B cell responses in the gut and mesenteric lymph nodes of mice to infection with Eimeria falciformis
by Patricia Varga Nash

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Science
Montana State University
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Abstract:
The B cell response to infection with Eimeria falciformis (Eimer, 1870) Schneider, 1875, was investigated in naive and immune mice after a single inoculation with the parasite. BALB/cByJ mice were divided into three groups. Group 1 remained untreated throughout the study. Group 2 received only one parasite inoculation of approximately.1000 oocysts on day 16, and Group 3 was given approximately 1000 oocysts on day 0, and 1000 oocysts on day 16. Four mice from each group were killed at 6 hr and 24 hr after inoculation on day 16, and then every other day through day 13 post-inoculation (PI). The large intestine, cecum and mesenteric lymph nodes (MLN) were collected and processed for histological examination. An avidin-biotin immunoper-oxidase procedure was used to stain for IgA-, IgM- and IgG-containing lymphocytes.

Primary and secondary IgA, IgM and IgG lymphocyte responses were seen in the large intestine of non-immune (group 2) and immune (group 3) mice, respectively. IgA-containing lymphocytes were the largest population of responding cells in this tissue. Cell numbers peaked 11 days PI in naive, inoculated mice (group 2), and 9 days postchallenge (PC) in immunized, challenged mice (group 3). IgM+ lymphocyte counts were highest 11 days PI in group 2 and 9 days PC in group 3, and IgG+ cell numbers were greatest on days 11-13 PI in non-immune mice (group 2), and on. days 9-11 PC in immune mice (group 3). There were no IgA or IgM responses in the cecum, but IgG+ cells exhibited both primary and anamnestic responses to infection. There were primary but no secondary responses in the MLN, and immunized mice had reduced IgA+ and IgG+ cell numbers in the nodes after challenge. The largest population of responding cells in the lymph nodes contained IgG. Peak IgG and IgA lymphocyte numbers were observed on day 11 PC in both groups of infected mice. IgM+ cell numbers peaked on day 9 PC in both groups. IgA appears to be the most important antibody in the mucosal response to E. falciform is infection, while IgG and IgM probably play a minor role in local immunity. IgG may contribute substantially to the systemic response.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABBREVIATIONS

ABC	avidin-biotin-peroxidase complex
cIg	cytoplasmic immunoglobulin
DAB	diaminobenzidine tetrahydrochloride
HPF	high power field
IEL	intraepithelial lymphocytes
IFA	immunofluorescent antibody
MeOH	methyl alcohol
MLN	mesenteric lymph nodes
PBS	phosphate buffered saline
PC	post-challenge
PI	post-inoculation
SRBC	sheep red blood cell

ABSTRACT

The B cell response to infection with Eimeria falciformis (Eimer, 1870) Schneider, 1875, was investigated in naive and immune mice after a single inoculation with the parasite. BALB/cByJ mice were divided into three groups. Group 1 remained untreated throughout the study. Group 2 received only one parasite inoculation of approximately 1000 oocysts on day 16, and Group 3 was given approximately 1000 oocysts on day 0, and 1000 oocysts on day 16. Four mice from each group were killed at 6 hr and 24 hr after inoculation on day 16, and then every other day through day 13 post-inoculation (PI). The large intestine, cecum and mesenteric lymph nodes (MLN) were collected and processed for histological examination. An avidin-biotin immunoperoxidase procedure was used to stain for IgA-, IgM- and IgG-containing lymphocytes.

Primary and secondary IgA, IgM and IgG lymphocyte responses were seen in the large intestine of non-immune (group 2) and immune (group 3) mice, respectively. IgA-containing lymphocytes were the largest population of responding cells in this tissue. Cell numbers peaked 11 days PI in naive, inoculated mice (group 2), and 9 days post-challenge (PC) in immunized, challenged mice (group 3). IgM+ lymphocyte counts were highest 11 days PI in group 2 and 9 days PC in group 3, and IgG+ cell numbers were greatest on days 11-13 PI in non-immune mice (group 2), and on days 9-11 PC in immune mice (group 3). There were no IgA or IgM responses in the cecum, but IgG+ cells exhibited both primary and anamnestic responses to infection. There were primary but no secondary responses in the MLN, and immunized mice had reduced IgA+ and IgG+ cell numbers in the nodes after challenge. The largest population of responding cells in the lymph nodes contained IgG. Peak IgG and IgA lymphocyte numbers were observed on day 11 PC in both groups of infected mice. IgM+ cell numbers peaked on day 9 PC in both groups. IgA appears to be the most important antibody in the mucosal response to E. falciformis infection, while IgG and IgM probably play a minor role in local immunity. IgG may contribute substantially to the systemic response.

CHAPTER 1

INTRODUCTION

Coccidiosis is a clinically severe intestinal disease in poultry and livestock resulting from infection with oocysts of one of several Sporozoan genera. Virtually every domestic animal will be exposed to these widespread coccidian parasites during its lifetime (24). Members of the genus _Eimeria_ cause morbidity and high mortality in young animals, and are responsible for heavy losses in the cattle and poultry industries. It is estimated that the annual monetary loss in the United States due to this disease is at least $90 million in domestic birds, and $30 million in ruminants (24). Approximately 5-20% of the cattle treated for bovine cocccidiosis die from the infection (23).

Eimeria bovis is a host-specific, intracellular parasite that causes severe intestinal disease, particularly in weaned calves. It also affects adult cattle when the parasite is present in sufficient numbers, as in a feedlot situation. Severely infected animals exhibit characteristic acute symptoms including copious hemorrhagic diarrhea, anorexia, dehydration, weight loss, fever, weakness, ataxia, and increased respiration. The disease may be chronic, with

less debilitating manifestations of the same symptoms, but most deaths occur during the acute stages (88).

The pathological changes associated with bovine coccidiosis primarily affect the large intestine and cecum (88). The epithelium erodes from the mucosal surface, and large hemorrhagic areas are present and may be surrounded by necrotic tissue. Extensive leukocyte infiltration of the mucosa occurs, and the mesenteric lymph nodes are often greatly enlarged.

The sexual stages of _E. bovis_ (microgametes, macrogametes, and oocysts) appear to do the most damage to the intestinal mucosa in cattle (88). It has been observed, however, that _Eimeria falciformis_ causes mucosal destruction in mice during the earlier asexual stages of merogony as well (66). Meronts of _E. bovis_ may also cause some erosion of the epithelium.

Practical, effective immunization against this parasite is not yet available and the mechanism of acquired resistance is poorly understood. Both antibody- and cell-mediated immunity appear to be involved in parasite rejection, but the role of each system is unclear. Investigation of the B cell response in mice to infection with _Eimeria falciformis_ (Eimer, 1870) Schneider, 1875, will provide information about the relative importance of the various antibody classes in protection. Changes in immunoglobulin-containing lymphocyte numbers in the gut and mesenteric lymph nodes may

indicate which parasite stages stimulate the immune response, and knowledge of the B cell mechanisms involved in resistance could lead to the development of an effective vaccine for bovine coccidiosis.

The mouse-E. falciformis host-parasite relationship is a useful model for bovine coccidiosis caused by E. bovis. The course of infection, pathogenesis, and development of immunity have been well documented in the mouse (16, 20, 65-69, 82, 115), and there are many parallels between the two systems. The life cycles of both eimerian species go through stages of merogony and gametogony in epithelial cells of the large intestine and cecum (16, 30). The subsequent release of oocysts from these cells results in desquamation and extensive mucosal damage in both hosts. There are differences between the species in the number of meront stages, and the first asexual generation of E. bovis occurs in endothelial cells of the central lacteals of the small intestine (28) while this stage of E. falciformis is found in glandular epithelial cells of the large intestine. However, the progression of the life cycle stages of sporogony, merogony, gametogony and oocyst formation in the two parasites is identical.

There are also similarities in the immune responses induced in these hosts. Both cattle and mice develop partial immunity to reinfection after one exposure to E. bovis or to E. falciformis, respectively (30, 67, 114). There may

be some parasite development and oocyst shedding after a second inoculation, but the clinical symptoms are inapparent or very mild, and oocyst production is sharply reduced. Resistance is incomplete though, and may be overcome by a large parasite dose.

.The objectives of this study were to characterize the immunoglobulin classes of B cells in the normal BALB/c mouse gut and mesenteric lymph nodes, and to identify the subsets of B lymphocytes responding in mice infected with E. falci-formis. The time course of changes in lymphocyte numbers in both naive and previously infected mice were examined after parasite inoculation. The relationships among parasite stage, B cell number, and morphological alterations in the gut are discussed. Investigation of immunity to an eimerian parasite in a murine system may clarify the role of one of the effector mechanisms responsible for resistance to E. bovis. It may then be possible to artificially augment the natural responses to protect cattle effectively from this disease.

CHAPTER 2

LITERATURE REVIEW

Immunity to eimerian parasites has been studied by a number of research groups, but is still not well understood. Much of the work has been done using chickens and the various chicken coccidia. This particular host is economically very important, and so has been the subject of numerous studies of naturally acquired resistance, and of possible methods of artificially inducing immunity. Birds are immunologically similar to mammals in that they possess both antibody- and cell-mediated systems. Chickens have a thymus for T cell development and a bursa for B cell maturation, and in addition are capable of making all of the classes of mammalian antibodies except IgE (35). Investigations of the immune response to _Eimeria_ in poultry, and the studies in mammalian hosts, have been helpful in elucidating some of the mechanisms involved in acquiring resistance to coccidia.

Species specificity of immunity

The _Eimeria_ are species specific in both their ability to infect an animal, and in the stimulation of a parasite-specific immune response by the host. The most extensive studies of host specificity have been done in chickens (reviewed by McLoughlin, 62), ruminants (reviewed by Levine

and Ivens, 54) and rodents (reviewed by Levine and Ivens, 53). _Eimeria_ _tenella_ from chickens could not be success-fully transmitted to turkeys, ducks, pheasants or quail (86), although there have been conflicting results in pheasants (26). Vetterling (127) also found E. _tenella_ to be infectious in chickens but not in other gallinaceous birds. Similarly, E. _acervulina_ from chickens did not infect quail (86, 125), pheasants (125) or turkeys (117). Mammalian coccidia also exhibit host specificity. _Eimeria_ _bovis_ and E. _cylindrica_ parasitize domestic cattle but will not complete their life cycles in pigs or goats (129), and E. _falciformis_ of mice does not infect rats or dogs (77). This high degree of selectivity between host and parasite is likely the result of immunological as well as physiological factors (102), since it can be circumvented through the use of immunosuppressants (62, 118).

The host immune response is also highly specific for the immunizing species and strain of coccidia. Vaccination with one species of _Eimeria_ does not usually confer protection against other eimerian parasites. Immunization of chickens with E. _tenella_ does not protect them from infection with E. _necatrix_ (45, 107, 126), and vice versa. Resistance to other avian (17) coccidia, and to the _Eimeria_ of rodents (7) and rabbits (5) has also been shown to be species specific. Strain specificity of the host response to variants of the same eimerian species has been demonstrated

with chicken coccidia (46, 55, 78), and may have important
consequences for vaccine development.

Immunity to specific parasite stages

Second generation meronts appear to be the most immuno-
genic stage of E. tenella in chickens (36, 37, 49), and can
provide protection from challenge when inoculated intra-
rectally (39). This has not been demonstrated with other
avian Eimeria, but it appears that sporozoites and gameto-
cytes of chicken coccidia do not induce a strong protective
response in the host (58, 109). Inoculation of first gener-
ation merozoites of E. bovis into the cecum of cattle pro-
tects them from oral challenge with the same parasite (31),
providing further evidence that it is the asexual stages
which are the most immunogenic.

The host response to an eimerian parasite occurs pri-
marily during the invasive sporozoite and merozoite stages.
After excystation, sporozoites may be prevented from enter-
ing intestinal epithelial cells, as suggested by studies on
Eimeria nieschulzi in the rat (72) and E. tenella in the
chicken (4, 51). Sporozoites that do penetrate epithelial
cells in a resistant host may be prevented from developing.
There are reports of abnormal sporozoite morphology follow-
ing cell penetration in immune chickens (38, 51, 126), and
this stage is inhibited in Eimeria-infected rabbits (76,
97). Hammond et al. (29) proposed that the protective

response to E. bovis in cattle acted primarily on the sexual stages, but later found that both meronts and gamonts were affected (30, 31).

There are neutralizing antibodies to sporozoites and merozoites in the serum of chickens infected with E. maxima and E. tenella (34, 59, 104), and the presence of both anti-sporozoite and anti-merozoite IgA in the ceca of immunized chickens has been demonstrated (19). Serum IgG antibodies to sporozoites, merozoites, and oocysts, and anti-sporozoite and anti-merozoite IgA from the cecum were observed in mice immunized with E. falciformis (128). Using an immunofluor-escent antibody (IFA) technique to evaluate serum reactiv-ity, Mesfin and Bellamy (70) reported that E. falciformis sporozoites and merozoites were more immunogenic than gamet-ocytes and oocysts. Thus, there appear to be host responses to every stage of the coccidian life cycle, but it is un-clear whether any of the antibodies produced are protective.

Antibody production

Both agglutinins and precipitins have been detected in the sera of cattle inoculated with E. bovis (2). The high-est titers of agglutinating antibody to merozoite antigens were observed 23-41 days post-inoculation, and some lysis of this parasite stage occurred at low serum dilutions. There were morphological changes in the parasite at higher dilu-tions. The amount of precipitating antibody to extracted

oocyst antigens also peaked 23-41 days after inoculation with E. bovis. Studies with Eimeria of chickens (13, 34, 44, 52, 56, 61, 92, 100) found serum agglutinins to sporozoites and merozoites, and precipitins to merozoite and oocyst antigens. Complement-fixing antibodies and precipitins to oocyst antigens have been detected in E. stiedai-infected rabbit sera (32, 98, 99) and Rose (100) described complement fixation with antibody to second generation merozoites of E. tenella.

There is evidence that serum opsonizing antibodies are also produced in infections with Eimeria. They have been observed in E. tenella-infected birds (104), and sporocysts and sporozoites incubated with serum from immunized chickens are more readily phagocytized by macrophages than untreated parasites (41, 87, 103).

Until recently, little work has been done on the gut mucosal antibody response because of its relative inaccessibility. Early attempts to show the negative effect on parasites of extracts of the cecum, or its contents, were unsuccessful (4, 34, 39). Orlans and Rose (81) did find some parasite-specific IgG and IgA in saline extracts of feces from infected chickens, and Movsesijan et al. (73) also describe IgG in the cecal contents.

Further evidence of the importance of local production of IgA was reported by Davis et al. (19), working with E. tenella in chickens. They found large numbers of

IgA-positive lymphocytes in the cecal tissue of infected chickens, but very few IgM- or IgG-containing cells. IgA was also the predominant antibody detected in cecal and intestinal secretions, and an anamnestic response in these IgA titers was observed after a second parasite inoculation.

Passive transfer of immunity

Complete immunity to oral challenge with *Eimeria* sp. is not transferable with serum. Some resistance to *E. nie-schulzi* and *E. maxima* can be conferred to naive hosts by the repeated injection of serum from infected rats (111) or chickens (101, 105), respectively, but most attempts to passively immunize chickens, mice and cattle have failed (8, 22, 114, 125). Other investigations using bursectomized (111) or cyclophosphamide-treated (47) chickens suggest that there is a role for antibody-mediated protection, since these deficient animals were somewhat more susceptible to reinfection. However, a cellular component of immunity also appears to be of equal or greater importance (69, 111, 112).

All of these antibody transfer studies involve the intravenous or intraperitoneal administration of a circulating antibody, probably of the IgG class, to protect against a parasite of the intestinal mucosa. This method of passive immunization does protect well against an intravenous parasite challenge (57), but is not as effective when the challenge is given orally. Since there are no known

extraintestinal stages of most <u>Eimeria</u> sp., serum transfer might not be expected to provide much protection. There may be some serum leakage into the mucosa due to the inflammatory processes associated with coccidial infections (108), but it is more likely that anti-parasite antibodies of the IgA class are stimulated and produced locally. Orlans and Rose (81) were able to confer some resistance to chickens by injecting IgA purified from the cecal contents of immunized birds, but again the antibody probably had very little contact with the parasites due to the route of administration.

B cells in normal gut and mesenteric lymph nodes

There are no studies which describe the isotypes of immunoglobulin-containing cells in murine or bovine large intestine. However, the distribution of B cell immunoglobulin classes in the normal mouse small intestine has been examined (18, 122, 123). Twenty-two percent of lamina propria lymphocytes contain cytoplasmic immunoglobulin (cIg), and 96% of these B cells are of the IgA heavy chain isotype. Approximately 3.7% of the total population were IgM- and IgG-containing cells.

B lymphocytes with cytoplasmic antibody have been isolated from the mesenteric lymph nodes (MLN) of BALB/c mice, and characterized (25, 122, 123). The total percentage of these cells in normal mouse MLN ranges from 0.18-2.1%,

57-80% of which contain IgA. Between 10-40% are of the IgM isotype, and 2.1-10% are positive for IgG.

IgA lymphocyte trafficking in the mouse gut and associated lymph nodes has been studied using radiolabelled lymphocyte and immunofluorescence techniques. Antigen stimulation is believed to occur in the Peyer's patches (15), where precursors of cIgA are the predominant cell type (121). Stimulated lymphocytes travel via the lymphatic system to the mesenteric lymph nodes where they mature and proliferate. They migrate from the efferent vessels of the nodes into the thoracic duct circulation, and are finally carried back to the intestinal lamina propria by the bloodstream. Here they complete the differentiation process to plasma cells (50). IgA-containing cells from Peyer's patches accumulate in the spleen as well, but that organ does not appear to be an obligatory part of the circulation route (120). Lymphocytes from the MLN (25, 63, 93, 110) and intestinal mucosa (124) have been found to home to the gut lamina propria in cell transfer studies in syngeneic mice.

Mattioli and Tomasi (60) report that there are two populations of intestinal IgA cells in neonatal mice, a large group with a half-life of approximately 4.7 days, and another smaller population with a longer, but undetermined, half-life. The life span of IgA lymphocytes may be different in adult mice or in antigenically stimulated animals. The half-life of lymph node plasma cells in rats given

antigen is approximately one-half the normal half-life (79).

B cell response to antigen

There is disagreement concerning the role of antigen in mucosal lymphocyte responses. Evidence for antigen-independent (83) and antigen-dependent (42, 43, 80, 93) cell migration to the gut has been reported, and it appears that both mechanisms may be operative. There is IgA lymphocyte accumulation in the mucosa as a result of organ-specific homing, and due to antigen-driven memory cell division (94, 95).

The intestinal plasma cell response of mice to oral administration of sheep red blood cell (SRBC) antigens is primarily of the IgA isotype (3), although IgM- and IgG-producing cells are also present. Andre et al. (3) observed peak numbers of IgA and IgM cells on day 11 PI in non-immune mice, and on day 9 PC in immune mice after an SRBC challenge. The anamnestic response was greater than the primary response. IgA-containing plasma cells predominate in the mesenteric lymph nodes after oral presentation of SRBC antigens (6), and IgM-containing lymphocytes were detected following a single SRBC inoculation.

Antigen form affects both the magnitude and the isotype characteristics of the mucosal response. Crude preparations of cholera toxin and toxoid are more effective in eliciting intestinal immunity in rats than are more purified forms of the antigen (91). Similarly, Brucella abortus, a

particulate antigen, is more immunogenic than ovalbumin, which is a soluble substance (9). Intraileal infusion of B. abortus results in the accumulation of IgA and IgM isotype plasma cells in the ilea of sheep, whereas administration of ovalbumin stimulates the production of IgG-containing lymphocytes (9). Adams et al. (1) report an increase in IgA and IgG plasma cells in the intestinal lamina propria of sheep infected with Trichostrongylus colubriformis, a nematode that may present both particulate surface antigens and soluble proteins in the form of enzymes or excretory-secretory products.

CHAPTER 3

MATERIALS AND METHODS

Parasite

Eimeria falciformis, originally obtained from John V. Ernst (Animal Parasitology Institute, USDA, Beltsville, MD), was maintained by periodic passage through BALB/c mice. Following inoculation by gavage with approximately 1000 oocysts, mice were placed in cages with wire mesh floors, and feces were collected on days 7, 8, and 9 PI. Fecal material was processed to remove the fresh oocysts by filtering it through successively smaller wire mesh screens (20, 48, and 115 mesh) into a collecting pan. The filtrate was then centrifuged for 10 min at 1500 rpm, and the sedimented material mixed 1:2 with Sheather's sugar solution, and stirred for 1 hr. The mixture was poured into petri dishes and allowed to stand uncovered for 1/2 hr. The top of the dish was inverted and placed on the solution for 1/2 hr, then removed and the oocysts rinsed off with 2.5% potassium dichromate. The last two steps were repeated.

The oocysts were sporulated in 2.5% potassium dichromate by aerating the solution for 3-5 days at room temperature. Sporulated oocysts were stored in the same potassiuum dichromate solution at 4°C for no more than two months, and

rinsed thoroughly with tap water before inoculation. All experimental mice received approximately 1000 oocysts/0.5 ml tap water, by gavage, for each scheduled inoculation.

Animals

Twelve-week old, age-matched female BALB/cByJ mice (Jackson Laboratories, Bar Harbor, ME) were raised in a specific pathogen- free environment, and housed in the Montana State University animal facility during the study. Control and infected mice were kept in separate rooms, and animals passing oocysts were placed in cages with wire mesh bottoms to minimize the possibility of retroinfection. Mice were checked for coccidia by sucrose fecal flotation prior to use in an experiment.

Experimental protocol

Ninety-six mice were divided into three groups of 32 animals, and given one of the following treatment schedules:

Group 1   Control mice- untreated throughout study

Group 2   Non-immune mice- inoculation with parasite on day 16

Group 3   Immune mice- inoculation with parasite on day 0 , inoculation with parasite challenge on day 16

Four mice from each of these groups were killed at 6 hr after inoculation on day 16, and at 1, 3, 5, 7, 9, 11, and 13 days PI. The experiment was repeated six months later with the non-immune and immune groups only.

Tissue collection and processing

Mice were killed by cervical dislocation and the cecum, large intestine and mesenteric lymph nodes removed. The intestine and cecum were pinned to a piece of cork, cut open longitudinally, and cleaned of fecal material with a cotton swab moistened with saline. The flattened tissues and the lymph nodes were fixed for 3 hr in Bouin's fluid and then dehydrated overnight in 50% ethanol. The large intestine was wrapped into a "Swiss roll" (71), and all tissues were placed in 70% ethanol for several hr. Fixed tissues were processed through a series of alcohol and xylene baths, infiltrated with Surgipath infiltration media, and embedded in Surgipath embedding media (Surgipath Medical Industries, Inc., Northbrook, IL).

The tissues were sectioned at 3 μm, and fixed with gelatin to glass slides coated with Histostik (Accurate Chemical and Scientific Corporation, Westbury, NY). Sections were air-dried, decerated, and stained with hematoxylin and eosin for examination of parasites and morphological changes, or the immunoperoxidase procedure for the identification of B cells. Following dehydration, stained sections were mounted with Permount (Fisher Scientific Company, Fairlawn, NJ).

Immunoperoxidase staining

An avidin-biotin-peroxidase complex (ABC) method (40) was used to stain tissue sections for the presence of IgA, IgG, and IgM-containing lymphocytes. Rabbit anti-mouse IgA primary antiserum was obtained from Miles Scientific (Naperville, IL) and was reconstituted, aliquoted, and frozen at $-20°C$. Heavy chain-specific rabbit anti-mouse IgG and anti-mouse IgM came from Jackson ImmunoResearch Laboratories, Inc. (Avondale, PA). Sodium azide was added to the IgG and IgM antibodies, to a final concentration of 0.1%. These antisera were kept refrigerated. The anti-IgA was thawed and diluted to a final concentration of 1:50, anti-IgG was used at a dilution of 1:300, and anti-IgM at a concentration of 1:800. Blocking serum, biotinylated anti-rabbit IgG, and the ABC reagent were provided by a Vectastain Kit (Vector Laboratories, Burlingame, CA). The biotinylated secondary antibody was diluted to a final concentration of 1:200 for the IgA stain, and 1:500 for the IgG and IgM stains. Diaminobenzidine tetrahydrochloride (DAB) (Sigma Chemical Company, St. Louis, MO) and hydrogen peroxide were used to develop the brown precipitate on positive cells.

The staining procedure was that outlined in the Vectastain Kit with the exception of the use of 0.5 M sodium chloride in the ABC reagent buffer to prevent non-specific staining of mast cells (14, Vector Laboratories, personal communication). Briefly, slides were washed in phosphate

buffered saline (PBS), pH 7.6, for 5 min after deceration
and rehydration. Diluted normal goat serum was applied to
block F_c receptors, followed 20 min later by the application
of the diluted primary antiserum for 30 min. The slides
were rinsed in PBS and sections were covered for 30 min with
the diluted biotinylated secondary antibody. After rinsing
in PBS, the tissue sections were immersed for 30 min in a
bath of 0.3% hydrogen peroxide in MeOH, to remove endogenous
peroxidase activity. Another PBS rinse was followed by the
application of the ABC reagent for 1 hr. The slides were
rinsed for 5 min in PBS, and the color was developed on
positive cells by immersing the slides for 3-4 min in the
DAB-hydrogen peroxide solution. A tap water rinse was
followed by counterstaining with hematoxylin.

Three immunoperoxidase control slides were stained each
day along with the experimental tissues. One section of
normal BALB/c mouse small intestine was treated with diluted
normal rabbit serum in place of the primary antibody. This
controlled for any cross-reactivity of normal rabbit serum
proteins. Positive and negative control slides for each
primary antiserum were also stained (Fig. 1). Normal BALB/c
mouse lung was the positive control for anti-IgA, and an
IgM-secreting mouse hybridoma (generously provided by Dr.
Diane Brawner) that had formed a solid tumor, was used as a
negative control. The same IgM-secreting tumor was the
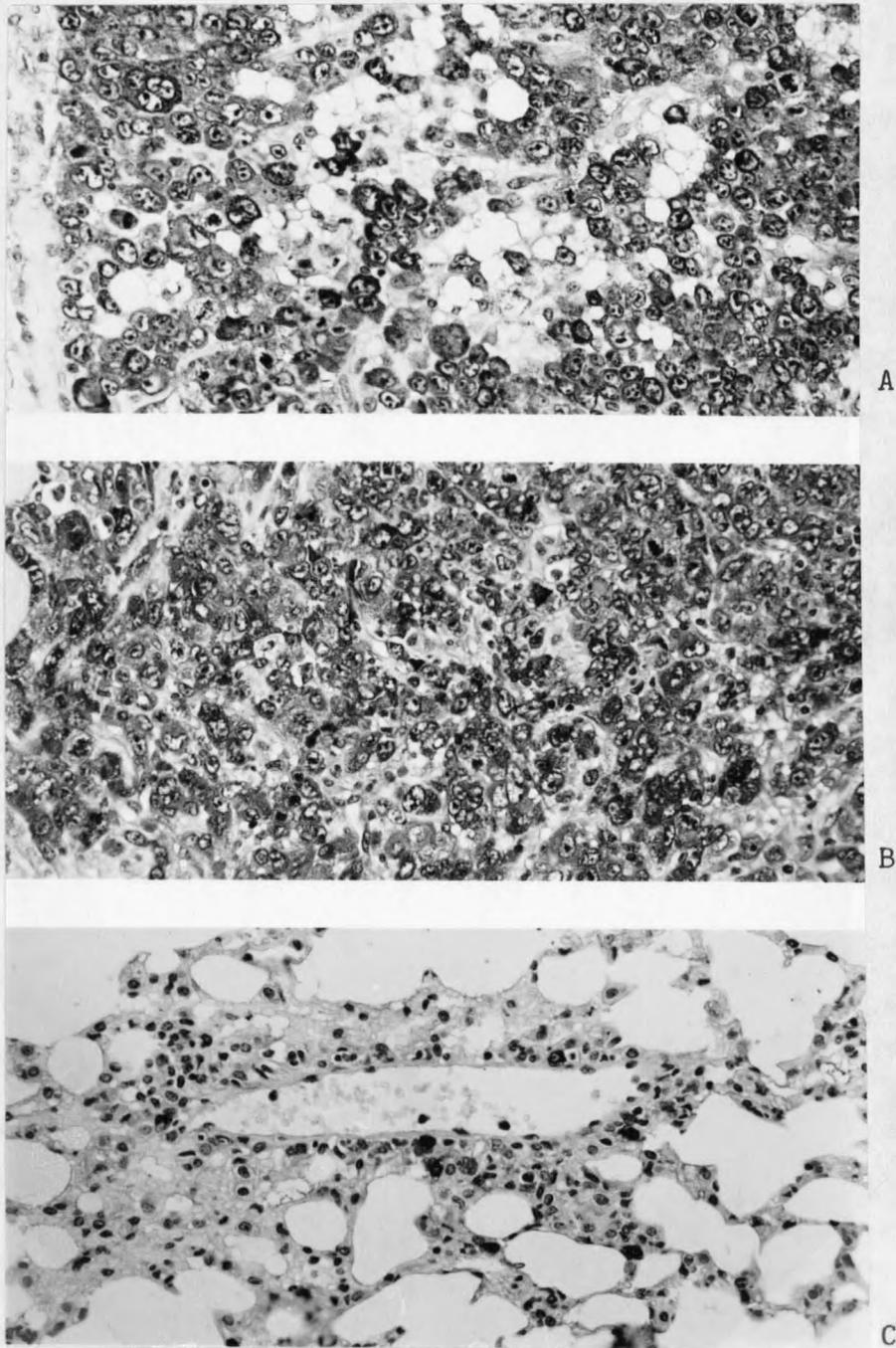positive control for the anti-IgM antiserum, and an IgG-

Fig. 1. Tissues stained with the immunoperoxidase procedure
and used as assay controls. A. IgM-secreting
hybridoma stained with anti-IgM. B. IgG-secreting
myeloma stained with anti-IgG. C. BALB/c mouse
lung stained with anti-IgA. x 250.

secreting solid tumor (MPC 11 OVA^r plasma cell tumor line, American Type Culture Collection, Rockville, MD) was the negative control (see below). The IgG-secreting tumor served as a positive control for the anti-IgG antiserum, and the IgM-secreting tumor was the negative control. The anti-IgA antiserum was also tested against the IgG-secreting tumor to rule out any cross-reactivity with IgG, and less than one percent of B cells in the lung control sections stained positively with either anti-IgG or anti-IgM.

Growth of IgG-secreting solid tumor

The MPC 11 OVA^r plasma cell tumor line was cultured in sterile RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 0.2% (w/v) sodium bicarbonate, and penicillin-streptomycin (5 U/ml), pH 7.4. The cells were subcultured every other day. Two female BALB/c mice were inoculated intraperitoneally with approximately 2000 tumor cells/0.2ml sterile magnesium- and calcium-free Hank's balanced salt solution (GIBCO, Grand Island, NY). A solid tumor, 1-2 cm in diameter, formed at the site of injection after two weeks. The tumor was removed and processed by the same methods used for the other mouse tissues.

Statistical evaluation

Positive cells in the intestine and cecum were counted in ten contiguous microscope fields of longitudinally sectioned gut. Only mucosal sections not immediately adjacent

to lymphoid nodules were selected. Each high power field (HPF) was 0.126 mm^2, and counts of the large intestine were made in the lower 2/3 of that tissue. Ten microscope fields, 0.2 mm^2, were counted for each section of lymph node medulla.

The counts/tissue were totaled separately for each animal, transformed to the square root of the total count, and analyzed by analysis of variance and Duncan's multiple range tests.

CHAPTER 4

RESULTS

Parasite stages

Parasites were concentrated in the cecum and anterior 1/3 of the large intestine, particularly in the early stages before oocyst formation. Six stages of parasite development were observed (Fig. 2). Sporozoites were present at 6 hr and 24 hr PI in non-immune mice, but only at 6 hr PC in immune mice. Due to the sampling schedule, first generation merozoites were not detected; however, second generation merozoites were observed in the non-immune group on day 3 PI. None were seen in immune animals. On day 5 PI, third generation merozoites were present in both groups of infected mice, and fourth generation merozoites were seen on day 7 PI only in non-immune mice.

Microgamonts and macrogamonts were present in the epithelial cells of non-immune mice on days 7, 9, and 11 PI, and in immune mice on days 3, 7, and 9 PC. Oocysts appeared on days 7, 9, and 11 PI in the non-immune group, but were observed only on days 7 and 9 PC in the immune group. There were greater numbers of all parasite stages in the non-immune mice.