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Passive Protection of Chickens against *Eimeria tenella* Infection by Monoclonal Antibody

MARK S. J. CRANE,* P. KEITH MURRAY,† MARK J. GNOZZIO, AND THOMAS T. MACDONALD‡

Department of Biochemical Parasitology, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

Received 8 September 1987/Accepted 11 January 1988

Monoclonal antibodies reactive with the surface of *Eimeria tenella* sporozoites were produced in mice. This paper concerns one of these monoclonal antibodies, designated 1073.10, which agglutinated sporozoites in vitro and lysed the parasite in the presence of complement. This treatment neutralized sporozoite infections when the treated parasites were injected into the ceca of normal chickens. Passive transfer of ammonium sulfate-precipitated 1073.10 ascites fluid into 2- to 3-day-old or 3-week-old chickens conferred protection against challenge infection with *E. tenella*. These studies show that serum antibody may play a role in immunity to coccidiosis and that the sporozoite surface epitope recognized by 1073.10 is a possible vaccine candidate antigen.

Coccidiosis of the domestic chicken, a disease involving severe enteritis with associated mortality and morbidity, is caused by several species of protozoan parasites of the genus *Eimeria*. To initiate infection, sporozoites are released, in the gut, from ingested sporulated oocysts and invade epithelial cells lining the intestine. The entire life cycle occurs in the mucosa and lamina propria, and although light infections are often subclinical, heavy infection can result in severe weight loss, growth depression, and even death (13). It is well established that chickens which survive an initial infection are resistant to reinfection (26). Such immunity is species specific (21) and possibly directed against the sporozoite stage (22). It has been shown recently that extracts from the sporozoites of *Eimeria tenella* containing no viable parasites can be used to successfully vaccinate against this infection (17). The effector mechanism(s) of protective immunity induced by live infection or by extract vaccine remains unclear, however. Although antibodies from various sources demonstrate a variety of antiparasitic activities, including sporozoite immobilization (9), sporozoite and merozoite agglutination and lysis (2, 14), neutralization (1, 2, 9, 14, 15, 22), and surface changes (31), the role of serum antibody in protective immunity is unclear (11, 18, 23–25, 28). Recently, emphasis has been directed towards cell-mediated effectors (11, 24, 25) or secretory antibody (immunoglobulin A [IgA]), which has been shown to neutralize sporozoites both in vitro and in vivo (5, 6). In an attempt to identify specific antigens which may be involved in the induction of host immunity, monoclonal antibodies reactive with the surface of *E. tenella* sporozoites (the major species causing cecal coccidiosis) were generated. This paper describes some important features, including passive protection, of one of these monoclonal antibodies, 1073.10.

* Corresponding author.

† Present address: Institute for Animal Disease Research, Pirbright Laboratory, Pirbright, Woking, Surrey GU24 ONF, United Kingdom.

‡ Present address: Department of Pediatric Gastroenterology, St. Bartholomew's Centre for Research, London EC1A7BE, United Kingdom.

MATERIALS AND METHODS

Source of parasites. *E. tenella* (Merck strain LS18) oocysts were produced and maintained by passage in chickens. Pure oocysts and sporozoites were obtained as described previously (30).

Monoclonal anti-*E. tenella* antibody production. Monoclonal antibodies were produced as described previously (2). Briefly, BALB/c mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) were immunized with *E. tenella* sporozoites, and splenic lymphocytes were isolated and fused with the murine myeloma SP-2/0 (Human Genetic Cell Repository, Camden, N.J.). Hypoxanthine-aminopterin-thymidine-resistant cells were obtained by standard procedures, and culture supernatants were assayed by solid-phase immunoradioassay, using sonicated *E. tenella* sporozoites as the target antigen. Antibody-producing cells were cloned by using a soft agar technique and expanded. For ascites production, BALB/c mice were injected intraperitoneally with 0.5 ml of pristane (2,6,20,14-tetramethylpentadecane; Aldrich Chemical Co., Inc., Milwaukee, Wis.) 4 days prior to injection with 2×10^6 hybridoma cells. Ascites fluid from pristane-treated mice bearing the 1073.10 hybridoma (of the IgG3 isotype) was precipitated with 50% (wt/vol) ammonium sulfate, washed, suspended in phosphate-buffered saline (PBS; pH 7.2), and extensively dialyzed against PBS. Protein concentration was determined by the Lowry method (16). Ascites fluid from mice bearing a hybridoma with specificity for a viral surface antigen was similarly treated and used as a control in some experiments. This control monoclonal antibody was of the IgG2b isotype. Monoclonal antibodies were also purified by protein A-Sepharose (Pharmacia Fine Chemicals, Piscataway, N.J.) immunoaffinity (7).

FACS analysis of sporozoites. *E. tenella* sporozoites (5×10^6) were incubated for 30 min at 4°C with either protein-A-purified 1073.10 or control monoclonal antibody (30 μg/ml). The parasites were washed in PBS containing 1% (wt/vol) bovine serum albumin (BSA) and 0.1% (wt/vol) sodium azide (PBS-BSA) and suspended in 0.1 ml of a 1:40 dilution of fluorescein-conjugated $F(ab')_2$ goat anti-mouse IgG (Organon Teknika, Malvern, Pa.). After incubation for a further 30 min at 4°C, the sporozoites were washed, suspended in 0.5 ml of PBS-BSA, and analyzed for surface fluorescence

on a fluorescence-activated cell sorter (FACS) (FACS IV; Becton-Dickinson, FACS Systems, Mountain View, Calif.).

Analysis of 20,000 sporozoites was carried out with an argon ion laser (Spectra-Physics model 164-05) operated at 300 mW and an excitation wavelength of 488 nm. Fluorescence was measured between 510 and 550 nm. Data was stored and subsequently analyzed by using a PDP11/34 computer (Digital Equipment Corporation, Marlboro, Mass.).

Passive transfer of monoclonal antibodies. Two-day-old chickens (Hubbard/Hubbard broilers; Avian Services, Frenchtown, N.J.) received 1, 5, or 10 mg of protein of ammonium sulfate-precipitated ascites fluid in two intraperitoneal injections of 0.5, 2.5, or 5 mg each, given on the day prior to infection and 2 h before infection. The chickens were infected with 5×10^3 *E. tenella* (Merck strain LS18) sporulated oocysts per os by feeding tube. After 6 to 7 days, the chickens were killed by CO_2 asphyxiation and the ceca were examined for lesions. Lesions were scored on a scale of 0 to 4 as described by Johnson and Reid (10). Lesion scores of 1 or 2 represent slight gross pathology, and scores of 3 or 4 represent very severe damage. As chickens have two ceca, the lesion scores from both were measured and averaged to give one score for each individual.

A second batch of 1073.10 antibody was prepared by using the same technique and was used for passive transfer experiments in 3-week-old chickens. The protocol was the same except that the control antibody was mouse gamma globulin (Organon Teknika) and the chickens were challenged with 10^4 sporulated oocysts. Statistical analysis was performed using the Student *t* test.

Sporozoite agglutination and lysis. Replicate samples of a sporozoite suspension were incubated for 1 h at 41°C with various dilutions of protein-A-purified 1073.10. The incubation was performed in Linbro round-bottom-well microtiter plates, and the mixtures contained 2×10^5 sporozoites in 100 μl of antibody diluted in Eagle minimal essential medium (MEM). At the end of the incubation, 10 μl of glutaraldehyde was added to a final concentration of 0.5% (vol/vol), which prevented agglutinates from disaggregating during pipetting. Quantitation of agglutination was achieved by counting the samples on a Coulter Counter (model ZBI; Coulter Electronics, Inc., Hialeah, Fla.). A reduction in counts relative to MEM controls indicated agglutination such that the count is inversely proportional to the degree of agglutination. Results are expressed as an agglutination index which is the ratio of the Coulter count of the test sample to the control (MEM) count. Similarly, lysis may be monitored for samples in which normal chicken serum (NCS) has been added, as a source of complement, at a dilution at which complement-mediated lysis occurs via the classical (antibody-dependent) rather than the alternative pathway of complement activation (2).

In vivo sporozoite neutralization. In vivo sporozoite neutralization was assayed as described previously (1). Briefly, 2- to 3-week-old Hubbard White Leghorns (Avian Services), were wing banded and placed in clean cages, and feed was removed on the day prior to inoculation. On the day of inoculation, purified *E. tenella* (Merck strain LS18) sporozoites were suspended in MEM containing NCS (as a source of complement) either alone or with monoclonal antibody 1073.10 (protein-A-purified stock containing 342 μg of protein per ml) at a dilution of 1:8. Sporozoites were incubated at 41°C for 1 h, and 0.5-ml aliquots of the suspensions containing 20,000 sporozoites were inoculated directly into the ceca (0.25 ml per cecum), using a syringe fitted with a straight intubation needle (18 g × 2 in. [1 in. = 2.54 cm] with a spherical ball tip). There were five chickens per group, including a group of uninfected controls. Feed was restored immediately after inoculation. Chickens were killed on day 7 postinoculation, and the severity of disease was assessed by cecal lesion scores and oocyst counts as described previously (1).

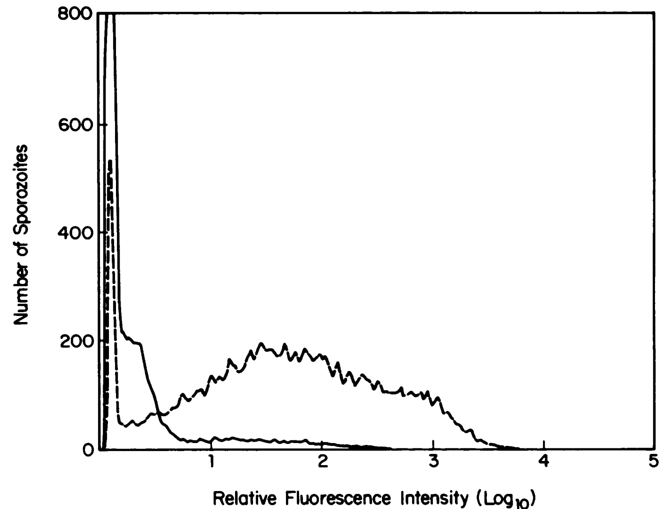

FIG. 1. Analysis of sporozoite surface fluorescence by FACS. The solid line represents sporozoites pretreated with control monoclonal antibody, and the dashed line is sporozoites pretreated with 1073.10.

RESULTS

The surface reactivity of 1073.10 was established by immunofluorescence, agglutination, and complement fixation assays.

FACS analysis of sporozoite surface fluorescence with 1073.10. Analysis of sporozoite surface fluorescence, using a FACS, demonstrated that 1073.10 bound to the sporozoite surface (Fig. 1). The majority of sporozoites (88.1%) incubated with 1073.10 followed by the fluorescein-conjugated antibody showed positive fluorescence with a peak channel (relative fluorescence intensity) of 85. Only 18.9% of the sporozoites incubated with control monoclonal antibody followed by the fluorescein-conjugated antibody showed positive fluorescence with a peak channel of 12. Sporozoites incubated with the fluorescein-conjugated antiserum only did not fluoresce (results not shown).

In vitro agglutination and lysis of *E. tenella* sporozoites by 1073.10. 1073.10 also agglutinated *E. tenella* sporozoites and, in the presence of chicken complement, lysed the sporozoites (Fig. 2). At the titer used, chicken serum alone (as a source of complement) had no effect on the parasites. Agglutination is shown by a decrease in the Coulter count relative to MEM controls, and lysis is demonstrated by an increase in Coulter counts relative to antibody-treated samples containing no complement. Lysed parasites with perturbed membranes do not agglutinate as well as intact parasites. Agglutination and lysis were also confirmed by visual examination of the samples.

In vivo sporozoite neutralization. Sporozoite neutralization activity of 1073.10 in the presence of chicken serum (as a source of complement) is shown in Fig. 3. NCS at the dilutions indicated and 1073.10, by themselves, had no effect on sporozoites as shown by the cecal lesion scores (means

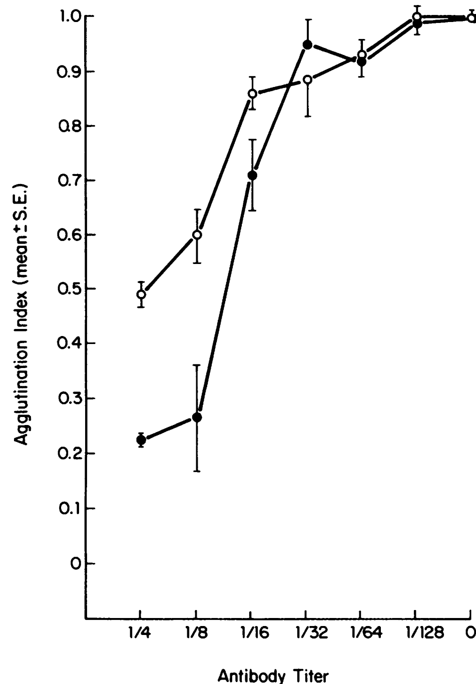

FIG. 2. Agglutination and lysis of *E. tenella* sporozoites by 1073.10. Sporozoites were incubated with protein-A-purified 1073.10 (stock contained 342 μg of protein per ml) at the dilutions indicated with (○) or without (●) NCS (at a dilution of 1/32 as a source of complement). Each point represents the mean and standard error (S.E.) of two to four determinations.

range from 2.4 to 3.0) and cecal oocyst counts (means range from 1.48×10^7 to 1.85×10^7 per chicken). However, incubation of sporozoites with 1073.10 in the presence of chicken complement reduced both the cecal lesion scores and oocyst counts. Using 1:8 dilutions of both NCS and 1073.10 reduced the mean lesion score to 0.5 and oocyst counts to a mean of 2.35×10^6 per chicken. As the NCS was titrated out, the severity of disease increased.

Passive protection of chickens with 1073.10. Two-day-old chickens received 1, 5, or 10 mg of protein of ammonium sulfate-precipitated ascites fluid in two intraperitoneal injections of 0.5, 2.5, or 5 mg each given the day before infection and 2 h before infection. After 6 days, the lesion scores were assessed. Table 1 shows the results of four experiments in which chickens received 1073.10 or a preparation of a control monoclonal antibody and were challenged with 5×10^3 *E. tenella* sporulated oocysts. Chickens receiving 5 or 10 mg of 1073.10 ascites fluid had significantly ($P < 0.05$) reduced lesion scores compared with untreated chickens. Neither the lower dose of 1 mg of 1073.10 nor any doses of control ascites fluid protected against lesions; there was no significant difference between these groups and untreated controls. In similar experiments with a second batch of 1073.10 and 3-week-old chickens, protection against disease was also observed (Fig. 4), demonstrating reproducibility between different antibody batches and between different ages of chickens.

The frequency distribution of lesion scores in 3-week-old chickens treated with 10 mg of 1073.10 or mouse gamma globulin (Fig. 5) also demonstrates the protection and provides additional information. In the group of chickens treated with 1073.10, 69% of the lesion scores were less than 2 (slight pathology). In contrast, in the group of chickens

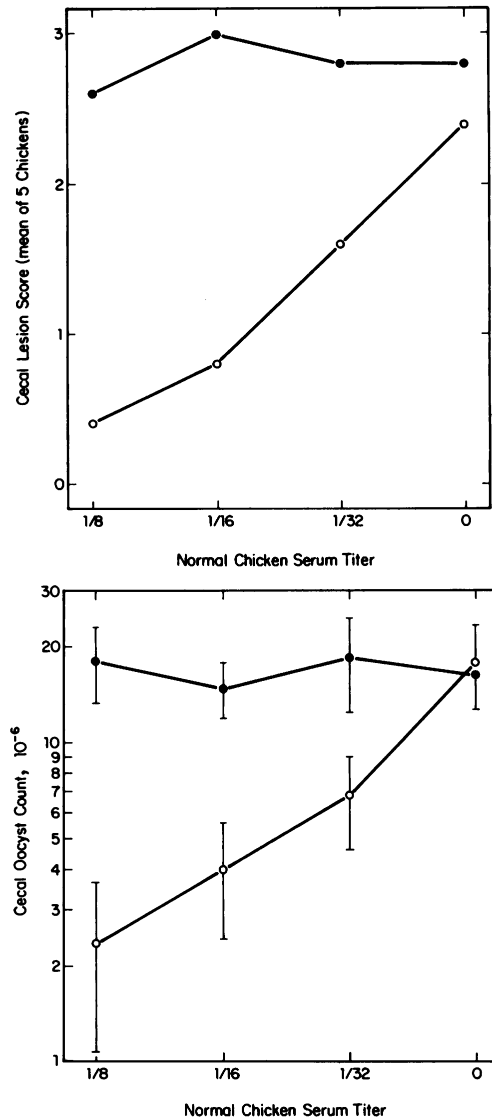

FIG. 3. Sporozoite neutralization by 1073.10 and NCS. Sporozoites were preincubated with protein-A-purified 1073.10 (at a 1/8 dilution of the stock solution containing 342 μg of protein per ml) with NCS that had been heat inactivated (●) or not heat inactivated (○). (A) Cecal lesion scores; (B) cecal oocyst counts. Each point represents the mean and standard error of five determinations.

treated with mouse gamma globulin, 62.5% of the lesion scores were greater than 2 (severe pathology).

DISCUSSION

Although other monoclonal antibodies have been raised against various species of chicken coccidia (3, 4), passive transfer of immunity with a monoclonal antibody has not been reported previously. The most important finding reported here is that a mouse monoclonal antibody (1073.10) which reacts with a surface determinant on the sporozoite of *E. tenella* can passively protect chickens against disease. The amount of monoclonal antibody ascites fluid necessary for protection was relatively large. The reason for this may be that since *E. tenella* sporozoites invade the cecal wall and complete their entire life cycle there and it is assumed that the effects of the antibody must occur locally at this site (27),

TABLE 1. Effect of passive immunization of 2-day-old chickens with monoclonal antibodies on cecal lesions caused by *E. tenella* infection

Expt no.	Dose (mg of protein)	Lesion score[a]		
		1073.10 ascites fluid	Control ascites fluid	Untreated controls
1	5	1.38 ± 0.24 (4)[b]	2.50 ± 0.50 (3)[c]	3.13 ± 0.13 (4)
2	1	2.63 ± 0.75 (4)[c]	ND[d]	3.50 ± 0.22 (5)
	5	1.60 ± 0.43 (5)[b]	2.38 ± 0.69 (4)[c]	
3	5	1.50 ± 0.30 (8)[b]	2.19 ± 0.28 (8)[c]	2.50 ± 0.19 (8)
4	1	2.63 ± 0.43 (4)[c]	2.75 ± 0.75 (2)[c]	3.36 ± 0.14 (7)
	10	0.90 ± 0.24 (5)[b]	3.17 ± 0.17 (3)[c]	

[a] Mean ± standard error (number of chickens per group). For scoring system, see Materials and Methods and reference 10.
[b] Significantly different ($P < 0.05$) from untreated controls.
[c] Not significantly different ($P > 0.05$) from untreated controls.
[d] ND, Not done.

it may be necessary to inject 5 to 10 mg of ascites fluid antibody intraperitoneally into the chickens to achieve biologically active amounts in the cecal wall.

We have, as yet, no data on the mechanism or site of the immunity induced by 1073.10. However we have shown that 1073.10, in the presence of complement, lyses the sporozoite in vitro and neutralizes sporozoite infection in vivo. Thus, it is possible that in chickens with passively transferred 1073.10, some of the antibody and complement leaked from the circulation into the cecal lumen or the lamina propria (12, 27) and sporozoites were lysed, resulting in reduced infection and disease.

The finding that passive transfer of mouse antibody protects against *E. tenella* infection is also significant in view of the failure by earlier workers to transfer immunity with immune serum from multiply infected, solidly immune chickens (18). There is, however, some species variation because it is possible to transfer resistance against *Eimeria maxima*, an intestinal species, with selected batches of sera from immune birds (20). Nevertheless, the actual mechanism of immunity against *Eimeria* spp. in the chicken is not well characterized (23). It is possible to protect bursectomized chickens by giving them multiple low-level infections (24), indicating that cell-mediated immunity is involved. However, it is our view, because of the protection with antibody found in this study and because sera can protect against *E. maxima* infection (20), that the failure to passively transfer resistance to *E. tenella* with serum (18) should not be taken as prima facie evidence that serum antibody is not the primary means of resistance.

In view of these results, it is clear that 1073.10 recognizes an epitope of importance on the parasite and could prove a useful tool for the isolation of a vaccine candidate antigen. By analogy with other infectious diseases, it is also possible that an anti-1073.10 idiotypic antibody could mimic the

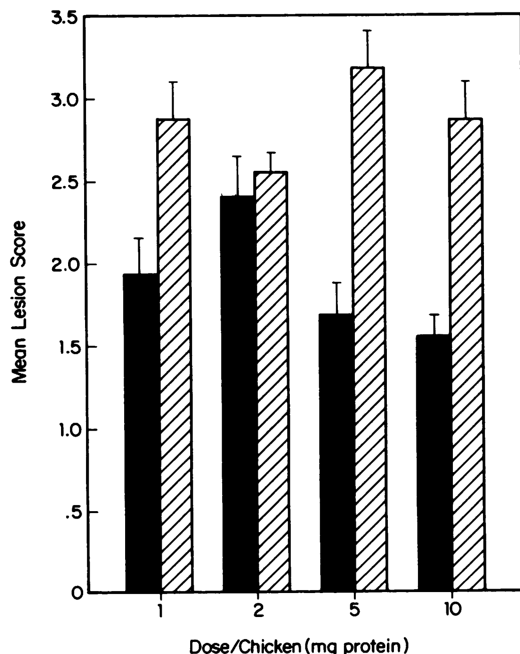

FIG. 4. Passive protection of 3-week-old chickens against *E. tenella* infection with 1073.10. Bars represent the lesion scores (mean and standard error) of chickens receiving 1073.10 (■) or mouse gamma globulin (▨). The groups of chickens receiving 5 or 10 mg of 1073.10 were significantly different ($P < 0.05$) from untreated controls which had a mean lesion score of 2.5. The groups of chickens receiving 1 or 2 mg of 1073.10 or 1, 2, 5, or 10 mg of mouse immunoglobulin were not significantly different ($P > 0.05$) from untreated controls.

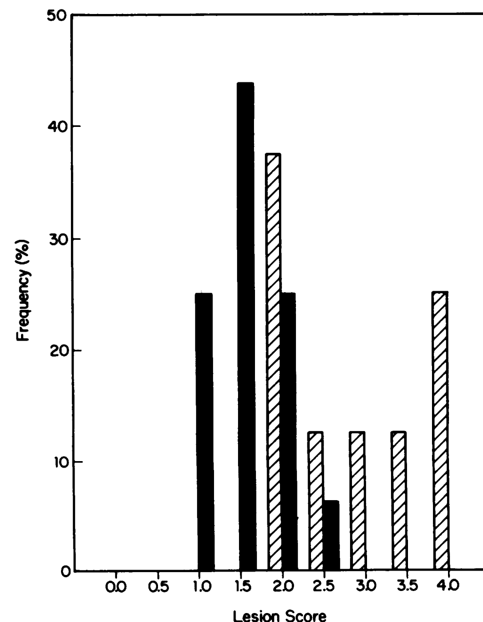

FIG. 5. Frequency distribution of lesion scores in 3-week-old chickens treated with 10 mg of 1073.10 (■) or mouse gamma globulin (▨) and infected with 10^4 *E. tenella* sporulated oocysts.

parasite antigen recognized by 1073.10 and thus be a surrogate parasite antigen for vaccination, as has been described for other pathogens (8, 19, 29).

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