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Cross-Protection against Four Species of Chicken Coccidia with a Single Recombinant Antigen

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A cDNA clone, SO7′, from an *Eimeria tenella* cDNA library was inserted into the high-expression vector pJC264 and was expressed in *Escherichia coli* as a fusion protein, CheY-SO7′, with a molecular mass of approximately 36 kDa. By using the purified recombinant antigen to immunize young chicks, it was demonstrated that a single dose, without adjuvant, not only protected against severe coccidiosis induced by infection with *E. tenella* but also protected chicks challenged with the heterologous species *Eimeria acervulina*, *E. maxima*, and *E. necatrix*. By using rabbit antiserum raised against recombinant CheY-SO7′, Western blot (immunoblot) analysis of sporulated oocysts of all seven major species of chicken coccidia showed that all species tested contained proteins characteristic of the B class of antigens, of which CheY-SO7′ is representative. It seems likely that a single B antigen could protect chickens against severe coccidiosis caused by infection with any of these *Eimeria* species. Although chicks exposed to prolonged, natural infection develop antibodies to B antigen, active immunization of young chicks with a protective dose of CheY-SO7′ does not elicit a humoral antibody response, suggesting that the partial protection results from cell-mediated effector mechanisms. In addition, the cross-protective nature of the immunity indicates that the response to B antigen is different from that induced by natural infection, which elicits a species-specific immunity. To date, the protection induced by B antigen immunization, although remarkable for a single recombinant protein, is not sufficient to compete with prophylactic chemotherapy.

Coccidiosis, which is caused by obligate, intracellular, protozoan parasites belonging to several species of the genus *Eimeria*, is a major problem for the poultry industry. Currently, the disease, which is controlled by the use of prophylactic chemotherapy, costs the broiler chicken industry worldwide an estimated $250 million per annum (29). Because of the expense of new drug development and the occurrence of drug-resistant parasites, an alternative method of disease control is desirable.

In recent years, modern approaches to vaccine development utilizing hybridoma and recombinant DNA technologies have been applied extensively to the problem of coccidiosis of the domestic chicken (6, 7, 10, 17). However, the recombinant antigens isolated to date either have not been tested in vivo or have been disappointing in their ability to protect broilers against coccidiosis (2, 11, 12, 24, 31). Nevertheless, the development of a subunit (recombinant) vaccine against coccidiosis for the broiler chicken industry still remains an attractive alternative to chemoprophylaxis.

Until recently, it was thought that a live coccidial infection was essential for an effective immune response to protect against a pathogenic challenge (37). There are live coccidiosis vaccines available, such as Immunocox (26) and Coccivac (Sterwin Laboratories), and others are under development for the broiler industry (27, 40). However, recent results from our laboratory and others have shown that live infection is not mandatory for protection but that dead antigen such as a crude parasite extract can also protect against severe coccidiosis (32). These results demonstrated that a dead (subunit) vaccine is feasible and would be economical if protection was induced by a protein which would allow vaccine production by recombinant DNA technology.

In subsequent studies, the crude, protective parasite extract was subfractionated by S200 chromatography, and a protective fraction (fraction V) which contained several polypeptides with a limited molecular weight range was identified (23). By using antisera raised against fraction V and against various other protective parasite extracts, a λgt11 cDNA library of *Eimeria tenella* was screened and clones representing the major polypeptides of fraction V were isolated (35). By using immunopositive clones from the *E. tenella* cDNA library and a novel bacterial expression vector, CheY (18), fusion proteins were engineered, expressed, purified, and tested for protective ability against coccidiosis with broilers in our battery model. Several recombinant proteins from five antigen classes (35) demonstrated partial protection against severe coccidiosis caused by *E. tenella* (8). Moreover, further results demonstrated that one of these recombinants, CheY-SO7′ of the B antigen class, was extremely efficacious and cross-protected against coccidiosis caused by *Eimeria acervulina* and *Eimeria maxima* (8). This report describes further characterization of the fusion protein CheY-SO7′ with respect to its construction, immunogenicity, and protective ability in broiler chicks against coccidiosis caused by four of the seven species of chicken coccidia. In addition, it is demonstrated that class B antigens are present in all seven species of chicken coccidia, indicating that this class of antigen could protect chickens against coccidiosis caused by any of these species.

MATERIALS AND METHODS

Induction and identification of the CheY-SO7′ fusion protein. A cDNA insert with *Eco*RI ends, SO7′ (28), was ligated

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to *Eco*RI-linearized pJC264 (18). *Escherichia coli* JM109 was transformed with this recombinant plasmid by following a standard protocol (39). Individual recombinant bacterial colonies were grown in Luria broth containing 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) to an optical density of 0.6 to 0.7 spectrophotometric units at 600 nm. Induction of the gene was achieved by adding 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG; Sigma Chemical Co.). Following 18 h of growth after induction, bacteria were harvested by centrifugation, resuspended in sample buffer (0.1 M Tris hydrochloride [pH 6.8], 2% sodium dodecyl sulfate [SDS], 5% β-mercaptoethanol, 5% glycerol, 0.001% bromophenol blue) and boiled for 5 min. The total soluble bacterial proteins were resolved in duplicate by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (10% polyacrylamide) (25). Following electrophoretic resolution of the proteins, one of the gels was stained with Coomassie blue and the other was processed for immunoblotting (41) and probed with a hyperimmune rabbit antiserum raised against *E. tenella* sporozoites (5).

Purification of recombinant B antigen for vaccination. A pellet of the recombinant bacterial clone CheY-SO7′/9 was disrupted with a French press. The cell lysate was centrifuged (12,000 × *g*, 45 min, 4°C), and the insoluble fusion protein was collected in the pellet. The insoluble recombinant antigen was washed with 1% Triton X-100, which removed a large proportion of pelleted bacterial proteins. The recombinant protein was solubilized in 6 M guanidine HCl–10 mM dithiothreitol at 50°C, diluted further with 7 M urea, and applied to a hydroxyapatite column previously equilibrated with 7 M urea. The column was washed in a stepwise fashion with portions of a 7 M urea solution containing sodium phosphate buffers (pH 6.5) of increasing molarity (10 to 160 mM). Recombinant fusion protein was eluted at 10 and 20 mM sodium phosphate and was stored at −20°C in elution buffer until use. Various modifications of this purification scheme have been used in the preparation of other batches of recombinant B antigen for vaccination studies (22).

Experimental vaccinations. Experimental vaccinations were carried out as described previously (8). Briefly, day-old broiler chicks (Petersen × Arbor Acre) were obtained (Avian Services, Frenchtown, N.J.) and housed in all-stainless-steel, 16-compartment, brooder-type cages (Hazleton Co., Aberdeen, Md.) in rooms where extreme precautions were taken to minimize adventitious exposure to avian coccidia. Experimental antigens diluted in phosphate-buffered saline (PBS, pH 7.4) were administered intramuscularly without adjuvant in the thighs of 2- to 4-day-old broilers. Following immunization, chicks were reared under the conditions described above, with feed and water ad libitum, until three weeks of age. At this time, chicks were transferred to four-compartment, all-stainless-steel poultry batteries (Allentown Caging Equipment Co., Allentown, N.J.), moved to a separate facility, and challenged with a single species of chicken coccidia. Twenty-four hours prior to challenge, feed was removed to ensure that the chicks were hungry. At the time of challenge, pathogenic doses of sporulated oocysts were administered to chicks via feed (15 g of feed per 2.5 ml of water per dose per chick). The inoculated feed was usually fully consumed within 2 h, after which normal feed was restored. The challenge dose, which was determined previously, was sufficient to induce a mean internal coccidial lesion score (19) of approximately 3.0 in nonvaccinated controls. The strains of the four species of chicken coccidia used were the Merck, Sharp and Dohme Research Laboratories strains of *E. tenella* (LS-18), *E. acervulina* (LS-3), *E. maxima* (FS-110) and *Eimeria necatrix* (AF1021).

Western blot (immunoblot) analysis. Sporulated oocysts from strains of all seven major species of chicken coccidia were obtained by standard procedures (13). The strains used in this analysis were *E. tenella* LS-18 and *E. necatrix* AF1021 and strains of *E. acervulina*, *E. maxima*, *Eimeria praecox*, *Eimeria mitis*, and *Eimeria brunetti* obtained from Peter Long, University of Georgia, Athens. (21, 30).

A crude antigen preparation was made from each of the strains by disrupting the oocysts fully by shaking them with glass beads (2-mm diameter) in Dulbecco's PBS (Ca^{2+} and Mg^{2+} free, pH 7.2) containing a cocktail of protease inhibitors (0.1 mM phenylmethylsulfonyl fluoride, 10 mM 1,10-phenanthroline, 48 μg of soybean trypsin inhibitor per ml, 48 μg of aprotinin per ml, 0.04 mM leupeptin, 13 mM benzamidine, and 5 mM EDTA). Low-speed centrifugation (700 × *g*, 10 min) pellets were obtained from each disrupted oocyst preparation and were solubilized by heating them to 100°C for 5 to 10 min in sample buffer (2% SDS, 5% β-mercaptoethanol, 10% glycerol, and 0.003% bromophenol blue in 62.5 mM Tris hydrochloride [pH 6.8]). Any particulate material was removed by centrifugation (13,000 × *g*, 5 min), and the protein content of the supernatant was determined by the modified Bradford method (38). The solubilized polypeptides of the seven samples were resolved by SDS-PAGE by using a Protean I chamber (Bio-Rad Laboratories) according to the manufacturer's instructions, which are based on the method of Laemmli (25). The samples, 50 μg of protein in sample buffer, were applied to a 10% polyacrylamide resolving gel with a 3% polyacrylamide stacking gel. Recombinant CheY-SO7′ (20 μg) and biotinylated SDS-PAGE molecular weight standards were also run in lanes adjacent to the oocyst samples. Following electrophoresis, the polypeptides were transferred to nitrocellulose (Hoefer Scientific Instruments) in a Transblot chamber (Bio-Rad Laboratories) according to the manufacturer's instructions, which are based on the method of Towbin et al. (41). The B antigens were localized in the transferred polypeptides by using a double antibody method (1) utilizing a Bio-Rad Immuno-Blot assay kit. Following incubation with the first antibody, a hyperimmune rabbit anti-CheY-SO7′ antiserum, the nitrocellulose sheet was incubated with the second antibody, a goat anti-rabbit immunoglobulin G (heavy and light)-alkaline phosphatase conjugate. B antigen localization was revealed with a color development substrate. The molecular weight standards were visualized by incubation with an avidin-alkaline phosphatase conjugate and then with the color development substrate (15).

Solid-phase fluorescence immunoassay. To determine whether chicken sera contained specific antibodies to either recombinant CheY-SO7′ or to whole sporulated oocyst antigen (SOA), a solid-phase fluorescence immunoassay was employed (44). Briefly, Immulon II 96-well microtiter plates (Dynatech Laboratories) were coated with antigen by incubating them with 100 μl of either B (CheY-SO7′) antigen or disrupted *E. tenella* SOA per well at protein concentrations of 10 μg/ml and 1 mg/ml, respectively, in coating buffer (0.02 M Tris buffer [pH 9.1] with 0.02% sodium azide). Plates were incubated at 37°C for 3 to 4 h, kept at 4°C overnight, washed with PBS containing 0.02% sodium azide (PBS/AZ), incubated with 200 μl of blocking buffer (1% bovine serum albumin [BSA] in PBS/AZ) per well for 2 h at 37°C, and washed with PBS/AZ. Various chicken serum samples (100 μl) diluted 1:50 with sample buffer (0.1% BSA in PBS/AZ)

were loaded into the wells and incubated for 2 h at 37°C. Following washes with PBS/AZ, phosphatase-conjugated goat anti-chicken immunoglobulin G (heavy and light) (Kirkegaard & Perry) at a concentration of 0.2 μg/ml in sample buffer was added to each well and incubated for 2 h at 37°C. After rinsing with Tris washing buffer (0.01 M Tris, 0.85% NaCl, 0.02% sodium azide), 100 μl of 0.01% 4-methylumbelliferyl phosphate in substrate buffer (50 mM sodium carbonate, 1 mM magnesium chloride [pH 9.8]) was added to each well, and the plate was incubated in the dark for 1 h at room temperature. Fluorescence intensity was quantified with a Titertek Fluoroscan microtiter plate reader (Flow Laboratories) with excitation and emission wavelengths of 455 and 480 nm, respectively.

RESULTS

Expression and purification of the CheY-SO7′ fusion protein. Figure 1A shows schematically the construction of the recombinant plasmid. Following transformation of the *E. coli* host, colonies were picked and the orientation of the SO7′ insert with respect to the CheY portion of the operon was determined by restriction enzyme mapping of the plasmid from each clone. The clone CheY-SO7′/9 had the correct orientation of SO7′ for the expression of a large fusion protein and was chosen for the purification of the recombinant antigen.

The fusion protein in the crude bacterial cell extract made from this clone, solubilized in guanidine HCl and urea, was applied to a hydroxyapatite column and was eluted with 10 and 20 mM sodium phosphate buffer washes. As shown in Fig. 1B, the clone CheY-SO7′/9 expressed a fusion protein with the appropriate molecular mass of approximately 36 kDa and was defined as a recombinant antigen of the B class on the basis of its reactivity with specific antisera (34a). Note that analysis of corresponding fractions from a bacterial lysate derived from a clone containing the CheY expression vector with SO7′ inserted in the reverse orientation did not reveal a protein of the characteristic size or immunoreactivity. For experimental vaccinations, purified recombinant antigen preparations were stored in buffer containing 7 M urea (22) at −20°C until use.

In vivo protection. Since its identification, several batches of the recombinant B antigen CheY-SO7′ have been produced and purified and have shown consistent protection in broiler chicks against coccidiosis caused by *E. tenella*, *E. acervulina*, and *E. maxima* infection (8). Over an extended period of time, several thousand broilers have been immunized with this antigen. The frequency distribution of lesion scores from large numbers of chicks provides a clear demonstration of the protective ability of CheY-SO7′ antigen. A comparison of the frequency distribution of lesion scores in immunized and nonimmunized broilers infected with *E. tenella* and *E. maxima*, pooled from several individual experiments involving several hundred chickens, is shown in Fig. 2A and B, respectively. In the nonimmunized group, >85% of the broilers infected with *E. tenella* developed lesion scores of ≥2.0 (mean lesion score = 2.90), while >80% of the broilers in the immunized group developed lesion scores of ≤2.0 (mean lesion score = 1.72). Similarly, in the nonimmunized group infected with *E. maxima*, >90% of the broilers developed lesion scores of ≥2.5 (mean lesion score = 3.04), while in the CheY-SO7′-immunized group, >85% of the broilers developed lesion scores of ≤2.5 (mean lesion score = 1.89). A similar frequency distribution of lesion scores was obtained from results with *E. acervulina* (results not shown).

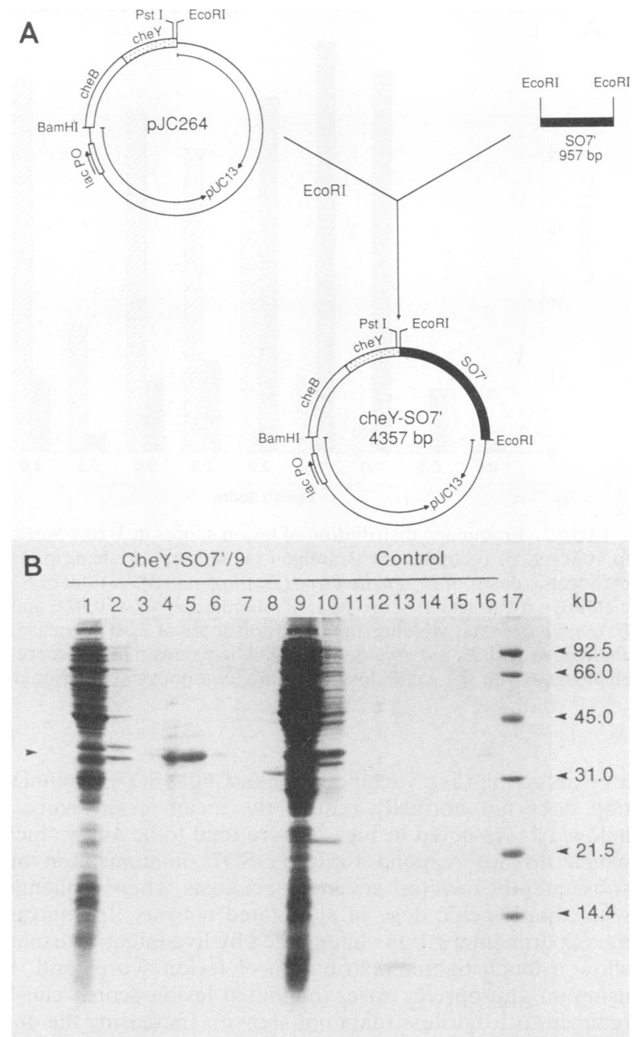

FIG. 1. Construction of plasmid CheY-SO7′ and expression of the CheY-SO7′ recombinant polypeptide antigen. (A) A cDNA fragment of 957 bp (28) containing a 216-amino-acid reading frame of the B antigen clone SO7′ was inserted, in frame, into the *Eco*RI site of the expression vector pJC264 (18). (B) SDS-polyacrylamide gel of fractions obtained during the purification procedure of the recombinant B antigen clone CheY-SO7′/9. Lanes 1 through 8: fractions from CheY-SO7′/9. The arrow on the left indicates the position of the recombinant B antigen. Lanes 9 through 16: fractions from a control clone containing the CheY expression vector with the SO7′ fragment inserted in the inverse orientation. Lanes 1 and 9: supernatant from whole bacterial lysate. Lanes 2 and 10: Triton X-100 wash of the insoluble pellet. Lanes 3 and 11: hydroxylapatite column flowthrough. The column was washed with 7 M urea containing sodium phosphate buffer (pH 6.5) at concentrations of 10 mM (lanes 4 and 12), 20 mM (lanes 5 and 13), 40 mM (lanes 6 and 14), 80 mM (lanes 7 and 15), and 160 mM (lanes 8 and 16). Lane 17: molecular weight standards.

In addition to protecting broilers against severe coccidiosis caused by *E. tenella*, *E. acervulina*, and *E. maxima*, the recombinant B antigen CheY-SO7′ also protects broilers against *E. necatrix*-induced coccidiosis (Fig. 3). The level of protection induced by CheY-SO7′ immunization is similar for all four species. However, unlike immunization by low-

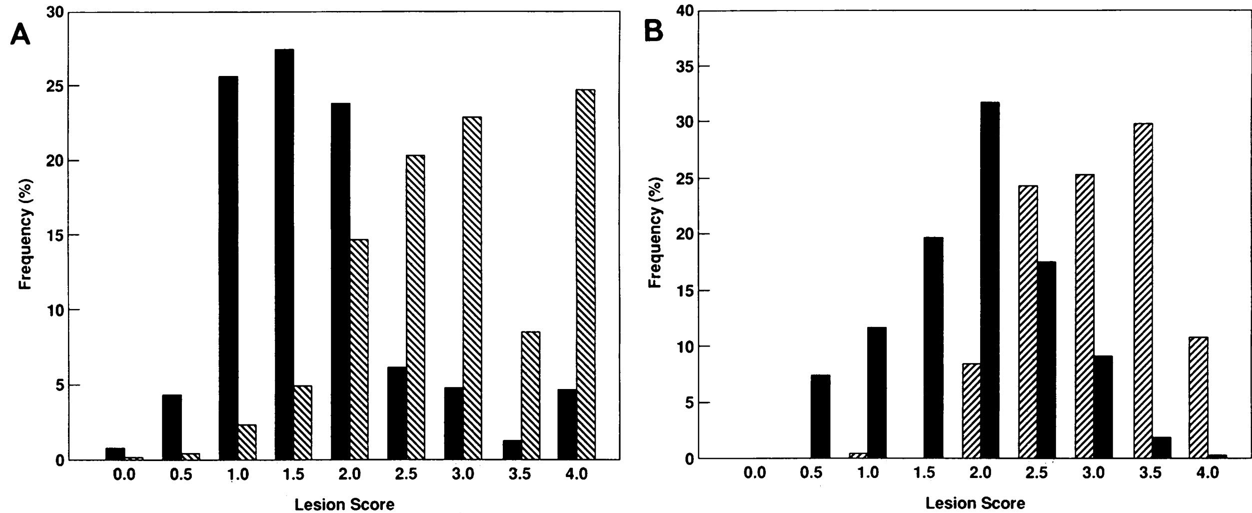

FIG. 2. Frequency distribution of lesion scores in 3- to 4-week-old chicks which were either immunized with various protective doses (0.1 to 10.0 μg) of recombinant B antigen (solid bars) or sham immunized with control buffer (hatched bars) and which were challenged with pathogenic doses of *E. tenella* (A) or *E. maxima* (B). A total of 626 broilers were immunized with CheY-SO7′ and challenged with *E. tenella*, yielding a mean lesion score of 1.72 (standard error = 0.02), and 317 broilers were sham immunized and challenged with the same dose of *E. tenella* oocysts, yielding a mean lesion score of 2.90 (standard error = 0.03). A total of 283 broilers were immunized with CheY-SO7′ and challenged with *E. maxima* oocysts, yielding a mean lesion score of 1.89 (standard error = 0.04), and 209 broilers were sham immunized and challenged with the same dose of *E. maxima* oocysts, yielding a mean lesion score of 3.04 (standard error = 0.04).

level infection (live vaccine groups), CheY-SO7′ immunization does not normally reduce the mean lesion score to below 1.5. As noted in Fig. 2, there tend to be a few chicks which do not respond to CheY-SO7′ immunization and subsequently develop severe coccidiosis when challenged with a pathogenic dose of sporulated oocysts. In contrast, the experimental groups immunized by live infection usually show a much tighter distribution of lesion scores and, by using an appropriate dose, individual lesion scores can be reduced to 1.0 or less (data not shown). Increasing the dose of CheY-SO7′ from 1 μg to 10 μg (Fig. 3) or increasing the number of doses (8) does not improve the observed protection.

Western blot analysis. CheY-SO7′ antigen was obtained from a cDNA library of *E. tenella* and has been shown to be present in the sporulated oocyst, localized in the refractile body of the sporozoite (34a). It is interesting that unlike live vaccination, which is species-specific, CheY-SO7′ cross-protects against at least four species of chicken coccidia (Fig. 3). Thus, to determine whether the B class of antigen is common to all species of chicken coccidia, immunoblots of SDS-PAGE-resolved polypeptides of sporulated oocysts of the seven species were undertaken. The localizing antibody preparation was a hyperimmune rabbit anti-CheY-SO7′ antiserum, and recombinant CheY-SO7′ antigen was used as the positive control. By using reactivity with the anti-CheY-SO7′ antiserum to define the B antigen class, all species of chicken coccidia were found to possess a predominant polypeptide band characteristic of B antigen (Fig. 4). As determined by the mobility of the molecular weight markers, the antigens from the various species have molecular masses of approximately 26 to 28 kDa, as shown previously for *E. tenella*, *E. acervulina*, and *E. maxima* (33). There is some interspecies variation in the relative mobilities of the B antigens, which may reflect differences in the molecular weight and/or other characteristics of the antigen between the different species of coccidia. As expected, the recombinant B antigen CheY-SO7′ has a higher molecular mass (approximately 36 kDa) because of the contribution of CheY in the fusion protein (22).

Chicken antibody to recombinant CheY-SO7′. It is known that eimerian sporozoites are very sensitive to the action of antibody and complement (5) and that chickens can be partially protected from coccidiosis by passive transfer of specific antibody (6, 36, 43). To ascertain whether the CheY-SO7′ immunization of broiler chicks induced antibody production, serum samples were obtained from chicks which had undergone a variety of immunization regimens. Since the observed protection was induced by purified antigen without any adjuvant, no adjuvant was used in any of these immunizations.

Young chicks, less than 14 days old, which had not been immunized with CheY-SO7′ had demonstrable levels of antibody to both CheY-SO7′ and the crude parasite extract, SOA (Table 1). Since these chicks were very young and were reared in isolation, it can be assumed that this reactivity is due to maternal antibody; sera from chicks older than two weeks of age did not demonstrate this reactivity. Immunization with CheY-SO7′ did not induce specific antibody in either young or older chicks. The only other chicks tested which demonstrated CheY-SO7′-specific antibody in their sera were those hyperimmunized with whole sporozoite antigen in Freund's adjuvant and the much older chicks from breeder flocks which had been exposed to live coccidia for extended periods of time.

DISCUSSION

Problems incurred because of the emergence of drug-resistant strains of *Eimeria* spp. and the difficulty in discovering and developing new anticoccidial agents have emphasized the need for novel approaches for the control of coccidiosis in the poultry industry (4). It is well established that infection with virulent (14, 42) or attenuated (20) strains

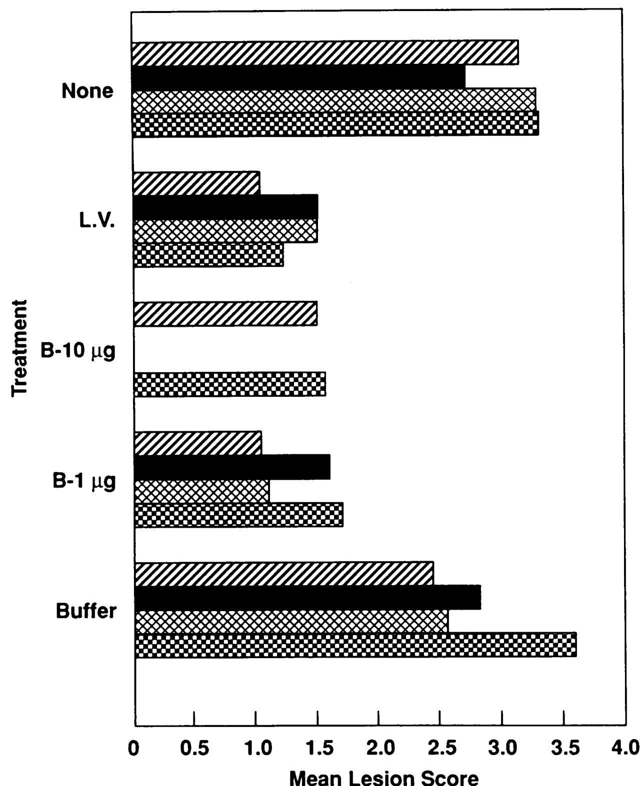

FIG. 3. Recombinant B antigen-induced cross protection of 3- to 4-week-old broilers against *E. tenella* (▨), *E. acervulina* (■), *E. maxima* (▩), and *E. necatrix* (▩). Groups (8 to 10 chicks per group) of 2- to 4-day-old chicks either were immunized with 1 μg (B-1 μg) or 10 μg (B-10 μg) of B (CheY-SO7′) antigen per chick, were sham immunized with buffer (Buffer), were inoculated with an immunizing dose of sporulated oocysts equivalent to a live vaccine (L.V.), or were untreated (None). The live vaccine dose was 100 sporulated oocysts per chick for *E. tenella*, 2,000 sporulated oocysts per chick for *E. acervulina*, 50 sporulated oocysts per chick for *E. maxima*, and 1,000 sporulated oocysts per chick for *E. necatrix*. At approximately 3 weeks of age, the chicks were challenged with pathogenic doses of sporulated oocysts of one of the four species tested, and lesion scores were evaluated 6 days postinfection for *E. acervulina* and 7 days postinfection for *E. tenella*, *E. maxima*, and *E. necatrix*. The bars represent mean lesion scores; standard errors of the mean ranged between 0.08 and 0.29.

of coccidia induces strong, species-specific, protective immunity in chickens. Nevertheless, the use of available live vaccines consisting of virulent strains for the control of coccidiosis is not widespread but is limited mostly to chicken breeder flocks, suggesting that such vaccines are not suitable for broiler production. Attenuated live vaccines for the broiler industry are being developed (3, 16, 34). However, as with other live vaccines, there are concerns about stability, quality control, cost-effectiveness, and efficacy against the vast array of field strains likely to be encountered in different geographical areas (4, 9). The development of a subunit vaccine would circumvent some of these problems.

The recombinant antigen (CheY-SO7′) described here is remarkable in that it induces cross-protective immunity against at least four major species of coccidia and is effective at relatively low doses (8). On the basis of the immunoblot analysis which demonstrates the presence of B antigens in all seven species, it is possible that a single recombinant antigen

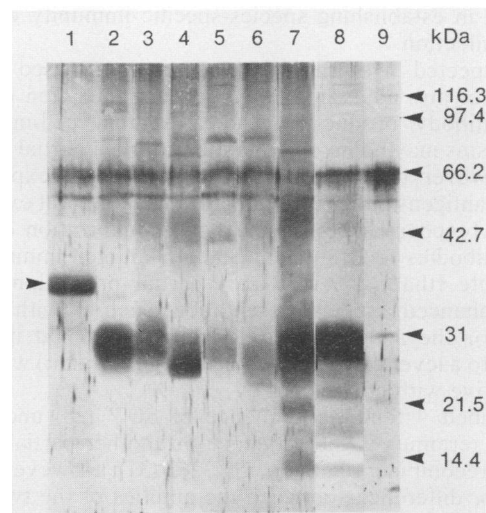

FIG. 4. Western blot analysis of sporulated oocyst preparations from all seven major species of chicken coccidia. The oocyst preparations (lanes 2 through 8), purified CheY-SO7′ (lane 1, arrow), and biotinylated molecular weight markers (lane 9) were resolved by SDS-PAGE, transferred to a nitrocellulose sheet which was incubated sequentially with hyperimmune rabbit anti-CheY-SO7′ antiserum, a goat anti-rabbit immunoglobulin G (heavy and light)-alkaline phosphatase conjugate, and the color development substrate. The molecular weight markers were visualized by incubation with an avidin-alkaline phosphatase conjugate and then with the color development substrate. Lanes: 2, *E. necatrix*; 3, *E. tenella*; 4, *E. acervulina*; 5, *E. maxima*; 6, *E. praecox*; 7, *E. brunetti*; 8, *E. mitis*.

could cross-protect against not only all chicken coccidia but also against other species. However, in contrast to live vaccination, B antigen, even at relatively high doses, does not induce sterile immunity nor does it fully prevent disease. These results indicate some fundamental differences between immunity induced by mild infection and that induced by the B antigen; it would appear that B antigen is not

TABLE 1. Reactivity of chicken sera with recombinant B (CheY-SO7′) antigen and *E. tenella* SOA[a]

Serum type	Antibody-antigen reactivity (fluorescence units)	
	CheY-SO7′	SOA
Preimmune from 2-day-old chicks	6,393	7,571
Preimmune from 2-wk-old chicks	1,570	1,343
Nonimmunized from 4-wk-old chicks	1,128	1,222
CheY-SO7′-immunized from 4-wk-old chicks[b]	872	628
Hyperimmune chick anti-sporozoite serum[c]	9,701	>10,000[d]
Breeder chick[e]	7,788	9,750

[a] Reactivity determined in a solid-phase fluorescence immunoassay.

[b] Chicks were immunized intramuscularly with 1 μg of CheY-SO7′ antigen 2 days, 2, 9, and 16 days, or 16, 23, and 30 days after hatching, and serum was taken at weekly intervals up to 7 weeks of age. These results are examples of several samples tested, none of which demonstrated positive reactivity.

[c] Serum from chicks immunized with disrupted sporozoites in Freund's complete adjuvant and then with sporozoite antigen in Freund's incomplete adjuvant at regular intervals until serum became hyperimmune (5).

[d] Fluorescence signal was beyond the upper threshold of the microtiter plate reader.

[e] Chick serum samples obtained from Spafas Inc., Storrs, Conn.

involved in establishing species-specific immunity effected by live infection.

As expected with the low antigen doses used in the absence of any adjuvant, B antigen immunization did not elicit antibody production, indicating that cell-mediated mechanisms may be involved in the observed partial protection. However, it is interesting to note that chicks exposed to parasite antigens either through prolonged, natural exposure (as with breeder chicks) or by hyperimmunization do produce antibodies to B antigen. Since B antigen immunity is incomplete (that is, it provides partial protection only), either enhanced antigen presentation, such as with a viral vector, or the addition of other antigens should improve efficacy to a level at which such a vaccine (subunit) would be competitive with chemotherapy.

Contained within the sequence of SO7′ (28) and at its carboxy terminus is the sequence of another partially protective, recombinant antigen, GX3262 (31). However, there are major differences between the abilities of the two antigens to immunize chicks against coccidiosis. In contrast to GX3262, a single low dose (10 ng) of CheY-SO7′ in the absence of any adjuvant is sufficient to protect 2-day-old chicks against severe coccidiosis caused by infection with any one of several species of coccidia for several weeks after immunization (8). Whether this is due to the larger size of SO7′, providing more, important epitopes (at the amino terminus), or due to the fact that CheY-SO7′ is a CheY fusion protein (rather than a β-galactosidase fusion protein) is under investigation. The ability to obtain CheY fusion protein with high purity (22) may also contribute to the low-dose efficacy (based on total protein per dose) of CheY-SO7′. These differences also exist between CheY-SO7′ and other refractile body recombinant antigens (11). Nevertheless, despite qualitative and quantitative differences, it is interesting that several protective antigens have been identified which originate in the refractile body of the parasite.

All of the studies reported here have been conducted in our cage model for chicken coccidiosis. In addition to studies aimed at improving the efficacy of the B antigen so that mean lesion scores would be reduced to below 1.0 in immunized chicks, other studies to demonstrate protection against coccidiosis, including productivity competitive with chemotherapy under floor-pen conditions, are ongoing.

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