Immunology 1994 **81** 468–474

Suppression by *Trypanosoma brucei* of anaphylaxis-mediated ion transport in the small intestine of rats

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SUMMARY

The hypothesis that failure of hosts infected with *Trypanosoma brucei* to express type 1 hypersensitivity is related to this parasite's ability to down-regulate IgE production, and not to an innate lack of allergenicity of *T. brucei* antigens, was tested by studying anaphylaxis-induced changes in net epithelial ion transport in rats. Transport changes were quantified electrophysiologically *in vitro*, as a change in transmural short-circuit current when sensitized intestine was challenged with homologous antigen. Rats injected parenterally with trypanosome antigen elicited intestinal anaphylaxis in response to antigenic challenge, whereas the intestine of rats infected with *T. brucei* failed to respond. Infection with *T. brucei* also suppressed the anaphylactic response in rats sensitized to and challenged with ovalbumin and *T. spiralis*-derived antigens. In these cases suppression was related to the ability of *T. brucei* to block production of IgE, and not to the physiological failure of the epithelial response. However, in rats sensitized by infection with *T. spiralis*, neither the anaphylactic response nor IgE production were inhibited by *T. brucei*. Furthermore, intestinal mastocytosis normally associated with trichinosis was unaffected by the trypanosome infection. Results support the conclusion that the failure to express anaphylaxis in *T. brucei*-infected rats is due to the inhibition of IgE production and not to the lack of allergenicity of trypanosome antigens.

INTRODUCTION

The protozoan parasite *Trypanosoma brucei brucei* is one of the causes of African trypanosomiasis in both wild and domestic animals and is very closely related to the two subspecies, *T. b. rhodesiense* and *T. b. gambiense*, the causative agents of sleeping sickness in humans.[1] Trypanosomes have diverse effects on the host's immune system, affecting almost all of the lymphoid cell subpopulations.[2] Although the exact nature of the interaction between the trypanosome and its host's immune system is not completely understood, a general immunosuppression is generally observed.[3,4] As well as modulating lymphoid cell functions, there is a widespread perception that trypanosomes and other protozoa, especially when compared with helminths, are unable to invoke the components of type 1 hypersensitivity or anaphylaxis, i.e. IgE, mast cells and eosinophils.[5,6] This apparent inability to prime for anaphylaxis is due either to the non-allergenic nature of trypanosome antigens or to the ability of *T. brucei* to inhibit the production and/or secretion of specific cytokines and growth factors necessary for type 1 mediator production and secretion.

Our objective was to test the hypothesis that the failure of hosts infected with *T. brucei* to express type 1 hypersensitivity is related to the parasite's ability to down-regulate immunoglobulin E (IgE) production and not due to an innate lack of allergenicity on the part of *T. brucei*-derived antigens. The hypothesis was tested by using a well-characterized anaphylactic response evoked in rats infected with the nematode *Trichinella spiralis*, i.e. anaphylaxis-mediated epithelial net Cl^- secretion in the intestine.[7,8] In this host–parasite system the intestinal epithelium of rats sensitized with various antigens or by infection with *T. spiralis*, can be induced to secrete Cl^- ions when the sensitized intestine is exposed to the homologous antigen. This local anaphylactic response can be quantified electrophysiologically as a change in transmural short-circuit current when the challenged tissue is mounted in Ussing chambers and exposed to the sensitizing antigen. The response is dependent on IgE homocytotropic antibodies and mast cells impinging on the epithelial cells via the mediators histamine, serotonin and prostaglandin E_2 (PGE_2).[9]

Received 13 July 1993; accepted 30 September 1993.

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MATERIALS AND METHODS

Experimental animals and parasites

Outbred male Sprague-Dawley rats (Hilltop Lab Animals, Scottsdale, PA), initial body weight 100–124 g, were used as experimental hosts. *Trypanosoma brucei brucei*, stock TREU 667, was kept as frozen stabilates in liquid nitrogen. For infection, stabilates were first grown in male CF-1 mice, 20–30 g

(Charles River, Wilmington, MA), and after 3–5 days the mice were killed and the trypanosomes isolated and injected into rats. Each rat received 10^5 trypanosomes.

The nematode *Trichinella spiralis* was used to sensitize the gut of some rats. The worms were isolated as infective larvae (L_1) by enzymatic digestion of skeletal muscle from infected CF-1 mice as previously described,[10] and each rat was infected with 3×10^3 larvae. The strain of *T. spiralis* used was one that has been maintained in this laboratory for ~20 years. The phenomenon of rapid rejection, as an example of functional immunity dependent on the expression of type 1 hypersensitivity, was investigated by infecting various immunized rats with 2×10^3 infective larvae via oral inoculation. After 24 hr the rat intestines were isolated and the worms counted using the method of Castro and Fairbarn.[10]

Immunological methods

Antigen preparations. Trypanosome antigen, larval somatic antigen of *T. spiralis* (LSA) and ovalbumin were used to sensitize the small intestine of rats. LSA was prepared from infective stage larvae as described previously.[11] To prepare trypanosome antigen, *T. brucei* was grown in rats to maximum parasitaemia (7–9 days) and separated from the blood by anion-exchange chromatography using DEAE cellulose.[12] They were then disrupted by rapid freezing and thawing in liquid nitrogen and a 37° water-bath, respectively, followed by homogenization. The homogenate was centrifuged at 10,000 ***g*** for 30 min at 5°. The final protein concentration was determined by the method of Lowry *et al.*[13] All antigens were administered to rats using an aluminium gel suspension as adjuvant. The antigens were adsorbed to $Al(OH)_3$ following the method outlined by Williams and Chase.[14] One hundred micrograms of *T. brucei* antigen and LSA, and 10 μg of ovalbumin were administered on days 0 and 21. The small intestine was removed for testing 5–9 days after the second or booster dose of antigen.

Passive cutaneous anaphylaxis (PCA). The PCA technique perfected by Watanabe and Ovary[15] was used to estimate the levels of circulating antigen-specific IgE in rat sera. Sequential double dilutions of 0·1 ml aliquots of pooled serum samples were administered intradermally into the shaved backs of anaesthetized male rats. After a sensitization period of 48–72 hr the rats were challenged intravenously with 2 mg of homologous antigen in 0·5 ml of 1·5% Evans Blue in phosphate-buffered saline (PBS). The anaphylactic reactions were measured 30 min later on the inner surface of the skin. Spots 5 mm or larger in diameter were considered positive PCA reactions. Titres were expressed as the highest dilutions eliciting a positive reaction.

Passive sensitization. The collection of hyperimmune serum and passive sensitization were carried out as described previously.[8] The level of *T. spiralis*-specific IgE was determined by PCA, and only serum with a PCA titre of 1:512 or more was used for passive sensitization. Sera from uninfected rats were used as controls.

Mast cells. The level of mastocytosis in the small intestine of *T. spiralis*-infected rats was studied at the light microscope level employing the mast cell stains, Alcian Blue/Safranin O, as described by Alizadeh and Wakelin.[16] Rats were infected with *T. spiralis* or *T. spiralis* and *T. brucei* for 35 days after which time 2-cm pieces of jejunum were removed and fixed in ice-cold Carnoy's fixative. After staining and mounting, the number of mast cells in the tissues was evaluated using a Nikon Optiphot binocular microscope. Mast cell numbers were enumerated per 20 villus crypt units (VCU) of the small intestine.[16]

Electrophysiological measurements

The proximal jejunum was removed from anaesthetized rats and immediately washed in Krebs–Ringer bicarbonate buffer (KRB) solution. The gut was then cut open lengthwise along the mesenteric border and washed in fresh KRB solution to remove any faecal material. Strips of jejunum, 2 cm in length, were mounted as a flat sheet between the two halves of an Ussing-type chamber and attached to a glass reservoir. Each reservoir was filled with 10 ml of KRB solution to bathe the mucosal and serosal sides of the jejunal tissue. The solutions were gassed continuously with 95% O_2–5% CO_2 at 37°. Due to the active transport of ions, mammalian small intestine is electrically polarized from serosa to mucosa with the serosal surface positively charged in reference to the mucosal surface. This transmural potential difference (PD) was measured using calomel electrodes connecting the serosal and mucosal solutions via agar bridges. The segments were then voltage-clamped at zero transmural potential difference by passing a direct current, equal and opposite to the spontaneous PD. This current required to nullify the spontaneous PD, the short-circuit current (I_{sc}), is an index of net active ion transport across the tissue. After reaching a steady-state short-circuit current, which occurred within 30 min after mounting the tissue, each segment was challenged on the serosal side with antigen or with various mast cell-derived Cl^- secretagogues. Following stimulation with these agents the maximal change in short-circuit current (ΔI_{sc}) from the steady-state condition was measured as an index of net ion transport.

Statistics

Statistical procedures outlined by Zar[17] were used. Results are expressed as the mean ± standard error of the mean (SE). When making comparisons between the means of two experimental groups, Student's *t*-test was performed. In cases where more than two means were compared, a single-factor analysis of variance (ANOVA) was used. Multiple comparisons between the means were subsequently analysed by the Tukey test. A *P*-value of 0·05 or less was considered statistically significant.

Materials

Histamine diphosphate, 5-hydroxytryptamine creatinine sulphate (5-HT) and PGE_2 were purchased from Sigma (St Louis, MO). Alcian Blue 8GX and Safranin O were purchased from Polysciences Inc. (Warrington, PA). DEAE cellulose (DE-52) was purchased from Whatman Labsales (Hillsboro, OR). Histamine and 5-HT were dissolved in KRB; PGE_2 was dissolved in ethanol.

Experimental design

Cl^- secretion was studied in jejunal tissues from rats which were either infected with *T. brucei* for a period not less than 35 days, or were injected parenterally with a soluble antigen preparation from *T. brucei*. The small intestine was then challenged in Ussing chambers with trypanosome antigen to measure and compare the expression of local anaphylaxis between the two different sets of sensitized rats. To assess the relative allergenicity of *T. brucei* antigens, a time curve of the change in short-circuit current induced by challenge with *T. brucei* antigens was

constructed and compared with the time curves for LSA- and ovalbumin-sensitized rat jejunum.

To investigate further the different effects on ion transport between trypanosome infection and antigen injection, and to confirm whether an infection with *T. brucei* can block or suppress antigen-induced Cl^- secretion, rats were first infected with *T. brucei* for 7 days before being parenterally sensitized with various antigens. Some rats were infected with *T. brucei* and then subsequently infected with *T. spiralis* in order to assess the effect of *T. brucei* on a much stronger inducer of type 1 hypersensitivity.

To differentiate whether the suppressive effect of *T. brucei* on Cl^- secretion was at the physiological or immunological level, the effects of various Cl^- secretagogues were studied. Physiologically, Cl^- secretion *per se* by the epithelial cells was examined in normal and *T. brucei*-infected rats by comparing their responses to histamine, serotonin and PGE_2. These agents are released by, or result from, antigen-stimulated mast cells in sensitized rats.[9] At the immunological level, we initially examined the effect of *T. brucei* on IgE production and mastocytosis. To study the possible impact of *T. brucei* on functional immunity, we evaluated acquired resistance to *T. spiralis* by measuring the rapid rejection of L_1 larvae from the intestine, an anaphylaxis-mediated immune response.[8,18]

RESULTS

Trypansoma brucei and sensitization for antigen-induced Cl^- secretion

Rats were infected with *T. brucei* for over 35 days, whereupon their small intestines were removed and tested in Ussing chambers. Other rats were sensitized by parenteral injection with trypanosomal antigen and $Al(OH)_3$ adjuvant. The ΔI_{sc} evoked by subsequent challenge are displayed in Fig. 1. Those animals infected with *T. brucei* elicited no increase in I_{sc} indicating a possible absence of gastrointestinal (GI) tract sensitization. In contrast, the trypanosomal antigen preparation was able to sensitize the GI tract as evident in the elevation in I_{sc}.

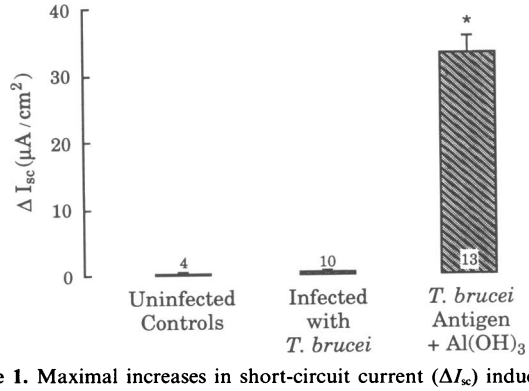

Figure 1. Maximal increases in short-circuit current (ΔI_{sc}) induced in jejunum from rats sensitized by infection with *T. brucei*, or by parenteral administration of *T. brucei* antigen. Rat jejunum was challenged in Ussing chambers with 100 μg/ml trypanosome antigen added to the serosal compartment. Values are mean ΔI_{sc} + SE; n = number at base of bar. Asterisk indicates significant difference ($P < 0.05$) compared with uninfected control using an unpaired t-test.

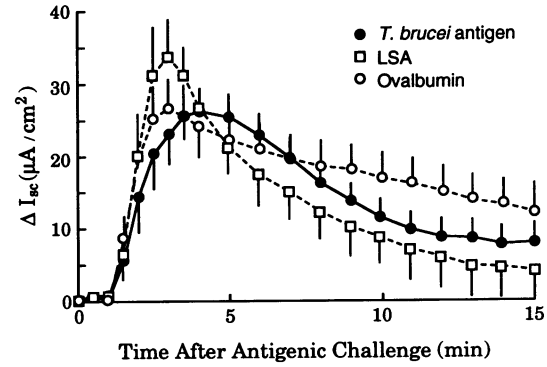

Figure 2. Time–course of change in short-circuit current (ΔI_{sc}) response to challenge with *T. brucei* antigen, LSA or ovalbumin, in jejunum from rats sensitized with the respective antigen. Each rat was sensitized by intraperitoneal injection with either 100 μg of *T. brucei* antigen or LSA, or 10 μg ovalbumin + $Al(OH)_3$. Tissues were challenged in Ussing chambers with 100 μg/ml antigen added to the serosal compartment. Values are mean ΔI_{sc} + SE; $n = 9$ rats for each group.

The increase in I_{sc} induced in rats sensitized to and challenged with *T. brucei* antigen was plotted over time and compared with the time curves for LSA and ovalbumin (Fig. 2). The ΔI_{sc} induced by *T. brucei* antigen in sensitized rats peaks at about 4 min after addition of homologous antigen to the Ussing chamber. This compares with a peak at ~3 min for LSA and ovalbumin. The similar overall profiles of the three time curves suggests that sensitization with *T. brucei* antigen is comparable to that for more common sensitizing antigens, indicating that even though a live infection with *T. brucei* may fail to sensitize rat jejunum, trypanosome antigens are clearly allergenic.

Trypanosoma brucei infection and suppression of Cl^- secretion

Due to the apparent inability of *T. brucei* infection to prime for antigen-induced Cl^- secretion, the possibility was explored that infection blocked or suppressed the sensitization to its own or other antigens. Rats infected with *T. brucei* for 7 days previously were parenterally injected with either trypanosomal antigen, LSA or ovalbumin (Fig. 3a), or infected with *T. spiralis* (Fig. 3b), and then challenged in Ussing chambers with homologous antigen. As evident, the intestines from animals infected with *T. brucei* failed to respond to antigenic challenge in contrast to uninfected counterparts, if the rats were sensitized with antigen. However, rats concomitantly infected with *T. spiralis* as the sensitizing agent were able to respond to challenge. There was no significant difference ($P < 0.05$) in ΔI_{sc} between those groups infected only with *T. brucei* and those concomitantly infected with both *T. brucei* and *T. spiralis*.

Potential suppression of Cl^- secretion mediators

To determine whether *T. brucei* might be exerting its effect by interfering with Cl^- secretion at the level of the epithelial cell, three Cl^- secretagogues, which have been previously determined to be the most important mast cell-derived mediators involved in Cl^- secretion in the rat,[9] were added to the serosal bathing fluid of Ussing chambers containing jejunum from *T. brucei*-infected rats (Fig. 4). The Cl^- secretory results (ΔI_{sc}) for animals infected with *T. brucei* were not significantly different

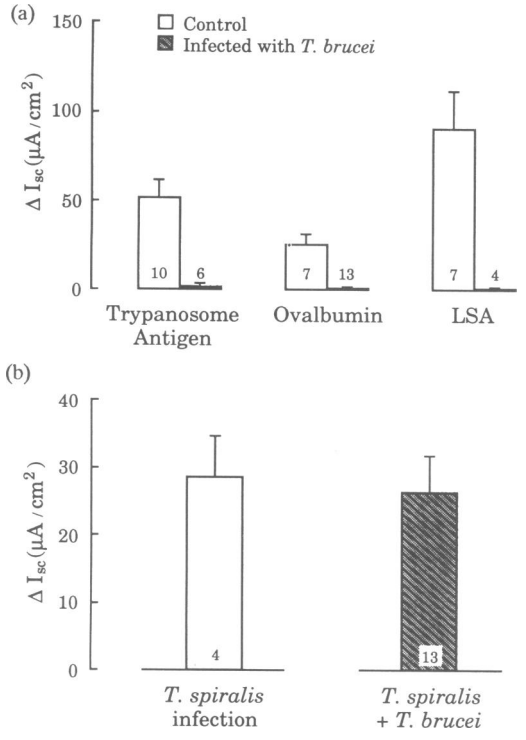

Figure 3. Effect of *T. brucei* infection on antigen-induced change in short-circuit current response (ΔI_{sc}) induced in jejunum from rats sensitized by (a) parenteral injection with *T. brucei* antigen, ovalbumin or LSA, (b) infection with *T. spiralis*. Challenge was carried out in Ussing chambers with 100 μg/ml of the homologous antigen added to the serosal chamber for those rats sensitized with antigen, and 25 μg/ml for rats infected with *T. spiralis*. Values are mean ΔI_{sc} + SE; *n* = number at base of bar.

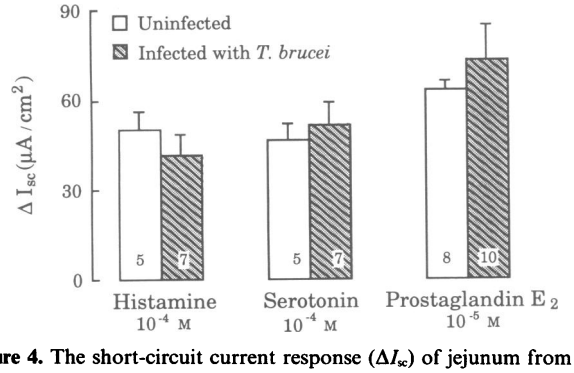

Figure 4. The short-circuit current response (ΔI_{sc}) of jejunum from *T. brucei*-infected rats, to exogenous Cl^- secretagogues. Histamine (10^{-4} M), serotonin (10^{-4} M) and PGE_2 (10^{-5} M) were added to the serosal bathing fluid of the Ussing chamber. Values are mean ΔI_{sc} + SE; *n* = number at base of bar.

($P < 0.05$) from those in uninfected animals. These results indicate that *T. brucei* does not interfere with immune-mediated ion transport by blocking or inhibiting Cl^- secretion at the level of the epithelium.

Effect of *T. brucei* on IgE production

Because *T. brucei* apparently has no adverse effects on the physiology of Cl^- secretion, relevant immunological aspects of antigen-induced Cl^- secretion were examined. In anaphylaxis-mediated Cl^- secretion, IgE and mast cells are key components in antigenic signal transduction. The amount of IgE in the serum of rats was subsequently determined by PCA (Table 1). Rats sensitized with various antigens all produced a PCA titre ranging from 1:16 to 1:32. However, when rats were concurrently infected with *T. brucei*, no detectable titre was recorded. As expected, rats infected with *T. spiralis* produced a much higher PCA titre than rats sensitized with LSA. Serum from rats infected with both *T. brucei* and *T. spiralis* also had a substantial IgE titre, even though it was lower than that in rats infected with *T. spiralis* only. This latter result explains why the intestines from rats with the double infection were still able to express antigen-induced Cl^- secretion in the Ussing chamber (Fig. 3b). Although *T. brucei* could suppress the amount of IgE produced compared to that in *T. spiralis*-infected rats, the level was still not low enough to prevent immune-mediated Cl^- secretion.

Table 1. IgE levels measured by passive cutaneous anaphylaxis (PCA) in serum from rats sensitized by parenteral administration of trypanosomal antigen, larval somatic antigen of *T. spiralis* (LSA) or ovalbumin, or by infection with *T. spiralis*

Method of sensitization	Serum PCA titre*	
	Uninfected rats	Rats infected with *T. brucei*
Adjuvant only	<1:2	—
Trypanosomal antigen	1:16	<1:2
LSA	1:16	<1:2
Ovalbumin	1:32	<1:2
T. spiralis infection	1:256	1:64

* Highest dilution eliciting an anaphylactic reaction.

Results from the prior experiments verified an apparent relationship between IgE and antigen-induced Cl^- secretion. To examine for a possible dissociation of this relationship in trypanosomiasis, rats infected with *T. brucei* for up to 35 days were administered 6 ml of serum containing IgE. The aim of this experiment was to determine if antigen-induced Cl^- secretion could be restored by passive transfer of homocytotropic antibody. Four days after serum transfer, intestinal segments from the recipient rats were mounted in Ussing chambers and challenged with *T. spiralis* antigen (Fig. 5). Antigen-induced Cl^- secretion was expressed in *T. brucei*-infected rats receiving exogenous IgE. This observation also confirms that *T. brucei* has no noticeable effect on the IgE/mast cell interaction because Cl^- secretion indicates that mast cell mediators are being effectively released upon challenge with antigen.

Effect of *T. brucei* on mast cells

To investigate the effects of *T. brucei* on mastocytosis, rats were initially infected with *T. brucei*, then 7 days later infected with *T. spiralis*. After a further 35 days the absolute numbers of mucosal mast cells were determined in the small intestines (Fig. 6). An infection with *T. brucei* did not significantly alter the number of mast cells generated by infection with *T. spiralis*. Also, the numbers of mast cells in rats infected with only *T. brucei* were

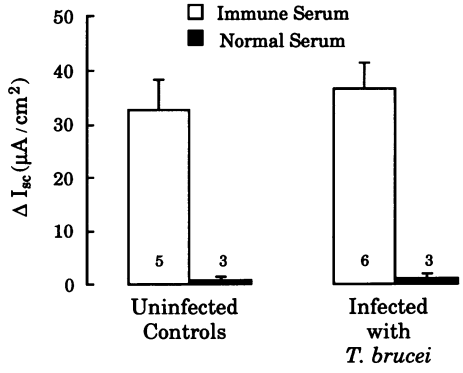

Figure 5. Maximal expression of the antigen-induced short-circuit current response (ΔI_{sc}) in *T. brucei*-infected rats receiving serum containing anti-*T. spiralis* IgE. Each rat was administered 6 ml of serum intraperitoneally. Four days later the jejunum was removed, mounted in Ussing chambers and challenged with *T. spiralis* antigen (100 μg/ml) added to the serosal compartment. Values are mean ΔI_{sc} + SE; n = number at base of bar.

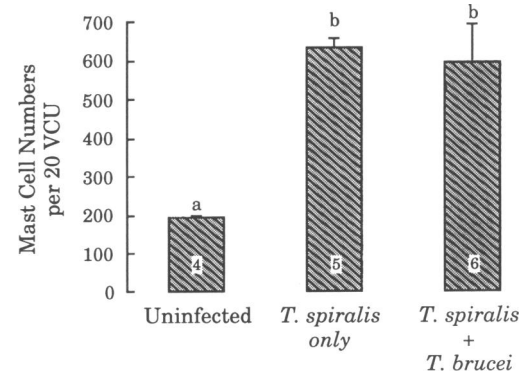

Figure 6. Mucosal mast cell numbers per 20 villus-crypt units (VCU) in the small intestine of rats infected for 35 days with *T. spiralis*, or with *T. spiralis* and *T. brucei*. Values are means + SE; n = number at base of bar. Comparisons among means were performed by ANOVA using the Tukey test for component analysis. Means identified by different letters are significantly different ($P < 0.05$).

very similar to those in uninfected rats (S. S. Gould and G. A. Castro, unpublished observation).

The effect of *Trypanosoma brucei* on larval rapid rejection

Because a *T. brucei* infection has suppressive effects on particular aspects of anaphylaxis-mediated Cl^- secretion, the potential outcome at the level of functional immunity was explored by investigating the effects of *T. brucei* on the rapid rejection of *T. spiralis* larvae in rats. The animals were immunized either through prior infection with *T. spiralis* or by intraperitoneal injection of LSA (Fig. 7). *Trypanosoma brucei* was able to suppress worm rejection in rats when immunity was induced by LSA, but had little effect when the rats were immunized through *T. spiralis* infection. These results parallel both the results of *in vitro* work in the Ussing chamber and the levels of IgE found with PCA, where *T. brucei* was able to suppress antigen-induced Cl^- secretion and IgE in LSA-immunized rats but had little or no effect on animals immunized through infection with *T. spiralis*.

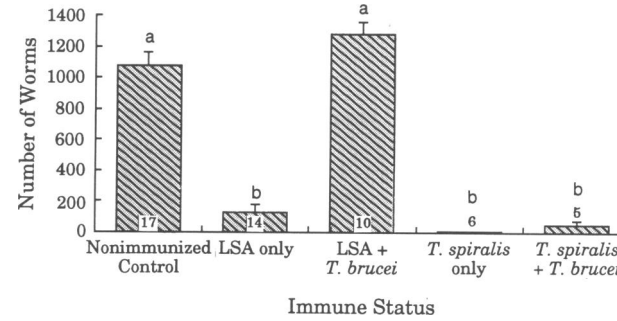

Figure 7. Effect of *T. brucei* infection on establishment of *T. spiralis* in the small intestine of rats immunized by parenteral administration of LSA or by infection with *T. spiralis*. Each rat was challenged with 2×10^3 *T. spiralis* L_1 larvae. Values are means + SE; n = number at base of bar. Comparisons among means were performed by ANOVA using the Tukey test for component analysis. Means identified by different letters are significantly different ($P < 0.05$).

DISCUSSION

Host–parasite systems have become well established as a useful tool to study immunological regulation of gastrointestinal functions.[7,19,20] The procedure used in most studies to induce sensitization of the local mucosal immune system has been infection with a helminth, *T. spiralis*, or by parenteral injection of helminth-derived antigens with an adjuvant. To delve further into immunoregulatory processes in the mucosa of the GI tract, studies were undertaken to examine the effects of a blood protozoan, *T. brucei*, which has potent systemic immunosuppressive properties.

In contrast to infection with *T. spiralis*, an infection with *T. brucei* was unable to sensitize the rat small intestine for antigen-specific Cl^- secretion. This failure indicated an inability to prime the GI mucosal tissues for type 1 hypersensitivity. Either *T. brucei* was incapable of appropriately stimulating the immune system, or it was blocking the transport function of the epithelium. We subsequently determined that a preparation of trypanosomal antigens, when adsorbed to alum and injected i.p., was capable of bringing about tissue sensitization as evident from specific antigen-induced Cl^- secretion. In this regard, trypanosomal antigen compared favourably with two other antigens employed for the same purpose, namely LSA and ovalbumin. Because an ion transport defect at the level of the epithelium was ruled out and because IgE production was inhibited by *T. brucei* infection, the conclusion is warranted that this protozoan blocks sensitization to its own antigens, hence expression of intestinal anaphylaxis, by inhibiting the synthesis of homocytotropic antibody. *Trypanosoma brucei* substantially decreased the amount of *Trichinella*-specific IgE produced, but not enough to prevent anaphylaxis-induced Cl^- secretion. The basis for this suppression of IgE production can not be ascertained at this point. A concomitant infection with *T. brucei* was also unable to alter significantly the development of mastocytosis induced by *T. spiralis* infection. Intriguingly, this

result was in contrast to our observations with *T. spiralis*-induced eosinophilia which a *T. brucei* infection was able to almost totally suppress (S. S. Gould and G. A. Castro, unpublished observation).

The various suppressive effects of *T. brucei* on rats immunized against *T. spiralis* were further investigated by focusing on the phenomenon of rapid rejection. Rats immunized through prior infection with *T. spiralis* exhibit a remarkable protective response against recurrent infection. Newly introduced infective larvae are expelled from the GI tract within minutes after their entry into the intestine. This example of immunity has become appropriately known as rapid rejection, involves IgE, and is considered to be mediated by an immediate hypersensitivity response.[8,18] Rats immunized with LSA are also partially protected against secondary infection, but not to such a dramatic degree as seen with immunity through prior infection. *Trypanosoma brucei* was able to almost totally inhibit the immunizing effects of LSA, as it had for antigen-induced Cl^- secretion in the Ussing chamber. However, *T. brucei* infection had little effect on rats immunized by prior infection with *T. spiralis*, which was consistent with findings that these rats still had a considerable amount of *T. spiralis*-specific IgE and an intense mucosal mastocytosis. A rational conclusion here is that *T. brucei*, having suppressed the *T. spiralis*-specific IgE invoked by LSA to undetectable levels, prevented rapid rejection. Although *T. brucei* can suppress the levels of other antibodies such as IgG and IgM,[4,21] it is doubtful that these antibodies, apart from certain homocytotropic isotypes, could induce such an immediate response as that seen in rapid rejection.

To determine the basis for this suppression of type 1 hypersensitivity, future work should concentrate on establishing the method of immune system stimulation during a *T. brucei* infection. Analysis of T-cell cytokine production may then reveal that cytokines necessary for IgE production are not being synthesized and secreted.

The properties of a particular antigen that enable it to induce type 1 hypersensitivity are under intensive investigation and the most important breakthrough, at least in mice, has come from work with various $CD4^+$ T-helper subsets and the particular cytokines each subset secretes.[22] T-helper 1 (Th1) cells secreting mainly interferon-γ (IFN-γ) and interleukin-2 (IL-2) are mostly involved in delayed-type hypersensitivity reactions and offer protection against viruses and other intracellular infections. The Th2 cells produce IL-4, IL-5 and IL-10, cytokines that induce the production of IgE, eosinophils and immediate hypersensitivity in general. These two subsets of helper cells are also able to down-regulate and interfere with the functions of each other. With regard to type 1 hypersensitivity, the Th1 cytokine IFN-γ is known to inhibit the proliferation of Th2 cells and impair production of IgE.[23] Substantial amounts of this cytokine are produced by infections with the *T. brucei* group.[24] Most protozoans studied to date such as *Leishmani*, *T. cruzi* and *Toxoplasma*[25-27] are, to one degree or another, associated with Th1-mediated protection, as one would expect with intracellular parasites. The African trypanosomes have not been studied in as much detail as the above protozoa, but judging by the amount of IFN-γ produced plus the results from initial studies,[28,29] it certainly appears as if Th1 cells are also the principle subset induced.

The suppression of IgE production (and eosinophilia) by *T. brucei* may involve the inhibition of Th2 cells, possibly mediated via Th1 cytokines such as IFN-γ. The situation with mast cells is not so obvious, probably because mastocytosis evoked by various cytokines and other growth factors is a more complex phenomenon than cytokine regulation of IgE production or eosinophilia.[30] IL-3, IL-4 and possibly IL-9 and IL-10 are the influential mast cell cytokines. IL-3 can be produced by both Th1 and Th2 cells, and the other mast cell growth factors such as stem cell factor (SCF) are produced by haemopoietic stromal cells.[30,31] Therefore, a lack of Th2 cytokines may not necessarily affect mast cell proliferation to the same degree as the other branches of type 1 hypersensitivity, IgE and eosinophils.

Under certain circumstances, protozoal parasites can induce the production of significant amounts of IgE, either under natural conditions,[32-34] or experimentally as with *Leishmania major* in BALB/c mice.[25] Therefore, the various African trypanosomiases, the particular species, stock or strain of *Trypanosoma*, and the mammalian host infected could have an important bearing on the ability to induce type 1 hypersensitization. The *T. brucei* stock used in these experiments, TREU 667, has a maximum infection period in Sprague-Dawley rats of about 50 days. Trypanosome species which can effect a much more chronic infection, such as *T. gambiense*, may prove to be better inducers of type 1 hypersensitivity in rats or other mammals. The same applies with some strains of *T. congolense* or *T. vivax* in either wild or domestic ruminants. The latter two species would be especially interesting to investigate because the immunosuppression due to trypanosomiasis is not so pronounced in ruminants as in rodents.[35]

ACKNOWLEDGMENTS

We would like to thank Dr A. Clarkson, New York University, for providing the *Trypanosoma brucei* stock TREU 667. This work was supported in part by Research Grants AI-11361 from the NIAID, and POI-DK 37260, NIDDKD, National Institutes of Health, and by the Initiative in Mucosal Biology, President's Office, The University of Texas Health Science Center.

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