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CENTRAL NERVOUS SYSTEM INVOLVEMENT IN EXPERIMENTAL TRYPANOSOMIASIS CRUZI

JOSÉ EYMARD HOMEM PITTELLA

Laboratório de Neuropatologia, Departamento de Anatomia Patológica e Medicina Legal, Faculdade de Medicina da Universidade Federal de Minas Gerais, Av. Alfredo Balena 190, 30130 Belo Horizonte, MG, Brasil

A review of the available literature on central nervous system involvement in experimental trypanosomiasis cruzi is undertaken. From a critical analysis of 26 works on experimental infections with Trypanosoma cruzi *(23 on the acute phase, 2 on the chronic phase, and one describing sequentially both phases), all supported by neuropathologic studies, it can be concluded that: 1) central nervous system involvement during the acute phase, in the form of encephalitis in multiple foci, with variable intensity of the parasitism and inflammatory changes, is frequent and well documented; 2) in animals with more severe central nervous system involvement death occurs as a result of the brain lesions or acute chagasic myocarditis, the latter being always present; 3) in animals with more discrete brain involvement death during the acute phase is due to complications not related to the nervous system, among which congestive heart failure secondary to acute chagasic myocarditis, a condition that is always present, regardless of whether or not the central nervous system is infected; 4) it is possible that in surviving animals that had mild encephalitis the inflammatory changes from the acute phase usually regress as the infection progress to the chronic phase.*

Key words: trypanosomiasis cruzi – chagasic encephalitis – brain

Since the susceptibility of dogs to *Trypanosoma cruzi* infection was established by Chagas (1909), many workers have used these and other animals to study central nervous system (CNS) involvement in experimental trypanosomiasis cruzi.

We have found in the available published literature on experimental infections with *T. cruzi* as many as 26 works in which CNS involvement, confirmed by histopathologic evidence, is described. No review or critical analysis of these works, however, has so far been undertaken. Thus we have decided to review and critically analyze the above reports and, based on this analysis, suggest the possible natural history of CNS involvement in experimental Chagas' disease.

Of the 26 published works on the subject, 23 contain observations on the acute phase (Torre & Villaça, 1919; Villela, 1923; Villela & Torres, 1926; Souza-Campos, 1927; Villela & Villela, 1932; Goble, 1952; Alencar & Elejalde, 1959; Menezes, 1964; Vichi, 1964; Menezes & Alcântara, 1965; Mosquera & Herrera, 1965; Jardim, 1967; Marsden & Hagstrom, 1968; Campos, 1969; Jardim, 1971; Kramer Jr, 1972; Amaral et al., 1975; Queiroz, 1975; Pires, 1978; Tanowitz et al., 1982; Cosenza et al., 1984; Costa et al., 1986; Falangola et al., 1988); only 2 reports are concerned with the chronic phase (Schwartzburd & Köberle, 1959; Britto-Costa, 1971); and only one work discusses sequentially both phases (Pittella et al., 1990). In this review each phase of experimental *T. cruzi* infection is considered separately.

Acute Phase

Four different species of animals were used as follows: dogs (12 times), rats (8 times), mice (5 times), and guinea pigs (once). All animals were susceptible to inoculation with *T. cruzi,* and parasites and inflammatory changes were seen in the CNS of almost all animals. Only one group of workers (Falangola et al., 1988) used

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the same strain of *T. cruzi* to inoculate different species of animals of the same age: the frequency and intensity of the parasitism and inflammatory changes varied according to the species, with mice showing greater susceptibility than guinea pigs and rats. Excluding a few works in which the number of animals examined was not mentioned (Souza-Campos, 1927; Costa et al., 1986; Falangola et al., 1988) or which used very few animals (Torres & Villaça, 1919; Villela, 1923; Villela & Villela, 1932; Mosquera & Herrera, 1965; Pires, 1978), the number of sample animals was adequate in all studies (Villela & Torres, 1926; Goble, 1952; Alencar & Elejalde, 1959; Menezes, 1964; Vichi, 1964; Menezes & Alcântara, 1965; Jardim, 1967; Marsden & Hagstrom, 1968; Campos, 1969; Jardim, 1971; Kramer Jr, 1972, Amaral et al., 1975; Queiroz, 1975; Cosenza et al., 1984; Pittella et al., 1990). Most of the inoculated animals were young and ranged from newborns to 4-month olds (Torres & Villaça, 1919; Villela, 1923; Villela & Villela, 1932; Goble, 1952; Alencar & Elejalde, 1959; Menezes, 1964; Vichi, 1964; Menezes & Alcântara, 1965; Jardim, 1967; Marsden & Hagstrom, 1968; Campos, 1969; Pires, 1978; Cosenza et al., 1984; Costa et al., 1986; Falangola et al., 1988; Pittella et al., 1990). Adult animals were used in three studies (Villela & Torres, 1926; Souza-Campos, 1927; Goble, 1952). In some of the reports the age of the animals is not mentioned (Mosquera & Herrera, 1965; Jardim, 1971; Amaral et al., 1975; Queiroz, 1975). Most of the animals died or were sacrificed within 40 days after inoculation. In some reports the survival time after infection is not indicated (Souza-Campos, 1927; Villela & Villela, 1932; Falangola et al., 1988).

In several works the strain of *T. cruzi* used was not specified (Torres & Villaça, 1919; Villela, 1923; Villela & Torres, 1926; Souza-Campos, 1927; Villela & Villela, 1932; Alencar & Elejalde, 1959; Menezes, 1964; Vichi, 1964; Mosquera & Herrera, 1965; Queiroz, 1975; Tanowitz et al., 1982). Strain Y was the most frequently used (7 times), followed by strain B (twice) and the Berenice, Peru, 21 SF (Type II), PNM, MR, FL, CL, A, W, 12 São Felipe and Colombiana strains (once each). In three works (Goble, 1952; Amaral et al., 1975; Costa et al., 1986) two or more strains of *T. cruzi* were used for comparative analysis; a more marked brain parasitism was induced by the PNM strain (Amaral et al., 1979).

Intraperitoneal injection was the most frequently used route of inoculation (Goble, 1952; Alencar & Elejalde, 1959; Vichi, 1964; Menezes & Alcântara, 1965; Mosquera & Herrera, 1965; Jardim, 1967; Campos, 1969; Jardim, 1971; Amaral et al., 1975; Queiroz, 1975; Pires, 1978; Cosenza et al., 1984; Costa et al., 1986; Pittella et al., 1990). Subcutaneous inoculation was used five times (Villela & Torres, 1926; Goble, 1952; Marsden & Hagstrom, 1968; Kramer Jr, 1972; Costa et al., 1986). Intracerebral inoculation (Menezes, 1964), instillation into the conjunctival sac (Marsden & Hagstrom, 1968), cell culture (Tanowitz et al., 1982), and congenital transmission (Villela, 1923) were each used once. In four works the route of inoculation was not mentioned (Torres & Villaça, 1919; Souza-Campos, 1927; Villela & Villela, 1932; Falangola et al., 1988).

The neuropathologic picture produced by experimental infections with *T. cruzi* is similar to that observed in patients with the acute nervous form of Chagas' disease (Queiroz, 1978). The presence of encephalitis in multiple foci of variable intensity and irregular, random distribution, with nodular arrangement of the inflammatory cells, is usually reported (Torres & Villaça, 1919; Villela, 1923; Villela & Torres, 1926; Souza-Campos, 1927; Goble, 1952; Alencar & Elejalde, 1959; Menezes, 1964; Mosquera & Herrera, 1965; Marsden & Hagstrom, 1968; Jardim, 1971; Kramer Jr, 1972; Amaral et al., 1975; Queiroz, 1975; Pires, 1978; Falangola et al., 1988; Pittella et al., 1990). Amastigotes are either not detected (Villela, 1923; Mosquera & Herrera, 1965; Pires, 1978; Pittella et al., 1990) or may be found within the inflammatory foci or inside glial and microglial cells and macrophages (Torres & Villaça, 1919; Villela & Torres, 1926; Souza-Campos, 1927; Villela & Villela, 1932; Goble, 1952; Alencar & Elejalde, 1959; Menezes, 1964; Menezes & Alcântara, 1965; Jardim, 1967; Marsden & Hagstrom, 1968; Campos, 1969; Jardim, 1971; Kramer Jr, 1972; Amaral et al., 1975; Queiroz, 1975; Tanowitz et al., 1982; Costa et al., 1986; Falangola et al., 1988; Pittella et al., 1990). The presence of parasitized neurons was reported in two studies (Villela & Villela, 1932; Tanowitz et al., 1982). According to most records, however, neurons located adjacent to the inflammatory foci are usually either preserved (Torres & Villaça, 1919; Queiroz, 1975; Pittella et al., 1990) or show unspecific degenerative changes (Villela & Torres, 1926; Souza-Campos,

1927; Pittella et al., 1990). A few authors, on the other hand, reported 1) unspecific degenerative neuronal changes bearing no topographical relation to nests of amastigotes or to inflammatory nodules (Menezes, 1964; Mosquera & Herrera, 1965); or else, 2) a reduction in the number of neurons in the anterior horn of the spinal cord (Vichi, 1964) and nucleus of the third cranial nerve (Jardim, 1971), as well as in the number of cerebellar Purkinje cells (Jardim, 1967). These findings should be interpreted with due care, since: 1) no control group was used by the authors who reported degenerative neuronal changes not related to parasites or inflammatory foci (Menezes, 1964; Mosquera & Herrera, 1965); 2) in addition to being unspecific, these degenerative neuronal changes could in part be of an artifactual nature, as is more commonly observable in very young animals, such as those used (Wertham, 1934; Friede, 1972). Even if, as a result of existing inflammatory changes, some degree of neuronal reduction may occur in the CNS of animals in the acute phase of experimental infection, neuronal losses in such levels and to such extent as described by these authors are hard to explain, specially if it is considered that chagasic encephalitis is known to be focal and of irregular distribution, with neurons almost never being parasitized and remaining preserved even when located close to inflammatory foci (Torres & Villaça, 1919; Queiroz, 1975; Pittella et al., 1990).

Acute chagasic nyocarditis was found in virtually all animals showing concomitant CNS infection that died or were sacrificed during the acute phase of experimental *T. cruzi* infection (Goble, 1952; Marsden & Hagstrom, 1968; Kramer Jr, 1971; Pittella et al., 1990).

Chronic Phase

Two animal species were used: rats (twice) and dogs (once). In two studies 10 and 5 animals were used, respectively (Britto-Costa, 1971; Pittella et al., 1990). In one work the number of animals examined was not mentioned (Schwartzburd & Köberle, 1959). The inoculated animals were all young, with ages ranging from 20 to 49 days (Britto-Costa, 1971; Pittella et al., 1990). In one report the ages of the animals were not specified (Schwartzburd & Köberle, 1959). As for the survival time, the animals were sacrificed at 6 months after the inoculation (Britto-Costa, 1971) and at different times during the chronic phase, with one animal surviving almost three years after being infected (Pittella et al., 1990). In one work the survival time after inoculation was not specified (Schwartzburd & Köberle, 1959).

In only one report was the strain (Colombiana) of *T. cruzi* used indicated (Pittella et al., 1990). Intraperitoneal injection was used in two studies (Britto-Costa, 1971; Pittella et al., 1990). In one work the route of inoculation was not mentioned (Schwartzburd & Köberle, 1959).

Unlike those for the acute phase, the results of histopathologic examination of the brain and spinal cord during the chronic phase of experimental trypanosomiasis cruzi are conflicting. Neuronal losses were observed in the anterior horns of the spinal cord (Schartzburd & Köberle, 1959) and in the supraoptic nucleus of the hypothalamus (Britto-Costa, 1971). However, no inflammatory changes, parasites or glial reaction (gliosis, for instance) were observed by Schwartzburd & Köberle (1959). In Britto-Costa's (1971) study, in addition to neuronal losses, there were also inflammatory infiltrate, granulomas, and gliosis foci, although no mention was made of the relation between the site of these findings and the loss of neurons. In connection with these results, the same critical observations applies as that made in respect of the degenerative neuronal changes and neuronal losses reported in the acute phase. Considering that chagasic encephalitis is known to be focal and of irregular distribution, with neurons being rarely parasitized and usually remaining preserved even when located close to the inflammatory foci (Torres & Villaça, 1919; Queiroz, 1975; Pittella et al., 1990), neuronal losses secondary to inflammatory changes present during or persisting from the acute phase, even if they might occur, would hardly be explainable during the chronic phase of experimental *T. cruzi* infections, particularly in such levels and to such extent as described by those authors.

In the third and most recent work on CNS involvement in the chronic phase of experimental Chagas' disease (Pittella et al., 1990), a detailed histopathologic study of the brain, including immunohistochemical examination for the demonstration of amastigotes, revealed no inflammatory changes or parasites in the nervous tissue. There are two possible expla-

nations for these findings: 1) the animals had no CNS involvement during the acute phase; 2) in some animals, any discrete inflammatory changes that had been present in the acute phase probably regressed as the infection progressed to the chronic phase.

In short, CNS involvement during the acute phase of experimental trypanosomiasis cruzi, similar to that observed in patients with the acute form of Chagas' disease, is undoubted and well documented. However, very few works are currently available that allow definitive conclusions to be made as to whether or not histopathologic changes occur in the brain and spinal cord during the chronic phase. The recently undertaken sequential study of CNS involvement during the acute and chronic phases of experimental *T. cruzi* infection (Pitella et al., 1990), combined with a critical analysis of the published literature on experimental infections with this organism, does allow us to conclude, however, that: 1) CNS involvement is frequent in the acute phase; 2) the intensity of the parasitism and related inflammatory changes in the CNS may vary in the acute phase; 3) some animals show severe brain involvement in the acute phase, with death ensuing in virtually all cases; 4) other animals may have more sparsely distributed lesions, with or without resident parasites. In these cases, death in the acute phase results from complications not related to the nervous system, among which congestive heart failure secondary to acute chagasic myocarditis, a condition that is always present, whether or not there is nervous system infection; 5) in surviving animals that had milder encephalitis, it is possible that the inflammatory changes tend to regress during evolution of the infection to the chronic phase.

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