

Effect of *Campylobacter*-Specific Maternal Antibodies on *Campylobacter jejuni* Colonization in Young Chickens

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Using laboratory challenge experiments, we examined whether *Campylobacter*-specific maternal antibody (MAB) plays a protective role in young chickens, which are usually free of *Campylobacter* under natural production conditions. Kinetics of *C. jejuni* colonization were compared by infecting 3-day-old broiler chicks, which were naturally positive for *Campylobacter*-specific MAB, and 21-day-old broilers, which were negative for *Campylobacter*-specific MAB. The onset of colonization occurred much sooner in birds challenged at the age of 21 days than it did in the birds inoculated at 3 days of age, which suggested a possible involvement of specific MAB in the delay of colonization. To further examine this possibility, specific-pathogen-free layer chickens were raised under laboratory conditions with or without *Campylobacter* infection, and their 3-day-old progenies with (MAB⁺) or without (MAB⁻) *Campylobacter*-specific MAB were orally challenged with *C. jejuni*. Significant decreases in the percentage of colonized chickens were observed in the MAB⁺ group during the first week compared with the MAB⁻ group. These results indicate that *Campylobacter*-specific MAB plays a partial role in protecting young chickens against colonization by *C. jejuni*. Presence of MAB in young chickens did not seem to affect the development of systemic immune response following infection with *C. jejuni*. However, active immune responses to *Campylobacter* occurred earlier and more strongly in birds infected at 21 days of age than those infected at 3 days of age. Clearance of *Campylobacter* infection was also observed in chickens infected at 21 days of age. Taken together, these findings (i) indicate that anti-*Campylobacter* MAB contributes to the lack of *Campylobacter* infection in young broiler chickens in natural environments and (ii) provide further evidence supporting the feasibility of development of immunization-based approaches for control of *Campylobacter* infection in poultry.

Campylobacter jejuni, a gram-negative bacterium, is the most commonly reported bacterial cause of human food-borne illness in the United States and other developed countries. Each year, an estimated 2.1 million to 2.5 million cases of human campylobacteriosis, characterized by watery and/or bloody diarrhea, occur in the United States (1, 5, 12). *Campylobacter* infection is also the most common antecedent of Guillain-Barré syndrome, a demyelinating disorder that causes acute neuromuscular paralysis of peripheral nerves and respiratory muscle compromise that can lead to death (25, 41). Epidemiologically, the majority of human *Campylobacter* infections are associated with the consumption of undercooked poultry or other food products that are cross-contaminated by raw poultry meat during food preparation (1, 8, 12, 16). Thus, reduction or elimination of poultry contamination by *C. jejuni* would greatly decrease the risk of human campylobacteriosis.

At present, no effective control measures are available for prevention of *Campylobacter* colonization of commercial broiler chicken flocks. The limited success of improved hygiene measures in reducing carcass contamination at slaughterhouses highlights the need for farm-based intervention methods to control *Campylobacter* (23). Due to the complexity of

Campylobacter transmission and the ubiquitous distribution of the organism in poultry production environments, management-based methods such as strict biosecurity measures have had limited success in preventing the introduction of *C. jejuni* into the poultry flocks (3, 37). Therefore, alternative intervention strategies, such as vaccination, are needed to control *C. jejuni* infection in the poultry reservoir. Although several studies were directed toward the investigation of poultry immunity to *Campylobacter* colonization (7, 24, 27, 42), the nature of the protective immune responses against *C. jejuni* colonization in chickens is still unknown.

A general observation, and a distinct characteristic of *C. jejuni* colonization in poultry, is that this organism is not detected in chicks less than 2 to 3 weeks of age under commercial broiler production conditions (11, 17, 26, 37). Infection of broiler flocks by *C. jejuni* usually starts from the third week, increases with age, and peaks at the market age (6 to 7 weeks) (8, 10, 26). This unique ecological feature suggests that young chickens may have age-related resistance to *Campylobacter* colonization. However, the resistance mechanisms have not been defined. Elucidation of the factors contributing to the lack of *Campylobacter* colonization is of particular interest, as this may provide valuable information for designing strategies to prevent *Campylobacter* colonization in broiler chickens. One possible contributing factor for the resistance may be related to *Campylobacter*-specific maternal antibodies (MAB), which are widely present in young chickens (7, 26, 27, 32, 33). In a previous study, we demonstrated that 100% of day-old hatchlings obtained from five different commercial broiler flocks

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TABLE 1. Shedding percentages and serum IgG levels in broiler chickens following challenge with *C. jejuni* strain S3B (experiment I) or 21190 (experiment II)

Expt and group	Age (day)	Serum MAB level (±SD) at challenge	% Shedding on day after challenge ^a :										Serum IgG level ^b after challenge on day ^c :				
			1	3	5	7	9	11	13	15	21	28	−1	7	15	21	28
I																	
1	3	1.565 ± 0.088	0*	0*	0*	100*	100*	100*	100*	100*	100*	100*	1.565*	0.546*	0.134*	0.112*	0.474*
2	21	0.087 ± 0.012	0*	90†	100†	100*	100*	100*	100*	100*	60*	10†	0.087†	0.336†	0.871†	0.838†	0.722*
II																	
1	3	1.846 ± 0.152	0*	0*	0*	0*	0*	0*	0*	0*	0*	0*	1.846*	NT ^d	NT	NT	NT
2	21	0.113 ± 0.033	0*	30*	40*	70†	100†	70†	30*	0*	0*	0*	0.113†	NT	0.266*	0.316*	0.730*

^a The numbers in the same column of each experiment with different superscript symbols differ significantly (Fisher's exact test).

^b Mean ELISA OD for 7 to 10 serum samples.

^c Means in the same column of each experiment with different superscript symbols differ significantly (one-way analysis of variance).

^d NT, not tested.

were positive for MAB against *Campylobacter* (32). In each flock, high levels of circulating *Campylobacter*-specific MAB were detected during the first week of age. Thereafter, the levels of MAB dropped substantially at 14 days of age and reached background levels by the third and fourth weeks of age. By using immunoblotting, MAB was shown to react strongly with multiple components of the outer membranes of *C. jejuni*, including flagellin, lipopolysaccharide, and other unidentified proteins (32). Moreover, *Campylobacter*-specific MAB was shown to be effective in complement-mediated killing of *C. jejuni* isolates in a strain-dependent manner (32). These findings suggested that MAB may protect young chickens from colonization by *C. jejuni* and prompted us to conduct this study to assess the potential protective role of anti-*Campylobacter* MAB.

Historically, the role of anti-*Campylobacter* MAB might have been underestimated, since many studies found that young chickens were susceptible to colonization by *C. jejuni* following experimental challenges (7, 13, 35, 39). However, these studies were not designed to determine the protective role of MAB, and the relatively high challenge doses might have overwhelmed any protection conferred by MAB. Thus, more defined experimental challenge studies with appropriate controls are necessary to better understand whether maternal immunity protects against *Campylobacter* colonization in young chickens. Toward this end, we conducted laboratory challenge studies to determine the effect of *Campylobacter*-specific MAB on the colonization of young chickens by *C. jejuni*. Infection of chickens with different doses and strains of *C. jejuni* revealed that *Campylobacter*-specific MAB delayed the onset of colonization and reduced the rate of horizontal spread of *C. jejuni* in young chickens. This finding indicated a partially protective role of anti-*Campylobacter* MAB and suggests that the MAB is a contributing factor to the lack of *Campylobacter* colonization in young chickens.

MATERIALS AND METHODS

Bacterial strains and culture. *Campylobacter jejuni* strains 21190 and S3B (both of poultry origin) were chosen for use in challenge experiments because of their ability to colonize chickens. Also, these two strains are genetically diverse as determined by pulsed-field gel electrophoresis and sequencing of the *cmp* gene (43). Bacterial cultures were grown for 48 h in Mueller-Hinton (MH) broth (Becton Dickinson, Sparks, Md.) in anaerobic jars under microaerobic condi-

tions produced by CampyPack Plus (BBL Microbiology Systems, Cockeysville, Md.) at 42°C.

Challenge experiments using broiler chickens. Day-old commercial broiler chickens were obtained from a local commercial hatchery. Birds were housed in wire-floored cages in steam-cleaned and formaldehyde-fumigated rooms and provided with unlimited access to feed and water. The feed (C-2-88; Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio) was custom made, *Campylobacter* free, and without any animal protein or antibiotic additives. Prior to challenge, all birds were confirmed to be free of *Campylobacter* as determined by cloacal swab culture. Since every 3-day-old broiler bird (total chicks tested, 200) was positive with anti-*Campylobacter* MAB, we were unable to find MAB-negative broiler chicks from commercial sources for control groups. Thus, it was not feasible to determine the role of MAB via challenge studies using 3-day-old commercial broiler chickens. However, it was known from our previous work that 21-day-old broiler chickens were free from *Campylobacter*-specific antibody (32). Therefore, some broiler chickens were kept under laboratory conditions for 3 weeks and then used for the challenge experiments with *C. jejuni*. In experiment I, 3-day-old (group 1 [with anti-*Campylobacter* MAB]) and 21-day-old (group 2 [with no antibody to *Campylobacter*]) broiler chickens (originating from the same hatch group) were inoculated with a relatively low dose (3×10^2 CFU/bird) of *C. jejuni* strain S3B via oral gavage (Table 1). To isolate *Campylobacter*, cloacal swabs were taken 1 day before challenge and every other day after the challenge until the end of the experiment. Also, the day before challenge and on days 7, 15, 21, and 28 post-challenge, a blood sample was collected from each bird, and the level of serum immunoglobulin G (IgG) specific for *Campylobacter* was determined by enzyme-linked immunosorbent assay (ELISA) as described below. Experiment II was performed in a similar way to experiment I, except that *C. jejuni* strain 21190 was used to infect both 3-day-old and 21-day-old broiler chickens, and collection of blood samples was not performed at each sampling time (Table 1).

Challenge experiments using specific-pathogen-free (SPF) chickens. To further assess the protective effect of MAB on *Campylobacter* colonization using a more defined system, fertile eggs from SPF White Leghorn hens were purchased from Hy-Vac (Adel, Iowa) and hatched in the laboratory animal facilities of our department to establish SPF flocks free of *Campylobacter*. At the age of 15 weeks, the birds were divided into two flocks (A and B), each consisting of 15 pullets and four cockerels. The absence of colonization of these birds with *Campylobacter* was confirmed by cloacal swab culture. Once the hens started laying eggs, birds in flock A were orally inoculated with *C. jejuni* strain S3B (ca. 10^7 CFU/bird) at the age of 22 weeks, while birds in flock B remained uninfected to serve as negative controls. Starting from the second week after the inoculation, eggs were collected from both groups and tested for the levels of *Campylobacter*-specific antibody by ELISA as described previously (32). When significant levels (an ELISA optical density [OD] of >1.0) of anti-*Campylobacter* antibody were observed in eggs from flock A (ca. 4 weeks after inoculation), hatchlings were obtained from eggs of both flocks A and B. The young hatchlings were tested for the level of serum antibodies specific for *Campylobacter*. As expected, the chicks that hatched from the eggs of flocks A were positive for *Campylobacter*-specific MAB, while the majority of chicks from the eggs of flock B were negative for the specific MAB. A few chicks in flock B with a serum antibody level slightly higher than the cutoff value were excluded from the study. The hatchlings were housed in wire-floored cages and used for challenge experiments.

TABLE 2. Shedding percentages in 3-day-old SPF chickens challenged with *C. jejuni* strain S3B (experiment I) or 21190 (experiment II)

Expt and group	Challenge dose (CFU/bird)	Serum MAB level ($\pm$ SD) at challenge	% Shedding on days after challenge ^a :										
			2	4	6	8	10	12	14	21	28	35	42
I													
1	5×10^3	1.544 ± 0.258	17*	17*	25*	50*	75	100	100	100	100	NT ^b	NT
2	5×10^5	1.515 ± 0.194	25*	33*	59†	92†	100	100	100	100	100	NT	NT
3	5×10^3	0.201 ± 0.058	27*	36*	55*	70†	100	100	100	80	100	NT	NT
4	5×10^5	0.206 ± 0.086	73†	91†	91†	100†	100	100	100	100	100	NT	NT
II													
1	2×10^4	1.592 ± 0.231	0*	27*	45*	73	82	100	100	91	82	100	100
2	2×10^6	1.608 ± 0.217	18*†	55*†	64*†	100	100	100	100	100	91	100	100
3	2×10^4	0.195 ± 0.071	45†‡	82†‡	91†	91	100	100	100	72	72	100	100
4	2×10^6	0.206 ± 0.076	73‡	91‡	91†	100	100	100	100	82	72	91	91

^a Numbers in the same column of each experiment with different superscript symbols differ significantly (Fisher's exact test).

^b NT, not tested.

In experiment I, 3-day-old SPF chicks were divided into four groups, each of which consisted of 11 or 12 birds. Birds in groups 1 and 2 (12 birds/group) originated from *C. jejuni*-infected hens (flock A) and thus had high levels of *Campylobacter*-specific MAB (i.e., MAB⁺) (ELISA OD values are shown in Table 2). In contrast, birds in groups 3 and 4 (11 birds/group) were obtained from *Campylobacter*-free SPF hens (flock B) and were negative for any specific antibody to *Campylobacter* (i.e., MAB⁻) (ELISA ODs are shown in Table 2). To determine the effect of MAB on colonization with a homologous strain, the chickens in experiment I were challenged with strain S3B, which was used to infect the SPF hens in flock A. The chicks in groups 1 and 3 were infected with a relatively low dose (5×10^3 CFU/bird) of S3B, while those in groups 2 and 4 received a higher dose (5×10^5 CFU/bird) of the same strain via oral gavage (Table 2). Cloacal swabs were taken once every other day during the first 2 weeks after challenge and once per week for an additional 2-week period. The swabs were used to isolate *C. jejuni* from feces. In addition, serum samples were collected from the birds on the day before challenge and on days 6, 14, 21, and 28 postchallenge for detection of anti-*Campylobacter* antibody. Experiment II was performed in the same way as experiment I, except that *C. jejuni* strain 21190 was used to infect chicks to determine the effect of MAB on colonization with a heterologous strain (Table 2). The challenge doses were 2×10^4 CFU/bird for groups 1 and 3 and 2×10^6 CFU/bird for groups 2 and 4, respectively. The birds in experiment II were monitored for 2 weeks longer than those in experiment I (Table 2).

Isolation of *Campylobacter* from cloacal swabs. Cloacal swabs were collected in order to enumerate *Campylobacter* cells in feces. By visual estimation, approximately 100 mg of feces was suspended and serially diluted in MH broth. One hundred microliters of each fecal suspension was directly plated onto MH agar (Becton Dickinson) plates containing *Campylobacter*-specific growth supplements (Oxoid, Hampshire, United Kingdom). Following incubation for 2 to 3 days in a microaerobic atmosphere at 42°C, *Campylobacter*-like colonies were counted. The isolates were confirmed as *C. jejuni* by using a species-specific PCR test (40). Both the percentage of colonized chickens and the estimated numbers of organisms shed in feces (CFU/gram of feces) in colonized birds were calculated and used to determine the degree of protection conferred by MAB. The limit of sensitivity of this detection method as determined by recovery of the organism from artificially inoculated fecal samples was approximately 200 CFU/g of feces.

Antibody detection by ELISA. *Campylobacter*-specific IgG antibodies in serum and yolk samples were measured by using an indirect ELISA as described previously (32). In the experiments involving broiler chickens, ELISA plates were coated with outer membrane components of *C. jejuni* strain 33291, whereas the plates in the experiments involving SPF chickens were coated with outer membrane components of strain S3B. For detection of *Campylobacter*-specific IgM and IgA antibodies, serum samples were diluted 1:100 in a blocking buffer containing 2% bovine serum albumin and 2% skim milk in phosphate-buffered saline-0.1% Tween 20. Goat anti-chicken IgM and IgA conjugated to peroxidase (Bethyl Laboratories, Montgomery, Tex.) were diluted 1:500 in the same blocking buffer as that used for the secondary antibodies. After a 30-min reaction with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (6) [ABTS]-peroxidase substrate (Kirkegaard and Perry Laboratories, Gaithersburg, Md.), absorbance values at 405 nm were recorded using a spectrophotometer (Emax; Molecular Devices, Sunnyvale, Calif.). For serum IgG, a cutoff absorbance value of 0.270 was used

to indicate a positive sample. This number was calculated by adding 3 standard deviations to the mean absorbance value of negative controls, which were 115 serum samples from 3-week-old broiler chickens as determined previously (32).

Serum bactericidal assay. To assess bactericidal activity of sera obtained from 2-day-old broilers or SPF White Leghorn chicks, serum bactericidal assay was performed using *C. jejuni* strains S3B and 21190. Briefly, stationary-phase cultures were diluted in MH broth to give approximately 2×10^4 organisms/ml. Sera collected from two *Campylobacter*-negative SPF chickens were used as the complement source. The sera were confirmed to lack *C. jejuni*-specific antibodies by immunoblotting and ELISA, filter sterilized using a 0.45- μ m-pore-size filter, and kept frozen in small aliquots at -80°C until use. To evaluate the role of anti-*Campylobacter* MAB in complement-mediated killing of *C. jejuni*, serum samples from 10 2-day-old *Campylobacter*-free SPF White Leghorns chicks with or without MAB were pooled separately and used as the antibody source in the bactericidal assay (Table 2). Pooled sera derived from 10 2-day-old *Campylobacter*-free commercial broilers having high levels of anti-*Campylobacter* MAB were also used. These sera were inactivated at 56°C for 30 min prior to use to abolish complement activity. The bactericidal assay was performed in sterile microfuge tubes as described previously (32).

Genetic characterization of *Campylobacter* isolates. To verify that the *Campylobacter* isolates recovered from the experimental chickens originated from the inoculated strains, genotyping of *Campylobacter* isolates was performed by sequencing the *cmp* gene (which codes for the major outer membrane protein) as described previously (43). The *cmp* alleles in strains S3B and 21190 (GenBank accession numbers AY083463 and AF285141, respectively) are distinct from the known *cmp* alleles in other strains. The forward primer F3 (5'-ATGAACTA GTTAACTTAGTTTA-3') and reverse primer R3 (5'-GAATTTGTAAAGA GCTTGAAG-3') were used in PCR to amplify the *cmp* gene from various isolates. The amplified PCR products were purified using the QIAquick PCR purification kit (Qiagen) and subsequently sequenced. DNA sequences were determined using an automated DNA sequencer (model 377; Applied Biosystems) and analyzed with the Omega 2.0 sequence analysis software package (Oxford Molecular Group).

Statistics. Fisher's exact test was used to measure the significant differences in the percentage of colonized chickens at each sampling point between groups (Statistical Analysis System; SAS Institute, Inc.). One-way analysis of variance followed by a least-significant difference test was used to calculate the significant differences in shedding level (log transformed) and ELISA OD values at each sampling point among groups. A *P* value of ≤ 0.05 was considered significant.

RESULTS

***Campylobacter* colonization in commercial broilers.** The 3-day-old commercial broiler chicks inoculated with a low dose (3×10^2 CFU/bird) of strain S3B (experiment I, group 1) did not shed the organism until day 7 postchallenge (Table 1). Thereafter, the proportion of colonized chickens abruptly reached 100% and stayed at this level until the end of the experiment on day 28 postchallenge. The numbers of *C. jejuni*

cells ($\log_{10}$ CFU/gram of feces) shed in feces of colonized chickens ranged between approximately 5 and 7.5 U (results not shown). In contrast to the 3-day-old broilers, *Campylobacter* shedding was detected on day 3 postinoculation in 90% of the 21-day-old birds following challenge with the same dose (3×10^2 CFU/bird) of the same strain (Table 1, experiment I, group 2). The percentage of colonized chickens decreased substantially 3 weeks after inoculation in the chickens challenged at 21 days of age (group 2). However, the proportion of colonized chickens remained at 100% in the chicks challenged at 3 days of age (group 1) until the end of the experiment.

In experiment II, in which strain 21190 was used as the challenging strain, infection of 3-day-old chicks with a low dose (3×10^2 CFU/bird) of strain 21190 did not result in shedding by any of the birds throughout the experiment (Table 1, experiment II, group 1). However, 21-day-old birds (group 2) became colonized as early as day 3 postchallenge following inoculation with the same dose (3×10^2 CFU/bird) of strain 21190. The numbers of *C. jejuni* cells shed in feces of colonized chickens ($\log_{10}$ CFU/gram of feces) ranged between 4 and 5.8 U (data not shown). Similar to what was observed in experiment I, the proportion of colonized birds in the group infected at the age of 21 days decreased over time. In fact, none shed the organism in the feces after day 15 postchallenge in group 2 (Table 1).

Together, these observations indicated that, although there was strain-associated variability in *Campylobacter* colonization of chickens, older chickens (3 weeks old) were consistently more susceptible to *C. jejuni* infection than younger ones (3 days old). This age-related disparity in susceptibility to *Campylobacter* colonization might be due to the difference in antibody levels, because the 21-day-old birds had no antibodies to *Campylobacter*, while 3-day-old chicks had high levels of MAB against this organism (Table 1). However, other differences between 3-day- and 3-week-old birds, such as intestinal development stage and composition of microflora, could also be responsible for the observed difference in susceptibility to *Campylobacter* colonization. Thus, a defined challenge system with SPF chickens was further used to determine the role of MAB in protecting chickens from *Campylobacter* colonization.

Campylobacter colonization in SPF White Leghorn chickens. Three-day-old SPF birds with or without anti-*Campylobacter* antibodies were obtained in our laboratory (detailed in Materials and Methods). The chicks were challenged either with strain S3B, which was used to infect the laying hens to examine the homologous protection or with strain 21190 to assess the heterologous protection. As shown in Table 2, following infection with a relatively low dose (5×10^3 CFU/bird) of strain S3B, the percentages of chickens shedding *Campylobacter* in the MAB⁺ group (group 1) were consistently lower than those in the MAB⁻ group (group 3) on days 2, 4, 6, 8, and 10 postchallenge (Table 2, experiment I), although the differences were not statistically significant. When challenged with a higher dose (5×10^5 CFU/bird) of the same strain (S3B), a similar trend was observed, with significant differences being noted between the MAB⁺ group (group 2) and the MAB⁻ group (group 4) on days 2 and 4 postchallenge (Table 2, experiment I). A 100% shedding rate in all groups was reached on day 12 postinoculation and then stayed at the same level

until the end of the experiment on day 28 postchallenge. The mean number of organisms ($\log_{10}$ CFU/gram of feces) shed in feces of colonized birds ranged between 4.9 and 6.3 U. In contrast to the shedding percentage, there were no significant differences (data not shown) in the mean number of *C. jejuni* cells shed in the feces of the colonized birds in groups infected with the same dosage level of the organism, regardless of the status of *Campylobacter*-specific MAB.

When 3-day-old MAB⁺ and MAB⁻ SPF chickens were infected with a relatively low dose (2×10^4 CFU/bird) of strain 21190, the percentages of birds shedding the bacterium in the MAB⁺ group (group 1) were significantly lower than those seen in MAB⁻ group (group 3) on days 2, 4, and 6 postchallenge (Table 2, experiment II). Notably, on day 2 postchallenge, none of the chicks shed the bacterium in MAB⁺ group, although 5 (45%) of 11 birds in MAB⁻ group excreted the organism in feces. Slightly lower rates of shedding in MAB⁺ birds were also noticed on days 8 and 10 postchallenge than those observed in MAB⁻ birds. Following infection with a high dose (2×10^6 CFU/bird) of strain 21190, significantly lower rates of shedding occurred on days 2 and 4 postchallenge in the group with MAB⁺ birds (group 2) than the group with MAB⁻ birds (group 4). Also, the shedding percentage in the MAB⁺ group on day 6 postchallenge was considerably less, but not statistically significantly so, than that in the MAB⁻ group (Table 2, experiment II). By day 12 postchallenge, a 100% shedding rate was observed in all groups. The mean numbers of *C. jejuni* cells excreted in feces ($\log_{10}$ CFU/gram of feces) of the colonized chickens were between 5.4 and 6.7 U (results not shown). Similar to the challenge with strain S3B, there were no significant differences at a given sampling point in the mean numbers of *C. jejuni* shed in feces of colonized birds between groups challenged with the same dose of strain 21190, regardless of the MAB status of the birds. Together, the results from the challenge experiments using SPF chickens further confirmed that *Campylobacter*-specific MAB was associated with the partial protection against *C. jejuni* colonization in chickens in the first week of life.

Age-related difference in active immune response to *Campylobacter* colonization in broiler chickens. To determine the development of active antibody responses elicited by *Campylobacter* colonization in broiler chickens at different ages, serum IgG responses were measured following experimental infection of broilers with strain S3B. Comparison of serum IgG responses in chickens infected with S3B on day 3 or 21 of age indicated that active immune response to *Campylobacter* occurred sooner and at significantly higher levels in older chickens than in younger ones (Table 1, experiment I). A noticeable level of active IgG response was not detected until the fourth week following the infection of 3-day-old chicks, whereas the chickens inoculated at 21 days of age mounted a marked response to the infection as early as 1 week after the challenge. These results indicated that the dynamics of active antibody response in broiler chickens to *Campylobacter* colonization differs with the age of the birds at the time of challenge. In chickens challenged on day 21 of age, the decreases in the percentage of birds colonized with either *Campylobacter* strain seemed to be correlated with increasing anti-*Campylobacter* serum IgG antibody titers, suggesting a potential role of active

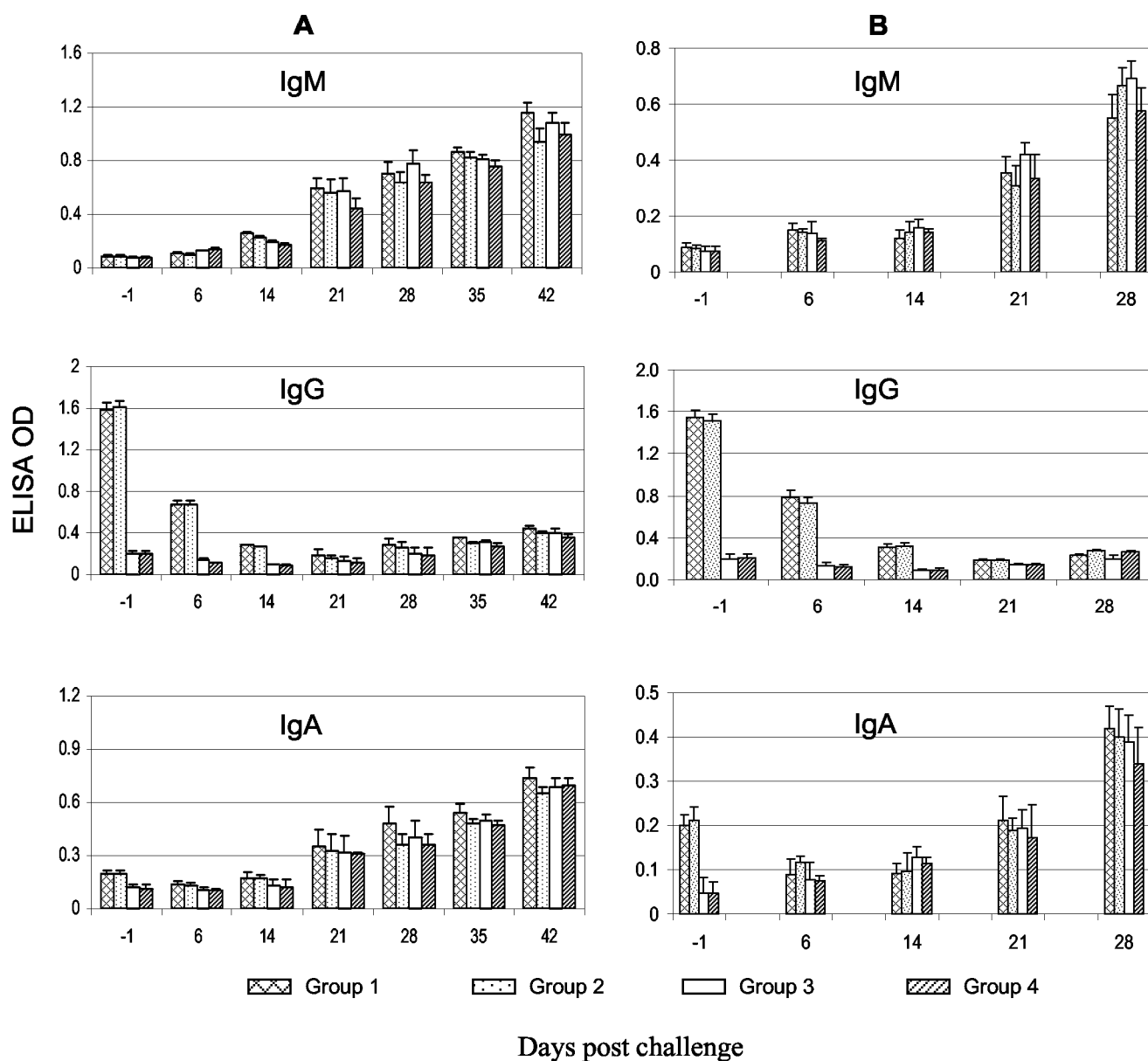


FIG. 1. Levels of *Campylobacter*-specific serum antibodies before and after challenging of 3-day-old SPF chickens with *C. jejuni* strain 21190 (A) or S3B (B). Each bar represents the arithmetic mean OD value $\pm$ the standard error of the mean for 5 to 10 serum samples.

antibody response in reducing colonization of chickens by *C. jejuni*.

Effect of MAB on active immune responses to *Campylobacter* colonization in SPF White Leghorns. To determine if anti-*Campylobacter* MAB affects the development of active immune response to *Campylobacter* colonization, the presence of *Campylobacter*-specific antibodies in serum samples was determined before and after challenging of 3-day-old White Leghorn chickens (Fig. 1). As expected, 2-day-old chicks from eggs laid by *Campylobacter*-infected hens (groups 1 and 2) had high levels of maternally acquired IgG antibodies prior to the challenge, whereas chicks from eggs of *Campylobacter*-free SPF hens (groups 3 and 4) were negative for this class of anti-*Campylobacter* antibody. The level of maternal IgG antibody

continually decreased on days 6, 14, and 21 postchallenge (Fig. 1). The maternal transfer of IgM and IgA antibodies from hens to the progeny was negligible (Fig. 1), although very low levels of maternal IgA were detected in chicks derived from *Campylobacter*-infected parent flock (groups 1 and 2). With respect to the production of active humoral immune response to *C. jejuni* colonization, only weak IgM and IgA responses were elicited during the first 2 weeks postinfection, followed by a gradual increase in the antibody levels during the next 2 weeks (Fig. 1). A low level of active IgG antibody response was observed only after 4 weeks postinfection, which is consistent with the slow development of active IgG antibody response in experimentally infected 3-day-old broiler chickens (Table 1, experiment I, group 1). Despite some variations in the IgM and IgA levels in

TABLE 3. Bactericidal activity of *Campylobacter*-specific maternal antibody on *C. jejuni* strains

Strain	Mean % reduction in CFU by maternal antibodies (SD)		
	SPF ^a	SPF ^{-b}	Broiler ^{+c}
S3B	100 (0)	0.8 (1.4)	22 (2.6)
21190	-1.5 (10.5)	-1.1 (7.9)	46 (9.2)

^a Pooled sera from 2-day-old *Campylobacter*-free MAB⁺ SPF White Leghorns whose hens were experimentally infected with strain S3B.

^b Pooled sera from 2-day-old *Campylobacter*-free MAB⁻ SPF White Leghorns whose hens were *Campylobacter* free.

^c Pooled sera from 2-day-old *Campylobacter*-free commercial broilers having high levels of anti-*Campylobacter* MAB.

active antibody response to *C. jejuni* colonization, the levels of antibodies at a given sampling point for these immunoglobulin isotypes did not differ significantly regardless of the MAB status of the birds at the time of challenge. The dynamics of active immune responses were similar in birds challenged with strain S3B or 21190 (Fig. 1). These findings demonstrated that, following *Campylobacter* infection, the IgA and IgM responses occurred earlier and at much higher levels than the IgG response and that the presence of MAB did not significantly affect the development of active antibody response to *C. jejuni*.

In vitro bactericidal effect of MAB on *Campylobacter*. When heat-inactivated sera from 2-day-old broilers (with high MAB level) were incubated with *C. jejuni* strain S3B in the presence of an exogenous complement source, a 22% reduction in CFU counts was observed. However, the same sera had a substantially higher rate (46%) of killing of strain 21190 (Table 3). On the contrary, sera obtained from MAB⁺ White Leghorn chicks, which were the progeny of S3B-infected hens, killed 100% of the homologous strain S3B in the presence of complement but had no effect on the heterologous strain 21190 (Table 3). These results indicated that chicken MAB was highly effective and strain specific in complement-mediated killing of homologous *Campylobacter* strains in vitro. These results also showed that specific maternal antibodies from different sources had various in vitro killing effects on different *C. jejuni* strains. Sera from MAB⁻ White Leghorn chicks, however, did not result in any reduction in CFU counts of either strain, further indicating the specificity of complement-mediated killing. Although the sera from the MAB⁺ SPF chickens had no bactericidal effect on strain 21190, the chickens were partially protected against colonization by strain 21190 (Table 2), suggesting either that the in vitro antibody-dependent complement-mediated killing is not an indicator for the in vivo protection or that the antibody-dependent complement-mediated killing is not the only mechanism mediating the protective immunity.

Genotyping of *Campylobacter* isolates from the chickens. To confirm that the isolates recovered from the experimental chickens were derived from the inoculated S3B and 21190 strains, representative *Campylobacter* isolates obtained at different sampling points were analyzed by PCR and sequencing of the *cmp* gene, which is diverse among different strains (43). With respect to each challenge experiment involving different *C. jejuni* strains, *Campylobacter* isolates recovered from the chickens showed a *cmp* gene sequence indistinguishable from that of the challenge strain (S3B or 21190; data not shown).

This result indicated that there were no extraneous sources of *Campylobacter* in the experimental chickens.

DISCUSSION

Results from the present study demonstrated that colonization of chickens with *C. jejuni* varies with different doses and strains and is affected by the age of the host at the time of oral challenge. These results are in agreement with findings obtained in previous studies (19, 39). More importantly, our results indicate for the first time that *Campylobacter*-specific MAB in young chickens confers a partial protection against colonization upon challenge with both homologous and heterologous *C. jejuni* strains. This conclusion is supported by two complementary observations. First, older broiler chickens (21 days old) that were negative for *Campylobacter*-specific antibody were much more susceptible to colonization with *C. jejuni* than were 3-day-old broilers that had high levels of anti-*Campylobacter* MAB (Table 1). Second, 3-day-old SPF White Leghorn chicks with high levels of *Campylobacter*-specific MAB showed significantly less colonization during the first week after challenge than did 3-day-old SPF chicks without *Campylobacter*-specific MAB (Table 2). The demonstrated protectiveness of MAB and its high prevalence in young chickens as shown in previous studies (7, 32, 33) suggest that anti-*Campylobacter* MAB is a contributing factor for the lack of *Campylobacter* colonization in young broiler birds under commercial production. It should be pointed out that the protection conferred by MAB is partial and that there may be other unidentified factors that also contribute to the lack of *Campylobacter* infection in young broiler flocks.

Antibodies present in the systemic circulation of breeder chickens can be readily transferred to the egg yolk. During hatching, the yolk immunoglobulins are absorbed into the embryonic circulation as MAB. The transferred maternal antibodies are mainly of the IgG class, and minimal passage of IgA and IgM from breeders to progenies occurs (20, 21, 29). In the present study, we observed similar findings regarding the maternal transfer of anti-*Campylobacter* antibodies. The predominant immunoglobulin class detected in 2-day-old chicks hatched from eggs from *Campylobacter*-infected hens was IgG, although maternal transfer of small amounts of IgA antibody also occurred (Fig. 1). Chicks hatched from eggs from *Campylobacter*-free SPF hens lacked detectable levels of specific anti-*Campylobacter* antibodies. Due to the fact that transudation of circulating maternal IgG antibody to the intestines of chicks only occurs during the first week of life (20, 21, 36), it was not surprising that the protective activity of MAB against *Campylobacter* colonization was only observed in the first few days after the challenge (Table 2).

Maternal antibodies in young birds are known to be protective against infectious agents, including viruses, bacteria, and parasites, which colonize the intestinal epithelia of poultry during the early stage of their lives (14, 15, 36, 38). Studies performed with mammalian hosts, including humans, monkeys, mice, ferrets, and rabbits, indicated that passively acquired *Campylobacter*-specific MAB or active anti-*Campylobacter* immunity induced by vaccination or natural exposure was protective against subsequent infection with *C. jejuni* (2, 4, 6, 9, 28, 30, 31). In humans, breast feeding of newborns was

associated with decreased numbers of cases and duration of diarrhea caused by *C. jejuni*, thereby indicating the protective effect of MAB (30). Also, intraperitoneal vaccination of female mice with killed whole-cell *C. jejuni* resulted in transfer of MAB to the pups, which were protected from infection with the homologous strain by oral challenge on days 4 to 6 after birth (9). The exact mechanisms by which MAB confers protection against *Campylobacter* colonization are unknown. It is possible that the MAB in the intestinal tract of young chickens during the first week of age may block bacterial adhesins on the surface of *Campylobacter* cells, agglutinate the organism, interfere with the chemotaxis toward receptor sites, or eliminate the organism via complement-mediated killing. We found that *Campylobacter*-specific MAB was also effective against challenge with the heterologous *Campylobacter* strain 21190, to which the breeders were not exposed. This observation suggests that naturally occurring anti-*Campylobacter* MAB in young broiler chickens on farms may confer a broad protection against different strains of *C. jejuni*.

The development of active humoral immune responses following *C. jejuni* infection of 3-day-old chickens was also monitored (Fig. 1). A slowly progressive humoral immune response was elicited against *C. jejuni* colonization, in which IgM and IgA responses preceded that of IgG. Despite some variations, the level of an immunoglobulin isotype did not differ significantly between groups at a given sampling point, regardless of the MAB status of the birds and the challenge dose (Fig. 1). These observations suggested that the presence of high levels of MAB in young chickens did not interfere with the development of a humoral immune response to *Campylobacter* colonization, in agreement with a previous observation (42). However, the effect of MAB on the development of active local immune responses in the intestinal tract was not investigated in this study and remains to be determined. The lack of interference by MAB on the development of an active systemic immune response to *Campylobacter* may have positive implications for the vaccination of young chickens, a possibility which needs to be explored in future studies. Our observation regarding the kinetics of active immune responses to *C. jejuni* colonization was consistent with previous findings reported by other investigators (7, 24, 27), in which circulating IgM and IgA antibodies occurred much earlier than the IgG response in chickens infected with *C. jejuni*.

After inoculation with *Campylobacter*, older (21-day-old) broilers mounted a substantially higher level of humoral immune response within a shorter period than did the young broilers (3 day old) (Table 1). Widders et al. (42) also reported the production of poor immune responses in day-old chicks immunized with flagellin protein, whereas 24-day-old chickens produced significantly higher levels of serum IgG antibodies specific for the flagellin of *C. jejuni*. The differences in the levels of active immune responses in different age groups of chickens are likely to have resulted from the fact that the immune system of newly hatched chicks is only partially developed (18, 34). Interestingly, the percentage of colonized chickens dropped dramatically 2 to 4 weeks after challenge of 21-day-old broilers, although such marked reductions were not noticed following infection of 3-day-old chicks (Table 1). Others also noted the shorter duration of shedding of *Campylobacter* in both experimentally and naturally infected older

chickens compared with younger birds (19, 22). The reduction in colonization was accompanied by the formation of anti-*Campylobacter* IgG antibodies in chickens infected with strain S3B (Table 1). Similar observations were also found in a previous study, which showed that the decrease in the number of viable *Campylobacter* cells in feces was associated with the development of specific serum agglutinin antibodies in experimentally infected chickens at the age of 3 months but not in chickens infected at 3 days or 5 weeks of age (19). However, a correlation between the clearance of *Campylobacter* and the development of specific IgG was not apparent in the 21-day-old chickens inoculated with strain 21190, since the birds stopped shedding of the organism before a substantial IgG response was observed (Table 1). Nonetheless, this does not exclude the possibility that an early IgA or IgM response contributed to the clearance of *Campylobacter* from the chickens challenged at 21 days of age with strain 21190. The role and nature of active immune responses in protecting chickens from *Campylobacter* colonization remain to be determined.

Previously, we showed that *Campylobacter*-specific MAB was active in killing *C. jejuni* in a strain-specific manner (32). In this study, MAB from commercial broilers, whose parents are likely to be infected with multiple strains of *C. jejuni* under natural conditions, were effective in the killing of strain 21190 but were less effective in the killing of strain S3B (Table 3). In contrast to the commercial broiler serum, MAB in 2-day-old White Leghorn chicks, whose SPF parents were artificially infected with S3B, completely killed the homologous strain (S3B) in the presence of complement but had no effect on the heterologous strain 21190 (Table 3). These results further indicate the strain-dependent specificity of complement-fixing MAB. It is likely that the *Campylobacter* surface antigens that induce complement-fixing antibodies are antigenically variable among different strains, resulting in variation in the MAB-dependent complement-mediated killing of different isolates. Despite the great differences in susceptibility to the in vitro bactericidal effects of MAB, both S3B and 21190 showed similar colonization characteristics in 3-day-old White Leghorns (Table 2). This suggests that the complement-fixing ability of MAB is not the only factor contributing to the protection. Thus, other unknown mechanisms are likely also responsible for the partial protection against *Campylobacter* colonization in young chickens conferred by MAB.

In conclusion, the results from this study demonstrated a partial protective role of *Campylobacter*-specific MAB in young chickens. As suggested by Newell and Wagenaar (26), the *Campylobacter* source for flock infection under commercial production is likely at a low dose and stressed condition. Therefore, the actual protection conferred by MAB in the natural environment may be higher than that observed with laboratory challenge studies. Although the MAB protection is short lived and does not last long enough to cover the entire production period (6 to 7 weeks) of broiler chickens, the observed protective activity of anti-*Campylobacter* MAB in this study indeed provides new evidence that boosting immunity may be a feasible approach for controlling *Campylobacter* at the farm level, as was suggested by other studies (7, 26, 27, 42). However, difficulties in developing effective vaccines are expected due to the short life span of broiler chickens and the fact that *Campylobacter* is nonpathogenic to the poultry host

and mainly resides in the intestinal tract. To overcome these difficulties, future efforts are needed to understand the protective immune mechanisms and develop innovative approaches for vaccine design and delivery.

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