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Enhancement versus Tumor Resistance Induced by Different Levels of Immunodepression in BALB/c Mice with Protozoan Infections*

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Abstract—*The relationship between the growth pattern of a spontaneous syngeneic adenocarcinoma and the immunodepression state caused by* Trichomonas vaginalis *and* Trypanosoma congolense *infection was evaluated in syngeneic BALB/c mice. We show that in* T. vaginalis *infected mice a slight and transient depression of both humoral and cellular immune reactivity induces an enhanced tumor growth. By contrast, a marked and long lasting depression of both humoral and cellular immune reactivity in mice infected with* T. congolense *occurs together with a delay in tumor take and a slower tumor growth.*

The various parameters affecting tumor growth in protozoan infected mice are discussed.

INTRODUCTION

One of the most constant features of protozoan infections is a resulting disorder of the host immunological system. A reduced humoral response towards several unrelated antigens, in fact, has been demonstrated in malaria patients [1, 2] as well as in animals experimentally infected with various protozoa [3–6].

By contrast, the relationship between cellular immunity and protozoan infections is still controversial and different results have been reported for various experimental systems [7, 8]. This alteration of the host immunological system may have important clinical and biological implications, e.g., decreased resistance to superinfection by several viruses and bacteria [5, 9, 10]. Moreover, protozoan infections may influence the incidence and development of human and animal tumors [11–13]. *Plasmodium berghei yoelii* infection increases the frequency of lymphoma induced by Moloney leukaemia virus [9]. Moreover, a significant correlation has been found between malaria infection and E-B virus induced lymphoma [14, 15].

In the present report we analyze the relationship between the immunodepression induced by systemic infection with *T. congolense* and peritoneal infection with *T. vaginalis* and the resistance *in vivo* to the growth of a transplantable syngeneic mammary adenocarcinoma.

MATERIALS AND METHODS

Animals

Brother–sister mated inbred BALB/c mice raised in our Department were used. This strain originated from the colony bred in the Animal Production Branch, National Institutes of Health, Bethesda, (Maryland) U.S.A.

Trichomonas

A *Trichomonas vaginalis* strain (AI_1) was isolated from a woman during an acute in-

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fection and cultured axenically in agar-free Diamond's medium [16] as described by Cappuccinelli *et al.* [17]. This strain was maintained by freezing at −80°C in 10% glycerol [18]. A virulence test was performed by eliciting subcutaneous abscesses in BALB/c mice as described by Honigberg [19]. The experiments, here reported, were performed injecting each mouse i.p. with 0.5 ml of a suspension containing 7×10^6/ml viable organisms.

Trypanosomes

The strain of *Trypanosoma congolense* used was kindly provided by Professor Ivo De Carneri (Carlo Erba Pharmaceutical Co.). Infected mouse blood was distributed into capillary tubes, frozen and stored in liquid nitrogen at −196°C [20]. To readapt the *T. congolense in vivo*, the content of one tube was injected into a mouse. Six days later, when a heavy parasitaemia was evident, blood was collected in Alsever solution and Earle's buffer (1:10 ratio). The experiments, here reported, were performed injecting each mouse intraperitoneally (i.p.) with 0.2 ml of a suspension containing 5×10^4/ml viable organisms.

Tumor

An adenocarcinoma (ADK/1t), that arose spontaneously in our BALB/c mice, was used [21]. It was transplanted by subcutaneous inoculations of 0.2 ml of a suspension containing 1×10^5 live cells in Earle's solution. The injection site was palpated for the presence of a tumor every third day. Growth rates were measured with a caliper.

Immune response to sheep red blood cells

Infected and control mice were routinely challenged i.p. with 10^8 sheep red blood cells (SRBC). Five days later the mice were exanguinated and their spleen removed. The sera were inactivated at 56°C for 30 min. Haemagglutinating antibodies were assayed as described by Murray *et al.* [22]. The test was performed using 25 µl of each serum dilution and 25 µl of a 2% suspension of washed SRBC. The haemolitic antibodies were assayed in microtiter "U wells" plates (Labtek, Eurobio, Paris, France). Briefly, 25 µl of a 1% suspension of washed SRBC were added to 25 µl of each antiserum. After 15 min incubation at room temperature, 50 µl of guinea pig complement were added and after 45 min incubation at 37°C, antibody titres were read.

Plaque-forming cells (PFC) to SRBC were assayed by the monolayer technique of Cunningham [23]. Briefly, 0.1 ml of a suspension containing 10^6 splenic lymphocytes, 0.1 ml of 6% SRBC suspension and 0.2 ml of undiluted guinea pig complement were incubated at 37°C in a capillary chamber. Thirty five minutes later the number of PFC was determined at low power magnification. The number of plaques in 10 capillary chambers was counted and related to the number of PFC per 10^6 spleen cells.

Contact hypersensitivity

Contact hypersensitivity to 2-phenyl-4-ethoxymethylene-oxazolone (BDH-Chemicals Ltd, Poole, England) was induced in BALB/c mice as described by Asherson and Ptak [24]. Briefly, the shaved belly was sensitized with 0.1 ml of 2% oxazolone in ethanol. Skin reactivity was tested one week later by application of 0.1 ml of 1% oxazolone in olive oil to both ears. The thickness of the ear was measured, immediately before and 24 hr after the challenge, with a dial gauge micrometer (Panter).

PHA response

The response of mouse spleen cells to phytohaemagglutinin (PHA-M) (Difco Laboratories, Detroit, Mich, U.S.A.) was determined as described by Hayri and Defendi [25]. Briefly, spleen cells were incubated in plastic Petri dishes (Falcon Plastic, Los Angeles, U.S.A.) for 4 hr at 37°C in 95% air and 5% CO_2. Non-adherent cells were removed by repeated washing; 0.9 ml of a suspension of 2×10^6 lymphocytes/ml were placed in 16 × 19 mm glass tubes, and 0.1 ml of PHA (1:150 final dilution) or 0.1 ml of the medium were added to each tube. After 48 hr, the cultures were pulsed with 1 µCi (^{3}H)-thymidine (specific activity 2Ci/mmol), (Sorin, Saluggia, Italy). Sixteen hours later the cells were washed, collected on Whatman GF/A filters and counted in a Nuclear-Chicago, Mark I, liquid scintillation counter.

RESULTS

Humoral reactivity

Mice infected with *Trichomonas vaginalis* or *Trypanosoma congolense* were challenged with 0.1 ml of a suspension containing 10^8 SRBC, 7, 20, 28, 39 days after infection. The PFC number, haemolytic and haemagglutinating titre were evaluated 5 days after each challenge, at day 12, 25, 33, 44 after infection.

Results are shown in Fig. 1. Animals infected with *T. vaginalis* showed a decrease of

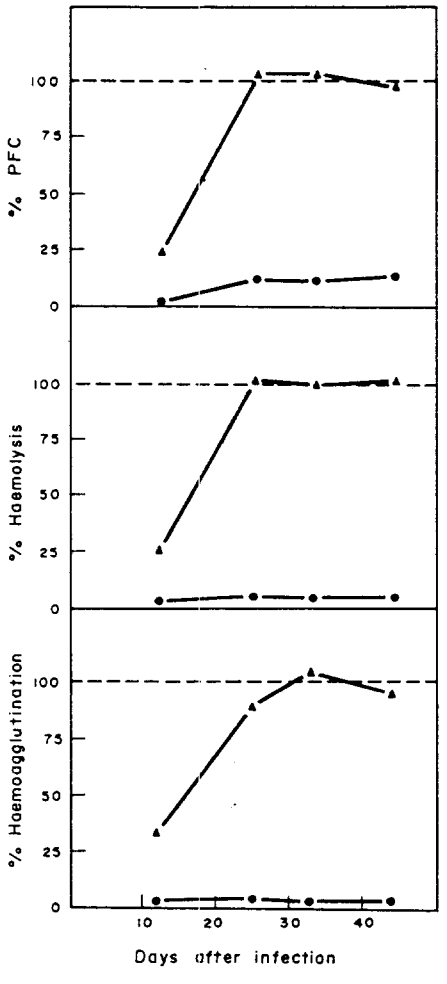

Fig. 1. *Effect of* T. Congolense *and* T. vaginalis *infection on the response to SRBC* in vivo. *PFC number, haemolytic and haemagglutinating antibody titres, expressed as percentages of the controls, are plotted against the number of days after protozoan infection.*

Each point is the mean ± S.D. *of* 5 *animals for each group.*
(● – –●) T. congolense *infected mice.*
(▲ – –▲) T. vaginalis *infected mice.*
(- - - -) *Uninfected control mice.*

the PFC number, the haemolytic and haemagglutinating antibody titre, 12 days after infection. Thereafter, the response returned to the same level as the non-infected controls.

On the other hand, animals infected with *T. congolense* showed a marked decrease of all three parameters throughout the experiment.

Contact hypersensitivity

The contact hypersensitivity to oxazolone was evaluated in normal, *T. vaginalis* or *T. congolense* infected mice sensitized 1, 8, 15 and 22 days after infection. As shown in Table 1, 8 days after infection, both *T. vaginalis* and *T. congolense* infected mice presented a marked depression of the cellular response to oxazolone compared with the controls. However, *T. vaginalis*-infected animals returned to the normal level on day 15 and *T. congolense* infected animals on day 29.

PHA response

Spleen cells responses to PHA in normal and *T. vaginalis* or *T. congolense* infected mice were evaluated 8, 15, 22, 29 and 36 days after infection. Figure 2 shows the ^{3}H-thymidine uptake of spleen cells from infected mice after 72 hr stimulation with the optimal dose (final dilution 1:150) of PHA expressed as percentage of uninfected animals response. A significantly weaker response in both infected groups was observed by day 15. However, while *T. vaginalis* infected mice reached normal levels by day 22, *T. congolense* infected animals did not return to normal levels until day 29. The same pattern of depression of the PHA response was observed using suboptimal doses (range 1:75–1:300 final dilution) of PHA (data not shown).

Table 1. *Increase in ear thickness, after sensitization with oxazolone in* T. vaginalis *and* T. congolense *infected mice*

	Days after infection			
Treatment	8	15	22	29
T. vaginalis	5.2*†	9.6‡	9.0‡	8.5‡
T. congolense	5.1*	4.3*	1.8*	8.4‡
—	13.4	11.4	12.0	8.8

Statistical significance of the difference between infected and uninfected mice was evaluated by "*t* Student" analysis.
*$P<0.01$.
†Increase in units of 10^{-3} cm. Each point is the mean of 10 animals for each group.
‡Not significant.

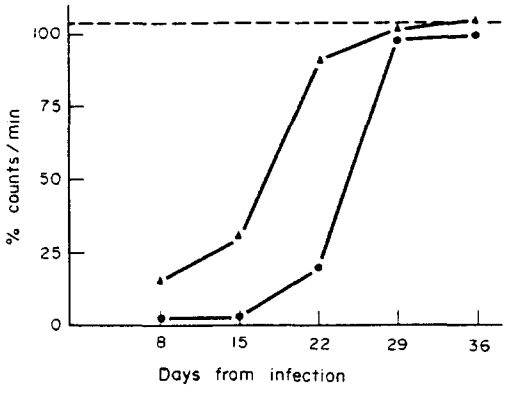

Fig. 2. Effect of T. congolense *and* T. vaginalis *infection on the spleen cells response to PHA.*
(^{3}H) -thymidine uptake (in counts/min*), expressed as percentage of the controls after PHA stimulation, is plotted against the number of days after protozoan infection. Each point is the mean* ±S.D. *of 5 animals for each group.*
(●——●) T. Congolense *infected mice.*
(▲——▲) T. vaginalis *infected mice.*
(----) *Uninfected control mice.*

Tumor incidence and growth

Tumor incidence and growth were evaluated in normal and *T. vaginalis* or *T. congolense* infected mice injected with 10^5ADK/1t live cells one week after infection. Tumors were detected by palpating the injection site every 3 days and diameters were measured with a caliper.

Initially, there was a slightly higher incidence of tumors in animals infected with *T. vaginalis* by comparison with the controls (Fig. 3). This difference gradually disappeared and an incidence of 100% was observed between 28 and 32 days after tumor injection in these two groups. Besides this acceleration of tumor taking, a significantly higher rate of tumor growth in *T. vaginalis*-infected group was observed (Fig. 4). In fact, the tumor mass was constantly higher in *T. vaginalis*-infected mice than in the controls, even after 100% tumor incidence was reached.

By contrast, in the *T. congolense*-infected mice, the tumor take was markedly lower than in the controls and reached 100% incidence only 18–20 days after the uninfected animals (Fig. 3). Moreover, as shown in Fig. 4, tumor growth in *T. congolense*-infected mice was consistently slower than in the other groups, even when the tumor incidence was 100%.

DISCUSSION

The present study has shown the effects of *T. vaginalis* and *T. congolense* infection on some parameters of the immune response and the influence on the growth of a transplantable syngeneic tumor.

T. vaginalis infection led to a relatively slight and short-lived (2 weeks) fall in either cellular or humoral immune reactivity. By contrast *T. congolense* infection induced a drastic and persistent depression of all the immunologic parameters evaluated. The humoral

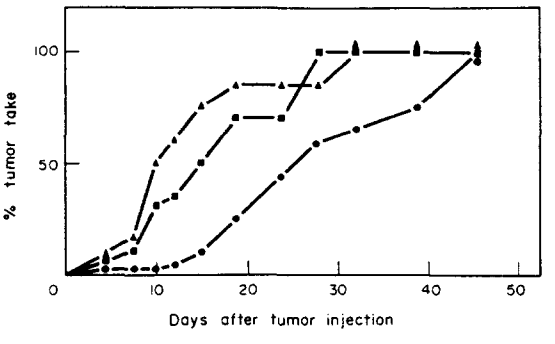

Fig. 3. Effect of T. congolense *and* T. vaginalis *infection on ADK/1t incidence. Experiments were carried on with* 20 *animals for each group.*
(● - -●) T. congolense *infected mice.*
(▲——▲) T. vaginalis *infected mice.*
(■——■) *Uninfected control mice.*
From the day 10th *to the* 32nd *after tumor injection, values of* T. congolense *infected mice* (●——●) *are significantly different* ($P<0.05$) *as evaluated by "chi square" analysis with Yates correction for small sample size.*

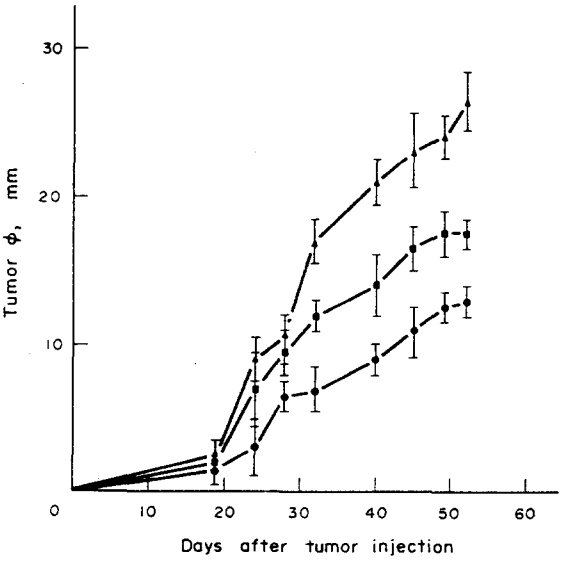

Fig. 4. Effect of T. congolense *and* T. vaginalis *infection on ADK/1t growth.*
Each point is the mean ±S.D. *of tumor diameter of ADK/1t bearing mice.*
(●——●) T. congolense *infected mice.*
(▲——▲) T. vaginalis *infected mice.*
(■——■) *Uninfected control mice.*
From 32nd *day after tumor injection, values are significantly different* ($P<0.05$) *as evaluated by "t" Student analysis.*

response was virtually abolished throughout the course of the experiments (10 weeks), while the cellular reactivity, as measured by skin reactivity and mitogen response, returned to normal levels only 3–4 weeks after infection. These results are in agreement with those of other investigators (1, 3, 26, 27). Greenwood *et al.* [28], using mice infected with *Plasmodium berghei yoelii*, observed a depressed humoral response to SRBC with no appreciable decrease in cellular reactivity as evaluated by skin graft rejection, contact hypersensitivity and mitogen response. Allt *et al.* [29] found that rabbits immunodepressed by infection with *Trypanosoma brucei* developed an autoimmune neuritis much weaker than controls.

Having shown the effects of protozoan infections on the immunologic parameters we evaluated, it was decided to analyze the growth of a transplantable tumor in animals infected with *T. vaginalis* and *T. congolense*.

The tumor used in our experiments shows tumor-specific and tumor-associated antigens, some of which can be employed to induce resistance in syngeneic animals [30, 31]. Previous work of this laboratory has indicated that its take and growth rate are influenced by spontaneous or artificially induced changes in immune reactivity of the host [21, 32]. Moreover other observations suggest that its growth in a syngeneic host is hindered by self induced immunological mechanisms that lead to a strong alteration of the immune response when the tumor mass reach critical diameters, several days after tumor induction [33].

The present results show different patterns of tumor growth related to various levels of immunodepression at the time of tumor inoculum when the immune system is completely unimpaired by tumor mass [34]. A transient and slight depression of the immune system after *T. vaginalis* infection, during the initial period of tumor-host relationship caused a significant enhancement in the tumor growth. By contrast, a delayed appearance and a decreased rate of tumor growth was observed after *T. congolense* infection which led to a marked and lasting depression of both humoral and cellular reactivity.

Some hypotheses to explain these observations may be advanced. Some investigators [35] found a massive splenomegaly and enhanced macrophage activation in animals infected with Trypanosoma. In two other parasitic infections, tumor resistance was correlated with the appearance of macrophages showing *in vitro* cytostatic activity against tumor cells [36, 37]. In the light of these results we would suggest that strong macrophage activation is responsible for the increased resistance to ADK/1t growth *in vivo*. The importance of activated macrophages in killing tumor cells both *in vivo* and *in vitro* has been shown by several investigators (for a review see [38]), even when such activation is not accompanied by lymphoid reactivity [32].

It may well be that systemic infection with *T. congolense* inhibits some of the weak initial cellular reactivity mechanisms that normally might induce immunostimulation of tumor growth [39–41]. Lastly, *T. congolense* infection may cause the formation of soluble factors that inhibit cell growth in general terms and in the same way inhibit neoplastic cell multiplication and lymphatic cell reactivity.

In conclusion low or high levels of immunodepression, probably related to low or high levels of macrophage activation, appear to induce different patterns of tumor growth, i.e., enhancement vs resistance. This could be in support to the hypothesis drawn by Prehn some years ago [42]: (a) stimulation of tumor growth whenever the immune reactivity is minimal, (b) inhibition of tumor growth at other times.

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