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Research Note

REVERSION OF CULTURE-INDUCED VIRULENCE-ATTENUATION IN *TRYPANOSOMA CRUZI*

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Attenuation of *Trypanosom cruzi* parasites by maintenance in axenic culture had been communicated (D. E. Bice & R. Zeledón, 1970, *J. Parasitol., 56:* 663-670; E. Chiari, 1974, *Rev. Inst. Med. trop. São Paulo, 16:* 61-67; F. Villalta & F. Kierzenbaum, 1987, *Am. J. Trop. Med. Hyg., 36:* 529-532). Following longterm maintenance in axenic culture, parasite infectivity may be markedly depressed and the longer the period after culture, the lower the infectivity reported, although it seems to depend on the strain tested (T. Pizzi, 1961, *Bol. Ofic. Sanit. Panam., 51:* 450-464; D. E. Bice & R. Zeledón, 1970, loc. cit.; E. Chiari, 1974, loc. cit.). Infection of host cells, either fish or mammal-derived, also results in parasite attenuation (S. Urdaneta-Morales, 1983, *Ann. Parasitol. Hum. Comp., 58:* 317-324).

To study virulence culture attenuation and its stability, we employed a highly virulent and lethal *T. cruzi* strain, RA, (S. M. González Cappa et al., 1981, *Medicina* (Bs. As.), *41:* 119-120) and an immunosuppressed model, the nude mice. The infective capacity of cultured trypomastigotes (c-tryp), was compared *in vivo* against bloodstream forms (b-tryp). Male 25 day-old Rockland mice were inoculated with the same number (10^5) of parasites taken either from infected mice or harvested at 17 (c-tryp-17) or 144 (c-tryp-144) days after Vero cell culture infection.

As can be seen in Fig. 1, mice inoculated with c-tryp-17 displayed a 7-day delay in the parasitemia peak (measured as reported by T. Pizzi, 1956, Prensa Universitaria Santiago de Chile p. 38) when compared with those inoculated with b-tryp, although similar parasitemia maximum values were found (7 x 10^6 parasites/ml). Even though mortality was 100% in both cases, mice infected with b-tryp died between days 8-12 pi while those inoculated with c-tryp-17 showed a delay in mortality (13-18 days pi). In contrast, mice inoculated with c-tryp-144 showed a parasitemia pattern that reached up to 10^5 parasites/ml, with a later steady drop in circulating parasites. In this group the mortality decreased to 20%, and showed a significant delay (18-34 days pi).

After one year of Vero cell culturing, c-tryp were employed to establish a subline in axenic culture. Six months later, at least 98% of the parasites were epimastigotes (c-epi). After two years of continuos maintenance in LIT medium (M. E. Camargo, 1964, *Rev. Inst. Med. trop. São Paulo, 6:* 93-100) 10^7 c-epi injected into 45 day-old male BALB/c mice failed to develop parasitemia. To evaluate the stability of these attenuated c-epi subline, suckling BALB/c, X-ray treated 45-day-old BALB/c (600 rads) and C3H/HeJ (400 rads) as well as 45-day-old C57BL (*nu/nu*) mice were injected intraperitoneally (ip) with c-epi (10^7) harvested after 15 days of culture. No parasitemia could be detected in adult irradiated or in suckling euthymic BALB/c mice. However, b-tryp were detected at day 13 pi in athymic mice, and were later maintained by serial passages in nude mice by ip inoculation of 10^5 parasites. After the second transfer, parasitemia grew linearly up to 3 x 10^6 parasites/ml at days 14-15 pi when all the animals died (data not shown).

The virulence for euthymic mice of c-epi subline after transfer to athymic mice was also

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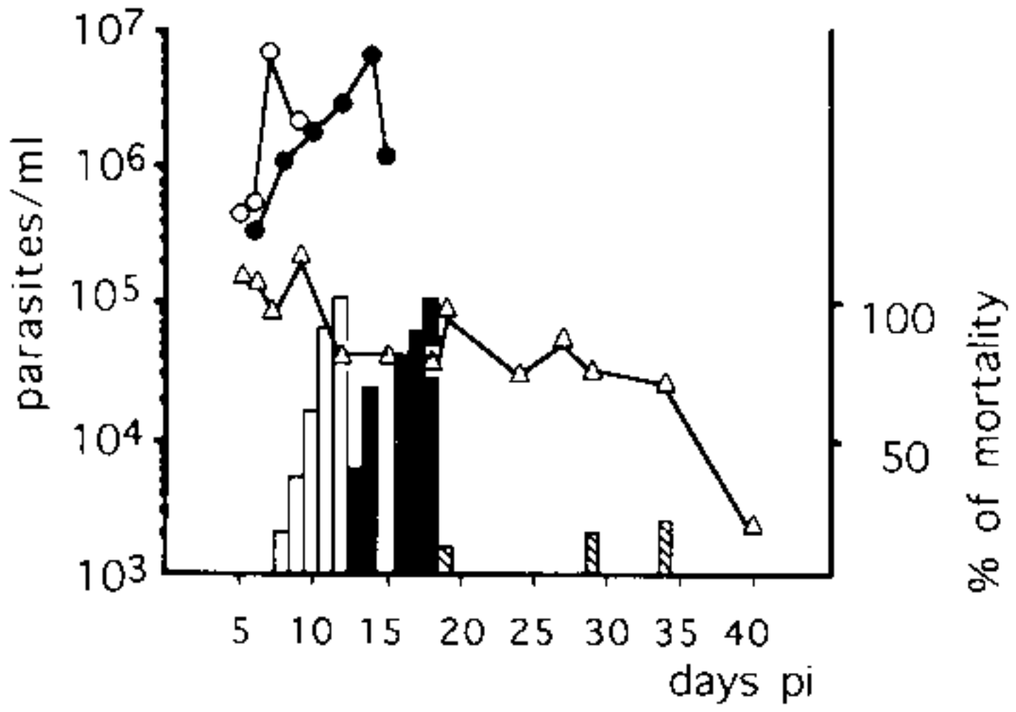

Fig. 1: attenuation of *Trypanosoma cruzi* by cell culturing. Trypomastigotes taken from infected mice (b-tryp) (○ □) or maintained in Vero cell culture 17 (c-tryp-17; ● ■) or 144 (c-tryp-144; Δ ▨) days were inoculated intraperitoneally into Rockland mice. Parasitemia curves and cumulative mortality percentage obtained are shown. Parasitemia values of c-tryp-144 at 7° and 9° days pi versus b-tryp: $P < 0.001$. Percentage of mortality of c-tryp-144 versus b-tryp: $P < 0.001$. Student's t test was employed.

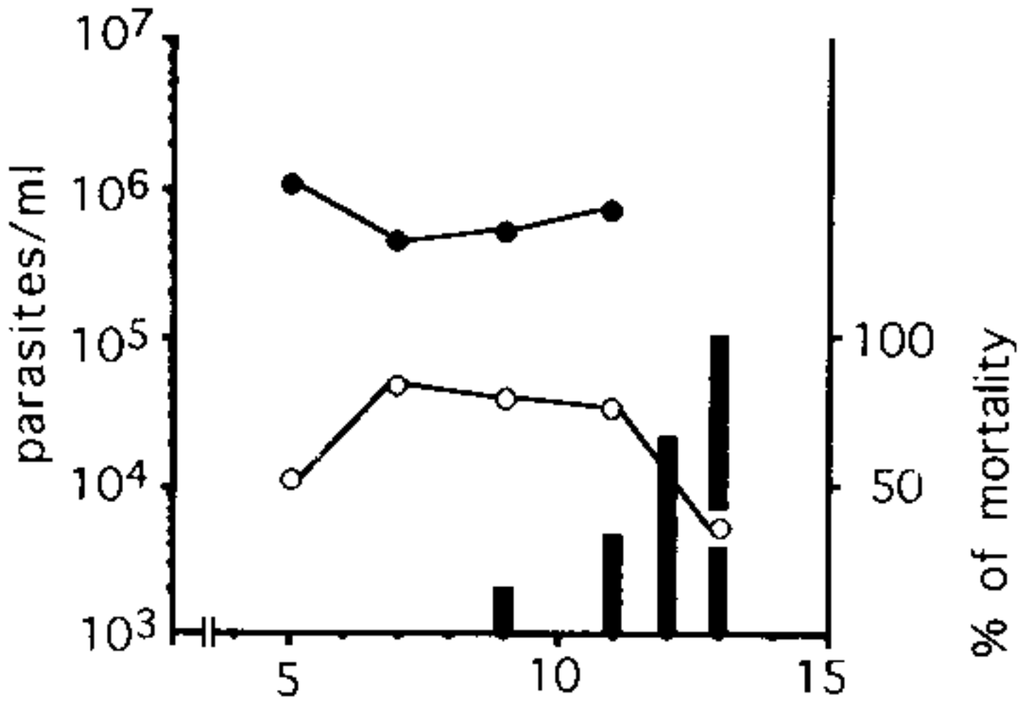

Fig. 2: reactivation of *Trypanosoma cruzi* after passage in nude mice. Trypomastigotes taken from C57BL nude mice inoculated 21 days (○) before with epimastigotes or taken after three serial passages in nude mice (75 days after epimastigotes inoculation (● ■) were given intraperitoneally into normal BALB/c mice. Parasitemia patterns and cumulative mortality percentages are shown. Parasitemia values at 7° day pi: $P < 0.01$; at 5°, 9° and 11° days pi: $P < 0.001$. Student's t test was employed. Percentage of mortality of euthymic mice inoculated with trypomastigotes taken after 21 days inoculation in nude mice was 0%.

studied. For this purpose, b-tryp (5×10^5) taken from nude mice after 21 days of c-epi injection (first passage), were inoculated into 45-day-old BALB/c mice. As shown in Fig. 2, parasitemia was readily detected reaching maximum values of 5×10^4 parasites/ml at day 7 pi. Thereafter, decreasing values were found until day 14 pi, when no parasites could be detected; the host survival was 100%. However, when parasites taken after three passages in nude mice (75 days after the c-epi injection) were inoculated into 45-day-old BALB/c mice, animals exhibited a dramatically different behavior; the lowest parasitemia value detected was one log greater (5×10^5 parasites/ml) than the highest obtained in the first assay with euthymic mice, reaching 10^6 parasites/ml, with 100% mortality between days 9-13 pi (Fig. 2).

In the results presented here a correlation between attenuation of infective capacity and the period of cell-culture maintenance was observed while the infectivity reactivation for euthymic mice increased with the number of passage in nude mice.

Different models were used to check the attenuated parasites infectivity including passages in triatome, axenic culture or inoculation of suckling, irradiated, prednisolone treated or splenectomized euthymic mice (H. Menezes, 1970, *Rev. Inst. Med. trop. São Paulo, 12:* 64-68; M. A. Basombrío et al., 1982, *Infect. Immun., 36:* 342-350). In our approaches we failed to recover the original virulence of the RA strain when employing irradiated adult or normal suckling mice. However, when employing nude mice which provide the highest immunosuppressed model, attenuation was readily reverted. Moreover, the behavior of the reactivated RA strain showed a similar parasitemia pattern and mortality rate for athymic mice than that reported for a highly virulent strain maintained by serial passages in euthymic mice (E. L. Segura et al., 1981, *Medicina* (Bs. As.), *41:* 328-332).

Basombrío and coworkers (1982, loc. cit.) were unable to select pathogenic parasites; based on their results they have suggested to use their "attenuated vaccine" in domestic reservoirs (1989, *Medicina.* (Bs. As.), *49:* 191-196). It has been also reported the inoculation of an attenuated strain in two volunteers (H. Menezes, 1971, *Rev. Inst. Med. trop. São Paulo, 13:* 144-154). The results presented here point that such procedures might develop untoward effects and emphasize the relevance of the nude model for further evaluation of the reversion of *T. cruzi* attenuated strains.