Effects of Trypanosoma brucei gambiense Infections in Microtus montanus on Susceptibility to Ehrlich’s Tumors

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Trypanosoma brucei gambiense infections in the field vole Microtus montanus increased susceptibility to Ehrlich’s tumor growth. Whereas uninfected voles were totally resistant to intraperitoneal Ehrlich’s ascites tumor cell challenge, over 78% of the animals infected with the trypanosomes developed tumors after challenge. Likewise, when Ehrlich’s ascites cells were injected subcutaneously to induce solid tumor formation, only 7% of uninfected controls developed tumors, whereas over 82% of trypanosome-infected animals exhibited malignancies after Ehrlich’s cell challenge. Finally, when solid tumors grown in albino CD-1 mice were implanted subcutaneously into uninfected voles, the tumor mass rapidly diminished in size and could not be found when animals were examined 2 weeks postimplant. However, in trypanosome-infected voles, implanted tumors exhibited pronounced expansion, and viable, solid tumors were recovered from over 70% of the challenged voles at 2 weeks postimplant. The implications of trypanosome-induced immunosuppression, especially toward susceptibility to neoplastic growth, are discussed.

It is now a well-established fact that certain protozoan infections depress the immunological responses of infected hosts. Malarial infections in rodents and humans suppress normal antibody levels to inert antigens, such as heterologous erythrocytes (1, 7-9, 15, 25, 28) and human gamma globulin (9). Similarly, antibody responses are depressed during trypanosomiasis in rodents and humans (5, 6, 10, 14, 16, 17).

The biological manifestations of protozoan-induced immunodepression are becoming evident, and the implications may be manifold. Impairment of humoral immunity probably underlies the increased susceptibility of malaria-infected mice to Salmonella typhi infections, as reported by Kaye and associates (13). Salaman et al. (21) reported that the pathological effects of mouse sarcoma virus in spleens of mice were increased by prior infection with Plasmodium berghei yoelii and Wedderburn (27, 28) reported that concurrent infections in mice with P. berghei yoelii and Moloney lymphomagenic virus resulted in increased incidences of malignant lymphomagenesis. These investigations arose from the increasing amount of evidence that implicates a connection between holoendemic malaria and Burkitt’s lymphoma in African children (2, 3).

Likewise, in trypanosome infections in rodents, immune resistance to other parasites is impaired. Expulsion of Nippostrongylus brasiliensis from the guts of mice and rats was delayed in Trypanosoma brucei-infected animals (4, 26). Also, Phillips and co-workers (20) reported that T. brucei infections in mice depress the expulsion of Trichuris muris from both primary infections and from hyperimmune animals. This present investigation was designed to examine the biological implications of T. brucei gambiense infections upon the immunological resistance of Microtus montanus to Ehrlich’s tumor growth.

MATERIALS AND METHODS

Experimental animals. M. montanus were obtained from an outbred colony maintained at Tulane University in New Orleans, La. The stock, care, and maintenance of the colony have been described by Seed and Negus (24). All animals used in this study were sexually mature and maintained on a 12-h light-12-h dark lighting regime. White mice (CD-1), used as controls, were obtained from Charles River Laboratories, Wilmington, Mass. Animals were always infected with trypanosomes, challenged with Ehrlich’s tumor cells, and sacrificed between 10:00 a.m. and 2:00 p.m. to obviate variations resulting from possible circadian rhythms.

Parasites. The Microtus were infected intraperitoneally with 5 x 10⁶ organisms of T. brucei gambiense Wellcome TS strain. The origin, maintenance, and pathogenicity of this strain in Microtus have been described by Seed and Negus (24) and Ackerman and Seed (submitted for publication). Prior to infecting Microtus, trypanosomes were har-
vested from the blood of mice as described by Seed and Baquero (22) and suspended to the appropriate concentration (10⁶ organisms/ml) in glucose-Ringer phosphate buffer (23).

Ehrlich's tumor cells. Ehrlich's ascites tumor cells were isolated from the peritoneal cavity of one mouse (kindly supplied by Sydney Craig of The University of South Carolina). Cells were suspended in Hanks balanced salt solution, pH 7.3, and maintained by syringe passage in 25-g mice every 10 days. Animals challenged with Ehrlich's ascites cells received one intraperitoneal injection of 1 × 10⁶ cells in Hanks balanced salt solution, as described below. Positive neoplastic growth was determined by a combination of observable factors, including significant weight increase (over 15%), peritoneal bloating, and the direct microscope observation of ascites cells in peritoneal fluid.

Solid Ehrlich's tumors were established in one mouse by injecting 1.0 × 10⁶ Ehrlich's ascites tumor cells (isolated from one mouse and suspended in Hanks salt solution) intramuscularly in each hind leg. When these tumors caused severe swelling, the mouse was sacrificed (chloroform overdose) and the tumors were surgically removed. This cancerous tissue was immediately immersed in cold (4°C) Eagle minimum essential medium and cut into 1-mm³ cubes.

Microtus, which were to receive these tumors, were anesthetized with Nembutal (0.05 mg/kg body weight) (Abbott Laboratories), and their backs were shaved. One piece of solid tumor was then implanted subcutaneously through a 1-cm incision placed immediately posterior to the right scapula. The incision was closed with two sutures (4-0 silk, Ethicon Inc.).

Another group of animals received subcutaneous injections of 1.0 × 10⁶ Ehrlich's ascites cells (from the peritoneal fluid of one mouse) also immediately posterior to the right scapula. For both solid and ascites tumor challenges, normal mice were the last animals to be challenged, thus constituting controls for the infectivity of the Ehrlich's cells. Positive solid tumor growth was determined by the presence of a visible or palpable "lump" of over 1 cm in diameter at the site of injection or implant after 2 weeks. Two weeks after implant, approximately 50% of the palpable tumors were surgically removed and implanted in mice (one Microtus tumor per mouse). All mice receiving these implants demonstrated positive tumor growth and ultimately died within 14 days, indicating the tumors contained viable cells and the original visual diagnoses were sufficiently valid.

**RESULTS**

Ehrlich's ascites tumor growth. Trypanosome infections in Microtus enhanced susceptibility to Ehrlich's ascites tumor cells administered via intraperitoneal injection (Table 1). In 25 control mice, 100% of challenged animals exhibited tumor growth and all died within 13 days postchallenge. On the other hand, none of the uninfected Microtus exhibited tumor growth after administration of Ehrlich's ascites tumor cells. But, trypanosome-infected Microtus were highly susceptible to Ehrlich's ascites tumor growth, in direct relation to duration of trypanosome infection.

Over 86% of Microtus infected with trypanosomes for 24 days (group D) exhibited positive tumor growth, whereas, in those infected for 1 day prior to Ehrlich's cell challenge (group B), only 66.7% demonstrated ascites tumor growth. Of all trypanosome-infected animals challenged with Ehrlich's ascites cells, 78.4% demonstrated positive neoplastic growth.

Ehrlich's solid tumor growth. Table 2 demonstrates the susceptibility of uninfected Microtus and T. brucei gambiense-infected Microtus to subcutaneous Ehrlich's ascites cell challenge. Control mice exhibited no resistance to Ehrlich's tumor growth, whereas of the uninfected Microtus (group A) only 1 of 14 animals possessed a small palpable "lump" 2 weeks after challenge with ascites cells. Upon surgical removal, the tumor of this uninfected Microtus appeared to be less compact (easily disintegrable when placed in medium) than any of the other solid tumors observed. Nevertheless, this tumor did contain viable cells, since the mouse that received the implant of this tumor did develop a solid growth and did subsequently die. In the remaining uninfected Microtus, no "lumps" appeared and no signs of cancerous growth were visible when animals were autopsied several months after these investigations.

However, in the Microtus infected with trypanosomes 1 day prior to Ehrlich's cell chal-

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**Table 1. Ehrlich's ascites tumor growth in uninfected and T. brucei gambiense-infected M. montanus**

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>Days infected*</th>
<th>No. of animals</th>
<th>% with Ehrlich's ascites tumor growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Uninfected Microtus</td>
<td>22</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Infected Microtus</td>
<td>1</td>
<td>12</td>
<td>66.7</td>
</tr>
<tr>
<td>C</td>
<td>Infected Microtus</td>
<td>7</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>D</td>
<td>Infected Microtus</td>
<td>24</td>
<td>15</td>
<td>86.7</td>
</tr>
<tr>
<td>Total</td>
<td>Infected Microtus</td>
<td>37</td>
<td></td>
<td>78.4</td>
</tr>
</tbody>
</table>

* Recorded as days infected with trypanosomes prior to Ehrlich's cell challenge.
challenge (group B), 75% developed solid tumors. Likewise, in Microtus infected for 14 and 22 days prior to Ehrlich's cell challenge (groups C and D), 85.7% and 83.7%, respectively, developed positive solid tumor growth. Of all the Microtus infected with trypanosomes, 82.1% developed solid tumors after subcutaneous Ehrlich's ascites cell challenge, compared with 7.1% in uninfected Microtus.

Solid mouse tumor implant. Table 3 shows the results of an investigation into the susceptibility of uninfected and T. brucei gambiense-infected Microtus to subcutaneous solid Ehrlich's tumor implant. Solid tumors, consisting of 1-mm³ "chunks" of solid Ehrlich's tumors grown in mice, successfully grew 100% of the time when implanted in control mice, but none of the uninfected Microtus (group A) demonstrated tumor growth, even when examined 2 months after implant. The implanted tumors in uninfected Microtus seemed to dissipate and eventually disappear within 2 weeks of the operation but, in Microtus infected with trypanosomes 1, 7, and 22 days prior to implant (groups B, C, and D), positive tumor growth was observed in 60, 75, and 80%, respectively, of the animals challenged. In these animals the size of the "lump" after implant exhibited pronounced expansion, and solid, viable tumors were found when the animals were autopsied 2 weeks after implant. Again, the percentage of animals demonstrating positive solid Ehrlich's tumor growth increased in direct relation to the duration of trypanosome infection. Among the trypanosome-infected Microtus, a total of 70.4% exhibited solid tumor growth after implant.

**DISCUSSION**

*T. brucei gambiense* infections in *M. montanus* result in pronounced humoral and cellular-mediated immunosuppressive effects (Ackerman and Seed, submitted for publication). We have noted a relatively higher incidence of spontaneous tumor formation in trypanosome-infected voles maintained in our facilities than in uninfected laboratory populations (unpublished data). The experiments described in the present paper were carried out to investigate the effects of trypanosome infection in *Microtus* upon susceptibility to Ehrlich's tumor growth. The results of this project suggest that, due to the immunosuppressive effects of trypanosomiasis, *Microtus* become highly susceptible to tumor growth. This enhanced susceptibility was exhibited within 1 day after trypanosome infection, and in two of three experiments (see Tables 1 and 3) susceptibility increased in direct relation to the duration of trypanosome infection prior to tumor challenge.

The relationship between artificially induced states of immunosuppression and the incidence of cancer is becoming increasingly obvious, especially due to the widespread practice of im-

### Table 2. Growth of subcutaneous Ehrlich's ascites cell challenge in uninfected and *T. brucei gambiense*-infected *M. montanus*

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>Days infected</th>
<th>No. of animals</th>
<th>% with Ehrlich's solid tumor growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Uninfected Microtus</td>
<td>14</td>
<td>7.1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Infected Microtus</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Infected Microtus</td>
<td>7</td>
<td>85.7</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Infected Microtus</td>
<td>22</td>
<td>83.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Infected Microtus</td>
<td>28</td>
<td>82.1</td>
<td></td>
</tr>
</tbody>
</table>

* Recorded as days infected with trypanosomes prior to Ehrlich's ascites cell challenge.

### Table 3. Growth of solid Ehrlich's tumors in uninfected and *T. brucei gambiense*-infected *M. montanus* after solid mouse tumor implant

<table>
<thead>
<tr>
<th>Group</th>
<th>Condition</th>
<th>Days infected</th>
<th>No. of animals</th>
<th>% with Ehrlich's solid tumor growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Uninfected Microtus</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Infected Microtus</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Infected Microtus</td>
<td>7</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Infected Microtus</td>
<td>22</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Infected Microtus</td>
<td>27</td>
<td>70.4</td>
<td></td>
</tr>
</tbody>
</table>

* Recorded as days infected with trypanosomes prior to surgical implant of solid mouse Ehrlich's tumor.
Immunosuppressive therapeutics utilized during transplantation surgery (for review, see 19). But, it is only recently that we have realized that certain microbial and protozoan diseases may be associated with neoplastic disease states, due to the immunosuppressive effects of parasitic organisms. Many viruses, both oncogenic and nononcogenic, can suppress various expressions of the immunological defenses of a host (18), and these effects can result in increased incidences in cancers arising spontaneously or after oncogenic virus infections (for review, see 11). Furthermore, there is good reason to believe that there is a causal connection between Burkitt’s lymphoma and holoendemic malaria (2, 3).

Immunodepression during trypanosomiasis has been substantiated to occur in naturally infected humans (10) and cattle (12). The import of this phenomenon has been stressed in relation to the enhancement of the trypanosome infection itself, to the predisposition to secondary bacterial and viral infections, and to the recommendation that vaccination campaigns should not be conducted during trypanosomiasis epidemics or in infected patients. The implications of the results of this present investigation suggest that one might expect to find higher incidences of neoplasia in subjects harboring trypanosome infections when compared with their uninfected counterparts.

ACKNOWLEDGMENTS

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LITERATURE CITED