IMMUNOGLOBULIN-CONTAINING CELL POPULATIONS IN TISSUES OF RATS INFECTED WITH *TAENIA TAENIAEFORMIS*

By

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ABSTRACT

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Immunoglobulin-containing inflammatory cells appear in murine tissues infected with migrating and sessile helminths. The purpose of this study was to characterize the appearance of IgE antibody-containing cells during the course of infection with the cestode, Taenia taeniaeformis.

We used immunofluorescence techniques and demonstrated that IgE-containing cell populations increased both in the intestinal mucosa and surrounding metacestodes in the liver. However, not all of these were plasma cells and many corresponded morphologically, histochemically and in their responses in vivo to dexamethasone and compound 48/80 to mucosal mast cells (Enerbäck, Acta Path et Microbiol. Scandinav., 66:303, 1966).

Mayrhofer, et al. (Eur. J. Immunol., 6:537, 1976) first described IgE-containing mucosal mast cells in the intestinal mucosae of rats infected with Nippostrongylus brasiliensis. Our results now extend these findings to

a different host-parasite system and demonstrate the appearance of IgE-positive mucosal mast cells in the liver of T. taeniaeformis-infected rats. The origin and possible function(s) of these cells are discussed.

To my parents and
my husband

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INTRODUCTION

Cysticercosis and hydatidosis were considered formerly to be of significant public health and economic importance in underdeveloped countries, but they are now becoming of increasingly greater concern to industrialized nations. A number of recent reviews of the literature on taeniid infections are available which provide a comprehensive coverage of the biology, epidemiology, and immunology of these parasites and which address the current public health and economic aspects of tapeworm infections in man and domestic animals [Gemmell and Johnstone 1977; Matossian et al., 1977; Hird and Pullen, 1979].

The life cycles of all these cestodes involve intermediate hosts (e.g., cattle, sheep, swine) in the musculature or visceral organs of which the larval parasites establish and grow. Their presence in food animals often results in partial or total carcass condemnation and in man they may cause serious diseases. The definitive hosts (e.g., man, dogs) ingest parasitized tissues and the cystic larvae develop into adults in the intestine.

Zoonotic cestode infections such as these pose special problems for experimental work, but Taenia

taeniaeformis is transmitted only between cats and rodents, and it therefore serves as a convenient and safe laboratory model for studies of immunity to cestode infection. Infective eggs from the cat are ingested by the rat, and hatch in the small intestine. The liberated activated oncospheres penetrate the intestinal wall and migrate to the liver. Metacestodes can survive in the liver for prolonged periods despite the early onset of marked inflammatory cellular reactions and protective antibody production by the host.

This thesis concerns the characterization of mast cell reactions in experimental taeniiasis in rats, and the findings have important implications for understanding the host-parasite relationship in other cestode infections. The literature review therefore summarizes the most salient characteristics of humoral and cellular immunity in taeniid parasitism, with emphasis on the protective responses of animals infected with T. taeniaeformis. Where no specific information is available for T. taeniaeformis infections, examples are drawn from studies of other intestinal or hepatic helminthiases which illustrate phenomena relevant to the discussion of mast cells and their function and role in cestodiasis.

Comprehensive reviews of the immune responses to cestode infection in general have been compiled (Weinmann, 1966, 1970; Gemmell and MacNamara, 1972; Williams, 1979),

and Leid (1977) recently reviewed the status of our knowledge of immunity in Taenia taeniaeformis infection. The following two sections focus upon those aspects of the immune responses to taeniid infection which are related to protection against challenge, and which form an important part of the background to the work reported below.

The third section deals with literature on histopathological responses in rodent taeniiasis with particular emphasis on the participation of mast cells and their relationship to immunoglobulins.

LITERATURE REVIEW

Humoral Immunity to Taeniid Infection

Circulating protective antibodies

There is substantial evidence for the development of active immunity to taeniid metacestodes (Urquhart, 1961; Blundell et al., 1968; Gemmell et al., 1969) and many workers have demonstrated passive transfer of resistance (e.g., Taenia hydatigena [Blundell et al., 1968, Gemmell et al., 1969); Taenia ovis (Rickard and Arundel, 1974), Taenia pisiformis and Taenia taeniaeformis (Miller and Gardiner, 1932, 1934; Campbell, 1938]). Leid and Williams (1974) confirmed and extended the studies on T. taeniaeformis infections, and showed that $7S\gamma_{2a}$ is the major protective antibody produced in infected rats by 14 days after infection (DAI). Although colostral IgA can protect weanling rats (Musoke et al., 1975) and intestinal IgA was found to be protective against a T. taeniaeformis challenge infection in mice (Lloyd and Soulsby, 1978), IgA is not essential for protection in rats (Leid and Williams, 1974; Leid, 1977).

Many factors have been found to influence the class of protective antibodies produced. Firstly, susceptible host species vary in the type of antibody produced. Mice

develop protective $7S_{\gamma_1}$ antibody to T. taeniaeformis, while infected rats produce protective $7S_{\gamma_{2a}}$ (Musoke and Williams, 1975a). Secondly, the route of infection is important because when rats are immunized by the intraperitoneal implantation of T. taeniaeformis, the protective antibodies produced are of the classes $7S_{\gamma_1}$ and IgM (Musoke and Williams, 1975b). Thirdly, the age of the parasites is a significant factor because other classes of protective antibody appear as infection progresses (Musoke and Williams, 1975a).

Varela-Díaz et al. (1972) postulated that antigenic changes occur on the surfaces of taeniid metacestodes during their development and suggested that this may be one way in which parasites survive in infection. Damian (1964) had proposed that the parasites may, in fact, produce surface host-like antigenic determinants. These kinds of antigenic changes may be responsible for the induction of different antibody classes over time, though there is little direct evidence for this at the moment. Rickard (1974) proposed that blocking antibodies may adhere to the parasites and mask previously exposed antigens, thereby protecting the parasites from lethal effects of attack mechanisms. However, again, direct evidence for this is lacking. In addition to altering surface antigens, metacestodes may protect themselves from antibody damage in other ways. Following the study

by Musoke and Williams (1975a) demonstrating that complement-fixing antibodies were involved in resistance to T. taeniaeformis, Hammerberg et al. (1976) showed that metacestodes release a complement-depleting substance which may act locally to prevent complement-dependent antibody-mediated parasite destruction (Musoke and Williams, 1975a). This may also be true for other taeniids (Kassis and Tanner, 1976; Herd, 1976; Rickard et al. 1977c). The concept of parasite interference with inflammatory mechanisms such as complement and coagulation cascades has been discussed recently in detail by Leid and Williams (1979).

Immunoglobulin-producing cells and gut immunity

The intestine and its associated lymphoid tissues are likely to be especially important sites for immune responses to cestodes, but few studies have been done on these systems. Increases in immunoglobulin-producing cell populations occur within lymphatic tissues of animals infected with intestinal nematodes such as Ascaris suum (Khoury and Soulsby, 1977; Crandall and Crandall, 1971), Trichinella spiralis (Ruitenberg and Duyzings, 1972; Crandall et al. 1967) and Nippostrongylus brasiliensis (Mayrhofer et al. 1976). In general, the changes in antibody-containing cell populations parallel the appearance of the corresponding immunoglobulins in the circulation. Moreover, antibody-producing cell increases appear

to occur primarily in lymphoid organs draining parasitized tissues.

Leonard and Leonard (1941) suggested that the intestinal wall acts as a barrier to the migration of T. pisiformis in rabbits because few oncospheres reach the liver in immune animals. Although no data are available on cell types that respond to T. taeniaeformis in the lamina propria, a marked influx of lymphocytes and eosinophils occurs in the intestinal mucosae of animals infected with other helminths such as T. spiralis (Larsh and Race, 1954; Zaiman and Villaverde, 1964), Trichostrongylus colubriformis (Rothwell and Dineen, 1972, Rothwell and Love, 1974; Dineen et al. 1968) and N. brasiliensis (Love and Ogilvie, 1977). It seems possible that antibody may be produced locally in response to T. taeniaeformis antigens and contribute to mucosal resistance to reinfection. The observations of Crandall et al. (1967) are especially relevant here because they demonstrated that a primary T. spiralis infection in rabbits resulted in an increase in IgM-producing plasma cells in the intestinal mucosa, followed by a relative increase in IgG-containing cells. This pattern paralleled the appearance of the corresponding antibodies in the serum.

Immunoglobulin E (IgE)

The production of circulating reaginic (IgE) antibody is a remarkably consistent host response to parasitic infection (Ishizaka et al. 1976). Ogilvie (1964) first demonstrated reagin in sera of rats infected with N. brasiliensis using passive cutaneous anaphylaxis (PCA) assays. IgE sensitizes mast cells in vitro for antigen-mediated histamine release, and may remain in skin sites of the rat for weeks after intradermal administration.

In the rat, reagins are first detectable by 19 days after infection (DAI) with T. taeniaeformis, peaking at 32 days and thereafter declining (Leid and Williams, 1974). IgE antibodies are also detectable in rabbits infected with T. pisiformis (Leid and Williams, 1976) and in rats infected with other hepatic parasites such as F. hepatica (Day et al., 1978) and Schistosoma mansoni (Ogilvie et al., 1966; Rousseaux-Prevost et al., 1977).

The function of IgE in resistance to parasitic infestation is not yet known. It has been proposed for some time that IgE sensitizes mast cells for antigen-induced amine release, thereby increasing vascular permeability and facilitating the influx of circulating protective antibody and immune cells (Murray, 1971; Murrell et al., 1975; Steinberg et al., 1974; Rousseaux-Prevost et al., 1977). Leid et al. (1975) demonstrated an

antigen-induced release of histamine *in vitro* by peritoneal cells from rats infected with *T. taeniaeformis*. In addition to influencing local vascular permeability, amines may have direct inhibitory effects on parasite survival as demonstrated in *in vitro* studies with *T. colubriformis* (Rothwell et al., 1974) and *in vivo* experiments in which amines were infused into occluded loops of small intestine containing activated *T. taeniaeformis* oncospheres (Musoke et al., 1978). Although IgE is not essential for protection against *T. taeniaeformis*, it may enhance parasite destruction by protective $7S\gamma_{2a}$ globulin (Musoke et al., 1978), or may function to potentiate $7S\gamma_{2a}$-mediated antigen-induced amine release (Leid and Williams, 1974).

IgE may also participate more directly in resistance to reinfection. Capron et al. (1975, 1977), have demonstrated an IgE-mediated cytotoxic macrophage adherence to schistosomulae *in vitro*. Also, Li Hsü et al. (1979) showed recently that IgE was fixed to the tegument of *Schistosoma japonicum* in the skin of challenged rhesus monkeys. Earlier, Ogilvie et al. (1966) had demonstrated that an intradermal injection of IgE antibodies in rats prevented schistosome cercarial development at that site - a resistance that was not attributable to increased vascular permeability.

The origin of the circulating reagin in *T. taeniaeformis*-infected rats has not been studied. IgE-producing

plasma cells are normally located in the respiratory and gastrointestinal mucosae and in the regional lymphatic organs (Tada and Ishizaka, 1969). Reagin production may be a regional lymphoid response to parasitism because large numbers of IgE-positive cells are found in the mesenteric lymph nodes of rats infected with intestinal parasites, such as N. brasiliensis (Ishizaka et al., 1976; Mayrhofer et al, 1976), and they are also found in the axial and bronchial lymph nodes draining the tissues affected by migrating N. brasiliensis larvae (Mayrhofer et al., 1976).

Cellular Immunity to Taeniid Infection

Cellular immune (CMI) responses are said to play important secondary roles in immunity to the intestinal parasites, N. brasiliensis (Wells, 1962; Ogilvie and Jones,1961), Ascaris suum (Taffs, 1968) and T. spiralis (Larsh, 1953; Markell and Lewis, 1957) and the hepatic parasite, F. hepatica (Lang et al., 1967). Although there is no direct evidence for cell mediated protection in larval taeniid infections, CMI responses have been demonstrated to occur in natural and experimental infections with taeniid larvae when in vitro or in vivo skin tests were performed (Kagan et al., 1966; Rickard and Outteridge, 1974; Kwa and Liew, 1977). Delayed type hypersensitivity skin responses were also elicited in rats vaccinated with

taeniid antigens (Kwa and Liew, 1977), and these were adoptively transferred to recipients with peritoneal cells from the vaccinated rats.

Friedberg et al., (1967) were able to transfer some resistance to the cestode Hymenolepis nana with immune spleen cells in mice, and Cook (1979) found that immune lymphocyte populations exert a partial but significant protective effect during the first twelve hours of T. taeniaeformis infection in the rat. In addition, an oral infection of T. taeniaeformis oncospheres appears to trigger cell defense mechanisms against cysticerci implanted in an abnormal site (Musoke and Williams, 1976). These reactions are at least partially non-specific because similar cellular responses are stimulated by complete Freund's adjuvant (Musoke and Williams, 1976).

Despite the considerable indirect support for protective cellular immunity, many workers have been less than successful in demonstrating cell-mediated responses to T. pisiformis in rabbits (Nemeth, 1970), and Mitchell et al. (1977) were unable to protect "nude" (athymic) mice against T. taeniaeformis with purified T cells from infected animals. In addition, Blundell et al. (1969) were unsuccessful in attempts to transfer immunity to Taenia ovis or Taenia hydatigena in sheep with cells from infected donors. The significance of cellular immunity in resistance to taeniid infections may be underestimated

because it can be difficult to correlate in vitro or in vivo CMI responses with actual cell-mediated resistance (Cook, 1979). For example, Rickard and Katiyar (1976) found that the antigens of T. pisiformis that were responsible for protection were those that induced CMI reactions.

Histopathology of Taenia taeniaeformis Infection

Hepatic cellular responses

The maximum cellular reaction occurs around hepatic metacestodes by 15-20 DAI when levels of humoral protective antibody are also rising to a peak at 28 DAI (Cook, 1979; Leid and Williams, 1974). The host capsules surrounding the parasites contain many cell types including numerous eosinophils, lymphocytes, plasma cells and fibroblasts (Singh and Rao, 1967; Ansari and Williams, 1976; Cook, 1979). Considerable numbers of mast cells have been noted in the fibrous capsule which results from chronic infections (Coleman and DeSilva, 1963; Varute, 1971; Cook, 1979). The mast cell is a common component of chronic inflammatory reactions in the rat (Smith et al., 1972) but there are relatively few accounts of their presence in local reactions to hepatic parasites (Cheever, 1965; Andrade and Barka, 1962; Rahko, 1973; Shirai et al., 1976). It is possible that under similar conditions different hosts respond to any given organism with a

distinct pattern of cellular infiltration, and that mast cells do not participate to any great extent in most cases. However, an alternative explanation for the paucity of data on mast cell involvement in hepatic helminthiases is that routine histopathological techniques (e.g., formalin fixation followed by haematoxylin-eosin staining) do not reveal mast cells reliably (Enerbäck, 1966).

Intestinal cellular responses

Cook (1979) recently described villar hyperplasia in the small intestines of rats infected with T. taeniaeformis with a concomitant increase in the mucosal mast cell (MMC) population. The reason for this MMC rise is as yet unknown and immunoglobulin-labelling assays have not been performed to determine whether or not they are coated with IgE. Increases in MMC numbers have been shown to occur in animals infected with the intestinal helminths, N. brasiliensis (Befus et al., 1979; Jarrett et al., 1967; Kelly and Ogilvie, 1972; Miller and Jarrett, 1971), T. colubriformis (Rothwell and Dineen, 1972) and T. spiralis (Tronchin et al., 1979). Of special importance are the observations of Mayrhofer et al. (1976) who demonstrated IgE on the surfaces and within the cytoplasm of MMC in N. brasiliensis-infected rats.

Since metacestodes of T. taeniaeformis reside in the liver, it is clear that the physical presence of parasites

in the intestine may not be the determining factor in the stimulation of mucosal mast cells. Mucosal mast cell precursors may reside in the lamina propria of the intestine and become stimulated locally to divide or they may be triggered to migrate to the mucosa by some parasitic stimulus much as lymphocytes preferentially migrate from the mesenteric lymph node to the small intestine (Parrott and Ferguson, 1974; Guy-Grand et al., 1974; McWilliams et al., 1974; Rose et al., 1976). A second hypothesis for the increase of MMC at a site distant from resident parasites is that a parasite-derived factor may trigger a hormonal and/or cellular reaction sequence which ultimately stimulates MMC precursors.

The intestinal mastocytosis is limited to the MMC, and these differ from serosal or connective tissue mast cell (CTMC) populations histochemically (Enerbäck, 1966, a, b, c; Miller and Walshaw, 1972), ultrastructurally (Miller, 1971) and in their responses to compound 48/80 (Soll et al., 1979; Veilleux, 1973; Heap and Kiernan, 1973; Enerbäck and Löwhägen, 1979; Enerbäck, 1966) and glucocorticoids (Räsänen, 1960; Heap and Kiernan, 1973). Therefore, it seems likely that CTMC and MMC represent separate or divergent cell lines (Ruitenberg and Elgersma, 1976).

There has been a great deal of speculation as to the origin of the intestinal MMC that respond to

parasitism. Currently, the most accepted proposal is that they derive from lymphoid cell lines (Burnet, 1965; Miller, 1971; Burnet, 1977) but whether the precursor cells are T or B lymphocyte-derived or are regulated by some thymic factor is not yet clear. Thymectomized mice and thymectomized T cell-depleted rats (Ruitenberg and Elgersma, 1976, 1979; Mayrhofer, 1979) do not respond with an intestinal mastocytosis to T. spiralis or N. brasiliensis infection, respectively, and the MMC response can be restored by reconstituting nude mice with thymic fragments (Ruitenberg and Elgersma, 1976). Although there is no direct evidence of an immunological basis for the MMC response, mast cells that appear in cultures of immune lymph node cells show a greater response to specific antigenic stimulation than do normal mast cells (Ginsburg et al., 1978). In addition, there is a heightened MMC response in rats secondarily infected with N. brasiliensis (Mayrhofer, 1979).

An intestinal mastocytosis could benefit both the host and the resident parasites if it contributed to resistance to superinfection. Because of the difficulties in isolating MMC, hypotheses on their functions are based upon extrapolations made from in vitro and in vivo studies of CTMC responses. These extrapolations are questionable because MMC and CTMC differ in many structural and staining characteristics, and it seems likely

that they may differ considerably in function. Mucosal mast cells, like CTMC, may serve as sites for antigen-antibody-mediated amine release and thus facilitate antibody access to the parasites. CTMC release chemotactic factors such as ECF-A which enhance inflammatory cell migrations and thereby potentiate inflammatory reactions, but whether this is true of MMC remains to be shown. Recently, Czarnetski et al. (1979) found that heparin, a constituent of CTMC, will inhibit chemotactic factors in vitro. Therefore, it is possible that tempering the inflammatory reaction is a function of the mast cells described in the liver in chronic infections of T. taeniaeformis.

A most interesting phenomenon from an immunological viewpoint is the presence of IgE within the cytoplasm of CTMC in the skin of parasitized and atopic individuals (Halliwell, 1973, 1975; Feltkamp-Vroom et al., 1975; Li Hsü et al., 1979; Schopfer et al., 1979) and within MMC in the intestinal mucosa of N. brasiliensis-infected rats (Mayrhofer et al., 1976). Specific IgE may play a significant role in resistance to challenge infections and MMC might serve at sites of parasitic invasion to absorb and store and/or synthesize reagin (Halliwell, 1975). Since plasma cells are of lymphocyte origin and MMC are suspected to be of similar lineage then it seems likely that these cells may share certain

characteristics, even to the extent of synthesizing immunoglobulin. Further *in vitro* and *in vivo* studies will be necessary to determine which of the functions of CTMC are shared by MMC and which of these contribute to intestinal resistance to parasitic invasion. The observations presented in this thesis open up several avenues by which to explore these questions experimentally.

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ARTICLE 1

IMMUNOGLOBULIN E-CONTAINING CELLS IN INTESTINAL AND LYMPHATIC TISSUES OF RATS INFECTED WITH TAENIA TAENIAEFORMIS

by

Martha C. Lindsay and Dr. Jeffrey F. Williams

SUMMARY

Immunglobulin-bearing cell populations in rats infected with Taenia taeniaeformis were studied using indirect immunofluoresence assays. Cells positive for IgE in the intestinal mucosa increased to maximum numbers at 60 days after infection (DAI) but had fallen by 90 DAI. This increase was due largely to IgE-positive mucosal mast cells, many of which showed evidence of specific fluorescence intracytoplasmically as well as on their membranes. IgG_{2c}-containing cell numbers also rose in infected rats when compared to uninfected controls, but to a lesser degree than those stained for IgE.

In lymphatic tissues, cells producing all classes of immunoglobulins had increased by 6 DAI. Small numbers of IgE-positive cells were found in Peyer's patches throughout the infection in all rats, however, IgE-positive cells were often detected in clusters in infected rat spleens through 60 DAI. By 6 DAI, mast cells had accumulated near fluorescent plasma cell clusters along splenic vessels, and by 21 DAI many showed specific fluorescence with IgE. In addition, IgG_{2a}-containing cells increased in infected rat spleens until 21 DAI, but fewer were detectable at 28 days. No comparable trends occurred in spleens of age-matched control animals. More IgE-containing cells (including fluorescent mast

cells) were observed in infected rat mesenteric lymph nodes, until 60 DAI after which time they decreased to the levels seen in uninfected controls.

The relationship between mucosal mast cells and IgE and their possible function(s) in immune responses to T. taeniaeformis are discussed.

INTRODUCTION

Rats infected with Taenia taeniaeformis become immune to secondary challenge and it has been known for many years that this resistance can be transferred with antibody by both natural and artificial means (1-3). The protective antibodies implicated in recent studies (4) are of the IgG_{2a} type, and the resistance manifested in challenged animals has been shown to be complement-dependent (5). Enhancement of immune attack on early stages of the parasite occurs in animals sensitized with IgE-containing serum (6). However, the sequence of events in challenged animals is not clear, and the cellular participants in protection have not been identified.

There is a prolonged increase in mast cell numbers in the intestinal mucosa of rats infected with Taenia taeniaeformis, even though parasites are no longer present in the gut after the first few hours post infection (7). It seems likely that these cells contribute to resistance to reinfection, but their relationship to the pattern of antibody production has not been investigated. Studies of immunoglobulin-containing cell populations have provided some important insights into immune responses to other helminthiases (11), and recent work has surfaced some new cell-immunoglobulin associations in relation to IgE (12-14). The present study was therefore undertaken

to investigate these relationships in the intestine and associated lymphatic tissues in rats infected with T. taeniaeformis. Evidence is presented for the appearance of IgE-containing gut mast cells, similar to those detected in parallel groups infected with Nippostrongylus brasiliensis.

MATERIALS AND METHODS

Parasites

The strain of Taenia taeniaeformis used in these experiments was obtained originally from Mr. C.E. Claggett in the Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland. The parasite has been maintained in our laboratory as described by Leid and Williams (4). Third stage larvae of Nippostrongylus brasiliensis were kindly supplied by Dr. Paul Weinstein, Notre Dame University, South Bend, Indiana.

Experimental infections

Infections with T. taeniaeformis were established in 28 day old female Sprague-Dawley-derived rats (Spb:SD) purchased from Spartan Research Animals, Haslett, Michigan. Animals were infected by gastric intubation under light ether anesthesia, and with a suspension containing 1000 eggs. Immunofluorescence and histochemical observations were made on a total of 37 infected rats, and on an equal number of age-matched control animals.

Six 15 week old female Wistar rats (Charles River Breeding Laboratories, Wilmington, Massachusetts) each received 2000 N. brasiliensis third stage larvae subcutaneously in the dorsum of the neck. Six age-matched

uninfected rats of the same strain served as controls.

Histology

At specified time intervals post infection with T. taeniaeformis, groups of 3 to 7 rats were killed by exposure to carbon dioxide vapor and exsanguinated. Uninfected control groups of matching sizes were treated similarly. Samples of the spleen, mesenteric lymph node, the proximal 2-3 cm of duodenum and Peyer's patches were removed from each rat. During the sampling procedure, an estimation was made of the numbers of metacestodes in each liver to ensure that control rats had not been accidentally infected and that infected livers carried expected parasite burdens. Approximately 20% of the infective eggs gave rise to viable hepatic parasites in this experiment.

Tissue specimens were fixed for 24 hr at 4° C in lead subacetate-ethanol-acetic acid (15), washed in 70% ethanol for 12 hr, dehydrated, embedded in Paraplast Plus (Scientific Products, Romulus, Michigan) and stored at 4° C. Four micron thick sections were cut, mounted on gelatin-coated glass slides, incubated at 56° C for 10 min and stored at 4° C until used in immunofluorescence or histochemical studies.

Fifteen and 20 days after receiving N. brasiliensis larvae, groups of 3 rats each were killed and

exsanguinated. Samples of the duodenum were removed from infected and control rats, fixed and processed as described above.

Antisera

Sheep anti-rat IgE (Miles Laboratories, Elkhart, Indiana) was tested in immunoelectrophoresis against both normal rat serum and IgE myeloma protein. A known specific Goat anti-rat myeloma IgE antiserum was used in order to establish specificity. The myeloma protein and monospecific anti-IgE-myeloma serum were made available through the generosity of Dr. E.E.E. Jarrett, University of Glasgow, Glasgow, Scotland.

Sheep anti-rat IgA was prepared in our laboratory according to the methods described by Leid and Williams (4). Goat anti-rat IgG_{2a}, Rabbit anti-goat IgG (FITC), Goat anti-rabbit IgG (FITC), and Rabbit anti-sheep IgG (FITC) were purchased from Miles Laboratories, Elkhart, Indiana. Rabbit anti-rat IgM, Rabbit anti-rat IgG_{2b}, Goat anti-rat IgG_{2c} and Sheep anti-rat IgG_1 were purchased from Pel-Freez Biological, Rogers, Arizona. All antisera were tested in immunoelectrophoresis before use in immunofluorescence assays.

Prior to use, antisera were diluted at least 1:10 with phosphate-buffered saline (PBS), pH 7.4, and absorbed with 10 mg of rat liver acetone powder (Sigma Chemical Company, St. Louis, Missouri) per 1 mg of protein for 1

hour at 25° C with constant gentle agitation. The suspension was centrifuged at 5000 X g for 10 minutes. The supernatant was then filtered through .22 micron Millipore filters (Millipore Corporation, Bedford, Massachusetts) and aliquots stored at -70° C. Final dilutions were prepared immediately prior to use in assays.

Immunofluorescent and histological staining

Sections were dewaxed in xylene, rehydrated and stained for 30 min with 0.1% Alcian blue (Alcian blue 8GX, Gurr, London), pH 1.0, to stain mucosal mast cell mucopolysaccharides (12). After a 10 min wash in PBS, indirect immunofluorescence assays were begun. Sections were incubated with a primary antiserum for 30 min at 25° C with occasional gentle agitation and then washed in 3 1-liter changes of PBS for a total of 30 min. They were incubated further with the corresponding FITC-conjugate for 30 min at 25° C and washed in 4 1-liter changes of PBS for a total of 60 min. Stained sections were mounted in 50% PBS/50% glycerol, pH 9.0, for examination of fluorescence.

Specificity of antisera for tissue immunoglobulins

The following tests were performed to determine the specificity of the fluorescent reactions observed with each antiserum. A series of control slides was prepared in which either normal serum was substituted for each

primary antiserum, the primary antiserum was omitted, or an unconjugated secondary antiserum was applied prior to the corresponding FITC-labelled antiserum in order to block binding of the conjugate.

Counting method

The numbers of fluorescent cells were counted in 5 duodenal villus crypt units (16-17) in each of 2 separate tissue sections giving a total count for 10 villus crypt units (VCU). The arithmetic mean was calculated, expressed as the number of fluorescent cells/VCU and served as the cell count for each rat in subsequent statistical analyses.

Statistical analysis and rationale

The total fluorescent cell numbers in infected and control rats were compared using a one-way analysis of variance method for data collected at 6, 42, 60 and 90 DAI.

We then wished to determine which cell populations were responsible for any differences. The fluorescent mast cell count was subtracted from the total fluorescent cell number for each rat and the remaining cells were designated the "E" population. Since fluorescent mast cell counts were zero in all control groups, the total IgE-positive cell counts were equal to the E populations in these animals. A comparison was made between the E

values of control and infected groups at each time interval, again using a one-way analysis of variance with the α level set at 0.01. If a difference was found between the total fluorescent cell counts of infected and control groups but their E levels were the same, then the difference was attributed to MMC in the parasitized rats.

Microscopy and Photography

Sections were examined for immunofluorescence with a Zeiss Photomicroscope III (Carl Zeiss, Oberkocken, West Germany) by dark field illumination with light from a halogen quartz lamp. A BG-38 3mm red suppressor filter placed over the FITC exciter filter and a 530 nm barrier filter were used for photography. Fluorescence photographs were taken with Kodak Ektachrome 400 ASA daylight film. Bright field photographs were taken on Kodachrome 64 ASA daylight film using the same microscope.

RESULTS

Duodenum

The specificity of the Sheep anti-rat IgE preparation was confirmed prior to use in immunofluorescence studies. This antiserum produced one precipitin arc in immunoelectrophoresis against normal rat serum, and recognized rat myeloma IgE protein. The arc was identical to that formed with monospecific goat anti-IgE. When normal sheep serum was substituted for sheep anti-IgE in immunofluorescence tests or if the primary antiserum was omitted, all fluorescent staining was lost. In addition, unconjugated Rabbit anti-sheep IgG blocked fluorescent staining by the FITC-conjugate. Sheep anti-IgE was the only reagent that bound to mucosal mast cells (MMC) in this study.

We chose to sample at 6, 13, 21, 28, 42, 60 and 90 DAI because MMC numbers are significantly elevated by 21 DAI and circulating IgE is detectable from day 19 to 50 DAI (18). Figure 1 illustrates the changes in IgE-positive cell populations in the duodenum through 90 DAI. From 6 through 28 DAI (Figure 1A), fluorescent cells occupied the lower half of each villus. Many were plasma cells with eccentric nuclei and homogeneously fluorescent cytoplasm, but there were also a few

FIGURE 1

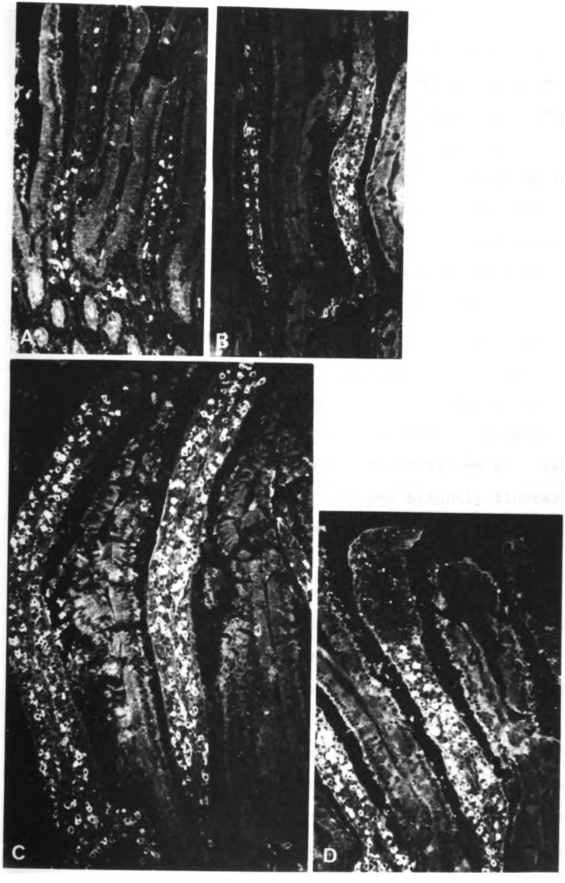
Fluorescence micrographs illustrating trends in IgE-positive cell populations in duodenal mucosae of infected rats (X 50).

A, 13 days after infection (DAI) showing positive cells located in the lower half of each villus.

B, 42 DAI. Cell numbers have increased and they are now distributed in the lower two-thirds of most villi.

C, 60 DAI. Maximum numbers of cells are visible at this stage, and they extend to the tips of the villi.

D, By 90 DAI, fewer cells are present and they are no longer detectable at the tips of villi.


A
B
C
D

unidentifiable round cells, some with granular cytoplasm, which were coated with IgE, and occasional Alcian blue-positive MMC with labelled surfaces. IgE-positive lymphocytes and granulocytes were difficult to identify, either because their morphological characteristics were not distinguishable or because they showed autofluorescence, respectively. However, both types could have contributed to the population of Alcian blue-negative cells whose surfaces appeared to bind anti-IgE.

At 42 DAI (Figure 1B), increased numbers of fluorescent cells were localized in the lower two-thirds of each villus and up to 50% of MMC either contained or were coated with IgE. Mast cells containing IgE were very similar to those described by Mayrhofer et al. (12) and Halliwell (19). The cytoplasm was brightly fluorescent around the unstained granules which appeared as dark inclusion bodies.

IgE-positive cell numbers peaked at 60 DAI (Figure 1C) and occupied the entire lamina propria of most villi. Approximately 90% of MMC became labelled with anti-IgE, and fluorescence was almost exclusively confined to cell surfaces. The fluorescent cell numbers declined by 90 DAI (Figure 1D) and IgE-binding cells were seldom found in the tips of villi. Only 50% of the MMC population became coated with anti-IgE at this time. Figure 2B illustrates the different fluorescent cell types found in the

FIGURE 2

IgE-containing MMC in the duodenal mucosa of a Taenia taeniaeformis-infected rat (X125).

A. Light micrograph showing Alcian blue-stained MMC with arrows (1) pointing to cells that contain IgE.

B. Fluorescence micrograph of the same field demonstrating IgE-positive cells with arrows (2) pointing to the two mast cells selected in A.

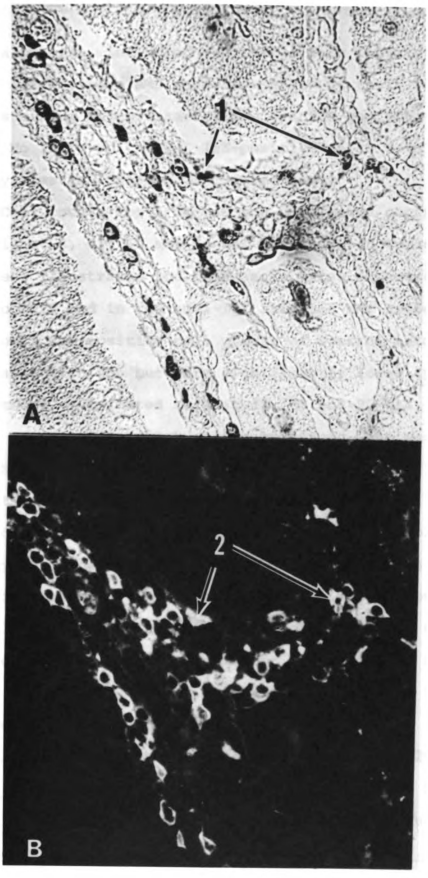

1
A
2
B

TABLE 1

Total IgE-containing cell counts and fluorescent MMC counts in rats at intervals after infection with *Taenia taeniaeformis* and in age-matched controls.

TREATMENT	TIME OF INFECTION (DAYS							
	6		42		60		90	
	Total Cells	Mast Cells	Total Cells	Mast Cells	Total Cells	Mast Cells	Total Cells	Mast Cells
INFECTED	17[a]	0	33	13	105	41	68	33
	14	0	35	15	116	92	62	23
	13	0	22	6	67	26	73	43
	12	0	37	28	98	61	77	44
	10	0	24	14	97	54	63	40
	10	0	32	18	87	47	63	36
	9	0	48	31	91	8	50	22
	12.14[b]	0	33.00	17.86	94.57	51.29	65.14	34.43
CONTROL	12	0	16	0	22	0	27	0
	16	0	16	0	21	0	45	0
	14	0	14	0	26	0	29	0
	12	0	19	0	24	0	20	0
	15	0	17	0	33	0	24	0
	23	0	22	0	34	0	35	0
	17	0	28	0	30	2	32	0
	15.57	0	18.86	0	27.14	0.29	30.29	0

[a] Each count is the arithmetic mean number of cells per VCU.

[b] Arithmetic mean.

FIGURE 3

IgE-positive fluorescent cells in the duodenal mucosae of rats infected with Taenia taeinaeformis and in age-matched controls. Coarsely-stippled columns represent total fluorescent cells in infected rats and white columns illustrate those which are mast cells. Finely-stippled columns correspond to total fluorescent cell numbers in control animals, and black columns represent those which are fluorescent mast cells. Vertical bars indicate standard error.

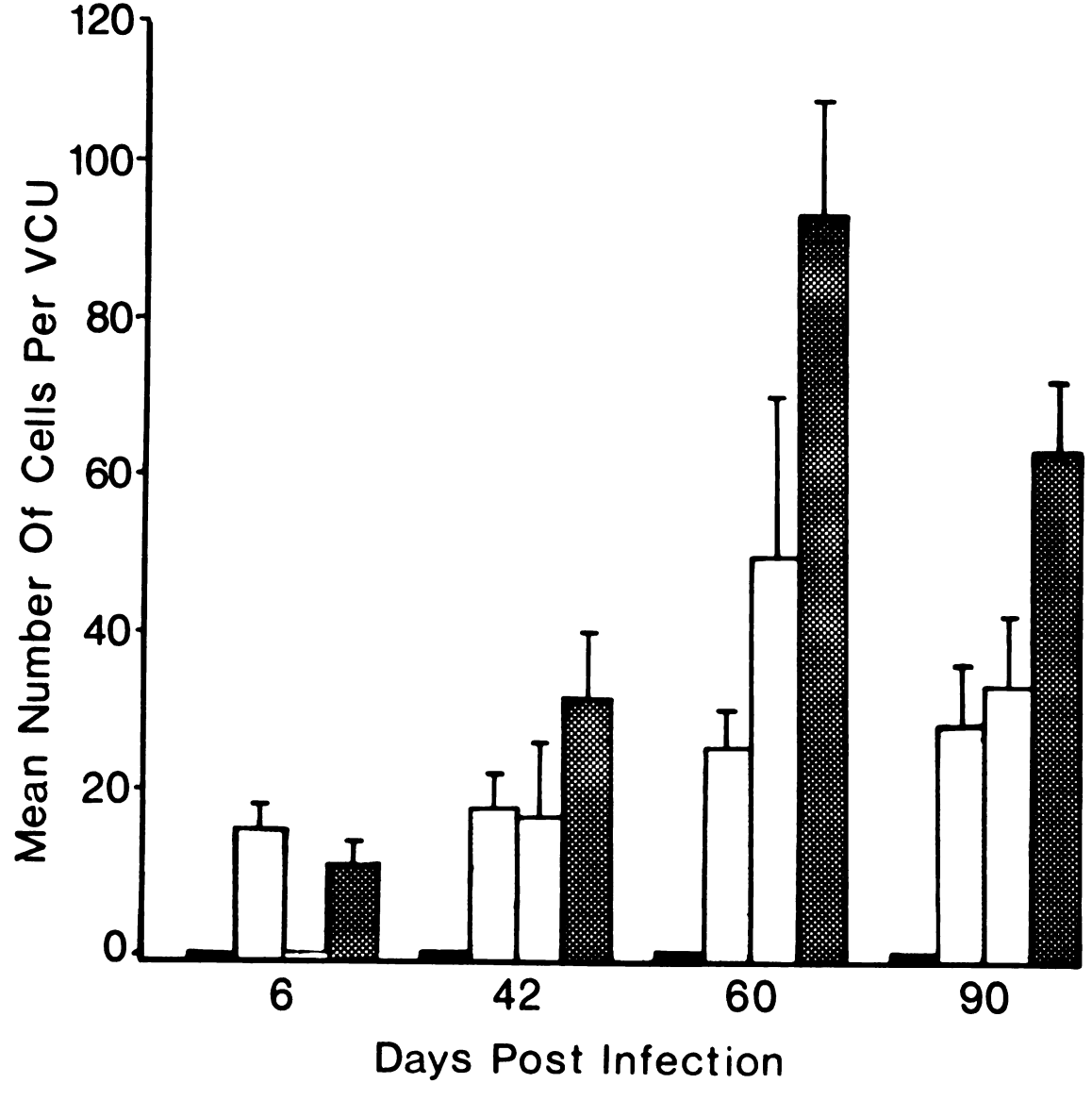
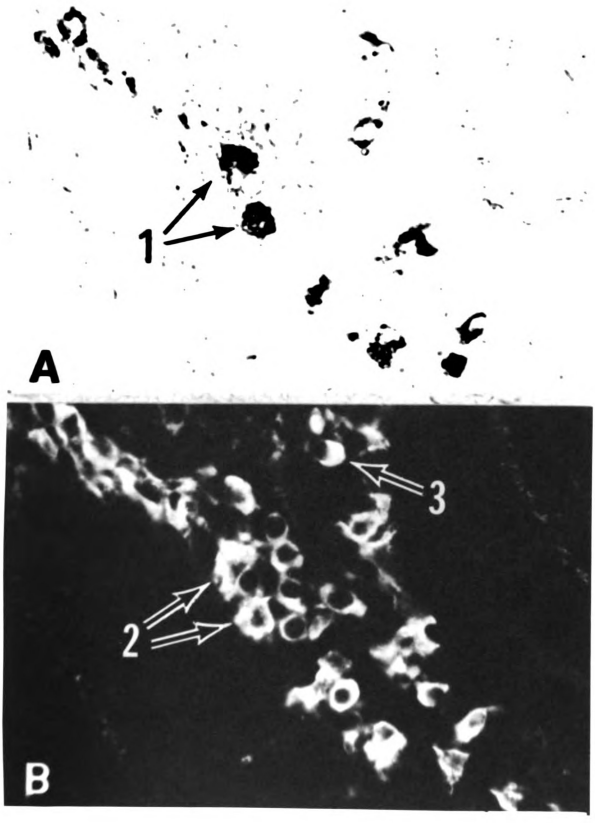

120
100
80
60
40
20
0
Mean Number Of Cells Per VCU
6
42
60
90
Days Post Infection

FIGURE 4

Cells containing IgE in the duodenal mucosa of a Nippostrongylus brasiliensis-infected rat (X 125).

A. Alcian blue-positive MMC with arrows (1) pointing to cells that contain IgE.

B. Fluorescence micrograph of the same field demonstrating MMC (2) and plasma cells (3) containing reagin.


1
A
3
2
B

taeniaeformis-infected animals, Alcian blue-positive cells showing bright intracytoplasmic fluorescence with anti-IgE were commonly seen.

All other antisera produced one precipitin arc in immunoelectrophoresis when tested against normal rat serum, with the exception of Goat anti-rat IgG_{2a}. Batches of this reagent always produced two arcs, one of which corresponded to IgG_{2a} and the other to IgG_{2b} or IgG_{2c}. However, the results of immunofluorescence assays for Goat anti-rat IgG_{2a} were distinctly different from those for the monospecific Goat anti-rat IgG_{2c} and Rabbit anti-rat IgG_{2b}. Control procedures in the indirect immunofluorescence tests confirmed the specificity of binding by antisera.

Cells containing IgG_{2a}, IgG_{2b} and IgM were detected in small numbers in the duodenal mucosae of infected and control rats throughout the observation period. IgG_1- and IgA-positive cell numbers increased steadily in all animals with age and counts were not elevated noticeably in infected rats. MMC showed no fluorescence with any of these reagents. Cells containing intracytoplasmic IgG_{2c} increased in number with time, and by 60 DAI there were greater numbers in infected rats than in non-infected controls, but the magnitude of these differences was much lower than for IgE-containing cell populations.

Peyer's patches

Cells containing all classes and subclasses of immunoglobulins were found in small numbers beneath the domes by 6 DAI. No consistent population trends were observed throughout the study, and no one immunoglubulin predominated. No remarkable differences were noted between infected and control rats.

At 6 DAI, a few Alcian blue-staining cells appeared in the domes but none was positive for IgE. Occasional IgE-positive mast cells were found at the periphery of the Peyer's patches by 42 DAI and they were no longer detectable at 60 days. Mast cells were found rarely in controls and none bound anti-IgE.

Spleen

All classes of immunoglobulins were represented in clusters of splenic cells by 6 DAI, and thereafter throughout the observation period in both control and infected rats. No single immunoglobulin-containing cell population was predominant, but IgA-positive cells were present in least numbers in all rats through 90 DAI. IgG_{2a}-containing cells in spleens of infected rats peaked at 21 DAI and there were noticeably fewer by 28 DAI. There were many IgE-positive cell clusters through 60 DAI, but by 90 DAI they had declined. These patterns did not occur in control animals.

In spleens of control rats few mast cells were seen until 13 DAI, when scattered clusters were found throughout the parenchyma. By 42 DAI, and during the remainder of the study, mast cells were found rarely. The pattern was markedly different in infected rat spleens. By 6 DAI, many more Alcian blue-staining cells were found singly and in clusters of 2 or more cells than had been detected in control spleens at any time. Mast cells were often present near IgE-positive cell clusters along small splenic vessels throughout the parenchyma. By 21 DAI, many individual IgE-positive mast cells appeared and clusters of more than 2 to 3 Alcian blue cells seldom fluoresced. Positive cells either contained or were coated with IgE, and the population increased until 28 DAI before declining. By 60 DAI only occasional fluorescent mast cells were detected although clusters of IgE-positive Alcian blue-negative cells were plentiful. Mast cells were rarely seen at 90 DAI and none was positive for IgE.

Mesenteric lymph node

Trends in each immunoglobulin-containing cell population were established by estimating the proportion of lymph node parenchyma containing positive cells at each time interval. Cells positive for each class of immunoglobulin were detected at 6 DAI in control and infected animals.

Approximately one-third of mesenteric lymph node parenchyma in infected rats showed IgE-positive cells (including occasional fluorescent mast cells) by 13 DAI. This situation persisted through 42 DAI, then decreased to control levels (less than 10%) by 60 DAI. Cell populations containing IgG_{2c}, IgG_{2a}, IgG_1 and IgM increased in all animals until 21 DAI, then decreased, whereas IgG_{2b}-positive cells were present to their greatest extent at 27 DAI, then declined. Except for IgE-staining cells, the trends did not differ in infected and control rats.

DISCUSSION

Mucosal mast cells increase in numbers in the lamina propria of rats infected with the intestinal parasites N. brasiliensis (20-21) and Trichinella spiralis (22), and also with the hepatic metacestode T. taeniaeformis (7). Our results show that the marked increase in IgE-positive cells in the intestines of T. taeniaeformis-infected rats was largely attributable to these MMC. Not only were MMC coated with IgE, but many also showed specific extragranular cytoplasmic fluorescence, indicative of intracellular IgE. There are precedents for this interpretation, because mast cells with cytoplasmic IgE have now been described in the dermis and intestinal mucosa of both atopic and parasitized individuals (23-26). It is unlikely that chance tangential mast cell sectioning had any effect on our observations (24), because the proportion of MMC showing fluorescent cytoplasm changed with time even though all tissues were processed and sectioned identically.

In view of the significance of this finding in terms of the relationship between MMC and IgE, we felt it important to validate our procedures by applying them to samples of duodenum from rats infected with N. brasiliensis (12). IgE-containing MMC have been described in the gut

at 15-21 DAI and we were able to confirm this in Charles River Wistar rats. However, we also found many IgE-producing plasma cells in the intestinal mucosae of rats infected with either N. brasiliensis or T. taeniaeformis. This represents a marked discrepancy between our results and those of Mayrhofer et al. (12) who observed very few plasma cells. It does not seem likely that the host strain contributed to this difference because both studies were done with Wistar rats from the same commercial supplier.

A further point of difference from the situation in nippostrongylosis was that MMC in T. taeniaeformis-infected animals were not subepithelial or epithelial in location. The distribution of IgE-positive MMC underwent some remarkable changes during the course of taeniiasis, perhaps related to the selectivity of location of precursors at different sites in the villi, or due to loss of IgE from established MMC population in a consistent pattern within each villus. Quantitative studies comparing total MMC and IgE-positive MMC within the same villi would be necessary to explore these possibilities experimentally. Our results certainly provide no evidence that IgE-positive MMC migrate to the intestinal lumen in primary infections, although this is generally believed to be their fate in other helminthiases (27, 28). The response of MMC in T. taeniaeformis-infected rats exposed to

secondary challenge would be particularly interesting in light of the recent observation that epithelial migration of mast cells occurs following re-exposure of rats after primary nippostrongylosis (29).

The increase in IgE-positive cells in the gut was preceded by a rise in similar populations in the spleen and mesenteric lymph nodes. There is considerable evidence for the preferential migration of mesenteric lymphoblasts to the intestinal mucosa (30-33), and our findings are consistent with this notion. Presumably some inducing factor, perhaps parasite-derived, could affect the local lymphatic tissues and stimulate plasma cell precursors to product IgE and MMC precursors to migrate to the intestine. In addition to plasma cells, the spleen and Peyer's patches also showed increased numbers of IgE-positive Alcian blue-staining cells. Antigen uptake by mast cells in lymphatic tissues has been described and it is possible that they increase in numbers in response to an infection in order to facilitate the processing of parasite antigens.

It is tempting to consider all these changes as part of an effort to develop a highly sensitized cell population in the gut lamina propria specifically prepared to respond to secondary challenge organisms as they migrate through the villar epithelium. Although no direct evidence exists, it has often been suggested that an intestinal immune

barrier is mounted to circumvent larval migration via this route (35) and the MMC may be an important component, even though their main contribution might be to enhance accessibility of protective IgG_{2a} antibodies (6). They could also accelerate the local accumulation of immune effector cells through the release of chemotactic factors after parasite antigen-induced triggering. The development of resistance to challenge infection and prevention of potentially debilitating superinfections could be advantageous both to the host and established parasites.

Results of immunofluorescence with other anti-immunoglobulin sera indicate that there is no marked increase in either IgG_{2a} or IgA-containing cells in the gut of infected rats. Antibodies of both these types have been implicated in acquired resistance in rodents (4, 36, 37). IgG_{2a}, in particular, is formed very rapidly after infection (4) and there was evidence in our study of increases in IgG_{2a}-forming cells in lymphoid tissues. The observation that lamina propria increases do not occur reinforces our view that mast cell modulation of vascular permeability in the intestine may be an integral part of serum antibody-mediated parasite destruction in challenged animals.

These proposals do not take into account other possible roles for IgE which have surfaced recently from

studies on anti-parasite immunity. IgE adheres to the surface of schistosomulae *in vivo* (25), and can sensitize macrophages *in vitro* for the destruction of *S. mansoni* (13). If IgE antibodies were released from intracytoplasmic sites in MMC after stimulation by *T. taeniaeformis*, these reports suggest that they could have a direct role in resistance effector mechanisms. It has also been suggested that IgE accumulated within mast cells represents a storage system (23) and this would be compatible with a protective role at mucosal or epithelial sites. Secretory cells do not generally store macromolecular products of other cell lines, and the possibility that certain MMC may have IgE-synthesizing capabilities cannot be discarded entirely in attempting to account for its presence within the cytoplasm. MMC are believed to be derived from a lymphoid cell lineage (38, 39, 40), and a subpopulation with functions similar to those of plasma cells could exist. However, the recent demonstration of peroxidase-labelled IgE within rat macrophages (41) establishes a convincing precedent for the hypothesis that preformed antibody can be selectively absorbed and retained for future use.

Our results shed no light on the question of how hepatic parasites influence the proliferation and/or accumulation of MMC at gut sites, but they do raise the possibility of using an additional discriminatory

parameter (IgE binding) in pursuing the characteristics of mastocytosis in this infection. Elsewhere we have shown that MMC increases are probably mediated via some circulating factor, and that prolonged hypergastrinemia accompanies chronic infection (42), although its role in gut-related pathologic changes is far from clear. More definitive accounts of the functions of MMC must await demonstration of their sensitivity to antigen and the nature of secretory responsiveness. In the meantime, hypotheses can only be developed based on the assumption that the findings for connective tissue mast cells also hold true for MMC. In fact, the two cell types differ morphologically, histochemically and in their responses to pharmacologic agents (40, 43, 44, 45, 46) and some divergences in function seem likely to emerge. The isolation of MMC from gut tissues (43) opens the way to exploration of these differences, but the hazards of *in vitro* characterization of enzymatically dispersed cells require that continued efforts be made to assess their roles *in vivo*. The situation in *T. taeniaeformis* in which reactivity of intestinal MMC can be investigated without the complicating presence of parasites in the gut offers unique advantages for research towards this goal.

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ARTICLE 2

MAST CELLS IN RATS INFECTED WITH *TAENIA TAENIAEFORMIS*

by

Martha C. Lindsay and Dr. Jeffrey F. Williams

ABSTRACT

Mast cells appeared in the liver around metacestodes of Taenia taeniaeformis by 13 days after infection (DAI). The population increased until 28 DAI, then gradually declined. These hepatic mast cells (HMC) were compared to intestinal mucosal mast cells (MMC) and connective tissue mast cells (CTMC) histochemically, morphologically and in their responses in vivo to compound 48/80 and dexamethasone. HMC were similar to MMC in that they stained positively with Astra blue, could not be demonstrated with .005% toluidine blue, and disappeared after 5 days of treatment with dexamethasone, but were unaffected by 48/80.

Immunoglobulin-containing cells in the liver were characterized using immunofluorescence assays. IgG_{2a}-, IgG_{2c}- and IgE-positive cell populations surrounding the parasites increased until 28 DAI then declined. Of these three populations, changes in anti-IgE-labelled cells were the most marked. Many IgE-positive cells were found to be HMC, and there was frequent evidence of intra-cytoplasmic IgE. The possible origin of these IgE-containing HMC and their potential role(s) in the local immune reactions to T. taeniaeformis are discussed.

KEY WORDS: mast cells, IgE, immunofluorescence, Taenia taeniaeformis, cestodes, immunoglobulins, liver.

INTRODUCTION

Experimental cysticercosis in the rats provides a useful laboratory model for study of host-parasite relationships and the persistence of tissue helminths in immune animals (38). In the early stages of infection with Taenia taeniaeformis there are intense inflammatory changes around the organisms (33). Eventually, however, the host response subsides and the growing foreign mass is accommodated remarkably well in the liver.

Histopathological accounts of inflammatory lesions around taeniid cestodes and other hepatic helminths seldom mention the participation of mast cells, (19, 23, 36), and in the few instances where they have been described their nature and possible function have not been investigated (2, 5, 6, 7, 33, 37). In view of the central role played by these cells in inflammatory processes and their known interactions with immunoglobulin E, we attempted to characterize the appearance of mast cells around developing larvae of T. taeniaeformis in the liver, and their relationship to IgE in the encapsulating response. The results provide evidence for the proliferation of a population of mast cells, similar in morphology and drug responsiveness to the mucosal mast cells (MMC) of the

intestine. Furthermore, these cells appear to exhibit not only surface sensitization by IgE, but also the capacity for intracytoplasmic accumulation of this immunoglobulin, described recently for intestinal MMC in the parasitized rat (22).

MATERIALS AND METHODS

PARASITE

The strain of Taenia Taeniaeformis used in this study was maintained as described by Leid and Williams (20).

EXPERIMENTAL INFECTION

Twenty-eight day old female Sprague-Dawley-derived rats (Spb: [SD]) were purchased from Spartan Research Animals, Haslett, Michigan. Animals were infected orally with 1000 eggs under light ether anesthesia.

COMPOUND 48/80 AND DEXAMETHASONE ADMINISTRATION

Groups of 6 rats infected for 27 days received intraperitoneal injections twice daily for 5 consecutive days of 0.1 mg compound 48/80 (Sigma Chemical Company, St. Louis, Missouri) per 100 gm body weight in 0.1 ml sterile saline (increased daily by increments of 0.1 mg/100 gm), 0.1 mg dexamethasone (Azium, Schering Corporation, Kenilworth, New Jersey) in .05 ml, or equivalent volumes of isotonic saline. The protocol described by Enerbäck (10) was followed for 48/80 treatments, and the regimen used by Heap and Kiernan (15) for dexamethasone administration.

HISTOLOGY AND HISTOCHEMISTRY

Animals which received compound 48/80, dexamethasone or saline were killed 4 hours after the first injection on day 5 by exposure to carbon dioxide vapor. Samples of tongue, skin, liver, duodenum and stomach (sampled according to the method of Heap and Kiernan, (15)) were placed in cold lead subacetate-ethanol-acetic acid fixative (26) for 24 hours.

Groups of 3 rats each were killed at 6, 13, 21, 28, 42, 65 and 90 days of infection. Groups of age-matched uninfected animals served as controls. Samples of the liver were taken from each rat and fixed for 36 hours at 4°C in either lead subacetate-ethanol-acetic acid or 10% neutral-buffered formalin.

After fixation, samples were treated for 12 hours in absolute ethanol before dehydration, embedding in Paraplast Plus (Scientific Products, Romulus, Michigan) and storage at 4°C. Sections were cut at 4μ, mounted on gelatine-coated glass slides, incubated at 56°C for 10 minutes and stored at 4°C until used in immunofluorescence or histochemical studies.

Tissue sections from dexamethasone-,48/80-, or saline-treated rats were dewaxed in xylene, rehydrated, stained for 30 minutes either with 0.5% Alcian blue (Alcian blue 8GX, Gurr, London), pH 1.0, and counter-stained with alcoholic acid fuchsin for 2 minutes, or with 0.5%

Astra blue (Astrablau FM, Roboz Surgical Instruments Company, Inc., Washington, D.C.), pH 1.0, and counterstained with 0.5% safranin (Safranin, Fisher Scientific Company, Fair Lawn, New Jersey) for 1 minute. Tissues were then dehydrated and mounted in Pro-Tex mounting medium (Scientific Products, McGaw Park, Illinois). Other sections were stained with .005% toluidine blue (Toluidine Blue O, Central Scientific Company, New York, New York), pH 0.5, to detect differences between affinities of connective tissue, mucosal or hepatic mast cells for thiazanine dye (16).

In order to determine the identity of immunoglobulin-containing cells, serial sections paired with those examined in immunofluorescence assays were dewaxed, rehydrated and stained with giemsa (May-Grunwald Giemsa method) or with an alcoholic Astra blue/acid fuchsin technique (Blaies and Williams, submitted for publication). Stained tissue sections were then dehydrated and mounted.

ANTISERA

All antisera were tested for specificity in immunoelectrophoresis prior to use in immunofluorescence assays. Sheep anti-rat IgE (Miles Laboratories, Elkhart, Indiana) were tested against both normal rat serum and IgE myeloma protein in parallel with a known specific goat anti-rat myeloma IgE. The monospecific anti-myeloma antiserum and the myeloma protein were generously provided by Dr. E.E.E.

Jarrett, University of Glasgow, Glasgow, Scotland.

Sheep anti-rat IgA was prepared in our laboratory according to the methods described by Leid and Williams (20). Goat anti-rat IgG_{2a}, Rabbit anti-goat IgG (FITC), Goat anti-rabbit IgG (FITC) and Rabbit anti-sheep IgG (FITC) were purchased from Miles Laboratories, Elkhart, Indiana. Rabbit anti-rat IgM, Rabbit anti-rat IgG_{2b}, Goat anti-rat IgG_{2c} and Sheep anti-rat IgG_1 were purchased from Pel-Freez Biologicals, Rogers, Arizona.

Prior to use in immunofluorescence studies, antisera were absorbed with rat liver acetone powder (Sigma Chemical Company, St. Louis, Missouri) as described previously (Lindsay and Williams, submitted for publication).

IMMUNOFLUORESCENCE ASSAY

Tissue sections were dewaxed in xylene, hydrated to distilled water than washed for 10 minutes in phosphate-buffered saline (PBS), pH 7.4. The slides were treated with a 0.01% solution of trypsin 1:250 (Difco Laboratories, Detroit, Michigan) in 0.1 M $CaCl_2$, pH 7.8, for 30 minutes at 37°C. They were then washed for 10 minutes in two changes of PBS prior to antiserum incubations. Indirect immunofluorescence assays were conducted according to the procedure described by Lindsay and Williams (submitted for publication).

SPECIFICITY OF IMMUNOFLUORESCENT REACTIONS

The following tests were performed to determine the specificity of the fluorescent reactions observed with each antiserum. A series of control slides was prepared in which either normal serum was substituted for each primary antiserum, the primary antiserum was omitted, or an unconjugated secondary antiserum was applied prior to the corresponding FITC-labelled anti-IgG in order to block the binding of the conjugate.

COUNTING METHOD

The numbers of duodenal mast cells were counted in 3 villus crypt units (VCU (17)) in each of two separate tissue sections giving a total number of 6 villus crypt units. The arithmetic mean was calculated, expressed as the number of mast cells/VCU and served as the cell count for each rat in subsequent statistical analyses.

STATISTICAL ANALYSIS

Mast cell counts of rats in each treatment group were analyzed using a one-way analysis of variance method, and the alpha level was set at .01 for all comparisons.

MICROSCOPY AND PHOTOGRAPHY

Tissue sections were examined for fluorescence using a Zeiss Photomicroscope III (Carl Zeiss, Oberkochen, West Germany) and dark ground illumination with a halogen quartz lamp. A BG-38 3mm red suppressor filter placed

over the FITC exciter filter and a 530 nm barrier filter were used for photography. Fluorescence photographs were taken with Kodak Ektachrome 400 ASA daylight film. Bright field microscopy was performed on the same microscope and photographs taken using Kodachrome 64 ASA daylight film.

RESULTS

HEPATIC MAST CELL POPULATIONS DURING THE COURSE OF INFECTION

Round to ellipsoidal, Astra blue-positive cells (5.5-13.2μm x 4.4-8.8μm) containing coarse to fine cytoplasmic granules and round to ovoid nuclei were present in small numbers around portal vessels and in clusters within the developing host capsules around metacestodes by 13 days after infection (DAI). This population of mast cells, hereafter designated as hepatic mast cells (HMC), increased markedly up to 28 DAI, then declined so that fewer cells were visible by 42 DAI. They were detectable throughout the host capsules, but the majority were localized in the outer capsular layer nearest normal hepatic tissue. Large numbers of HMC were observed in all portal areas through 28 DAI, but subsequently they disappeared from these areas, even though Astra blue-staining cells were still detectable around the encapsulated parasites at 90 DAI.

COMPOUND 48/80 - DEXAMETHASONE EXPERIMENT

In order to compare the responses of HMC with mast cells of connective tissue and mucosal types, rats were treated with compound 48/80 or dexamethasone for 5

consecutive days. Connective tissue mast cells (CTMC) of the tongue and skin were unaffected by dexamethasone administration but very few could be found in 48/80-treated animals. Gastric and duodenal mucosal mast cells (MMC) (Figures 1A, 1B, 1D, 1E) were unaffected by 48/80 treatment and they were almost undetectable in dexamethasone-treated rats. When the lamina propria mast cell numbers were recorded (Table 1) and analyzed, MMC counts in the dexamethasone-treated group were significantly lower ($p \leq .01$) than counts in 48/80- or saline-treated animals.

Hepatic mast cells were not detectable in the host capsules around parasites in rats that received dexamethasone (Figure 1F). However, those in saline- and 48/80-treated animals were not noticeably different from mast cells in untreated infected rat tissues (Figure 1C).

The CTMC and MMC exhibited different affinities for toluidine blue in lead subacetate-fixed tissues. Mast cells of the tongue and skin stained darkly, whereas MMC were seldom seen in the gastric or duodenal mucosae. Toluidine blue-staining mast cells were found occasionally in host capsules surrounding hepatic metacestodes and rarely around portal vessels.

TABLE 1

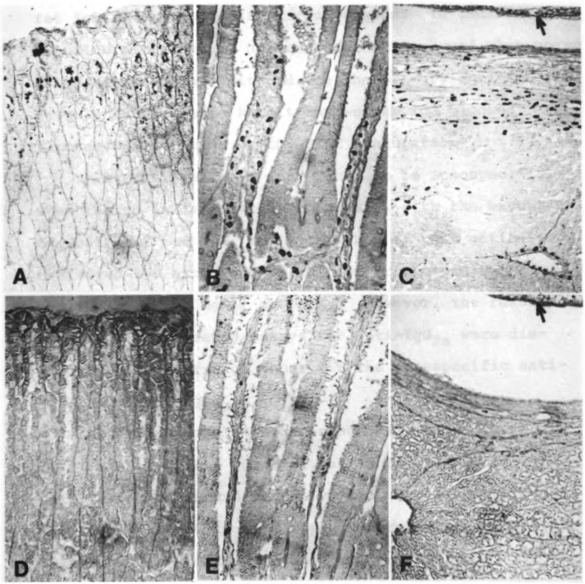
TREATMENT

COMPOUND 48/80	DEXAMETHASONE	ISOTONIC SALINE
18	1	15
30	2	17
19	2	14
20	2	14
18	6	17
14	0	16
MEAN ± SE 19.8± 5.38	2.2 ± 2.04	15.5 ± 1.38

Effect of treatment with Compound 48/80, Dexamethasone or saline on mucosal mast cells in rats infected with Taenia taeniaeformis.

FIGURE 1

Comparison of mast cell responses in rats infected with T. taeniaeformis and treated with Compound 48/80 (A,B,C) or dexamethasone (D,E,F) on days 27-32 after exposure. Alcian blue/acid fuchsin stain. (A) and (D), gastric mucosae. (B) and (E), duodenal mucosae. (C) and (F), host capsules surrounding hepatic metacestodes. Parasite membrane is at the top in (C) and (F) (arrows). X50.


A
B
C
D
E
F

IMMUNOGLOBULIN-CONTAINING MAST CELLS

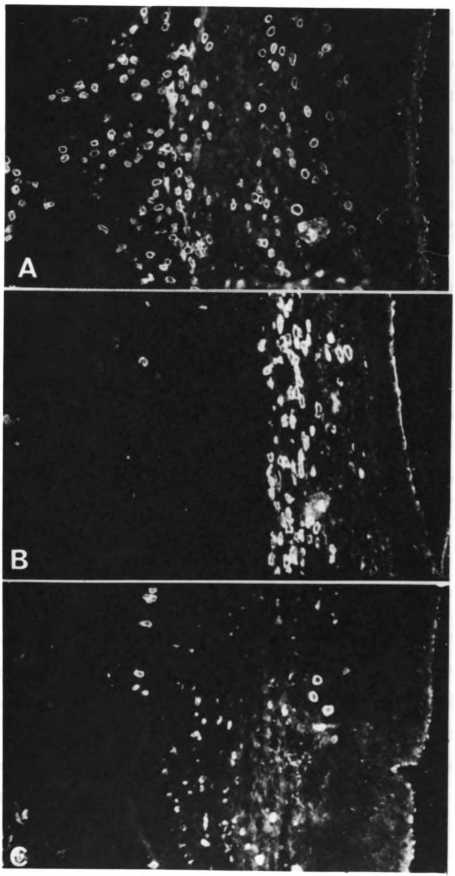
Sheep anti-rat IgE was found to be monospecific for rat IgE in immunoelectrophoresis tests. In indirect immunofluorescent tests no fluorescence occurred when normal sheep serum was substituted for the primary anti-IgE serum. Unconjugated Rabbit anti-sheep IgG blocked fluorescent staining by the FITC conjugate.

All other antisera were found to be monospecific when tested in immunoelectrophoresis, with the exception of Goat anti-rat IgG_{2a}. All batches of this antiserum exhibited two arcs, one of which corresponded to IgC_{2a} and the other to IgG_{2b} or IgG_{2c}. However, the results of immunofluorescence assays for anti-IgG_{2a} were distinctly different from those for the monospecific anti-IgG_{2c} and anti-IgG_{2b}.

No immunoglobulin-containing cells were detectable in livers at 6 DAI. However, at 13 DAI, IgE-positive cells appeared around portal vessels and by 21 DAI they were also present in host capsules around the parasites. IgE-containing cells were most evident at 28 DAI (Figure 2A) with fewer present by 42 DAI (Figure 2B). Some of these were plasma cells with eccentric nuclei and uniformly-fluorescent cytoplasm, but others were round to ovoid with granular cytoplasm (5.5-13.2μm x 4.4-8.8μm). IgE-fluorescent cells were found around portal vessels through 65 DAI, and at 90 DAI a few cells positively

FIGURE 2

Fluorescence micrographs demonstrating the decline in IgE-positive hepatic cell populations around metacestodes of Taenia taeniaeformis (to the right in each micrograph). (A), 28 days after infection (DAI) showing positive cells around portal vessels as well as within the host capsule. (B), 42 DAI illustrating fewer positive cells primarily within the host capsule. (C), 90 DAI when few cells are detected around parasites. X50.


A
B
C

labelled with anti-IgE were still evident in host capsules (Figure 2C). Many more cells were positive for IgE than for other immunoglobulins at each time sampled. Immunoglobulin-containing cells were detectable occasionally in portal areas of uninfected control livers throughout the study.

Plasma cells containing IgG_{2a} and IgG_{2c} appeared around portal vessels near parasites by 13 DAI. At this time, a few IgG_{2a}-positive plasma cells were also found around metacestodes within the developing host capsules. Cells positive for these and all other immunoglobulin classes were detected around parasites at 21 DAI, increased by 28 DAI, and declined thereafter, with few cells detected at 42 DAI. Positive plasma cells were not scattered randomly but were distributed in one or two layers within the host capsule.

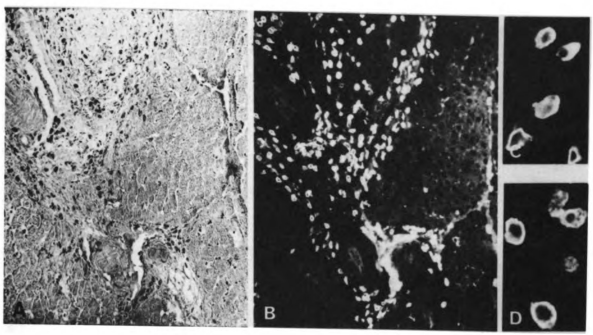
IDENTIFICATION OF IgE-POSITIVE CELLS

The vast majority of the IgE-positive cells in infected livers were round to ovoid, brightly-fluorescent irregularly-outlined cells containing intracytoplasmic granules of various sizes. Most were coated with IgE (i.e. outlined with fluorescein), and a large proportion of this population contained fluorescent cytoplasm surrounding the darker granules (Figures 3C and 3D).

In order to identify the IgE-positive granular cells,

FIGURE 3

Distribution patterns of IgE-positive cells in the liver of a Taenia taeniaeformis-infected rat. (A), light micrograph showing Astra blue-positive mast cells. (B), fluorescence micrograph demonstrating IgE-positive cells in the same field. (C), IgE-containing plasma cell (top right) and four Astra blue-positive granular cells, two coated with IgE and two containing intracytoplasmic IgE. (D), two granular IgE-containing cells (top right) and two positive cells coated with IgE. Figures 3A and 3B, X50; Figures 3C and 3D, X200.


B
D

paired sections of livers at 28 DAI were stained, one of each pair with anti-IgE and the other with either Astra blue/acid fuchsin or giemsa stain. Figures 3A and 3B illustrate the identical distribution patterns of IgE-positive cells and mast cells which were remarkably consistent in all infected rat livers. At higher magnifications, individual granular IgE-containing cells were found to be Astra blue-positive mast cells. No other cell types in giemsa-stained sections were similarly distributed or corresponded with IgE-positive granular cells.

DISCUSSION

The objective of this study was to characterize the course of the mast cell responses to developing metacestodes of T. taeniaeformis, and to establish their relationship to IgE and other immunoglobulins in the local hepatic lesions. The results provide evidence for the prompt appearance and persistence of a population of mast cells around the parasites which resemble the MMC of the intestinal lamina propria in terms of their histochemical traits, their affinity for IgE, and the accumulation of intra-cytoplasmic IgE.

The briskness and intensity of the hepatic mast cell reaction was unexpected. Mast cells are generally few in number in parenchymatous organs containing little connective tissue such as the liver (34). However, they have been shown to increase in certain pathological conditions including cirrhosis (1) and some chronic parasitic inflammatory diseases. There is a hepatic mast cell response in schistosomiasis (2,5) and fascioliasis (29, 32), and mast cells have been described in the thick fibrous host capsules around metacestodes of T. taeniaeformis in long-term infections (6, 7, 33, 37). The proportion of these increases has not been defined, and the time course of their occurrence has not been investigated

previously.

Our results suggest that mature mast cells or precursors of HMC may migrate from the periportal areas to the site of metacestode establishment, because they were detected around portal vessels before appearing in the developing capsule. Moreover, the accumulation of mast cells in clusters around the encapsulating fibroblastic response raises the possibility of local proliferation and/or differentiation (18). Since HMC and MMC reacted so similarly to dexamethasone and Compound 48/80, and shared affinities for copper phthalocyanine and thiazanine dyes which were quite distinct from those of CTMC, we conclude that most HMC are likely to be related to the intestinal mast cells often implicated in pathologic responses to gut helminths and their rejection in immune hosts (9,10,15,25). Nevertheless, the fact that a small minority of cells in T. taeniaeformis-infected livers did resemble CTMC is consistent with the possibility that representatives of both these populations respond to invading metacestodes.

Recent evidence for the existence of specialized mast cell types in species other than the rat (16) has stimulated interest in their origin and function in a variety of pathologic states. There are observations which suggest their derivation from lymphoid cell lineage (4, 24), and the preferential migration of lymphoblasts

from draining lymph nodes to sites of inflammation (3) may be important in the induction of hepatic mastocytosis. Whether specifically reactive lymphocytes can differentiate into mast cells or elaborate factors which stimulate the development or migration of HMC precursors remains to be seen. The parasites themselves may release chemical agents which induce differentiation or direct an accumulation of mature cells at sites of predilection. Both mechanisms are compatible with the observation that non-infected rats parabiotically united with those bearing larvae of T. taeniaeformis exhibit intestinal mastocytosis to the same degree as their infected partners (7). Local increases may be accentuated still further by the degranulation of mast cells which first arrive at the infection site. This has been proposed to account for mucosal cell increases induced by chemical degranulation experimentally (11).

The results of immunofluorescence tests show that the marked increase in IgE-positive cells around the parasites was largely due to HMC. Many of these, in addition to beraing surface IgE, contained extragranular cytoplasmic IgE. Plasma cells producing this and other immunoglobulins also accumulated locally, and they may be the source of bound antibodies in and on mast cells. There are several recent reports of IgE within mast cells in atopic and parasitized individuals (12,13,14,21,22,30,31), and

MMC showing specific anti-IgE fluorescence intracellularly also appear in the intestines of rats with T. taeniaeformis (Lindsay and Williams, submitted for publication), observations which suggest that MMC may be antigen-reactive, but there is, as yet, no direct evidence to support this. Specific sensitization to parasite products could place local mast cells in the role of controlling the influx of other inflammatory cells through the antigen-triggered release of vasoactive amines and chemotactic factors (27, 28, 35). On the other hand, they may also be responsible for modulating the intensity of the host response via the secretion of heparin, a potent inactivator of chemotactic agents (8). Such a mechanism might favor the eventual subsidence of inflammatory rejection efforts on the part of the host, and contribute to prolonged parasite survival.

Clearly, further work is required to define the functional characteristics of the HMC in order to substantiate hypotheses on their influence on immune evasion. Their presence in such large numbers in the infected liver provides an opportunity for such studies, either through organ perfusion, or by means of isolating viable mast cells from the lesions for in vitro experimentation. In the meantime, the observation that IgG_{2a}-producing cells, in particular, are plentiful in the immediate vicinity of the parasites is especially challenging,

because antibodies of this type are extremely potent protective factors (20). The nature of the relationship between the limiting plasma membrane of the metacestode surface and these humoral and cellular immune effector systems is the key to cestode parasite persistence in tissues. The results of our study emphasize the value of the rat-T. taeniaeformis model as a tool for research on this phenomenon in the future.

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ARTICLE 3

A SUPERIOR FIXATIVE FOR THE DETECTION OF IMMUNOGLOBULIN E-CONTAINING MAST CELLS IN IMMUNOFLUORESCENCE STUDIES

by

Martha C. Lindsay and Dr. Jeffrey F. Williams

KEY WORDS:

mast cells

immunofluorescence

fixatives

immunoglobulins

To the editor: — In a previous report, Dorsett and Ioachim[1] compared the effects of different fixatives on the detection of intracellular immunoglobulins in human tissues. There is now evidence for the existence of two populations of mast cells that appear to be active in ulcerative proctocolitis and which are analogous to the connective tissue and mucosal mast cell populations observed in the rat intestine.[3] Rat mucosal mast cells have been found recently to contain intracytoplasmic IgE antibody,[4,5] and because IgE-mediated hypersensitivity reactions may be involved in ulcerative proctocolitis,[3] we were interested in finding a fixative suitable for identifying IgE-containing intestinal mucosal mast cells in immunofluorescence assays. Lead subacetate-ethanol-acetic acid,[6] Carnoy's fluid and isotonic-formaldehyde-acetic acid (IFAA) preserve mucopolysaccharides in mucosal mast cells such that copper phthalocyanine dyes can be used to stain these cells selectively.[2] On the other hand, formalin is not a suitable fixative for the detection of mucosal mast cells, although it is effective in preserving the antigenicity of tissue immunoglobulins.[7] Therefore, we compared the effects of the former three fixatives with those of formalin on the intracellular immunoglobulins of mast cells and morphologic integrity

of rat duodenal mucosa.

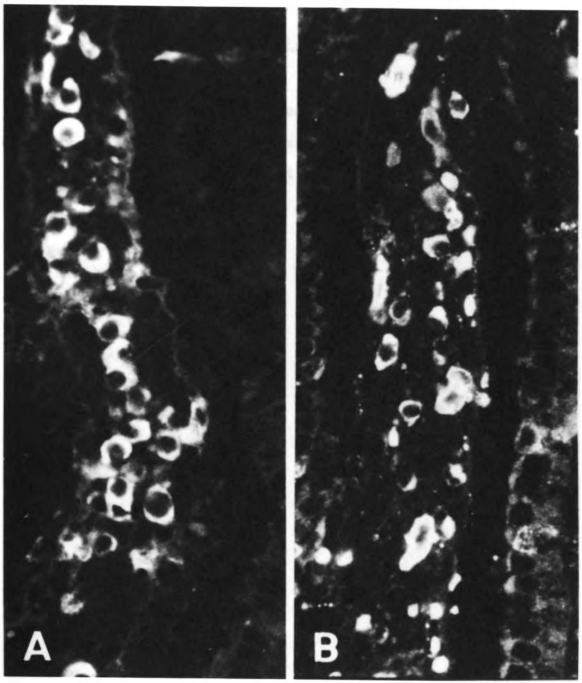
Sections 4 microns thick were deparaffinized, stained for 30 minutes with 0.1% Alcian blue (Alcian blue 8GX, Gurr, London), pH 1.0, and then incubated with sheep anti-rat IgE 1:50 and FITC-conjugated rabbit anti-sheep IgG 1:10 for 30 minutes each. Reagents were purchased from Miles Laboratories, Elkhart, Indiana and were tested in immunoelectrophoresis for specificity before use in immunofluorescence assays. Prior to antiserum incubations, formalin-fixed tissue sections were treated with 0.1% trypsin 1:250 (Difco Laboratories, Detroit, Michigan) as described by Lindsay and Williams.[4]

All four fixatives preserved satisfactorily the antigenicity of IgE; however, tissues fixed with lead subacetate showed superior mucosal morphology compared to those fixed with either Carnoy's fluid or IFAA. Figure 1 illustrates the bright fluorescence of IgE-containing cells that was achieved with both formalin and lead subacetate. Alcian blue-staining mast cells were only detected in tissues fixed with the latter.

This result suggests that lead subacetate fixation would be particularly useful in studies designed to identify and enumerate mucosal mast cells in pathological conditions of the gut, and to establish their relationship to IgE.

FIGURE 1

Fluorescence micrographs of duodenal villi. (A) Lead subacetate-fixed tissue section and (B) formalin-fixed trypsin-treated tissue section showing specific binding with anti-IgE. X55.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