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CONTACT HYPERSENSITIVITY TO DNFB IN TRYPANOSOMA CRUZI INFECTED MICE

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SUMMARY

C57B1/10J and A/Sn strains mice were infected with **Trypanosoma cruzi** (strain Y) and skin tested in the ear for contact hypersensitivity (CH) to DNFB seven days following sensitization. A/Sn mice were highly susceptible to strain Y infection and showed a transitory suppression of CH to DNFB, coincident with the highest levels of parasitaemia observed during the infection. No suppression of DNFB CH reactions was observed for C57B1/10J mice infected with **T. cruzi** inocula leading to levels of parasitaemia and mortality comparable to those observed in A/Sn mice. This approach indicates that the suppression observed in A/Sn mice cannot be ascribed solely to the higher parasitaemia and mortality rates observed in this strain. An intrinsic impairment of the ability of A/Sn mice to mount a cellular response to DNFB seems also to be excluded since non-infected A/Sn developed higher CH reactions to DNFB than C57B1/10J mice.

INTRODUCTION

We have recently demonstrated[1] that C57B1/10J mice infected with **Trypanosoma cruzi** express low levels of delayed type hypersensitivity (DTH) to **T. cruzi** antigens during the first weeks of infection. **Mycobacterium bovis** (BCG) enhanced DTH responses in animals immunized with disrupted epimastigotes while no enhancement of DTH was observed in animals infected with living blood trypomastigotes. Moreover, infection abrogated intense DTH responses in mice pretreated with BCG and disrupted epimastigotes. These results suggest strongly that infection with **T. cruzi** intervenes with the host's ability to mount and/or express cell mediated immunity (CMI) to the parasite's antigens. However, a general nonspecific decrease or suppression of CMI during the initial phase of infection cannot be discarded.

Indeed decreased DTH responses to oxazolone[5] and PPD[7] or BCG[5] during **T. cruzi** infection have been described.

In this work we chose a contact sensitizing drug, dinitrofluorobenzene (DNFB), in order to assess DTH responses during the infection with **T. cruzi** in mice.

MATERIAL AND METHODS

Animals

Inbred C57B1/10J and A/Sn mice weighing 23 to 25 g (8-12 week old) of both sexes were used.

Sensitization and testing for DTH

DNFB (2,4 dinitro-1-fluorobenzene-Merck, Germany) sensitization and testing of mice was carried according to the method of PHANUPHAK et al.[4] with minor modifications.

Briefly, 0.25% DNFB in acetone/olive oil was applied (200 μl), on two consecutive days,

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on the unshaven abdomen of the animals. Seven days following sensitization 20 μl of the same solution were applied to the dorsal aspect of the right ear. The left ear received only acetone/olive oil as a control for irritation caused by the solvent. Ear thickness was measured with a dial gauge micrometer (precision 0.01 mm) under light ether anesthesia before application and 24 hours after testing. Results were expressed as net increase in thickness over pretest values. Student's test was used to analyse the data.

Experimental design

Within each experiment both control and infected animals of either strain were of the same sex. A large number of animals were simultaneously infected. On the same day and at intervals thereafter groups of animals were sensitized with DNFB and tested seven days later for contact sensitivity. Results are refered on the day when skin testing was performed and are expressed as the mean ± standard deviation of the mean (SD) or as the mean ± standard error (SE).

Infection

The strain Y of **Trypanosoma cruzi** maintained by weekly passaging in mice was used. Infected mice were bled by cardiac puncture and the number of blood trypomastigotes was determined by the method of BRENER [2]. The concentration of parasites was adjusted by dilution in sterile saline to allow the intraperitoneal injection of the desired number of forms in a total volume of 0.2 ml.

Parasitaemia was determined in groups of six to eight mice bled from the tail [2].

RESULTS

Table I shows that both C57Bl/10J and A/Sn mice became sensitized with either 0.25% or 0.5% DNFB concentration.

TABLE I

Contact sensitivity to DNFB in C57Bl/10J and A/Sn uninfected mice

Sensitization with	24 hr ear swelling (x10^{-2} mm) *			
	C57Bl/10J		A/Sn	
	Right ear	Left ear **	Right ear	Left ear
0.5% DNFB	[a] 18.7 ± 6.8 (n = 19) [§]	1.5 ± 1.4	[c] 22.0 ± 2.6 (n = 5)	0.8 ± 1.0
0.25% DNFB	[b] 10.1 ± 3.9 (n = 17)	0.8 ± 0.9	[d] 14.8 ± 5.7 (n = 16)	2.1 ± 2.6
Unsensitized controls	3.1 ± 1.2 (n = 9)	0.3 ± 0.5	2.4 ± 2.8 (n = 8)	00.0

* Tests done in the right ear with 0.25% DNFB in acetone-olive oil 7 days following sensitization. Data express arithmetic means ± S.D.
** Left ear painted with acetone-olive oil
§ Number of animals per group in parenthesis
p values for tests comparing the levels of reaction between the experimental groups:
a vs b $p < 0.01$
a vs c $p > 0.05$
c vs d $p < 0.0005$
b vs d $p < 0.01$

The 24 hours ear swelling levels following contact sensitivity tests performed on the 7th day post sensitization with 0.5% DNFB do not differ significantly between both strains of mice. When DNFB was used at a concentration of 0.25% for sensitization, A/Sn mice expressed higher levels of DTH than those of C57Bl/10J animals.

Neither strain developed nonspecific swelling to the DNFB solvent, namely the acetone/oil mixture applied to the left ears.

0.25% DNFB caused minimal irritation (24 hours readings) when applied to the ear of normal C57B1/10J or A/Sn mice.

Based on these data we chose the concentration of 0.25% DNFB for sensitization and for testing in all the following experiments.

0.25% DNFB allows adequate sensitization, without the deep ulceration of the abdominal skin observed with 0.5% DNFB.

Table II shows that following infection with 50 **T. cruzi** blood forms, C57B1/10J mice developed enhanced contact sensitivity to DNFB when compared to identically sensitized uninfected controls. A/Sn mice challenged on the 8th day following infection with the same inoculum also developed higher levels of response. There was, however, a marked reduction of the levels of contact sensitivity exhibited by infected A/Sn mice on the 15th day post infection. The mortality rate for A/Sn mice inoculated with 50 blood forms of **T. cruzi** was 80% on day 20 post infection, while all C57B1/10J mice survived.

TABLE II
Contact sensitivity to DNFB in C57B1/10J and A/Sn mice infected with 50 forms of **T. cruzi**

Days after infection	24 hr ear swelling (x10^{-2} mm) *	
	C57B1/10J	A/Sn
8	[a] 13.5 ± 1.0 (n = 6)[§]	[c] 25.0 ± 7.2 (n = 6)
15	[b] 13.6 ± 2.5 (n = 7)	[a] 6.6 ± 2.5 (n = 5)
22	[c] 13.1 ± 3.9 (n = 7)	Not Done
29	[b] 14.4 ± 3.9 (n = 7)	Not Done
Uninfected sensitized controls	8.8 ± 2.2 (n = 6)	16.2 ± 4.8 (n = 8)

* Tests done on day 7 after sensitization with 0.25% DNFB. Mean ± S.D.
§ Number of animals per group in parenthesis
p values for tests comparing infected vs uninfected sensitized controls of the same strain:
a $p < 0.005$
b $p < 0.001$
c $p < 0.02$

Contact sensitivity to DNFB obtained for both uninfected and infected (8th day post infection) A/Sn mice were significantly greater ($p < 0.005$) than the corresponding values obtained for C57B1/10J mice.

Next we proceeded to look for an inoculum of **T. cruzi** which would lead to parasitaemia levels of the same order in both strains of mice and allow the survival of the majority of A/Sn and C57B1/10J mice.

Figure 1 shows that an inoculum of 2 x 10^4 blood forms for C57B1/10J mice and an inoculum of 2 x 10^1 blood forms for A/Sn mice fulfills these criteria.

DTH to DNFB, however, was not impaired for C57B1/10J mice infected with 2 x 10^4 forms while A/Sn mice still exhibited a significant decrease around the 16th day post infection when inoculated with only 20 forms (Table III). Moreover, both strains exhibited enhanced responses from the third week post infection on. Uninfected sensitized A/Sn controls showed higher responses than C57B1/10J controls ($p < 0.03$), as was also observed on Table II. No enhancement of the responses could be observed for either strain during the first two weeks of infection.

C57B1 mice infected with 1 x 10^5 forms failed to show depressed contact sensitivity reactions to DNFB even immediately before their death (Fig. 2). The levels of parasitaemia were higher than those observed for infection with 2 x 10^4 forms. Mortality rates were comparable to those observed for A/Sn mice inoculated with 50 forms (Fig. 2).

DISCUSSION

Our results show that strain Y **T. cruzi** infection did not impair the ability of C57B1/10J mice to mount and express contact hypersensitivity (CH) to DNFB. However, A/Sn mice which are a much more susceptible strain[8] exhibited significant, albeit transitory, suppression of CH during the infection with the same strain of **T. cruzi.**

The observed suppression occurred when parasitaemia levels were reaching their highest levels during the infection.

Suppression of DNFB reactivity in A/Sn infected mice cannot be ascribed solely to the higher parasitaemia and mortality rates observed

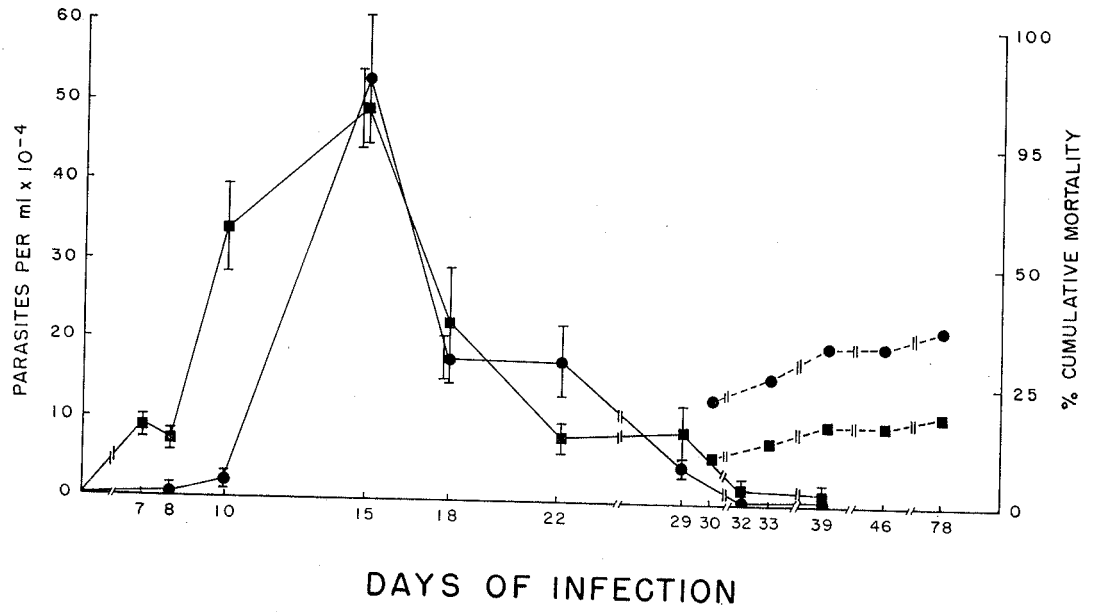

Fig. 1 — Course of parasitaemia in C57B1/10J mice infected with 20,000 forms (■——■) and A/Sn mice infected with 20 forms (●——●) of **T. cruzi**. Means of 8 animals ± SE. Cumulative mortality rates (%) for groups of 52 C57B1/10J (■) and 60 A/Sn (●) mice are indicated by the interrupted lines.

TABLE III

Contact sensitivity to DNFB in C57B1/10J and A/Sn mice infected with **T. cruzi** *

Days after infection	24 hr ear swelling (x10⁻² mm) §	
	C57B1/10J	A/Sn
9	8.4 ± 4.1 (n = 8) §§	13.0 ± 4.6 (n = 8)
16	11.1 ± 5.3 (n = 8)	6.6 ± 4.3 (n = 7) c
23	12.0 ± 3.3 (n = 13) b	15.8 ± 7.2 (n = 4)
30	20.0 ± 8.1 (n = 10) a	21.4 ± 7.0 (n = 12) b
45	Not done	28.7 ± 6.8 (n = 6) a
Uninfected sensitized controls	7.0 ± 4.2 (n = 6)	13.2 ± 6.4 (n = 8)

* C57B1/10J mice infected with 20,000 parasites A/Sn mice infected with 20 parasites

§ Tests done on day 7 after sensitization with 0.25% DNFB. Mean ± S.D.

§§ Number of animals per group in parenthesis

p values for tests comparing infected vs. uninfected sensitized controls of the same strain

a $p < 0.001$

b $p < 0.01$

c $p < 0.02$

in this strain. A/Sn mice infected with inocula of **T. cruzi** which allowed survival for 45 days or more, still exhibited depressed DNFB responses around the 16th day post infection. Parasitaemia and mortality rates comparable to those observed in A/Sn mice were obtained by injecting C57B1/10J mice with a thousand fold greater inoculum of **T. cruzi** without any suppression of DNFB reactivity. Infection of C57 B1/10J mice with 1 x 10^5 blood forms, which leads to mortality rates close to those obtained in A/Sn mice with 50 forms, failed to suppress CH responses to DNFB.

On the other hand, the virulence of the strain of **T. cruzi** used for determining the presence or absence of immunosuppression is also quite important. REED et al.[6] have reported suppression of CH to oxazolone in C57B1/10J mice infected with 1 x 10^3 Tulahuen strain blood forms leading to 100% mortality on the 18th day post infection.

Our results are in agreement with those related by CORSINI et al.[3] who worked with C3H He/J and (CBA x C57B1/10)F_1 mice and the strain Y of **T. cruzi.**

Suppression of CH reactions in A/Sn mice coincident with recovery from the first parasitaemia peak might be related to a number

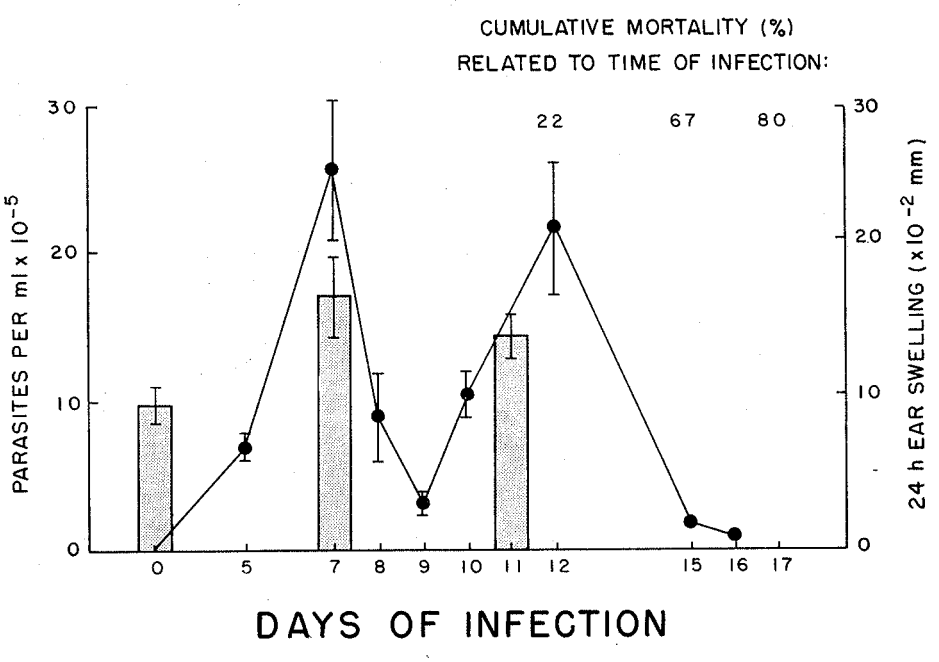

Fig. 2 — Parasitaemia (●——●), cumulative mortality rates (%) and contact hypersensitivity to DNFB (▨) in C57Bl/10J mice infected with 1 x 10^5 blood forms of **T. cruzi**. Means of 9 animals ± SE.

of yet unknown factors. Our results indicate that these animals albeit more susceptible to **T. cruzi** developed higher CH reactions to DNFB than C57Bl/10J mice. Thus it seems that an intrinsic impairment of the ability to mount a cellular response cannot be invoked to explain A/Sn susceptibility to infection.

The requirement of intact monocytes/macrophages for expression of CH reactions in infected animals sensitized with oxazolone was already stressed [6]. Thus differences in bone marrow tropism, rate of destruction of parasites or kinetics of the humoral responses may all be responsible for the transitory suppression of CH responses to DNFB in A/Sn mice.

We have also observed increased levels of CH to DNFB as infection progressed for both strains of mice. This finding might reflect nonspecific cooperation between independent immune responses or stimulation of nonspecific inflammatory responses.

RESUMO

Hipersensibilidade de contacto a DNFB em camundongos infectados pelo Trypanosoma cruzi

Camundongos das cepas C57Bl/10J e A/Sn infectados com a cepa Y de **Trypanosoma cruzi** foram sensibilizados com dinitrofluorbenzeno (DNFB) e testados na orelha 7 dias após para detecção da reação de hipersensibilidade de contacto (HC) a DNFB.

Camundongos da cepa A/Sn, que se mostraram muito mais suscetíveis à infecção pela cepa Y de **T. cruzi,** apresentaram supressão das reações de HC a DNFB, de caráter transitório e coincidente com a fase de alta parasitemia. Entretanto, não se detectou supressão das reações de HC a DNFB para camundongos da cepa C57Bl/10J infectados com inóculos de **T. cruzi** que levaram a níveis de parasitemia e mortalidade comparáveis aos obtidos em animais A/Sn. Esse tipo de comparação leva a crer que a supressão observada em camundongos A/Sn não está relacionada diretamente a níveis de parasitemia ou a um estado pré-agonico. Por outro lado, camundongos A/Sn não infectados exibiram níveis mais altos de reatividade do que camundongos C57Bl/10J, afastando uma eventual deficiência intrínseca daqueles animais na resposta ao DNFB.

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