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# *Trypanosoma cruzi:* variation in susceptibility of inbred strains of rats

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## Summary

Eight strains of male rats (AUG, BN, LEW, LIS, WAG, F 344, LOU/M, DA) and 3 strains of female rats (LEW, F 344, KGH) were challenged with $15 \times 10^4$ trypomastigotes of the Tehuantepec strain of *T. cruzi*. Parasitemia and mortality were observed for 60 days. Varying degrees of susceptibility were demonstrated between strains: complete resistance (R), no parasitemia; low resistance (LR), mild parasitemia; and no resistance (NR), high parasitemia. The differences in susceptibility to *T. cruzi*, using inbred strains of rats, were unrelated to Rt-1 haplotype. However, the level of parasitemia and host survival are not necessarily related; both male and female F 344 hosts are susceptible (NR) to *T. cruzi* but only females survive.

**Key words:** *Trypanosoma cruzi;* Chagas' disease; rats, inbred strains; major histocompatibility complex, MHC; genetic locus, Rt-1 haplotype; genes for immune response, Ir; susceptibility, host survival.

## Introduction

Susceptibility to infection with *Trypanosoma cruzi* varies depending on the species and strain of host (Goble, 1970; Pizzi et al., 1954; Trischmann et al., 1978; Wrightsman et al., 1982). The immunological system plays a role in natural resistance against *T. cruzi* infection, but other factors are probably involved. A major histocompatibility complex (MHC) has been demonstrated in the majority of vertebrates (Vaiman, 1978) and two systems have been studied in depth: the H-2 system of mice (Colombani, 1975) and the human HLA

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system (Dausset, 1976). Although the functions of these systems are similar for these two species, the organisation of loci within the MHC is different. The MHC of rats, known as Rt-1 (Report of the first international ..., 1978), seems to resemble the human MHC more closely than the MHC of mice (Gill et al., 1978). It also represents a polygenic control (Gill and Kunz, 1971) and plays an important role in the defence mechanism of the animal, with regard to different biological processes. It is currently though to contain two regions, A and B (Gill et al., 1978).

Most studies on *T. cruzi* infection have been carried out on mice (Hauschka, 1947; Pizzi et al., 1954; Goble, 1951), a highly susceptible host. On the other hand, rats are relatively resistant to this infection and have the advantage of developing a chronic phase of Chagas' disease, which permits the analysis of a certain number of immunological phenomena that appear during the course of the infection (Pizzi et al., 1953).

It has been observed that the resistance generally exhibited by rats does not always occur and that strains of male F 344 rats are particularly susceptible to infection (Rodriguez et al., 1981). Consequently, the susceptibility of different inbred strains of rats to *T. cruzi* infection has been investigated.

### Materials and Methods

*Animals.* Eight different inbred strains of male rats which differed in their Rt-1 haplotype were used for the investigation: August (AUG), Brown Norway (BN), Lewis (LEW), Hooded Lister (LIS), Wistar Ag (WAG) obtained from Service de Génétique, CNRS, Orléans, France; Fischer (F 344) obtained from Iffa Credo, L'Arbresle, France; Louvain (LOU/M) and Dark Agouti (DA) obtained from Institut Pasteur, Lille, France. Each group of animals to be infected was carefully matched for weight (220–300 g) and age (12–16 weeks).

In the second part of the study, three strains of female rats with very close genetic characteristics on their MHC were chosen: Lewis (LEW) obtained from CNRS, Orléans, France; Fischer (F 344) obtained from Iffa Credo, France; and KGH obtained from Institut Pasteur, Lille France. These also were matched for weight (150–200 g) and age (12–16 weeks).

*Parasites.* The Tehuantepec strain of *T. cruzi* (isolated from *Triatoma* from Mexico by Brumpt) was maintained by serial passage in male F 344 rats by the method described for mice by Pizzi and Prager (1952). For infection of the animals, blood was drawn by cardiac puncture from male F 344 rats, mixed with heparin and diluted in Alsever solution. Rats were inoculated intraperitoneally (i.p.) with 1 ml of this diluted blood containing $15 \times 10^4$ trypomastigotes/ml.

*Parasitemia:* In order to determine the course of parasitemia, tail blood (5 $\mu$l) was taken with a micropipette and smears prepared. 60 microscope fields were examined and the number of trypanosomes per milliliter was estimated by the method of Pizzi and Prager (1952) and Philipps (1960).

## Results

### *Variation in susceptibility of inbred male rat strains*

The course of parasitemia obtained in the different inbred male rat strains studied is shown in Fig. 1. Susceptibility was classified into three groups: the first group (AUG, LOU/M and DA) showed resistance and did not develop a

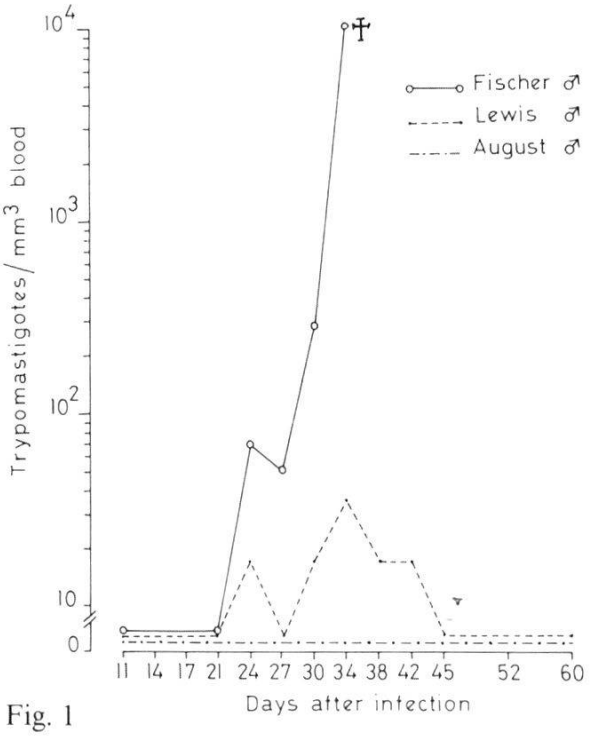


Fig. 1

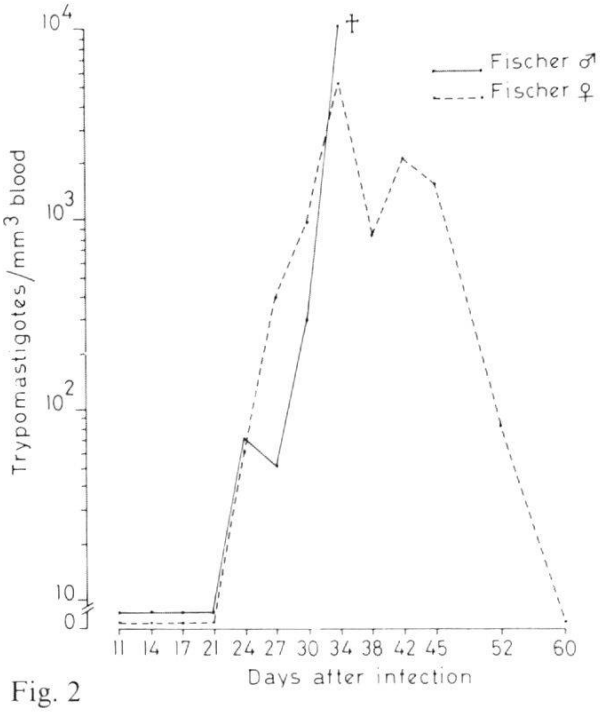


Fig. 2

Fig. 1. Course of *Trypanosoma cruzi* infection in different male rat strains. Male rats aged 3–4 months, given $15 \times 10^4$ trypanosomes i.p.; (○——○) NR = no resistance: male F 344 rats; (·----------·) LR = low resistance: no statistical difference (variance analyses) was found between strains with low parasitemia (LEW, WAG, BN, LIS), LEW was chosen as representative for the four strains; (–·–·–·–) R = resistant: male AUG, LOU/M and DA rats. Parasitemia expressed as geometric mean. † Death in $34 \pm 4$ days after infection.

Fig. 2. Course of *Trypanosoma cruzi* in F 344 rats. F 344 rats aged 3–4 months, given $15 \times 10^4$ trypanosomes i.p. Male rats (·——·), female rats (·– – – –·). Parasitemia expressed as geometric mean. † Death in $34 \pm 4$ days after infection.

parasitemia: it was considered as "resistant (R)". The second (LIS, LEW, WAG, BN) showed low resistance and developed a mild parasitemia between the 11th and 42nd day: it was considered as "low resistant (LR)". In contrast, the third group (F 344) was exceptional, showing no resistance; it presented a high level of susceptibility with high parasitemia: it was considered as "no resistance (NR)".

All the inbred male rat strains survived except for the F 344 strain all of which died between the 34th and 42nd day of the infection. The mortality and susceptibility is shown in Table 1. Strains with the same MHC (Rt-$1^c$: AUG and LIS, or Rt-$1^u$: WAG and LOU/M) differed in the outcome of infection.

*Variation in susceptibility of inbred female rat strains*

Following the preliminary results obtained from the different inbred male rat strains, 3 female strains were used. As shown in Table 2, all had indentical MHC B regions and different A regions (A region of the F 344 strain is a variant of the Rt-$1^l$ haplotype of the LEW strain and the KGH strain shows a Rt-$1^g$ haplotype) (Gill and Kunz, 1978).

Table 1. Male rat strains aged 3–4 months given $15 \times 10^4$ trypanosomes of *T. cruzi* i.p.

| Strains .... | AUG | LIS | LEW | F 344 | WAG | LOU/M | DA | BN |
|---|---|---|---|---|---|---|---|---|
| Rt-1 ..... | C | C | l | lvl | U | U | a | n |
| Outcome of infection ... | R | LR | LR | NR | LR | R | R | LR |
| Mortality .. | 0/6 | 0/6 | 0/6 | 6/6 | 0/6 | 0/6 | 0/6 | 0/6 |

Six rats per group; R = resistant; LR = low resistance; NR = no resistance.

Table 2. Female rats aged 3–4 months given $15 \times 10^4$ trypanosomes *T. cruzi* i.p.

| Strains ..... | F 344 | LEW | KGH |
|---|---|---|---|
| Rt-1 ..... | lvl | l | g |
| Region A ..... | lvl | l | g |
| Region B ..... | l | l | l |
| Outcome of infection ..... | NR | LR | LR |
| Mortality ..... | 0/7 | 0/7 | 0/7 |

Seven rats per group; LR = low resistance; NR = no resistance.

The analysis of the infection of these strains is shown in Table 2. The LEW strain and KGH strain demonstrated a low level of parasitemia (low resistance: LR), whereas the F 344 strain showed a high parasitemia (no resistance: NR). All female F 344 rats survived the infection and showed the highest parasitemia between the 34th and 42nd day; subsequently the parasite numbers diminished progressively until they totally disappeared (Fig. 2).

## Discussion

*Biological aspects*

Most of the inbred strains were either resistant (AUG, LOU/M and DA) or showed a low level of parasitemia (LIS, LEW, WAG, BN). This confirms former observations with regard to the resistance of rats to *T. cruzi* infection (Kolodny, 1940; Pizzi et al., 1953). Experiments carried out with the male F 344 rats indicate that this strain develops an acute phase of the disease and dies during this phase. However, the other strains may exhibit a chronic phase. This should be investigated by physiological and histopathological examination of their hearts.

*Genetic aspects*

Strains with the same MHC responded differently (LOU/M and WAG, AUG and LIS). In order to show the role of the MHC haplotypes more precise-

ly, a further experiment was carried out, in which 3 strains with genetically close MHC haplotypes were infected. According to Table 2, the hypothesis was that if the B region played an important role in the control of *T. cruzi* infection, the F 344 and LEW strains should show the same susceptibility; this was not the case. The other possibility was that the A region played an important role, which would mean that the LEW and KGH strains should show different susceptibilities; and this was not the case.

In conclusion, it may be stated that the MHC does not seem to play an important role in *T. cruzi* susceptibility. These results agreed with those of Trischmann et al. (1978), using inbred strains of mice, which showed that the principal genetic determinant of resistance was not linked with an H-2 haplotype. But another paper (Wrightsman et al., 1982) shows that the level of parasitemia was not linked with the H-2 region and H-2-linked gene(s) was involved in survival of the infection. We have clearly demonstrated that the level of parasitemia and host survival are not necessarily related. The course of *T. cruzi* infection in female and male inbred rats of strain F 344 was not the same (see Fig. 2). Both showed a high degree of parasitemia, but all the males died between the 34th and 42nd day of the infection, whereas all the females survived the infection, with total disappearance of the parasitemia.

Both male and female F 344 hosts are highly susceptible to *T. cruzi* but the host survival are different. This distinction between susceptibility and host survival corroborate date on *T. cruzi* infected mice in a recent paper of Wrightsman et al. (1982); the MHC is probably not important for susceptibility but the MHC could still be of value in survival in mice.

The difference of host survival in F 344 rats does seem to depend on sex (a hormonal factor or a sex-linked genetic factor). Finally, complementary experiments (the use of F1 and back crosses) might lead to the discovery of the mechanisms that control infection with *T. cruzi.*

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