal genotypes in artificial mixtures (De Lana et al. 1998). Macedo and Pena (1998) have recently proposed a 'clonal-hystotropic model', which states that *T. cruzi* clonal genotypes infecting the same host have each a specific tropism for given organs. These proposals as well as our results dealing with interactions of clonal genotypes lead to consider that the idea: 'one strain, one pathology' is possibly too simplistic. Still the fact remains that convergent lines of results suggest a profound impact of the phylogenetic diversity of *T. cruzi* natural clones on their relevant biomedical properties.

For studies dealing with the integrated genetic epidemiology, *T. cruzi c*onstitutes an ideal model, for it is clearly subdivided into clear-cut discrete entities: upper and lesser DTUs, and at a lower level of phylogenetic divergence, the natural clones. The RAPD technique is an abundant source of markers for designing probes and PCR diagnoses specific of either DTUs or natural clones. These specific molecular tools can be conveniently used in the context of integrated genetic epidemiology of Chagas' disease.

THE VECTOR

Although less known than the parasite's genetic diversity, triatomine bugs have been the material for various evolutionary genetic analyses. These studies were based mainly on multilocus enzyme electrophoresis, and have focused either on the intraspecific level (population genetics analysis; Tibayrenc et al. 1981a, b, Dujardin & Tibayrenc 1985, Dujardin et al. 1998) or on between-species comparisons (phylogenetic analysis; Pereira et al. 1996, Solano et al. 1996). These data provide a fine starting basis to include the study of the vector in the integrated genetic epidemiology of Chagas'disease. Nevertheless, it will be necessary to complement isoenzyme typing with more modern molecular tools such as RAPDs or microsatellites, in order to increase the resolution power of triatomine bug genetic characterization.

THE HOST

From the genetic point of view, of the three links of Chagas transmission chain, man is the less known. As a matter of fact, contrary to other parasitic diseases such as malaria or schistosomiasis (Abel & Dessein 1997), nothing is known about possible human genetic susceptibility to Chagas'disease and its different clinical forms. Now the genetic variability of the human species has been widely explored (HLA and microsatellite typing, gene mapping), which should make easier to explore the parameter of host genetic susceptibility in the specific case of Chagas' disease.

INTEGRATED GENETIC EPIDEMIOLOGY OF CHAGAS' DISEASE: EXPERIMENTAL APPROACH

Chagas' disease constitutes a very fine model for experimental studies, since it is possible to establish a complete artificial cycle in the laboratory. The parasite is relatively easy to culture, under epimastigote, trypomastigote and amastigote forms. Rearing the vector is easy too, including through artificial feeding devices, which makes it easier to monitor the experimental parameters (Pinto et al. 1998). Lastly, many mammiferous models (mainly mice) can be used as vertebrate hosts. The principle of an experimental approach of integrated genetic epidemiology is to have only one parameter vary at the same time, while the two other ones are kept as constant as possible. For example, if the impact on Chagas' disease of *T. cruzi* is to be explored (either with pure clonal genotypes or artificial mixtures of genotypes), homogenous triatomine bug and mice strains will be used. When the influence of the vector is explored (both at the level of subspecific and interspecific variability), this will be done, in a given experiment, with only one *T. cruzi* clonal genotype and with a unique mouse strain. Lastly, when the host is considered, various populations of a given strain and various strains (males and females) will be used with the same *T. cruzi* clonal genotype and the same triatomine bug strain. Apart from the empirical observation of the respective impact of the host's, the vector's and the pathogen's genetic diversity on Chagas transmission and pathogenicity, it will be possible to identify the genes that are implied in the infectious process, and to analyze gene regulation phenomena through the analysis of mRNAs with the RNA AP-PCR technique (Welsh et al. 1992). For example, it will be possible to analyse gene expression of given *T. cruzi* clonal genotypes (amastigote, epimastigote and trypomastigote forms) before and after passage through given vector and host populations, or before and after infection of cell cultures, or to compare infected vs non-infected cardiac or digestive cells of dissected mice. Again in these RNA AP-PCR analyses, only one parameter will be allowed to vary at a given time, while the other ones are kept as constant as possible.

FIELD STUDIES

The experimental step is the easiest one to master, and is indispensable. Nevertheless, it definitely has to be completed with a more ambitious approach, which is field studies. This involves the joint analysis of man, triatomine bug and parasite populations. When man is considered, the now well-codified screening with microsatellite markers will have to be used. This makes it possible, through the study of families and control, Chagas-free, populations, to look for possible associations between given parts of the human genome and susceptibility to Chagas' disease and its various clinical forms, through a statistical analysis of linkage disequilibrium. In the same time, isolation of *T. cruzi* stocks from the same populations of patients gives the opportunity to explore possible associations between *T. cruzi* clonal genotypes and clinical forms of Chagas' disease. Lastly, the joint analysis of the genetic variability of triatomine bug populations and of the *T. cruzi* stocks isolated from them could make it possible to increase by far the level of resolution of genetic epidemiological tracking. As a matter of fact, genetic evolution of the vector and of the parasite do not have the same patterns and the same speed, although they are linked. They can give therefore non-redundant, complementary indications on the spread of Chagas'disease epidemics.

CONCLUSION; PERSPECTIVES

IGEID is a very ambitious endeavor, that will need the joint efforts of many different teams having complementary expertises. These various competences are difficult to find in only one country. For these reasons, it is a typical field of research that should be launched in the recently-proposed project of 'European Centre for Control of Infectious Diseases' (ECCID; Tibayrenc 1997a, b). The Chagas model gives the opportunity to launch the first bases of this approach, with the advantages of easy-to-master experimental protocols, and aboundant amount of knowldege on the genetic diversity of the pathogen, and, to a lesser extent, of the vector. The general methodologies developed for Chagas'disease will be applicable to a large extent to other infectious models, especially to the ones that involve related parasites (*Leishmania* and African trypanosomes). The comparative approach advocated for in the case of evolutionary genetics of pathogens (Tibayrenc 1995, 1996) should be retained for IGEID. Indeed, only a comparative IGEID approach will permit to draw the general laws that govern pathogen/host/vector coevolution, and in the same time, to enlighten the specificities of each model.

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