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Simulation of parasite-induced gut hypersensitivity: implications for vaccination

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SUMMARY

Antigenic challenge of jejunum from rats infected with *Trichinella spiralis* evokes a biphasic pattern of epithelial Cl^- secretion, as measured *in vitro* by electrophysiological methods. Peaks of secretion occur at ~1·5 and ~5·0 min post-challenge. Challenge of jejunum from hosts passively immunized with serum containing anti-*Trichinella* anaphylactic antibody evokes the late phase but not the early phase of Cl^- secretion. Since the early phase is mediated by 5-hydroxytryptamine and histamine from mast cells, we hypothesized that the failure to express that phase was due to a decrease in mast cell-derived mediators secondary to a deficiency in mucosal mast cell numbers. The hypothesis was tested by correlating mast cell numbers with patterns of antigen-induced Cl^- secretion using several immunization regimes. Rats actively immunized by infection produced anti-*Trichinella* IgE and had a mucosal mastocytosis. Rats passively sensitized with serum containing anti-*Trichinella* IgE had normal numbers of mast cells in their mucosa. Inducing mastocytosis in rats, by infecting them with *Nippostrongylus brasiliensis* prior to passive sensitization with anti-*Trichinella* serum, primed for the expression of a biphasic Cl^- secretory response upon subsequent challenge with *Trichinella* antigen. Rats actively sensitized by injection with *Trichinella* antigen elicited an IgE response without mastocytosis and expressed only the late phase of antigen-induced Cl^- secretion. Results (i) support our hypothesis, (ii) emphasize the importance of the cellular state of the mucosa in the functional expression of local anaphylaxis; and (iii) provide a physiological explanation for the general failure of vaccination and passive sensitization to induce functional immunity equivalent to that induced by natural infection.

INTRODUCTION

Our study is based on the observation that transfer of serum immunizes rats against *Trichinella spiralis* and prepares the host to respond physiologically to antigenic challenge. However, the level of immunity expressed is relatively low and the physiological response is qualitatively different compared with rats immunized actively by infection (Harari, Russell & Castro, 1987). Our objective was to determine if passively immunized rats could be conditioned to respond to antigenic stimulation like their infected counterparts.

Antigenic challenge of jejunum from rats sensitized to *T. spiralis* by infection elicits local anaphylaxis that is expressed in the secretion of Cl^- ions (Harari *et al.*, 1987; Castro, Harari & Russell, 1987). That secretion, which can be quantified electrophysiologically as a change in transmural short circuit current (ΔI_{sc}), is biphasic. The ΔI_{sc} peaks at ~1·5 min and ~5·0 min, respectively. The chemical mediators of fast Cl^- secretion are 5-hydroxtryptamine (5-HT) and histamine derived from mucosal mast cells. Prostaglandin E_2 (PGE_2) synthesized *de novo* following mast cell activation mediates the slow phase of Cl^- secretion (Castro *et al.*, 1987). Antigenic challenge of rats passively sensitized by transfer of anti-*Trichinella* serum elicited only a monophasic ΔI_{sc}, resembling the slow phase in actively immunized hosts (Harari *et al.*, 1987).

The failure of transferred serum to prime for expression of fast Cl^- secretion is the focus of this communication. We hypothesized that failure to observe fast Cl^- secretion was due to insufficient concentrations of 5-HT and histamine being released upon antigenic stimulation. This is attributed to insufficient numbers of mast cells responding to antigenic stimulation. The objective was to test our hypothesis by examining the relationship between mucosal mast cell numbers and patterns of Cl^- secretion induced by *Trichinella* antigen in rats (i) actively immunized by infection with *T. spiralis*; (ii) passively sensitized by transfer of anti-*Trichinella* serum; (iii) infected with a heterologous parasite prior to passive sensitization against *Trichinella*; and (iv) actively immunized by parenteral injection with *Trichinella* antigen. Results, part of which have been published in abstract (Harari & Castro, 1988),

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indicate that the full expression of antigen-induced epithelial Cl^- secretion is dependent on mucosal mast cell hyperplasia in challenged jejunum.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley rats (Hilltop Lab. Animals, Scottsdale, PA) were used as experimental hosts. Some rats were immunized against *T. spiralis* by inoculation with $4{\cdot}5 \times 10^3$ infective larvae obtained by enzymatic digestion (Castro & Fairbairn, 1969a) of skeletal muscle from CF-1 mice (Charles River, Wilmington, MA). Infective *Nippostrongylus brasiliensis* larvae (L_3) were acquired from Dr Lillian Mayberry, University of Texas at El Paso. Rats were injected subcutaneously with 2×10^3, larvae and were used 30–40 days later. The course of infection with *N. brasiliensis* was monitored by following the appearance and disappearance of eggs in the faeces.

Electrical measurement of jejunal ion transport

Twenty centimetres of rat jejunum, beginning ~5 cm distal to the ligament of Treitz, were removed, rinsed free of luminal contents with Ringer's solution and slit open along the mesenteric border. Two-centimetre segments of full-thickness jejunum were mounted as a flat sheet between two Ussing half chambers with an aperture of 1·13 cm^2. Six segments from each rat, studied simultaneously, were bathed on their mucosal and serosal sides with 10 ml Ringer bicarbonate buffer. The composition of the buffer and the specific methods to calculate transmural potential (PD), electrical resistance and short circuit (I_{sc}) were published in detail elsewhere (Harari *et al.*, 1987; Castro *et al.*, 1987). The antigen-induced changes are presented as (i) the maximal elevation in short circuit current (ΔI_{sc}) and are expressed as $\mu A/cm^2$, or (ii) the total amount of the univalent charge transferred across the tissue during a 24-min period after the addition of the antigen. This parameter is expressed as $\mu eq/cm^2$ and is calculated by integrating the area under the curve of ΔI_{sc} versus time and applying Faraday's constant (Baird *et al.*, 1984).

Immunological methods

Antigen preparations. Trichinella antigen was prepared from infective stage larvae as described in prior publications (Harari *et al.*, 1987; Castro *et al.*, 1987). To prepare antigen from *N. brasiliensis*, worms were collected from the small intestine of rats infected with 2×10^3 larvae using the method described by Castro & Fairbairn (1969b). Worms were cleaned and homogenized in sterile saline. The homogenate, which had a final protein concentration of 6·28 mg/ml, as determined by the method of Lowry *et al.* (1951), was used as the antigen preparation.

Serum collection and passive sensitization. Rats were inoculated twice with 8×10^3 infective *T. spiralis* larvae at 6-week intervals. Six days after the second inoculation rats were anaesthetized with a intramuscular injection of xylazine (Rompun®, Haven-Lockhart, Shawnee, KS) at a dose of 5 mg/kg and ketamine hydrochloride (Ketaset®, Bristol Laboratories, Syracus, NY) at 75 mg/kg. Blood was collected by cardiac puncture and serum was separated and stored at −70°. Rats were passively sensitized by intraperitoneal infection of 6 ml of serum. Blood from uninfected rats provided control serum.

Passive cutaneous anaphylaxis (PCA) assay. All sera were titred for IgE levels using the PCA assay. The specific procedures which employ the methods originally described by Watanabe & Ovary (1977) have been published elsewhere (Harari *et al.*, 1987).

Active immunization with T. spiralis *antigen.* Rats weighing 250–300 g were injected i.p. with *Trichinella* antigen, 500 μg protein, mixed with complete Freund's adjuvant in a total volume of 0·1 ml. Some rats received adjuvant only. Two additional injections were given 7 and 14 days after the first. On Day 21 following the initial injection the jejunal response to challenge with *T. spiralis* antigen was measured.

Histological techniques

In preparing tissue for examination by light microscopy, jejunal segments were fixed in Carnoy's fixative, embedded in paraffin and cut into 5-μm sections. Sections were stained with 1% Alcian Blue 8GX, as described by Alizadeh & Wakelin (1982), with 1% Safranine 0 as a counterstain. Some tissues were processed using an alternate procedure. Jejunal segments were opened with a longitudinal incision and immersed in a modified Karnovsky's fixative buffered with 0·1 M sodium cacodylate, pH 7·4, containing the following: glutaraldehyde, 1%; paraformaldehyde, 1%; dextran (MW 38,400) 3%; $CaCl_2$, 2·5 mM. After 90 min at room temperature samples were stored at 4°. Buffer washes were followed with post-fixation in $0s0_4$(1%) reduced with potassium ferrocyanide (1·5%) for 90 min, washed in tap water, and dehydrated through a graduated series of ethanol. Infiltration was with Spurr's media (Spurr, 1969) polymerized at 69° overnight. Semi-thin sections were cut with a Sorvall MT2–B ultramicrotome and stained with Methylene Blue-Azur II (Richardson, 1960).

Tissue histamine levels

Mucosa from jejunal segments 2 cm long was scraped from underlying musculature and suspended in 2 ml of Ringer's solution. The mucosa was heated for 15 min in a boiling water bath to abolish the activities of histamine-degrading enzymes and endogenous S-adenosyl methionine. The mucosa was homogenized and centrifuged at 2000 *g* at 5° for 30 min. Histamine was measured in the supernate using the enzymatic isotopic assay of Shaff & Beaven (1979). Histamine-*N*-methyl transferase was prepared from rat kidneys. Histamine content was expressed as ng/mg mucosal protein.

Materials

Serotonin creatinine sulphate, histamine diphosphate, diphenhydramine hydrochloride, indomethacin and S-adenosylmethionine were purchased from Sigma (St Louis, MO). Cinancerin was obtained from E.R. Squibb (Princeton, NJ). Alcian blue 8GX and Safranine 0 were purchased from Polysciences Inc., (Warrington, PA). [^{3}H]S-adenosylmethionine was acquired from ICN chemical (Radioisotope Division, Irvine, CA).

Experimental design

The jejunum of rats actively immunized by infection with *Trichinella* was examined histologically for mast cells. Mast cell numbers were also estimated by measuring tissue histamine content. This procedure is based on the premise that with enteric helminthiases increased mucosal histamine is due to an increase in mast cell number and not histamine content per cell (Wells, 1962; Wingrin *et al.*, 1983). Histological and chemical findings

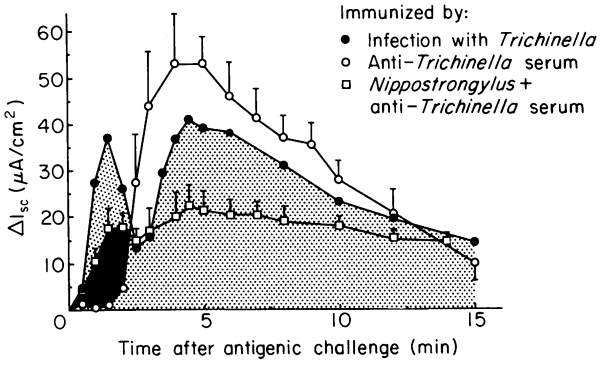

Figure 1. Change in jejunal short circuit current (ΔI_{sc}) induced by a maximally stimulating concentration of *T. spiralis* antigen in rats immunized by three different regimens. The shaded area represents the biphasic response induced by antigen in gut segments from rats actively immunized by infection (previously reported; Castro *et al.*, 1987) and with which responses of passively sensitized tissues are compared. Values for passively sensitized groups are means and SE for six to seven animals. Rats passively immunized with anti-*Trichinella* serum expressed only a slow phase response upon antigenic challenge, while *N. brasiliensis*-primed recipients of antiserum expressed a fast response (solid area) in addition to the slow-phase response.

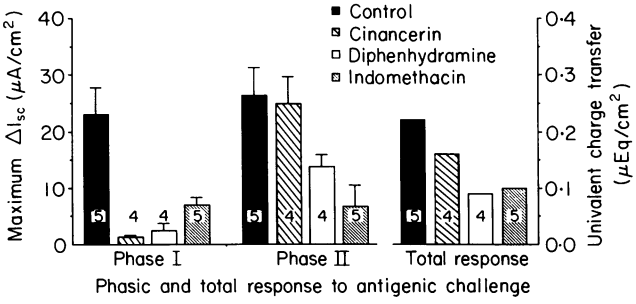

Figure 2. Action of inhibitors of Cl^- secretion on the biphasic response (maximum ΔI_{sc}) evoked by *Trichinella* antigen in rats primed with *N. brasiliensis* and passively sensitized to *T. spiralis* through transfer of serum. The control represents untreated tissue. Values for the fast (Phase I) and slow (Phase II) responses are means and SE; n = number at base of each bar. The univalent charge transfer represents the mean value calculated by applying Faraday's constant to the cumulative change in short circuit current during the 24-min period following antigenic challenge.

were correlated with the antigen-induced Cl^- secretory response. Similar correlations were made in rats passively sensitized with anti-*Trichinella* serum and examined 4 days after serum transfer. Rats actively immunized by injection with *Trichinella* antigen were examined 3 weeks after the initial antigen injection. The Cl^- secretory response was evoked by challenge with a maximum stimulatory dose of *Trichinella*-derived antigen (100 μg protein/ml Ussing chamber fluid).

Rats inoculated with *N. brasiliensis* larvae 30–40 days previously were passively sensitized by transferring anti-*Trichinella* serum. Four days after serum transfer histological, chemical and physiological measurements were made. In addition to *in vitro* challenge with *Trichinella* antigen, *Nippostrongylus*-primed rats were challenged with *Nippostrongylus*-derived antigen to demonstrate homologous sensitization of the tissue and to compare the response to that induced by *Trichinella* antigen in rats actively immunized by infection with *Trichinella*.

To help substantiate that a particular phasic response to antigenic challenge was characteristic of fast or slow Cl^- secretion, the action of 'inhibitors' of the fast phase (diphenhydramine and cinancerin) and slow phase (indomethacin) on the observed response was determined. These agents were added to the serosal solution 15 min prior to antigenic challenge (Castro *et al.*, 1987).

RESULTS

The biphasic Cl^- secretory response expressed by jejunum from rats actively immunized to *T. spiralis*, and to which responses of other experimental groups are compared, is illustrated in Fig. 1. The failure of rats passively sensitized with anti-*Trichinella* serum to display the fast phase of antigen-induced secretion (Fig. 1) was overcome if the recipients of serum were first infected with *N. brasiliensis* (Fig. 1). Challenge with *Trichinella* antigen of *Nippostrongylus*-primed rats passively sensitized with anti-*Trichinella* serum elicited a biphasic change in I_{sc}. Although the magnitude of both phases was reduced compared with the biphasic response evoked by antigen in rats actively sensitized by infection with *Trichinella* (Fig. 1), the temporal expression of the biphasic response was similar. The antigen-induced fast phase (Phase I) of the jejunal ΔI_{sc} in the *Nippostrongylus*-primed rats was sensitive to 5-HT and H_1 receptor antagonists (cinancerin and diphenhydramine) and the slow phase (Phase II) was sensitive to indomethacin (Fig. 2).

Jejunal segments from rats infected with *N. brasiliensis* and challenged with a maximally stimulating dose (45 μg protein/ml) of *Nippostrongylus* antigen elicited a ΔI_{sc} somewhat similar in configuration but attenuated as compared with that evoked by *Trichinella* antigen in jejunum of rats actively sensitized by infection with *T. spiralis* (Fig. 3). Although the homologous *Trichinella* and *Nippostrongylus* systems had corresponding early ΔI_{sc} responses, the well-defined late phase in the *Trichinella* system was matched only by a very tentative response in the *Nippostringylus* model. Challenge of the *N. brasiliensis*-infected rats with *Trichinella* antigen elicited no secretory response [$0{\cdot}35 \pm 0{\cdot}4$ and $-1{\cdot}31 \pm 0{\cdot}7$ μA/cm² ($n = 10$)] at times corresponding with peak expression of Phase I and II, respectively.

The I_{sc} response of jejunal segments from rats actively sensitized to and challenged with *Trichinella*-derived antigen was monophasic, resembling the slow phase of the biphasic response observed in rats actively immunized by infection (Fig. 3). That monophasic response was insensitive to cinancerin and diphenhydramine, but was almost completely blocked by treatment with indomethacin (Fig. 4).

All groups of *Trichinella*-sensitized animals that expressed the slow Phase of the ΔI_{sc} upon antigenic challenge had anaphylactic antibodies conveyed through serum or generated such antibodies *de novo* (see PCA titres, Table 1). *Trichinella*-sensitive animals capable of expressing the fast phase of the antigen-induced ΔI_{sc} possessed anaphylactic antibodies and, in addition, elevated levels of mucosal mast cells. Rats actively immunized by infection with *T. spiralis* produced anti-*Trichinella* IgE (Table 1) and had a large number of mast cells in their intestinal mucosa compared with uninfected controls. Mastocytosis was indirectly evident from a high level of tissue histamine (Table 1) and directly evident from the histological picture

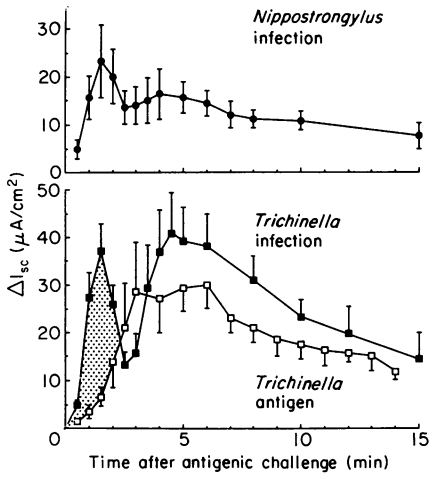

Figure 3. Change in short circuit current (ΔI_{sc}) in jejunum from rats infected with *N. brasiliensis* ($n=4$–7 for each point) or *T. spiralis* ($n=11$) or injected with *Trichinella* antigen ($n=7$). Data for the *Trichinella*-infected group were from a previous study (Castro *et al.*, 1987). *Nippostrongylus*-infected rats were challenged with homologous antigen and the two groups of *Trichinella*-sensitized rats were challenged with *Trichinella*-derived antigen. Values are means and SE.

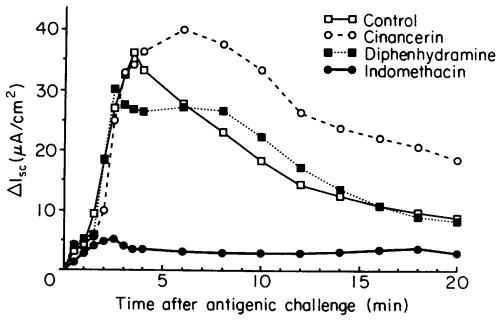

Figure 4. Action of inhibitors of Cl^- secretion on the monophasic response evoked by *T. spiralis* antigen in rats actively sensitized by parenteral injection with *Trichinella* antigen. The control represents untreated tissue. Values are means for seven animals in the control group and five animals in all other groups.

Table 1. Serum PCA titres and mucosal histamine levels

Group	Serum PCA titre	Histamine conc. (ng/mg protein)*
Uninfected	<1:2	22·8±1·5 (5)
Infected (*T. spiralis*)	1:256†	83·4±18·3 (6)‡
Infected (*N. brasiliensis*)	—	118·1±5·9 (6)‡
Sensitized with *T. spiralis* antigen	1:64	33·9±4·1 (4)
Adjuvant control	<1:2	33·1±3·6 (3)

* Values are means ± SE (*n*).
† PCA titre previously reported (Harari *et al.*, 1987).
‡ indicates significant difference compared with uninfected group as determined by the unpaired *t*-test.

presented (Fig. 5a, d). The number of mast cells in tissues from recipients of only anti-*Trichinella* serum was similar to that observed in jejunum from uninfected, control rats (Fig. 5a, b). The capacity of jejunum from *Nippostrongylus*-primed rats passively sensitized with anti-*Trichinella* serum, to express the first phase of the ΔI_{sc} was paralleled by an obvious abundance of mucosal mast cells (Fig. 5e). The histologic picture was similar to that observed in jejunal mucosa from rats infected with *Trichinella* (Fig. 5d). The failure of jejunum from rats actively sensitized with *Trichinella* antigen to express the fast ΔI_{sc} was associated with the failure of these animals to express mucosal mast cell hyperplasia (Fig. 5c). The cells, identified by Alcian blue staining as mast cells, clearly presented the granular characteristics of mast cells when stained with methylene blue and observed at higher magnification (Fig. 5f).

DISCUSSION

Infection with *T. spiralis* in rats induces a form of immunity reflected physiologically as a biphasic, antigen-induced Cl^- secretion. Only the slow phase of secretion is replicated through passive immunization with serum or through active immunization with antigens derived from L_1 larvae. Infecting rats with *N. brasiliensis* prior to transfer of anti-*Trichinella* serum primed for the expression of the biphasic secretory response. The priming infection induced mast cell hyperplasia, apparently providing more cells that could be armed with anti-*Trichinella* IgE supplied through transferred serum. Thus, the qualitative reproduction of the biphasic secretory response requires IgE and mucosal mast cell proliferation. More generally, our results support the conclusion that the quantity of muscosal mast cells responding to antigenic stimulation can dictate the quality of the physiological response.

The biphasic response to *Trichinella* antigen expressed in *N. brasiliensis*-infected recipients of anti-*Trichinella* serum was similar to that expressed in *Trichinella*-infected rats challenged with *Trichinella* antigen with regard to the time at which the two phases are expressed, and the sensitivity of the two phases to pharmacologic agents. The fast phase in the *N. brasiliensis*-primed rats was sensitive to 5-HT and H_1 antagonists (cinancerin and diphenhydramine), and the slow phase was sensitive to a blocker of prostaglandin synthesis (indomethacin). The capacity of indomethacin to significantly blunt the fast phase of Cl^- secretion (Fig. 2) is explained by its anti-histamine action (Castro *et al.*, 1987).

The reduction in the magnitude of the biphasic response in passively sensitized hosts with nippostrongylosis may be reconciled in part by the fact that mast cells in *N. brasiliensis*-primed recipients would have already bound homologous IgE elicited against the parasite, leaving relatively few Fcε receptor sites available to bind passively transferred anti-*Trichinella* IgE. Ishizaka *et al.* (1975) reported that in *N. brasiliensis*-infected rats only 10% of Fcε receptors on mast cells remain unbound.

The result that cannot be readily reconciled by the above explanation is the reduced magnitude of the slow phase of Cl^- secretion in *Nippostrongylus*-primed rats compared with that in passively sensitized, unprimed hosts (Fig. 1). A possible explanation for the difference between the two groups may hinge on differences in parasite life cycles. *Trichinella* is an intra-epithelial parasite in the intestinal phase (Gardiner, 1976; Wright, 1979), while intestinal stages of *Nippostrongylus* are lumen dwellers (Kassai, 1982). Different life cycles might influence local

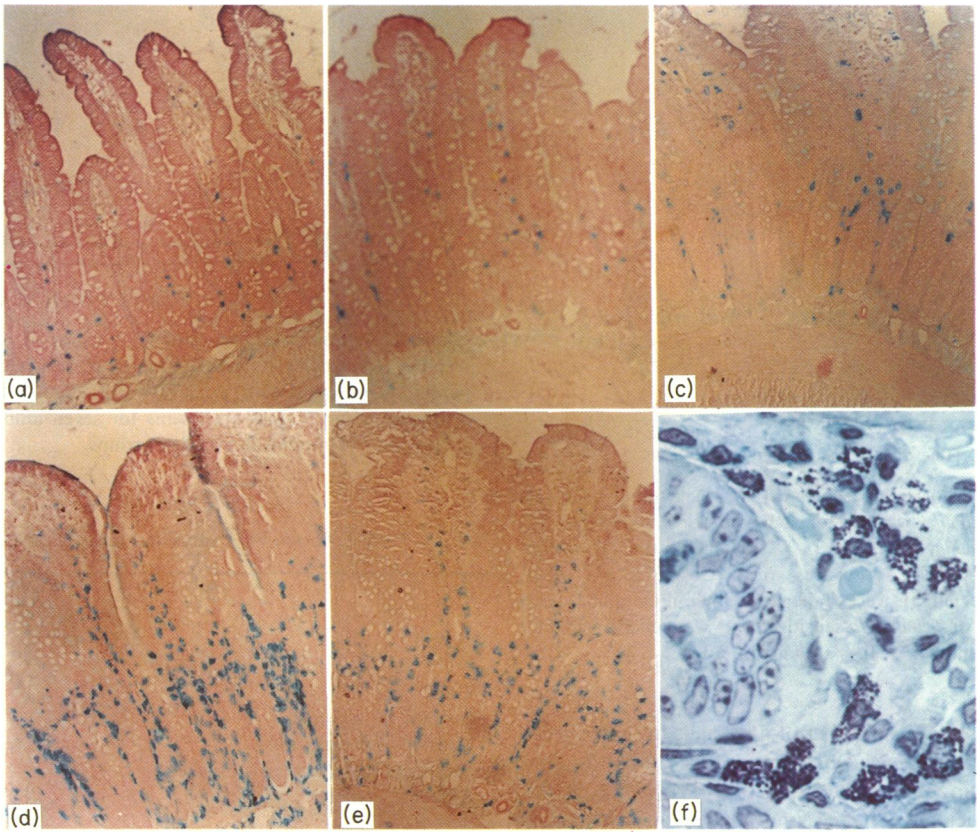

Figure 5. Histological sections of jejunum stained to visualize mast cells [blue-staining cells (a–e) and granulated cells (f)]. Paraffin-embedded tissues (a–e) were from the following hosts: (a) uninfected, (b) sensitized with anti-*Trichinella* serum, (c) sensitized by inoculation with *Trichinella* antigen, (d) infected with *T. spiralis* (35 days post-inoculation), (e) infected with *N. brasiliensis* (40 days post-inoculation). Pastic-embedded tissue (f) from *T. spiralis*-infected rat. Original magnifications: (a–e) × 100; (f) × 400.

immunization, which in turn may affect the response to antigenic challenge. Another explanation relates to possible differences in mediators of the ΔI_{sc} representative of the slow phase of secretion. Whereas the antigen-induced ΔI_{sc} in the *Nippostrongylus*-primed host is assumed to be mediated by PGE_2, other mediators of anaphylaxis may contribute to or modulate electrical changes. Examples of such mediators are mast cell protease II (Miller *et al.*, 1983), various prostaglandins (Kelly & Dineen, 1976) and leukotrienes (Moqbel *et al.*, 1987). The role of these mediators in antigen-stimulated changes in epithelial functions is unknown. Although mast cell protease II is an enzyme, its effect on anaphylaxis-mediated epithelial cell extrusion (Miller *et al.*, 1986) could be preceded by functional changes.

Infecting rats with *N. brasiliensis* to prime for the expression of the biphasic Cl^- secretory response is analogous to infecting antigen-sensitized rats with *Heligmosomoides polygyrus* to 'non-specifically' prime for expulsion of intestinal stages of *T. spiralis*. Bell & McGregor (1980) demonstrated that rats immunized by parenteral injection of antigens from *T. spiralis* became partially resistant to reinfection. That resistance was greatly potentiated if, during the time between antigen administration and *Trichinella* challenge, rats were infected with *H. polygyrus*. Although the specific mode of action of *H. polygyrus* could not be explained it was speculated that this nematode generated local changes in the parasite's microenvironment that interacted synergistically with immunologic factors (antibody or cells) to effect rejection of *T. spiralis*.

Passive immunization conveyed experimentally through transfer of cells or serum is generally less effective than infection-acquired immunity in preventing re-infection (Wakelin, 1978) with parasites. Likewise, limited success has been achieved in vaccinating humans and domestic animals against protozoans and helminths (Taylor & Muller, 1980; Lloyd, 1981). Failures have been attributed in part to a lack of success in identifying and obtaining purified, parasite-derived antigens that are functional in evoking powerful immune responses. In the long-standing effort to produce anti-parasitic vaccines, only two vaccines are widely acclaimed as successful in immunizing against parasitic worms (cattle lungworm, *Dictylcaulus viviporus* and dog hookworm, *Ancylostoma caninum*). Both vaccines involved administration of irradiated, infective larvae rather than purified antigens (Peacock & Poynter, 1980; Miller, 1978). Thus, it may be unreasonable in some cases to expect a highly purified antigen to duplicate the level of immunity that is acquired by infection.

It appears fair to state based on our results that an understanding of physiological behaviour of immune hosts

could lead to methods whereby specific physiological processes can be simulated by extrinsic intervention. If antigen-induced changes in physiological behaviour can be taken as correlates of functional immunity, simulation of such changes might be a useful criterion to be met in vaccine development. A major step toward improving chances for producing successful vaccines in which protection is effected indirectly may be to characterize key physiological processes associated with immunity in infected hosts, determine how closely those processes can be simulated by artificial immunization and determine if such simulation associates with the development of resistance to infection. The experiments described in this paper represent an initiative toward that approach.

ACKNOWLEDGMENT

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