# THE AVIRULENCE OF THE CULTIVATED PF STRAIN OF TRYPANOSOMA CRUZI. VI — INDUCED IMMUNOTOLERANCE IN MICE BY A VERY HIGH INOCULUM.

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*Immunosuppression by immunotolerance was obtained in mice by inoculating* Trypanosoma cruzi *avirulent PF strain in high doses to mice.*

*Direct and indirect laboratory tests were used to demonstrate the above statements.*

Since our first works on avirulence of the *T. cruzi* PF strain we were surprised to find some vaccinated animals with positive blood cultures and only rarely with positive parasitemias (11, 13, 14).

Although we couldn't define precisely the real nature of the fact, by then, we had already concluded that the observed parasites should be the same inoculated as vaccine. These trypanosomes did not infect young mice.

Among the various circumstances in which the fact happened, one specially called our attention — the relationship between it and the number of parasites inoculated as vaccine.

For this reason we decided to verify if the phenomenon could be reproduced and explained as due to immunological tolerance.

## MATERIAL AND METHODS

Ninety three albino mice weighing 10g each were divided in three groups — the first group with 34 animals, the second one with 35 and the third with 25.

The first group was vaccinated, subcutaneously, with $10^7$ living trypanosomes of the PF strain from a culture 19 days old in Warren medium.

The second group was vaccinated with $10^{10}$ parasites just the same and by the same route.

The third group was held as control.

Eight and fifteen days after vaccination a peripheral blood search was performed for trypanosomes in the vaccinated mice.

On the fifteenth day, from each vaccinated group, 10 animals were selected at random and bled to death. From the first

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TABLE 1

MICE VACCINATED WITH $10^7$ PF PARASITES AND CONTROLS

| Animal | Parasitemia After Vaccination | | Blood Culture | | Parasitemia After Infection | | | Blood Culture | Controls Parasitemia After Infection | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | 8 days | 15 days | 15 days | Four Weeks After Vacc. | 8 days | 15 days | 30 days | 30 days | 8 days | 15 days | 30 days |
| 1 | 0 | 0 | | Intraperitoneal injection of virulent Y strain blood forms of *T. cruzi* (5.000 parasites/g body weight) | 0 | 35 | 0 | Negative (Blood Pool of the Group) | 3.260 | 25.340 | |
| 2 | 0 | 0 | | | 105 | 0 | 0 | | 3.570 | | |
| 3 | 0 | 0 | | | 35 | 0 | 0 | | 3.570 | | |
| 4 | 0 | 0 | | | 0 | 0 | 0 | | 3.710 | | |
| 5 | 0 | 0 | | | 0 | 0 | 0 | | 3.920 | | |
| 6 | 0 | 0 | | | 140 | 0 | 0 | | 4.375 | 28.000 | |
| 7 | 0 | 0 | | | 0 | 0 | 0 | | 5.355 | | |
| 8 | 0 | 0 | | | 0 | 35 | 0 | | 5.830 | | |
| 9 | 0 | 0 | | | 70 | 70 | 0 | | 6.300 | 1.015 | |
| 10 | 0 | 0 | | | 0 | 0 | 0 | | 6.680 | | |
| 11 | 0 | 0 | | | 140 | 0 | 0 | | 7.350 | | |
| 12 | 0 | 0 | | | 0 | 70 | 0 | | 7.980 | 4.375 | |
| 13 | 0 | 0 | | | 0 | 35 | 0 | | 10.500 | | |
| 14 | 0 | 0 | | | 70 | 210 | 0 | | 11.600 | | |
| 15 | 0 | 0 | | | 35 | 35 | 0 | | 11.900 | | |
| 16 | 0 | 0 | | | 0 | 0 | 0 | | 12.670 | | |
| 17 | 0 | 0 | | | 0 | 0 | 0 | | 14.000 | | |
| 18 | 0 | 0 | | | 0 | 0 | 0 | | 15.750 | | |
| 19 | 0 | 0 | | | 35 | 70 | 0 | | 17.500 | | |
| 20 | 0 | 0 | | | 0 | 35 | 0 | | 17.710 | | |
| 21 | 0 | 0 | | | 35 | 0 | 0 | | 18.620 | | |
| 22 | 0 | 0 | | | 210 | 0 | 0 | | 22.750 | | |
| 23 | 0 | 0 | | | 4.830 | | | | 24.500 | | |
| 24 | 0 | 0 | Negative (Blood pool of the group) | | | | | | 32.200 | 3.290 | |
| 25 | 0 | 0 | | | | | | | | | |
| 26 | 0 | 0 | | | | | | | | | |
| 27 | 0 | 0 | | | | | | | | | |
| 28 | 0 | 0 | | | | | | | | | |
| 29 | 0 | 0 | | | | | | | | | |
| 30 | 0 | 0 | | | | | | | | | |
| 31 | 0 | 0 | | | | | | | | | |
| 32 | 0 | 0 | | | | | | | | | |
| 33 | 0 | 0 | | | | | | | | | |
| 34 | 0 | 0 | | | | | | | | | |
| Median | 0 | 0 | | | 0 | 0 | 0 | | 9.240 | 4.375 | |
| Mortal. | 0% | 0% | | | 0% | 4% | 4% | | 0% | 79% | 100% |

group a blood pool was made and the serum, after being centrifuged at 1.500 r.p.m., had its sediment cultivated in 10 Warren medium tubes.

From the second group a total blood culture was done in the same medium (two test tubes for each animal).

The blood pool serum of each group was used for the agglutination test using the technique described by Muniz (18).

The blood cultures readings were made after 30 days. The positive ones, after centrifugation, were inoculated intraperitoneally in 10 baby mice.

After 15 days of this inoculation all 10 animals were bled to death and from the sediment of the serum pool a new culture in 10 test tubes of Warren medium was made.

Four weeks later, all remaining mice, from the original vaccination, including those kept as controls were infected with blood forms of the Y virulent strain of *Trypanosoma cruzi*. The inoculation was made intraperitoneally at a dose of 5.000 parasites per gram of body weight.

Eight, fifteen and thirty days after the infection, parasitemias by the Pizzi-Brener technique (3) were detected in the mice of all the three groups.

Ten animals of the first vaccinated group and fourteen of the second one were killed by heart puncture at the 30th day of infection.

The blood serum pool of each group was centrifuged and cultivated as in the previous cases.

## RESULTS

As observed previously (10, 15) all the PF strain vaccinated animals had negative parasitemias 8 and 15 days after vaccination (Tables 1 & 2).

The blood culture of the mice vaccinated with $10^7$ trypanosomes was negative 15 days after vaccination, while 2 out of 10 of the $10^5$ trypanosomes vaccinated mice were positive.

The parasites from these positive blood cultures weren't able to produce infection in 10 baby mice which had negative blood cultures after being inoculated with them (Table 2).

The agglutination test for *T. cruzi* presentend titres of 1/320 for the first group of mice, 15 days after vaccination, and 1/160 for the second one.

The median of the parasitemias performed in the first group of animals 8, 15 and 30 days after infection was negative.

That of the second group was positive at the 8th day (35 parasites for $5mm^3$ of blood) and negative on the 15th and 30th days of infection.

The blood cultures made 30 days after infection were negative in the mice vaccinated with $10^7$ parasites and positive in those previously vaccinated with $10^5$ trypanosomes.

The mortality rate was alike in both vaccinated groups (4%).

The control animals presented very high parasitemias and mortality percentages that attained 100% on the 30th day of infection.

## DICUSSION AND CONCLUSIONS

Metchnikoff in 1883 (*in* 7) had already mentioned that a very high infection dose of yeast, in daphnia, impaired phagocitosis and permits the multiplication of their spores.

Bordet in 1897 (*in* 7) observed that guinea pigs infected with high inoculum of *Streptococcus* do not form antibodies against these bacteria but could do it against other micro-organisms injected in optimal quantity.

Felton in 1949 (7) verified that high doses of Pneumococcus polysaccharides have the same effect and designated the phenomenon — *immunologic paralysis*.

In a work that became a classic in Immunology, Fischman in 1961 (8), demonstrated that the antibody production against T2 bacteriophage, in cultures of lymph node cells from normal animals, failled to occur if more than an optimal amount of T2 bacteriophage was added to the macrophages.

Similar phenomenon has been observed by the use of other antigens (6, 17, 20).

Immunotolerance is a word used with different significances.

For some authors (2, 19) it represents the complete lack of immunologic response to a certain antigenic substance.

For the majority, on the other hand, this unresponsivity can be of short or long duration (1, 4, 9, 22) and denotes a com-

TABLE 2

MICE VACCINATED WITH $10^{6}$ PF PARASITES AND CONTROLS

| Animal | Parasitemia After Vaccination | | Blood Culture | Sub-Inoculation In Mice (10) | Blood Culture | Four Weeks After Vacc. | Parasitemia After Infection | | | Blood Culture | Controls Parasitemia After Infection | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number | 8 days | 15 days | 15 days | | 15 days | | 8 days | 15 days | 30 days | 30 days | 8 days | 15 days | 30 days |
| 1 | 0 | 0 | | | | Intraperitoneal injection of virulent Y strain blood forms of *T. cruzi*. (5.000 parasites/g body weight) | 0 | 0 | 0 | Positive (Blood pool of the group) | 3.260 | 25.340 | |
| 2 | 0 | 0 | | | | | 35 | 0 | 0 | | 3.570 | | |
| 3 | 0 | 0 | | | | | 385 | 105 | 0 | | 3.570 | | |
| 4 | 0 | 0 | | | | | 210 | 0 | 0 | | 3.710 | | |
| 5 | 0 | 0 | | | | | 105 | 0 | 0 | | 3.920 | | |
| 6 | 0 | 0 | | | | | 0 | 35 | 0 | | 4.375 | 28.000 | |
| 7 | 0 | 0 | | | | | 1.930 | 420 | 0 | | 5.355 | | |
| 8 | 0 | 0 | | | | | 0 | 35 | 0 | | 5.830 | | |
| 9 | 0 | 0 | | | | | 240 | 0 | 0 | | 6.300 | 1.015 | |
| 10 | 0 | 0 | | | | | 35 | 0 | 0 | | 6.680 | | |
| 11 | 0 | 0 | | | | | 0 | 0 | 0 | | 7.350 | | |
| 12 | 0 | 0 | | | | | 245 | 105 | 0 | | 7.980 | 4.375 | |
| 13 | 0 | 0 | | | | | 0 | 35 | 0 | | 10.500 | | |
| 14 | 0 | 0 | | | | | 0 | 0 | 0 | | 11.600 | | |
| 15 | 0 | 0 | | | | | 0 | 0 | 0 | | 11.900 | | |
| 16 | 0 | 0 | | | | | 70 | 0 | 0 | | 12.670 | | |
| 17 | 0 | 0 | | | | | 35 | 70 | 0 | | 14.000 | | |
| 18 | 0 | 0 | | | | | 0 | 35 | 0 | | 15.750 | | |
| 19 | 0 | 0 | | | | | 105 | 70 | 0 | | 17.500 | | |
| 20 | 0 | 0 | | | | | 0 | 70 | 0 | | 17.710 | | |
| 21 | 0 | 0 | | | | | 35 | 0 | 0 | | 18.620 | | |
| 22 | 0 | 0 | | | | | 35 | 0 | 0 | | 22.750 | | |
| 23 | 0 | 0 | | | | | 0 | 0 | 0 | | 24.500 | | |
| 24 | 0 | 0 | | | | | 0 | 0 | 0 | | 32.200 | 3.290 | |
| 25 | 0 | 0 | | | | | 70 | | | | | | |
| 26 | 0 | 0 | | Negative | Negative (Pool) | | | | | | | | |
| 27 | 0 | 0 | + | | | | | | | | | | |
| 28 | 0 | 0 | + | | | | | | | | | | |
| 29 | 0 | 0 | 0 | | | | | | | | | | |
| 30 | 0 | 0 | 0 | | | | | | | | | | |
| 31 | 0 | 0 | 0 | | | | | | | | | | |
| 32 | 0 | 0 | 0 | | | | | | | | | | |
| 33 | 0 | 0 | 0 | | | | | | | | | | |
| 34 | 0 | 0 | 0 | | | | | | | | | | |
| 35 | 0 | 0 | 0 | | | | | | | | | | |
| Median | 0 | 0 | 0 | | | | 35 | 0 | 0 | | 9.240 | 4.375 | |
| Mortal. | 0% | 0% | | | | | 0% | 4% | 4% | | 0% | 79% | 100% |

plete break or a reduction of the immunologic response (5).

The degree and duration of the non response depends of the host, of the antigen and sometimes of the via of the injection and of the methods used to investigate the incapacity of the immunologic response (21).

In this paper immunotolerance means a state of complete lack or reduction of an antigen specific immunologic response, of short or long duration, to a substance that otherwise is antigenic.

Consequently it is synonymus of immunologic paralysis, immunologic suppression and immunologic unresponsiveness.

In our experiments when we vaccinated animals with doses equal or superior to $10^{6}$ avirulent PF strain trypanosomes we sometimes had positive blood cultures, while all those performed with smaller amount were negative (14).

The lower agglutination titre for *T. cruzi*, 15 days after vaccination and positive parasitemia at the 8th day of infection in the animals vaccinated and challenged, although not statistically significant seems to be rather more than mere coincidence when it occurs exactly in the mice vaccinated with $10^{6}$ trypanosomes, i.e., in the group of animals that presented positive blood cultures after vaccination and after challenge.

This means that these animals became tolerant to a further virulent infection with trypanosomes.

We have already shown that this tolerance is low and of short duration (13, 14). None of the tolerant animals died of the virulent infection, nor do all of them show ulterior positive blood cultures (14).

An excellent degree of immunity we have obtained in mice with only the injection of $10^{5}$ PF strain trypanosomes (12).

Inocula one million higher constitute a factor of immunodepression and are responsible for the positive results observed in such circumstances.

We belive that, besides the chemical immunosuppression already demonstrated with the PF strain (16), biological immunosuppression by immunotolerance can also be induced in mice.

With the present results we are sure that, once more, the avirulence of the *Trypanosoma cruzi* PF strain is confirmed as well its immunogenic capacity, when the vaccine is used in appropriated dosage.

*RESUMO*

*Imunosupressão por imunotolerância foi obtida em camundongos pela injeção de altas doses da cepa avirulenta PF do* Trypanosoma cruzi.

*Testes parasitológicos, diretos e indiretos, foram utilizados com o objetivo de demonstrar a referida tese.*

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