

ASPECTS OF SUCCESSFUL HOST-PARASITE ASSOCIATION: OPOSSUM AND *TRYPANOSOMA CRUZI*.

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A host-parasite relationship may be qualified as successful when it grants the survival of both partners. This means that neither organism threatens the life of the other in the course of their joint existence and that, at the same time, each one has assured its permanence as species. This type of balanced relationship is the consequence of a process of co-evolution and indicates an ancient association.

The parasitism of opossums by *Trypanosoma cruzi* may be taken as an example of such a successful relationship, as we shall see.

It is now a well established fact that natural populations of *T. cruzi* are highly heterogeneous and may include any number of sub-populations with different biological and biochemical characters^{1,2,3}. Different selective pressures may favour one or more such sub-populations in detriment of others^{4,5}.

The work with opossums started with the idea of investigating how *T. cruzi* populations behave in these most important of all known reservoirs of the agent of Chagas' disease.

The first thing was to study, in the opossum *Didelphis marsupialis*, the course of experimental infections by some old "laboratory strains" of *T. cruzi* of which the behaviour in mice is well known^{6,7,8}. The strains chosen were Y, F and Fl. Various schemes of inoculation were followed, using either one strain or two strains in succession after various intervals, or two strains in equivalent numbers of trypomastigotes in the same syringe.

These were followed by another series of experimental infections with wild strains recently isolated from naturally infected opossums, the G-N (studied by Yoshida⁹) and G-49, both producing only sub-patent infections in mice.

The data here presented are the result of the work of a team of researchers and students of the Dept. of Protozoology, with the collaboration of other Depts. of the Inst. Oswaldo Cruz.

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In all cases the opossums were either born in captivity or reared from pouch young captured with their mother. The follow-up was by fresh blood examinations, xenodiagnoses, hemocultures and immunofluorescent antibody tests (IFAT)¹⁰, made at monthly intervals. Throughout the experiments the reisolated parasites were identified by their zymodemes and schizodemes.

Results may be summarized as follows^{11,12,13}.

1. Since very early age - 75 days, 45 g, i.e, as sucklings still strictly marsupium dependent, opossums are able to control chagasic infection, even after receiving high inocula (10^3 /g b.w.) of bloodstream trypomastigotes. The only deaths occurred in 24 day, 2.4 g babies just passing the embryonic stage; even so their survival was longer than among adult mice with equivalent inocula.

2. The strains most virulent for mice, Y and F1, produced only light and transient infections in the opossum; parasitological examinations soon became negative and IFAT titres were low and tended to negativation. The eventual complete elimination of these strains was ascertained by repeated blood sub-inoculations in mice and by gamma irradiation.

3. Early elimination of the Y strain was also evident in the experiments with mixed infections, whatever the scheme of inoculations; indeed the strain did not even appear in reisolates when it had been inoculated after the F strain.

4. Infections with the F strain and with the opossum strains G.N and G.49 resulted in patent parasitemia for the first 2-3 months, xenodiagnoses and hemocultures positive and high IFAT titres throughout the periods of observation which were over 2 years in some cases.

A number of natural infections were accompanied in the laboratory and found to follow the same pattern displayed by the F, G.N and G.49 strains. It should be mentioned here that all the isolates of *T. cruzi* from opossums that we have examined so far belong in the same zymodeme and schizodeme and have a similar behaviour in mice.

Up to this point it was quite clear that the opossum is able to eliminate certain sub-populations (or strains) of *T. cruzi*, while keeping others indefinitely. However, even among the stable, lasting infections there were cases in which the parasite seemed to disappear and reappear later, sometimes after quite long periods while IFAT titres were main-

tained at high levels. Very detailed post-mortem examinations of such cases revealed the possible explanation for the discordant results - the presence of *T. cruzi* multiplying as extracellular epimastigotes and transforming into metacyclic trypomastigotes in the lumen of the anal scent glands of the opossum^{14, 15}.

Later, another trypanosome, this time a *Megatrypanum*, *T. (M.) freitasi*, was found undergoing a similar double cycle in naturally infected opossums^{16, 17} and the natural occurrence of *T. cruzi* in the scent glands was reported by various investigators, in *Didelphis marsupialis* and *D. albiventris*^{18, 19, 20}.

The biological and epidemiological implications of these findings have been discussed²¹. The accumulated data indicate that the presence of *T. cruzi* in the opossum scent glands is uncommon in Nature, contrarily to what occurs in experimental infections.

Many questions arise about the factors that are instrumental in leading *T. cruzi* to colonize the glands. It might be a matter of strain or clones within a strain; however in our experimental infections strains obtained from naturally infected opossums were used. It might be a problem related to size of inocula which are probably artificially high in experimental infections; indeed, when comparing two series of experiments, with low and high inocula, and subsequent sub-patent or high parasitemia we found the glands negative in the first group and a high rate of gland involvement in the latter; yet the glands are colonized by *T. freitasi* that produces only very low and intermittent parasitemia

To investigate if the exclusive presence of parasites in the glands could account for the highly positive serological tests aforementioned and, at the same time if the glandular environment could support the growth of other flagellate species, the monogenetic insect trypanosomatids, *Crithidia deanei*, *Leptomonas samueli* and *Herpetomonas samuelpeessoai* were injected directly in the glands lumen. All three yielded large and lasting populations, as in axenic culture media; as expected, repeated hemocultures of the inoculated opossums were negative. However, within 10 days IFAT started to give positive results at significant titres with the specific antigens.

This was the first time that infections of a mammal by monogenetic trypanosomatids were reported^{22, 23} and it was suggested that scent glands could have been an intermediate step between the digestive tract

and the tissues of mammalian hosts in the adaptative pathway of digenetic species²⁴.

Of course arguments can be offered against the above speculations. It could be argued that, once settled in the host tissues, the trypanosomes may invade the glands as they can invade any other territory, and the type of cycle they perform in the glands is simply favoured by such factors as temperature and the coincidental presence of adequate nutrients. Colonization of the glands would thus be secondary to a previous adaptation of the parasite to the mammal host.

Anyway, two things are quite clear:

1. The glands offer excellent conditions for the maintenance of trypanosomatids that spend their whole life-cycle, or part of it, in the intestinal tract of insects;

2. The glands offer a good protection for trypanosomes that, as *T. (M.) freitasi*, seem to have been unable to develop evasion mechanisms against the immune defences of the host.

In a later experiment, the Y strain of *T. cruzi* was injected directly in the opossum scent glands; a positive hemoculture was obtained soon after but all subsequent parasitological tests were negative, indicating that the involvement of the host tissues had been transient, exactly as we had seen after subcutaneous inoculation of the strain. However, IFAT was consistently positive at high titres and the trypanosome continued to multiply as epimastigotes in the glands for at least one year. This was one more proof that, inside the glands, flagellates - mono or digenetic - are entirely protected from the host defences.

Finally, the findings here reported, give support to our introductory statement, that parasitism of the opossum by *T. cruzi* is an example of a balanced and, most probably, very ancient association. Indeed, the 70 million years old didelphid marsupials seem to support quite well the presence of the trypanosome, at the same time being able to eliminate from their tissues the more virulent clones of the parasite. On the other side, for the parasite, permanence in the host is guaranteed, and even a refuge for its more virulent progeny when it is expelled from the host tissues. Furthermore, transmission of the parasite to new hosts is granted by two different means - the insect vectors and the scent glands secretion.

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