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Homogeneity of *Trypanosoma cruzi* Strains of Different Virulence Derived from a Single Strain

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Abstract: We have been using the strain of *Trypanosoma cruzi* maintained in mice as a strain of high vurulence and the strain derived from the same strain but maintained for a long time *in vitro* as a strain of low virulence. To examine population homogeneity of virulence in each strain, we compared virulence of 48 isolates (nearly clones) from each strain obtained by limiting dilution method, that is, trypomastigote dilution at a concentration of 0.6/well was cultured with fibroblast cells from ICR newborn mice on a microwell plate (96F). Infectious experiment to mice showed that all 48 isolates from a highly virulent strain produced parasitaemia in infected mice within one week and 60% mortality within three weeks, whereas none of them from a low virulent strain produced parasitaemia within 10 days and mortal case within three weeks. This indicated that both strains of different virulence consisted of considerably homogeneous population.

Key words: Trypanosoma cruzi, Virulence, Population homogeneity, Strain difference

Generally, isolates of *Trypanosoma cruzi* from mammals or insects do not consist of homogenous populations but heterogenous. Goble (1951) and Pizzi and Prager (1952) reported that *T. cruzi* strains decreased in virulence during prolonged maintenance in culture media and Norman and Kagan (1960) reported an increase in virulence in weanling mice of several strains of relatively low virulence. Postan *et. al.*, (1984, 1986) pointed out that change of virulence was caused by mixed populatoins with different virulence in uncloned strains and cloned stocks of the parasite showed stable virulence in inbred mice. Deane *et. al.*, (1984) stated that more than one strain of *T. cruzi* can coexist in the same host, and the timing and method of parasite isolation from the vertebrate host act as selective factors, and further passage (in mice or cultures) may completely eliminate one (or more) strain from originally mixed trypanosome populations.

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The Tulahuen strain used in the present work had been maintained mainly in mice or cultures since its isolation in 1945 (Taliaferro and Pizzi 1955). We obtained it from the National Institute of Health, USA, through Keio University, Japan in 1971, since then have maintained it in mice by syringe passage and in culture using modified NNN medium. The strain maintained long in culture has decreased in virulence and has been used as a strain of low virulence for comparison with a strain of high virulence maintaned in mice (Hermosura *et. al.*, 1985). To examine homogeneity of these two strains derived from a single strain, we compared difference of virulence between isolates (nearly clones) obtained from them by limiting dilution. Trypomastigotes of high virulence were obtained from a severely infected mouse (ICR, 5−6 weeks old). Trypanosomes were separated from blood cell components by repeating centrifugation at 200 *g* for five min, finally sedimented by centrifugation at 1,100 *g* for 10min and suspended in Eagle's MEM supplemented with 10% fetal bovine serum (FBS) at a dilution of 0.6 cells/200 μl. An amount of 200 μl trypomastigote suspension was inoculated into each well of a Microwell Plate 96F (Nuncron) where fibroblasts derived from skin of newborn ICR mice (Kanbara *et. al.*, 1987) were previously prepared. Cultures were incubated at 37℃ in 5% CO_2 atmosphere with daily exchange of culture medium (MEM with 10% FBS).

Trypomastigotes grown in L-cell cultures after transfer of culture forms long maintained in a modified NNN medium (Hermosure *et. al.*, 1985) were used as a strain of low virulence. Trypomastigotes were isolated using a CM-cellulose column (Kanbara and Nakabayashi, 1983) and finally diluted in MEM with 10% FBS at a concentration of 0.6 cells/200 μl. Following steps were same as above-mentioned.

After two weeks incubation, free trypomastigotes in overlays were observed in some wells and finally became detectable in more than 50 wells of both culture systems.

During 20 to 35 days after the inoculation, trypanosomes grown in 48 wells of each culture system were transferred to two 24-well tissue culture plate (Nuncron) in which fibroblasts were prepared previously, and incubated in the same condition as before. More than 10^6 trypomastigotes obtained from each well of the second tissue culture plates were intraperitoneally injected into a 5-week-old ICR mouse. Thereafter, parasitaemia was examined in one drop of tail blood on 5, 7 and 10 day of infection and the number of dead mice was checked every day. As shown in Table 1, 94% of mice

Table 1 Parasitaemia and mortality of mice infected with isolates from strains of high and low virulence.

	Duration of infection				
	5 days	7 days	10 days	3 weeks	8 weeks
Source of isolates	Parasitaemia			Mortality	
high virulence	45 (93.8%)	48 (100%)	ND	29 (60.4%)	35 (72.9%)
low virulence	0	0	0		14* (29.2%)

*Only 4 mice proved to have parasitaemia just before their death.

infected with virulent isolates showed positive parasitaemia after 5 days of the inoculation and 100% were positive after 7 days, and 60% of mice died within three weeks of the infection. In contrast to that, none of the mice infected with low virulent isolates showed positive parasitaemia even 10 days after the inoculation and none died till 3 weeks after the inoculation. However 14 mice (29%) died afterwards till 8 weeks and in 4 of those parasitaemia were confirmed when they were dying.

Although only one mouse was used for one isolate infection in this work, the results clearly showed both strains of high and low viurlence consist of considerably homogenous population because all isolates from the former induced acute infection and all from the latter could not induce visible infection in th early phase of infection. Mortality showed some variation among isolates from both strains, but early deaths till 3 weeks of infection were observed only in mice infected with virulent isolates. Judging from the distinct difference between the strains of high and low virulence, it is conceived that change of the virulence of *T. cruzi* in some strains during long-term *in vitro* maintenance may occur because of individual phenotipic change and not because of selection of paticular clone. This idea was proposed in the old reports (Bice and Zeledon 1970) and recently seems not to be acceptable, but it should be reexamined using cloned stocks from proper strains.

REFERENCES

1) Bice D. E. & Zeledon R. (1970): Comparison of infectivity of strains of *Trypanosoma cruzi* (Chagas, 1909). J. Parasit., 56, 663−670.

2) Deane, M. P., Souza, M. A., Pereira, N. M., Goncalves, A. M., Momen, H. & Morel, C. M. (1984): *Trypanosoma cruzi*: Inoculation schedules and re-isolation methods select individual strains from doubly infected mice, as demonstrated by schizodeme and zymodeme analyses. J. Protozool., 31, 276−280.

3) Goble, F. C. (1951): Studies on experimental Chagas' disease in mice in relation to chemotherapeutic testing. J. Parasit., 37, 408−414.

4) Hermosura, M. E., Yanagi, T., Fukuma, T. & Kanbara, H. (1985): Comparative study on high and low virulent trypomastigotes of *Trypanosoma cruzi*: Infectivity to mouse macrophages, L-cells and newly cultured fibroblasts from mouse heart. Trop. Med., 27, 211−219.

5) Kanbara, H. & Nakabayashi, T. (1983): Improved method for separation on CM-cellulose of the trypomastigote form of *Trypanosoma cruzi* from forms grown in fibroblast cell cultures. Biken J., 26, 63−65.

6) Kanbara, H., Yanagi, T., Nakazawa, S. & Fukuma, T. (1987): Comparison of virulent trypomastigotes with low-virulent ones derived from a single strain of *Trypanosoma cruzi*: Infectivity to new fibroblasts from various organs of the mouse. Zbl. Bakt. Hyg. A 264, 407−413 (in press)

7) Norman, L., & Kagan, I. G. (1960): Immunologic studies on *Trypanosoma cruzi.* II Acquired immunity in mice infected with avirulent American strains of *T. cruzi*. J. Infect. Dis., 107, 168−174.

8) Pizzi, T. & Prager, R. (1952): Immunidad a la sobreinfección inducida mediante cultivos de

Trypanosoma cruzi de virulencia atenuada. (Communicación preliminar). Bol. Inform. Parasit. Chilen., 7, 20−21.

9) Postan, M., Mc Daniel, J. P. & Dvorak, J. A. (1984): Studies of *Trypanosoma cruzi* clones in inbred mice. II. Course of Infection of C57/BL6 mice with singlee-cell-isolated stocks. Am. J. Trop. Med. Hyg., 33, 236−238.

10) Postan, M., Mc Daniel, J. P. & Dvorak, J. A. (1986): *Trypanosoma cruzi*: constancy of clone pathogenicity for inbred mice during long-term *in vitro* maintenance. Trans. R. Soc. Trop. Med. Hyg., 77, 126−129.

11) Taliaferro, W. H. & Pizzi, T. (1955): Connective tissue reactions in normal and immunized mice to a reticulotropic strain of *Trypanosoma cruzi*. J. Infect. Dis., 96, 199−226.

同一株由来の病原力の異るクルーズトリパノソーマ株の均質性

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私達はこれまで一つの株（Tulahuen　株）由来でありながら，長期試験管内培養によりマウスに対する病原力を減じたものを弱毒株，マウスにて継代接種されてきたものを強毒株として使用してきた．この実験ではそれぞれの株の病原力について均質性を確めるため，それぞれの原虫を限定希釈（0.6/well に調整）により，前もって ICR 新生児マウス皮膚由来の線維芽細胞を培養しておいた96穴のマイクロプレートに移植しクローン化した．これにより得たそれぞれ48分離株のマウスに対する感染性を検討した．強毒株由来のものはすべて感染後７日目までに血流中に原虫を認め，３週目までに60％のマウスが死亡する急性感染を生じた．一方弱毒株由来のものは10日目までの検査で血流中に原虫は認められず，３週目までに死亡するマウスは皆無であった．このことにより病原力の異る二株は，それぞれかなり均質な病原力をもつ集団よりなっていると考えられる．

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