Intestinal Changes Associated with Expression of Immunity to Challenge with *Eimeria vermiformis*

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To provide more information on the mechanisms involved in the immune inhibition of eimeria infections, NIH mice were adoptively immunized against infection with *Eimeria vermiformis* by the transfer of mesenteric lymph node cells from primed animals and homologously challenged. Subsequent changes in the architecture and cellular composition of the intestine were compared with those observed in similarly challenged susceptible control mice and correlated with the development of the parasite in the two groups. Actively immunized mice were also examined. In adoptively immunized mice, the development of *E. vermiformis* was inhibited within 3 days of administering the challenge inoculum. Concurrent changes in the intestine included lymphocytic infiltration, crypt hyperplasia, flattening of the crypt epithelium, and a reduction in the number of Paneth cells. Hyperplasia of goblet and pyroninophilic cells in response to challenge, although accelerated and enhanced in adoptively immunized hosts, occurred after the inhibition of the parasites, and mastocytosis was not observed in these animals, findings which suggest that the activities of goblet, pyroninophilic, and mast cells were not instrumental in reducing the numbers of parasites. The intestines of immunized mice contained fewer intraepithelial lymphocytes at the time of inhibition of the parasites than did those of the controls. The protective effects and intestinal changes described above did not differ appreciably from those seen after challenge of mice that had been immunized by infection.

The changes in intestinal morphology and cellular composition induced by primary infections with *Eimeria* spp. have been well documented (for a review, see reference 10), particularly in chickens, a host in which these parasites are of prime economic importance. Most of these investigations have concentrated on the pathologic effects of primary infections, although a comparative study of events in euthymic and athymic rats (which do not develop resistance to reinfection) aimed to explore possible immune aspects of the response (31). There is little information on the effect of challenge of immune animals on the architecture of the intestine. Changes in intestinal permeability (17, 36, 37), absorption (44), and cellular infiltration (33, 44) within 24 h of challenge have been described, as has the lack of subsequent pathologic effects such as villous atrophy and malabsorption (44), normally associated with the acute stage of the primary infection.

The prompt cellular infiltration which occurred in the lamina propria and villous epithelium of primed chickens within hours of challenge with *Eimeria maxima* could be correlated with immunity (33), but this did not apply to mucosal mastocytosis (38) or to circulating mucosal mast cell protease, which were investigated in rats (11), despite their association with the activities of T cells, which are known to be essential for the expression of immunity to eimerian infections (for a review, see reference 30) and to be responsible for its adoptive transfer by suspensions of mesenteric lymph node cells (MLNC) from primed donors (34). In an attempt to obtain further correlations between T-cell function, the expression of immunity, and changes in the intestinal tissues, we have examined responses to challenge

with *Eimeria vermiformis* in the intestines of adoptively immunized mice. This approach was adopted in the belief that freedom from the effects of the initial infection, known to persist for some time (44), would allow better observation of those specific to challenge and identification of the responses that correlated with the inhibition of the parasite. Tissues from actively immunized mice were included for comparison.

MATERIALS AND METHODS

Parasite. The strain of *E. vermiformis*, originally obtained from K. S. Todd, University of Illinois, has been maintained at Houghton Laboratory for many years by passage through NIH or MF1 mice. The methods used, including counting of oocysts for inoculation and for estimating infection by examination of fecal samples, have been described previously (39).

Animals. Female NIH mice, aged 7 to 8 weeks, were obtained from Harlan Olac Ltd., Shaws Farm, Bicester, Oxford, United Kingdom, and kept as previously described (43).

Immunization. Mice were immunized by infecting them with 10^3 sporulated oocysts per os. For adoptive transfer, suspensions of MLNC were used as described before (42). Briefly, MLNC prepared from nodes obtained on day 11 postinoculation (p.i.) with oocysts (IMLNC) were injected intraperitoneally (i.p.) into mice that had been irradiated 3 days previously. Controls were coccidium-free mice, either untreated or injected with MLNC from naive mice.

Histologic examinations. The distal third of the small intestine, measured from the stomach to the ileocecal junction, was taken for fixation. A segment (approximately 1.5 cm long) was removed from the middle, opened, pinned on a card, and fixed in Carnoy's solution. The remaining two (longer) segments were flushed through with formal saline,

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TABLE 1. Protocols for experiments 1 to 4

Expt no.	Immunization	Irradiation (rads)	No. of MLNC inoculated (10^6)	No. of oocysts given for challenge ^a	Sampling for histologic examination	
					Days after challenge	No. of mice per time interval
1	Adoptive	200	1.3	10^3	1-9	2
2	Adoptive	200	2.5	5×10^5	1-4	3
3	Adoptive	600	1.0	5×10^5	1-4	3
4	Active	NA	NA	5×10^5	1-4	3

^a For mice (four to eight per group) in which fecal oocyst production was determined, 10^3 oocysts were given for challenge.

opened longitudinally, wound (Swiss roll fashion, mucosa side outwards) on an orange stick, secured with a pin, and immersed in formal sublimate for 24 to 48 h before transfer to 70% alcohol. After being blocked in paraffin wax, the longer (Swiss roll) pieces of gut were bisected longitudinally, and approximately 3- μ m-long sections were cut from each half for mounting and staining with hematoxylin and eosin (for morphometric analysis and counting of intraepithelial lymphocytes [IEL], Paneth cells, and parasites) or with periodic acid-Schiff stain (for counting of goblet cells). Sections cut from Carnoy-fixed tissues were stained with alcian blue or safranin for counting of mast cells or with methyl green pyronin for counting of pyroninophilic (plasma) cells. The lengths of villi and crypts were measured at a magnification of $\times 100$ with an eyepiece micrometer; care was taken to select only complete villi and crypts that had been longitudinally sectioned. This criterion was also applied to the counting of mast, goblet, and plasma cells, which was done on a villus-crypt basis with at least 20 villus-crypt units examined on each of two sections per mouse. The numbers of intestinal IEL (I-IEL) (identified as described in reference 9) are given relative to the numbers of enterocyte-nuclei, and at least 200 enterocytes were counted on each of two sections per mouse. Paneth cells were identified by their characteristic morphology and fluorescence of the granules after staining with eosin (25); for counting, sections were examined by UV light and the numbers of crypts which contained Paneth cells were recorded. At least 100 crypts were counted in each of two sections per mouse, and the data are presented as the percentage of crypts which contained Paneth cells (19).

For the enumeration of parasites, the total numbers of sporozoites and of first-generation schizonts were counted in each of two sections from each mouse. (A section constituted the sum of one cut from the proximal segment and one from the distal segment, i.e., it represented the length of the distal third of the small intestine minus the medial 1.5-cm segment that was removed for fixation with Carnoy's solution.) Subsequent developmental stages were too numerous to count in unimmunized animals, and they were quantified on the basis of the numbers of infected crypts per section.

Design of experiments. Immunized and control mice were challenged with oocysts, and their intestines were sampled at intervals thereafter; in some mice, the infection was allowed to run its course, and the production of oocysts in the feces after administration of a challenge inoculum of 10^3 oocysts was monitored. Adoptively immunized mice were challenged 1 day after they received MLNC; the interval between primary and challenge inocula in the actively immunized mice was 28 days. Details are given in Table 1.

RESULTS

Developmental stages affected by immunity. In the preliminary experiment (experiment 1), which was intended to determine the stage at which the antiparasite effects of the transferred cells became apparent, the inoculum of oocysts (10^3) was too small to allow enumeration of parasites in the tissues during the first few days of the life cycle (i.e., before replication); therefore, the intensity of the infection was evaluated by comparative scoring. Examination of the feces for oocysts confirmed the protective capacity of the cells since IMLNC-injected mice passed a total of $(0.01 \pm 0.0003) \times 10^6$ oocysts over 1.2 ± 0.5 days, as compared with control mice passing $(101.0 \pm 8.3) \times 10^6$ over 11.5 ± 0.6 days (values are means $\pm$ standard errors of the means for groups of four or five mice; $P < 0.001$). The results of a histological examination (Table 2) show that the IMLNC were effective by approximately day 3 to 5 p.i., indicating that attention should be focused on the early stages of the challenge infection. For a quantitative histologic examination of this early phase of the life cycle, a dose of at least 5×10^5 oocysts is required. The results given in Table 3 show that the transfer of approximately 10^8 IMLNC provided adequate protection (98%) against an inoculum of this size.

Table 4 (experiment 2) shows the effects of transferring 2.5×10^8 IMLNC on the developmental stages present in the intestinal mucosa on days 1 to 4 p.i. with 5×10^5 oocysts. In samples taken on days 1 to 3 p.i., there were fewer parasites in mice given IMLNC (38, 43, and 4%, respectively, of numbers seen in controls), and on day 4 p.i., none was

TABLE 2. Development of *E. vermiformis*^a in adoptively immunized^b and control mice

Time sampled (days p.i.)	Infection in intestinal epithelium ^c			
	Immunized		Control	
	Intensity ^d	Development stages seen	Intensity ^d	Development stages seen
1	—		—	
3	(+)	Schizonts	++	Schizonts
5	—		+++	Schizonts, some gametocytes
7	—		++++	Some schizonts, gametocytes
9	—		++++	Some gametocytes, oocysts

^a All mice were challenged with 10^3 oocysts.

^b For adoptive immunization, 1.3×10^8 IMLNC were inoculated.

^c Two mice were sampled at each time interval; two sections were scanned per mouse.

^d Intensity was graded from few parasites seen [(+)] to many parasites present (++++), or as no parasites detected (—).

TABLE 3. Ability of IMLNC to control challenge infections of differing magnitude in naive recipients

No. of oocysts inoculated at challenge	IMLNC injected (1.3×10^8)	Total fecal oocyst production in recipient mice ^a	
		Number (10^4)/mouse	Patent period (days)
1×10^3	—	$8,500 \pm 67^b$	10.0 ± 0.4^b
1×10^3	+	0.3 ± 0.2^c	1.0 ± 0.5^c
1×10^4	+	0.5 ± 0.4^c	1.0 ± 0.3^c
1×10^5	+	1.4 ± 0.6^c	$2.1 \pm 0.5^{c,d}$
5×10^5	+	110.0 ± 60.0^c	$3.7 \pm 0.7^{c,d}$

^a Values are means for groups of six to eight mice $\pm$ standard errors of the means. Those followed by different superscript letters are significantly different ($P < 0.014$).

detected in the sections examined from these mice. On the other hand, most of the crypts of the control mice contained large numbers of asexual developmental stages. The highly protective effect of the IMLNC was confirmed by the results of the examination of feces of mice challenged with 10^3 oocysts: IMLNC-injected mice passed a total of $(0.3 \pm 0.3) \times 10^6$ oocysts over 0.6 ± 0.6 days, compared with $(37.7 \pm 3.8) \times 10^6$ over 9.1 ± 0.4 days for the controls (values are means $\pm$ standard errors of the means for groups of eight mice; $P < 0.001$; seven of eight IMLNC-injected mice passed no oocysts). Although the numbers of oocysts of all developmental stages, including sporozoites, were reduced in the protected mice, this effect was most apparent at stages subsequent to first-generation schizogony. Thus, the mechanisms responsible were active by 24 h p.i. and had become fully effective between days 3 and 4 p.i. Similar findings were obtained in actively immunized mice (data not shown).

An increase in the amount of irradiation (from 200 to 600 rads) given prior to the inoculation of 10^8 MLNC (experiment 3) did not appreciably affect the results (data not shown), although rather more parasites were found in the tissues and feces. For example, oocyst production was $(18.2 \pm 11.4) \times 10^6$ (97% of the total contributed by two of five mice) in the group given IMLNC and $(93.4 \pm 4.5) \times 10^6$ in the group given normal MLNC.

Histologic changes in the intestine. Changes in the architecture and cellular composition of the intestine accompa-

nied infection with *E. vermiformis*, and these changes included increased length of crypts, decreased length of villi, decreased height of crypt enterocytes, decreased numbers of crypts containing Paneth cells, and cellular infiltration of the intestinal mucosa. Differences were evident between the control and immunized groups, either in the presence of the changes or in their time of onset. Some of these changes are illustrated in the photomicrographs (Fig. 1) of intestines sampled on days 2 and 3 p.i. (experiment 2).

In the preliminary experiment 1, in which samples were examined throughout the infection, the well-documented changes in crypt or villus lengths, usually caused by coccidiosis, were evident in the unprotected mice. Crypt depth increased from day 5 to day 9 p.i., and villous height was decreased on days 5 and 7. In the immunized group, there was no shortening of the villi and crypt depth was not markedly affected, although the latter value was significantly greater than that of the uninfected control on day 3 p.i. (Fig. 2A). In the two subsequent experiments, in which samples were taken daily from days 1 to 4 p.i., the depth of the crypts was maximal on day 2 (experiment 2) or 3 (experiment 3) in the protected groups, but there was no increase until day 4 in the control groups which received no (experiment 2) or normal (experiment 3) MLNC. Villous height over the 4 days sampled did not change significantly within either the protected or unprotected groups, but, on day 2 (experiment 2) or 3 (experiment 3) p.i., it was significantly less in the recipients of IMLNC than in the controls. Data from experiment 2 are presented in Fig. 2B. The findings obtained on challenge of actively immunized mice were similar with regard to crypts, and, in these mice, there was also shortening of the villi on days 1 and 2 p.i. (data not shown).

The percentage of crypts in which the epithelium appeared to be thinner than normal was greater in the recipients of immune cells than in the controls on days 1 (experiment 2), 2 (experiments 2 and 3), and 3 and 4 (experiment 3) p.i.; within the control groups, this number was greater on days 3 and 4 than on days 1 and 2 (data not shown; Fig. 1B).

The number of crypts containing Paneth cells decreased with infection and was accompanied by a change in the appearance of the granules (which became more prominent and appeared to coalesce) and an accumulation of amorphous material in the lumen of the crypts. While these changes were seen in both protected and unprotected mice, they occurred earlier in infection and were greater (over the four time intervals sampled) in the former. Data from experiments 2 and 3 are presented in Fig. 3. In actively immunized mice, the incidence of Paneth cells was similarly significantly decreased on days 3 and 4 p.i. (data not shown).

Cellular infiltration, consisting mainly of lymphocytes but with some granulocytes (in the 200-rad groups), was marked in the recipients of immune cells from day 2 (Fig. 1B and D). In the corresponding controls, it was becoming apparent to some degree on day 3 (experiment 3) or 4 (experiment 2).

Certain types of intestinal cell, namely, goblet cells, IEL, pyroninophilic cells, and mast cells, were quantified in experiment 1, and the results are presented in Fig. 4. In the control mice, the numbers of pyroninophilic and mast cells increased with time p.i. and the numbers of goblet cells were maximal on day 5 p.i.; the numbers of IEL were increased on days 3 to 7 p.i. but decreased on day 9 p.i. Hyperplasia of goblet and pyroninophilic cells was greater in the protected mice, but these mice did not show a mast cell response nor an increase in the numbers of IEL at 3 days p.i. In view of this difference between control and protected mice on day 3 p.i., the effect of infection on the numbers of IEL was also

TABLE 4. Development of *E. vermiformis* in adoptively immunized and control mice (experiment 2)

Time sampled (days p.i.)	IMLNC injected (2.5×10^8 /mouse)	No. of parasites in intestinal epithelium/section ^a		No. of crypts containing subsequent developmental stages/section
		Sporozoites	First-generation schizonts	
1	+	18.8 ± 12.1^b	0.8 ± 0.8	0
	—	51.7 ± 16.1	0.5 ± 0.3	0
2	+	2.5 ± 1.4	42.3 ± 18.5	6.3 ± 5.4
	—	3.5 ± 2.0	107.0 ± 45.3	8.3 ± 7.6
3	+	0.2 ± 0.2	0.7 ± 0.4	29.8 ± 21.8^b
	—	0	1.3 ± 0.7	716.7 ± 158.8
4	+	0	0	0
	—	0	0	903.7 ± 8.0

^a Values are means $\pm$ standard errors of the means for groups of three mice; two sections were scanned per mouse ($n = 3$). Challenge inoculum was 5×10^5 oocysts.

^b Differences between IMLNC-injected and control mice are significant ($P < 0.05$).

FIG. 1. Photomicrographs of sections of intestine of control (A and C) and adoptively immunized (B and D) mice sampled on day 2 (A and B) or 3 (C and D) p.i. with 5×10^5 oocysts of *E. vermiformis*. Immunized mice received 2.5×10^6 IMLNC i.p. (experiment 2). Arrows indicate parasites within enterocytes. In immunized mice (B), note cellular infiltration, crypt hypertrophy, and shallow epithelium on day 2 p.i.

examined in experiments 2 and 3 in which the tissues were sampled daily on days 1 to 4 inclusive. The data, shown in Fig. 5, confirm an early increase in the numbers of IEL in susceptible infected mice. There were no significant changes in the numbers of IEL in the protected mice over this time period.

Actively immunized mice (experiment 4) had higher values for mucosal mast and pyroninophilic cells than the susceptible controls, both before and on days 1 to 4 after challenge. The numbers of mast cells differed significantly at all times of sampling but did not change significantly in

response to challenge, whereas the numbers of pyroninophilic cells were increased after challenge of the immunized group (Fig. 6). The numbers of goblet cells did not differ significantly between the groups, and the numbers of IEL only did so on day 0 (and were lower in the immunized group) (data not shown).

DISCUSSION

The ability of MLNC, obtained from mice convalescing from infection with *E. vermiformis*, to transfer immunity to

FIG. 2. Lengths of villi and crypts in uninfected mice (□) and after challenge with oocysts of *E. vermiformis* in adoptively immunized (■) and control (▨) mice. (A) Oocysts (10^3) were inoculated and tissues were sampled (two mice per time point) on or between days 1 to 9 p.i.; immunized mice received 1.3×10^8 IMLNC i.p. (experiment 1). (B) Oocysts (5×10^5) were inoculated, tissues (three mice) were sampled daily, on days 1 to 4 inclusive, and immunized mice were given 2.5×10^8 IMLNC i.p. (experiment 2). The significance of differences within (compared to day 1) or between groups is indicated by asterisks placed within or between columns, respectively. *P* value symbols: *, <0.05; **, <0.01; ***, <0.001.

challenge with this parasite was confirmed. As before, the recipients of IMLNC passed fewer oocysts in their feces than did untreated controls or the recipients of cells obtained from naive mice (42), and irradiation of the recipients with 600 rads did not abrogate this protective effect (43), the mice being able to withstand challenge with a very large number of oocysts.

An examination of the intestines of both the adoptively and actively immunized mice revealed that the parasite was affected early in its development since numbers of sporozoites and of first-generation schizonts were reduced and no, or very few, parasites were detected on day 4 p.i. These findings resemble those seen in other hosts after challenge of actively immunized animals with the homologous *Eimeria* spp. (reviewed in reference 30) and indicate that it is likely

FIG. 4. Numbers of goblet cells, IEL, pyroninophilic (plasma) cells, and mast cells in the intestines of uninfected mice (□) and after challenge with 10^3 oocysts of *E. vermiformis* in adoptively immunized (■) and control (▨) mice. Immunized mice received 1.3×10^8 IMLNC i.p. Tissues were sampled (two mice per time point) on and between days 1 to 9 p.i. (experiment 1). The significance of differences within (compared to day 1) or between groups is indicated by asterisks placed within or between columns, respectively. *P* value symbols: *, <0.05; **, <0.01; ***, <0.001.

that any changes associated with the expression of immunity would be manifest within 4 days of challenge. The similarity of the degree and timing of protection in the two groups of mice, together with the paucity of the effect of increased irradiation, suggests that the immunity transferred by

FIG. 3. The incidence of crypts containing Paneth cells in adoptively immunized (■) and control (▨) mice after challenge with 5×10^5 oocysts of *E. vermiformis*. (A) Immunized mice received 2.5×10^8 IMLNC (experiment 2). (B) Immunized mice received 1.0×10^8 IMLNC, and control mice received the same number of normal MLNC (experiment 3). Three mice were sampled per time point; the significance of differences within (compared to day 1) or between groups is indicated by asterisks placed within or between columns, respectively. *P* value symbols: *, <0.05; **, <0.01; ***, <0.001.

FIG. 5. Numbers of IEL in adoptively immunized (■) and control (▨) mice on days 1 to 4 after challenge with 10^5 oocysts of *E. vermiformis*. (A) Immunized mice received 2.5×10^8 IMLNC i.p. (experiment 2). (B) Immunized mice received 1.0×10^8 IMLNC i.p., and control mice received the same number of normal MLNC (experiment 3). Three mice were sampled per time point; the significance of differences within (compared to day 1) or between groups is indicated by an asterisk (*P* < 0.05) placed within or between columns, respectively.

FIG. 6. Numbers of pyroninophilic (plasma) and mast cells in intestines of actively immunized (■) and control (□) mice on days 1 to 4 after challenge with 5×10^5 oocysts of *E. vermiformis*. In immunized mice, day 0 equals day 28 after the priming inoculum of 10^3 oocysts. The significance of differences within (compared to day 1) or between groups is indicated by asterisks placed within or between columns, respectively. *P* value symbols: *, <0.05; **, <0.01; ***, <0.001.

IMLNC, like that induced by infection (43), did not require the cooperation of other cells.

Although, because of the exigencies of the experimentation, the numbers of animals examined at each time point were small, there were significant differences between the histopathologic findings of the intestines from immunized mice and those of control mice. Those events that could be correlated with the immune state and that occurred at the time that the parasites were being affected were as follows: an early increase in the depths of the crypts, a rapid decrease in the numbers of crypts containing Paneth cells, and a marked lymphocytic infiltration of the mucosa. Most of the histopathologic changes noted in the immunized mice were also seen in the controls but at a later time p.i. Comparisons of actively and adoptively immunized mice confirmed the usefulness of the latter as a model which had the advantage of the absence of residual effects from the priming infection (see, for example, data for mucosal mast cells [Fig. 6B]).

Crypt hypertrophy, accompanied by villous atrophy, is a well-known histopathologic consequence of enteric disease, including coccidiosis (reviewed in reference 10). In eimerian infections, it is usually seen during the acute phase of primary infections, as in the unprotected mice in experiment 1 (Fig. 1). This lesion has been shown to be thymus dependent (7) for several enteric conditions, including coccidiosis (31), and an increase in the mitotic activity of the epithelial cells of the crypts of Lieberkühn, resulting from the local release of cytokines by sensitized T cells, is now taken to be an indicator of cell-mediated immune responses in the intestine (8). In the adoptively immunized mice examined in this study, crypt hyperplasia was observed as early as 2 days postchallenge (Fig. 2B), when the numbers of parasites were very low (Table 3), and it had practically resolved by day 4, at which time no, or very few, parasites were seen in the tissues. In vitro, crypt hyperplasia has been shown to occur within 18 h of activation of local T cells (6). The thinning of the crypt epithelium, which accompanied crypt hyperplasia in the immunized mice, may be a reflection of the changes in the kinetics of the crypt cells. Early villous atrophy was seen in the actively, but not in the adoptively, immunized mice; this response is not invariably seen in other conditions of the intestinal tract (26), including parasitic infections (15, 21). It was, however, present in the unprotected mice at the height

of infection (Fig. 2A), in accordance with a previous study (3).

A reduction in the numbers of crypts containing Paneth cells, presumably resulting from degranulation, has been noted in many inflammatory conditions of the small intestine (19), but there is little reference to this in connection with enteritis caused by parasites or by other infectious agents, although Blagburn and Todd (3) remarked on the prominence of the eosinophilic granules of Paneth cells in *E. vermiformis*-infected mice on day 4 p.i. Of a largely unknown function, Paneth cells are considered to be involved in defense because they appear to be phagocytic and contain lysozyme and immunoglobulins A and G (45). More recently, the presence of mRNAs for α 1-antitrypsin (16), for a defensin-like peptide (28), and for tumor necrosis factor (12) has led to the suggestion that they may constitute a significant exocrine source of immunoregulatory peptides (12). Whether the activities of Paneth cells played any part in the inhibition of replication of *E. vermiformis* observed in our experiments is not known.

Changes in the numbers and types of cells in the intestinal mucosa of the unprotected animals resembled those previously reported in rodent coccidiosis (3, 22, 31). The immunized animals differed in that (i) the lymphocytic infiltration and increased numbers of pyroninophilic and goblet cells occurred earlier, and (ii) there was neither a mast cell response nor an early increase in the numbers of IEL.

The accelerated hyperplasia of goblet cells resembled that found in rats adoptively immunized against *Nippostrongylus brasiliensis* (24), but in this system, there was also an earlier mast cell response (2). It would seem that the activities of goblet cells and plasma cells were not involved in the anti-eimeria effects described here because, despite being accelerated, hyperplasia of these cells postdated the reduction in numbers of parasites. The relative lack of importance of antibodies in controlling this parasite (41) and lack of correlation of mastocytosis with resistance have already been noted (11, 38, 43).

Increases in the numbers of IEL have been reported to be associated with mucosal cell-mediated immune responses (26), but this response was not evident at the time of *E. vermiformis* inhibition (experiments 2 and 3; Fig. 5). Instead, it was noted soon after challenge of the susceptible mice (Fig. 4C) that the onset of immunity to this primary infection was accompanied by a decrease in numbers (Fig. 4C, day 9 p.i.). A similar reduction in the numbers of I-IEL has been reported in rats recovering from infection with *Eimeria nieschulzi* (31). In chickens, the NK activity of I-IEL has been reported to decrease in the early stages of a primary infection with *Eimeria* spp. and to increase during the recovery phase and shortly after the administration of a challenge inoculum, but no correlation between NK activity and the numbers of IEL recovered from the intestine was reported (20). I-IEL are involved in the early stages of the developmental cycle of some eimeria (1, 18, 23), and it is known that sporozoites of *Eimeria tenella* (35) and *E. maxima* (29) are arrested within these cells in immunized hosts. These factors may complicate the normal traffic of I-IEL and thus explain the findings with respect to numbers and their association with immunity to the parasite.

The histopathologic changes associated with the inhibition of parasite development were probably induced by the rapid accumulation of the transferred IMLNC in the proximity of the invading sporozoites. Such localization has been demonstrated with radioactively labelled lymphocytes in chickens and rats challenged with *E. maxima* and *E. nieschulzi*,

respectively (32). Presumably, the release of cytokines resulting from contact of these sensitized cells with parasite antigen (5, 26) led to the changes noted in the crypt epithelium and may also have been responsible for the malabsorption (44) that occurred 6 to 8 h after homologous challenge of chickens highly immunized with *Eimeria acervulina*. The identity of these mediators is not presently known, but several, including gamma interferon, are thought to influence epithelial structure and function (4, 27). Gamma interferon is known to have anti-eimeria effects in vivo (41), and the results of work carried out in vitro (13, 14, 40) suggest that its action is mediated via the host cell. Whether the changes in the structure of the epithelium noted here themselves had an adverse effect on the establishment of the parasite or merely accompanied a more direct effect of the mediators is not known.

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