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PERGAMON

Comparative Immunology, Microbiology
& Infectious Diseases 25 (2002) 127–138

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The effect of immunosuppression on protective immunity of turkey poults against infection with turkey coronavirus

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Received 30 March 2001; received in revised form 12 September 2001; accepted 26 September 2001

Abstract

The objective of the present study was to evaluate the protective effect of humoral and cellular immunities on turkeys infected with turkey coronavirus (TCV). Two trials were conducted with two separate hatches of turkey poults. Turkeys were experimentally immunosuppressed with cyclosporin A (CsA) or cyclophosphamide (CY) and infected with TCV. Prior to infection, treatment with CsA selectively suppressed T cell activity as revealed by 2–3 fold decreased ($p < 0.1$) lymphocyte proliferation responses to a T cell mitogen, concanavalin A (Con A). Treatment with CY mainly induced B cell deficiency as indicated by significant reductions ($p < 0.05$) in antibody responses to sheep erythrocytes 7 days after injection. Body weight gain of turkeys treated with CY was significantly lower ($p < 0.05$) than that of untreated turkeys at 9 days post-infection (PI). Turkeys treated with CY had 1–2 fold higher immunofluorescent antibody assay (IFA) scores for TCV antigens ($p < 0.05$) in the intestine than untreated turkeys at 9 or 14 days PI. These results suggested that humoral immunity against TCV infection may be important in turkeys. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Cyclophosphamide; Cyclosporin A; Enteritis; Immunity; Immunosuppression; Turkey coronavirus

Résumé

L'objectif de cette étude était d'évaluer l'effet protecteur des immunités humorales et cellulaires sur des dindes infectées par le coronavirus (TCV) des dindes. Deux essais ont été conduits sur deux différentes portées de dindes. Les dindes étaient immunosupprimées de façon expérimentale avec de la cyclosporine A (CsA) ou avec du cyclophosphamide (CY) et infectées par le TCV. Les effets des

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traitements avec l'immunosuppression expérimentale sur l'infection du TCV étaient déterminées par le gain de poids corporel et par l'analyse d'anticorps immunofluorescents (IFA) pour l'antigène du TCV dans l'intestin. Avant l'infection, le traitement par la cyclosporine a sélectivement diminué l'activité des cellules T comme cela a été révélé par une diminution de 2 à 3 fois de la réponse à un mitogène, la concanavaleine A. Le traitement avec du CY a principalement provoqué une déficience de la cellule B comme indiqué par le déclin significatif ($p < 0.05$) plus bas que celui des dindes non traitées à 9 jours après l'infection. Les dindes traitées par le cyclophosphamide présentaient des taux d'immunofluorescence dans l'intestin vis à vis du coronavirus de la dinde 1 ou 2 fois plus élevés que les dindes non traitées 9 et 14 jours après l'infection. Ces résultats suggèrent que l'immunité humorale contre l'infection TCV pourrait être importante pour les dindes. © 2002 Elsevier Science Ltd. All rights reserved.

Mots-cléf: Cyclophosphamide; cyclosporine A; enteritis; immunité; immunosuppression; coronavirus des dindes

1. Introduction

Turkey coronaviral enteritis is an acute and highly infectious disease, which has contributed to significant economic losses in the US turkey industry, particularly in Indiana and North Carolina, for the last decade [1,2]. The clinical signs (diarrhea, decreased body weight gain, inappetence, and ruffled feathers) of the disease usually appear in turkey poults at 7–28 days of age. The morbidity is usually high and the mortality varies. In an epidemiological study, turkey coronavirus (TCV) was identified in 63% of poult enteritis mortality syndrome (PEMS)-affected flocks in North Carolina (Guy and Barnes, unpublished data). The coronavirus isolated from affected turkey flocks was found to be antigenically cross-reactive with antibodies specific to infectious bronchitis virus (IBV), a chicken coronavirus [3].

Once coronaviral enteritis is introduced into areas with high concentrations of turkeys, it is not easily eliminated and is encountered frequently in turkey flocks [4,5]. Treatments of the disease are often unsuccessful and there are currently no effective vaccines or medications to prevent viral infection. It is very likely that the disease will continue to negatively impact the turkey industry until effective strategies for prevention and control of the disease are established.

Infections of host animals with other coronaviruses, bovine coronavirus (BCV) [6], transmissible gastroenteritis virus (TGEV) [7], or IBV [8], stimulate protection of animals against subsequent challenge with the same virus. Although the detailed mechanisms for such protection are not clear, the protective immunity induced by the corresponding coronavirus during the primary infection is essential. Both serum IgG and fecal IgA antibody responses increased following secondary exposure with BCV. The virus-neutralizing antibody responses in serum were also increased following secondary challenge with BCV [6,9]. The presence of high numbers of antibody-secreting cells and high lymphocyte proliferation activity was correlated with protection against subsequent challenge of TGEV [7]. Antibody responses to IBV were detected in serum, tracheal washes, and tears of chicken infected with IBV. Vaccination studies on IBV generally correlated neutralizing serum antibody responses to protection [10].

Turkey flocks recovered from coronaviral enteritis (bluecomb disease) may develop

life-long immunity against challenge infection [11]. However, the nature of such resistance is not completely understood. Serum from recovered turkeys could neutralize the infectivity of TCV-containing intestinal contents [11,12]. Serum antibodies to TCV were detectable by immunofluorescent antibody assays (IFA) from 9 to 160 days post-infection (PI) [13]. Antibodies to TCV in the intestine and bile were detectable by immunodiffusion test [14] or a sandwich IFA [15] up to 6 months PI. The lymphocyte proliferation responses to mitogen was reported to be enhanced in turkeys infected with TCV [16]. In addition, sequential kinetic studies of humoral and cellular immune responses of turkey poult infected with TCV isolated in our laboratory have shown that activation of specific immune responses (humoral and cellular) are associated with the decrease of TCV antigens in turkey intestines (unpublished data). However, which immunity, humoral or cellular, provides the necessary protective immunity for turkey poult against infection with TCV is not clear. Such information is critical in developing strategies for the diagnosis, control, and prevention of turkey coronaviral infection in turkey flocks.

The purpose of the present study was to determine the protective immunity against turkey coronaviral infection in turkeys infected with TCV. Turkey poult were treated with selective T cell immunosuppressant (cyclosporin A, CsA) or B cell immunosuppressant (cyclophosphamide, CY) and subsequently challenged with TCV. The severity of infection was evaluated and compared.

2. Materials and methods

2.1. Turkeys

One-day-old turkey poult (British United Turkey of American, BUTA) of both sexes were obtained from Perdue Farm (Washington, IN). They were free of recognized pathogens for turkeys, including TCV. They were housed in Horsfall–Bauer isolation units under negative-pressure ventilation. Food and water were provided *ad libitum*.

2.2. Virus preparation

TCV was isolated from intestines of turkey poult with outbreaks of acute enteritis. Affected intestines were homogenized with 5-fold volume of phosphate-buffered saline (PBS) solution, clarified by centrifugation at $3000 \times g$ for 10 min, and filtered through 0.45 and 0.22 μm membrane filters (Millipore, Bedford, MA 01730). Twenty-two day-old embryonated turkey eggs were inoculated with 200 μl of the filtrate via amniotic sac route. Embryo intestines were harvested in 3 days. Intestines of serially propagated TCV-infected turkey embryos were homogenized with 5-fold volume of PBS buffer. The mixture was centrifuged at $3000 \times g$ for 20 min. The supernatant was used as the inoculum for experimental infection of turkey poult. The inoculum further examined by electron microscopy (EM) and immuno-EM contained TCV but not other pathogens.

2.3. Experimental design

Two trials with two separate hatches of turkey poult were conducted. The experimental

Table 1
Experimental design

Treatment	Trial	Days of age						Days PI					
		1	2	3	4	7	10	10 h	3	6	9	12	14
CsA ^a	1					x	x		x	x			
	2				x	x	x		x	x	x	x	
CY ^b	1,2	x	x	x									
SRBC ^c	1,2						x						
Con A ^d	1,2						x						
TCV ^e	1,2						x						
BW ^f	1,2						x					x	
IFA ^g	1							x	x		x		
	2								x				x

^a Turkey poult were treated with CsA, 100 mg per kg body weight, at every third day as indicated.

^b Turkey poult were treated with CY, 5 mg per turkey poult per day on the first 3 days post-hatching.

^c Turkey poult were intramuscularly injected with SRBC at 10 days of age. Antibody titers to SRBC were determined by hemagglutination at 7 days after injection.

^d Lymphocyte proliferation responses to Con A.

^e Turkey poult were infected with TCV at 10 days of age.

^f Body weight (BW) gain was determined from 10 (prior to infection) to 19 days of age.

^g IFA for TCV antigen in the intestine.

design of trials 1 and 2 is outlined in Table 1. In trial 1, 110 turkey poult were randomly separated into four groups. Thirty turkey poult in group I were treated with CsA at 7 days old and every third day thereafter. Thirty turkey poult in group II were treated with CY at 1, 2, and 3 days old. Thirty turkey poult in group III were not treated with any immunosuppressant. At 10 days old, five turkey poult from each of these groups were used for evaluation of humoral antibody responses to sheep red blood cells (SRBC). Another five turkey poult from each of these groups were used for evaluation of cellular immunocompetence by *in vitro* lymphocyte proliferation assay. The remaining turkey poult in each of the above groups were infected with TCV at 10 days old. Twenty turkey poult in group IV were neither treated with immunosuppressant nor infected with TCV and served as normal control group. Five poult from each of these four groups were weighted at 10 days of age (prior to infection). The same five poult were weighted at 19 days of age (termination of the experiment). The body weight gains of individual poult were calculated. Five poult were randomly selected from each group and necropsied at 10 h, 3, and 9 days PI. Intestines were collected from each bird and the duration of TCV in the intestine was examined by IFA.

In trial 2, 75 turkey poult were randomly separated into three groups. Twenty-five turkey poult in group I were treated with CsA at 4 days old and every third day thereafter. Twenty-five turkey poult in group II were treated with CY at 1, 2, and 3 days old. Twenty-five turkey poult in group III were not treated with any immunosuppressant. At 10 days old, five turkey poult from each group were used for evaluation of humoral antibody responses to SRBC. Another five turkey poult from each group were used for evaluation of cellular immunocompetence by *in vitro* lymphocyte proliferation assay. The remaining

turkey poults in each group were infected with TCV at 10 days old. Five poults from each of these four groups were weighted at 10 days of age (prior to infection). The same five poults were weighted at 19 days of age (termination of the experiment) and the body weight gains of individual poults were calculated. Five poults were randomly selected from each group and necropsied at 3 and 14 days PI. Intestines were collected from each bird and the duration of TCV in the intestine was examined by IFA.

2.4. Suppression of T cells with cyclosporin A

CsA (BIOMOL, Plymouth Meeting, PA) was dissolved in mineral oil and administered intramuscularly every 3 days as previously described [17]. The dose was 100 mg per kg of body weight. Turkey poults were given the first dose at 7 days old (3 days prior to challenge infection) in trial 1 and at 4 days old (6 days prior to challenge infection) in trial 2 and received subsequent doses every third day thereafter throughout the experimental period.

2.5. Suppression of B cells with cyclophosphamide

CY (Sigma, St. Louis, MO) was administered intraperitoneally at a daily dose of 5 mg per turkey poults for the first 3 days post-hatching as previously described [18].

2.6. Antibody response to sheep red blood cells

Ten-day-old turkey poults from groups treated with CsA or CY and untreated groups were injected intravenously with 1 ml of 7% saline suspension of SRBC (Sigma) prior to infection with TCV. Blood samples were collected at 7 days after injection. Anti-SRBC antibody titers were quantified by hemagglutination assay [19]. The titer was expressed as the \log_2 of the reciprocal of the highest dilution in which visible agglutination was observed.

2.7. Lymphocyte proliferation of whole blood cells

Blood was obtained from 10-day-old turkey poults from groups treated with CsA or CY and untreated groups prior to infection with TCV. Heparinized blood was diluted at 1:40 and dispensed in a 96-well plate. To each well of three sequential triplicate cultures were added 100 μ l of RPMI medium without mitogen or containing 40 μ g/ml of concanavalin A (Con A) (Sigma). Cell cultures were incubated at 41 °C for 48 h in a humidifying air atmosphere at 5% CO₂. Cultures were pulsed with 1 μ Ci of ³H-thymidine in 50 μ l of RPMI medium per well for the final 5 h of incubation. Cells were harvested onto glass filter papers with an automatic cell harvester (Packard, Downers Grove, IL). The counts per minute (CPM) values of individual cell samples were measured in a Packard TopCount Scintillation Counter (Model B9904, Packard). The CPM values of the triplicate cultures within each sample were averaged. Stimulation index (SI) was calculated by dividing the average CPM value in mitogen-stimulated cultures by the average CPM value in unstimulated culture.

2.8. Immunofluorescent antibody assay for TCV antigen in the intestines

The jejunum and ileum distal to the Merckel's diverticulum from each turkey poult at each time interval was obtained for IFA. The IFA used for detection of TCV was described previously [13]. Briefly, intestines were frozen immediately at -20°C and embedded in Tissue-Tek O.C.T. compound (Miles Laboratories, Elkhart, IN). Intestinal sections were cut serially with a cryostat into $6\ \mu\text{m}$ thickness pieces and fixed in absolute acetone for 10 min. Tissue sections were incubated with turkey antisera specific for TCV [2,3] at a dilution of 1:40 in dilution buffer containing 150 mM phosphate buffer, 0.85% NaCl, 1% BSA, and 0.02% Tween-20 in a humidifying chamber at room temperature for 30 min. After washing three times with PBS buffer, intestinal sections were incubated with fluorescein isothiocyanate (FITC) conjugated goat anti-turkey IgG (H + L) antibodies (Kirkegaard and Perry Laboratories, Gaithersburg, MD) at a dilution of 1:40 in dilution buffer at room temperature for 30 min. Slides were coverslipped and examined on a fluorescent microscope (Nikon, Tokyo, Japan). The IFA staining positive for TCV antigen appeared in the cytoplasm of enterocytes. The intensity of IFA staining was scored on a scale of 1–4 with 4 being the most intense staining. The distribution of area with positive staining within section was categorized into diffuse, multifocal, and focal and scored as 3, 2, and 1, respectively. The scores of intensity and area distribution were added. The final value indicated the overall amount of staining in a section, with higher value indicating more positive staining. The consistency of IFA is assured by inclusion of positive controls with intestinal sections from turkey embryos infected with TCV and negative controls with intestinal sections from normal turkey embryos in the assay.

2.9. Statistical analysis

Calculated means from each group were compared by one-way analysis of variance (ANOVA). A pooled t-test was used to determine significant difference at $p < 0.05$ or $p < 0.1$.

3. Results

The effects of treatments with CsA or CY on antibody response to SRBC and lymphocyte proliferation response of whole blood cells to Con A in 10-day-old turkey poults prior to infection with TCV are shown in Table 2. Turkeys treated with CY had significantly lower ($p < 0.05$) antibody response to SRBC than untreated controls in both trials. The differences in antibody response to SRBC between turkeys treated with CsA and untreated controls are not statistically significant in both trials. There were considerable variations in the lymphocyte proliferation responses. Nevertheless, the lymphocyte proliferation responses of turkeys treated with CsA were significantly lower ($p < 0.1$) than that of untreated controls in both trials. The lymphocyte proliferation responses of turkeys treated with CY were significantly lower ($p < 0.1$) than that of untreated controls in trial 1 but not in trial 2.

Effects of CsA- or CY-induced immunosuppression on body weight gains of turkeys experimentally infected with TCV at 10-day-old are shown in Table 3. The body weight

Table 2

Effect of CsA and CY on humoral and cellular immune responses of turkeys (* the difference between groups treated with CsA or CY and untreated group is significant ($p < 0.1$); ** the difference between groups treated with CsA or CY and untreated group is significant ($p < 0.05$))

Treatment	Antibody response ^a	Mitogenic response ^b
Trial 1		
None	3.20 ± 0.45	140.01 ± 101.98
CsA ^c	3.00 ± 0.00	61.37 ± 37.80*
CY ^d	2.25 ± 0.50**	35.46 ± 7.37*
Trial 2		
None	5.40 ± 2.30	60.89 ± 26.63
CsA	3.00 ± 1.00	21.04 ± 4.82**
CY	0.00 ± 0.00**	54.46 ± 24.31

^a Turkey poults were intramuscularly injected with SRBC cells at 10 days old prior to infection with TCV. Data are expressed as mean ± standard deviation (SD) of hemagglutination titers (\log_2) from five turkey poults in each group at 7 days after injection.

^b Data are expressed as mean ± SD of stimulation index from five turkey poults in each group at 10 days old.

^c Turkey poults were treated with CsA, 100 mg per kg body weight, at 7 days old in trial 1 or 4 days old in trial 2 and every third day thereafter.

^d Turkey poults were treated with CY, 5 mg per turkey poult per day on the first 3 days post-hatching.

gains of all treated or untreated groups were significantly decreased ($p < 0.05$) after infection with TCV as compared with that of turkeys neither treated with any immunosuppressant nor infected with TCV. The body weight gain of turkeys treated with CY was significantly lower ($p < 0.05$) than that of untreated controls after experimental infection with TCV in both trials. The differences in body weight gain after experimental infection with TCV between turkeys treated with CsA and untreated controls were not statistically significant in both trials.

Effects of CsA- or CY-induced immunosuppression on the severity of TCV infection in the intestine of turkey poults are shown in Table 4. At 10 h PI, the scores on IFA staining for TCV antigen were below 3.2 in all infected groups and the differences among groups treated or untreated with immunosuppressant were not significant. At 3 days PI, the scores on IFA staining for TCV antigen were above 5.6 in all infected groups and the differences among groups treated or untreated with immunosuppressant were not significant. At 9 (trial 1) and 14 (trial 2) days PI, turkeys treated with CY had significantly higher ($p < 0.05$) scores on IFA staining for TCV antigen than untreated turkeys and the differences of scores between turkeys treated with CsA and untreated controls were not statistically significant. Non-infected control turkeys remained negative for TCV antigen in intestines by IFA throughout the entire experimental period.

4. Discussion

CsA is a selective T cell immunosuppressant [17,20,21]. Activation of T cells by mitogen or by mixed lymphocyte reaction is inhibited by CsA. This compound exerts

Table 3

Effect of CsA and CY on body weight gain of turkey poult experimentally infected with TCV

Treatment	TCV ^a	Body weight gain ^b
Trial 1		
None	–	369.20 ± 47.74 ^c
None	+	211.20 ± 29.43 ^c
CsA ^d	+	195.20 ± 20.38 ^c
CY ^e	+	140.60 ± 42.59 ^c
Trial 2		
None	+	291.80 ± 64.16 ^c
CsA	+	262.40 ± 78.41 ^c
CY	+	172.80 ± 46.82 ^c

^a Turkey poult were infected with TCV at 10 days old.

^b Data are expressed as mean ± SD of body weight gains from five turkey poult in each group from 10 to 19 days old.

^c Values within a column of each trial with different superscript are significantly different ($p < 0.05$).

^d Turkey poult were treated with CsA, 100 mg per kg body weight, at 7 days old in trial 1 or 4 days old in trial 2 and every third day thereafter.

^e Turkey poult were treated with CY, 5 mg per turkey poult per day on the first 3 days post-hatching.

its effect by acting as a prodrug, only becoming active when complexed to endogenous, intracellular receptor, and the resulting complex blocks a late stage in the signalling pathway initiated by the T cell receptor [20]. The blocking effect leads to a failure of transcriptional activation of T cell genes, such as those encoding the cytokines, particularly interleukin 2 (IL-2). As a consequence, all cellular immune responses driven by IL-2 are influenced.

As expected, treatment of turkeys with CsA caused a deficiency in lymphocyte proliferation response to Con A while it had a limited effect on antibody response to SRBC. The effect of treatment with CsA on infection of turkey poult with TCV could not be determined in the present study. However, the results could not rule out the involvement of cellular immunity against TCV infection. The T cell function was not completely inhibited by CsA as revealed by considerable lymphocyte proliferation responses to Con A after the treatment. It is possible that the protection effect provided by T cells was very efficient and not affected by partial inhibition, or the effect of partial inhibition of T cell function by CsA on infection of turkey poult with TCV was not detectable by the evaluation methods in the present study.

The considerable variations in the lymphocyte proliferation responses between individual birds may be caused by variation in the number of lymphocytes in blood samples at the time of sample collection from different birds or resulted from the genetic diversity. The ability of chicken lymphocytes to respond to Con A was under genetic control [22]. Lymphocytes from chickens with genetic difference had different proliferative response to Con A. In addition, the genetic selection of turkeys for increased body weight or egg production affected the mitogenic responses of turkey lymphocytes to Con A [23,24]. The turkeys in the present study were obtained from commercial turkey flocks originate from outbred breeder stocks and had diverse genetic backgrounds. Similar

Table 4

Effect of CsA and CY on the severity of TCV infection as determined by immunofluorescent staining intensity and distribution of TCV in intestines of turkey poult experimentally infected with TCV. (Turkey poult were infected with TCV at 10 days old. Frozen turkey intestine sections were incubated with turkey antiserum specific for TCV and subsequently incubated with fluorescein isothiocyanate (FITC) conjugated goat anti-turkey IgG (H + L) antibody. Non-infected control turkey intestines were negative for TCV by IFA throughout the entire experimental period (data not shown). * The difference between groups treated with CY and untreated group is significant ($p < 0.05$)

Treatment	10 h PI ^a	3 days PI	9 days PI	14 days PI
Trial 1				
None	2.6 ± 2.2 ^b	6.2 ± 1.1	4.2 ± 2.3	ND ^c
CsA ^d	2.6 ± 1.5	6.4 ± 0.9	5.8 ± 0.8	ND
CY ^e	3.2 ± 2.3	5.6 ± 1.1	6.4 ± 0.9*	ND
Trial 2				
None	ND	6.4 ± 0.9	ND	3.0 ± 1.2
CsA	ND	6.4 ± 0.9	ND	3.2 ± 0.4
CY	ND	6.2 ± 0.8	ND	6.8 ± 0.4*

^a PI = post-infection with TCV.

^b The intensity of IFA staining was scored on a scale of 1–4 with 4 being the most intense staining. The distribution of area with positive staining within section was categorized into diffuse, multifocal, and focal and scored as 3, 2, and 1, respectively. The scores of intensity and area distribution were added. Data were expressed as mean ± SD of scores of IFA stainings from five turkey poult in each group. Higher value indicated more intense staining.

^c ND = not done.

^d Turkey poult were treated with CsA, 100 mg per kg body weight, at 7 days old in trial 1 or 4 days old in trial 2 and every third day thereafter.

^e Turkey poult were treated with CY, 5 mg per turkey poult per day on the first 3 days post-hatching.

mechanism(s) of genetic control as demonstrated for chicken lymphocytes may also contribute to the observed individual variation of lymphocyte proliferation responses of these turkeys.

In comparison, cellular immunity against avian IBV has been demonstrated by antigen-specific proliferation of T lymphocytes, delayed-type hypersensitivity, and increased populations of CD4 or CD8 T cells [10]. Experimental suppression of chicken T cells with CsA enhanced virus titers in the kidney. Spleen mononuclear cells of chicken vaccinated with live IBV showed antigen-specific lymphocyte proliferation and produced IL-2 and interferon- γ in response to *in vitro* stimulation with homologous or heterologous strains of IBV [25]. Cytotoxic T lymphocyte (CTL) responses to IBV was demonstrated to be correlated with initial decrease of amount of virus in kidney and lung and clinical respiratory signs of illness [26]. Adoptive transfer of CD8 T lymphocytes from chicks infected with IBV, protected chicks from challenge infection [27]. The functional CTL epitopes were probably present in spike or nucleocapsid structural proteins of IBV [28,29].

Treatment of newly hatched turkey poult with CY, an alkylating agent, has been shown to cause deficiency in antibody responses to SRBC and persistent cellular degeneration in bursa and bursa-dependent areas in all major lymphoid organs [18]. Results from the present study revealed significantly decreased antibody responses to SRBC in turkey

poults treated with CY. These data suggested that B cells were the major targets of CY in turkey poults. The undetectable antibody response of turkey poults treated with CY to SRBC in trial 2 is not caused by any reagent or operation problem because all samples from different groups were treated and examined in the same way simultaneously. This also does not necessarily reflect a complete elimination of humoral B cell activity. The antibody response to SRBC may not be detectable by the evaluation method, hemagglutination assay. Nevertheless, treatment of CY in the present study did significantly suppress the humoral B cell activity and, subsequently, significantly enhanced the severity of TCV infection in turkey poults as revealed by markedly decreased body weight gain and higher scores on IFA staining for TCV antigens in the intestine.

Humoral antibody responses to avian IBV in chicken are measurable by enzyme-linked immunosorbent assay, hemagglutination-inhibition test, or virus neutralization test. Vaccination studies of IBV generally correlated humoral antibody responses with protection. The protective role of B cells against IBV infections has been shown by selective experimental suppression with the chemical CY, the hormone testosterone propionate, or surgical bursectomy [10]. Chickens treated with CY had longer persistence of IBV and, subsequently, showed increased clinical signs and more severe histopathological lesions in the kidney. High titers of humoral antibodies were correlated with the absence of virus recovery from kidney and genital tract and seemed to protect the tracheal epithelium from challenge infection. Spike protein of IBV was considered the major structural protein that induces protective humoral antibody responses. Antibodies generated in mice using a recombinant spike protein as inoculum could neutralize IBV infectivity in ciliostasis tests with tracheal organ cultures [30]. Immunization of a recombinant fragment (S1) of spike glycoprotein protected chickens from challenge infection of IBV [31].

Decreased body weight gain is one of the major characteristics of turkey coronavirus enteritis [32]. Infection with TCV causes rapid histopathologic changes in the mucosa of the intestinal tract of turkey poults. Ultrastructural changes included granularity of epithelial cells, loss of microvilli, margination of chromatin in the nucleus, and increased cellularity of the lamina propria [32,33]. The alteration in the intestinal tract causes failure of absorption and accumulation of fluids and gases resulting in diarrhea and decreased body weight gain. The body weight gain of turkey poults treated with CsA or CY or without treatment of CsA or CY were all significantly decreased after infection with TCV as compared with that of non-infected controls. Treatment of CY increased the severity of TCV infection in turkey poults because the body weight gain of turkeys treated with CY was significantly lower than that of turkeys not treated with CsA or CY after infection with TCV.

Because considerable lymphocyte proliferation responses were detectable in turkey poults treated with CsA in trial 1, the initial treatment with CsA of turkey poults (group I) beginning at 7 days old in trial 1 was changed to 4 days old in trial 2 in order to enhance the suppression effect on cellular immunity of turkey poults at 10 days old when they were infected with TCV. For detection of TCV in the intestine by IFA, the time point of 10 h PI in trial 1 was not repeated in trial 2 because significant difference in the amount of staining between groups at this interval was not observed in trial 1. The difference in the amount of staining between groups at 10 h PI was not expected in trial 2 as suggested by the similar amount of staining among groups at 3 days PI in trial 1. In contrast, the time point of 9 days

PI in trial 1 was changed to 14 days PI in order to examine if there were greater differences between groups treated with CsA or CY and group not treated with CsA or CY.

The level of TCV antigen was high in the intestines of turkey poult within the first week following infection. The level of TCV antigen declined after 7 days PI while the virus-specific antibody responses rose markedly (Loa et al., unpublished data), suggesting the protective role of humoral immunity against TCV infection in turkey poult. Consistent with these observations, the results of the present study also revealed the declines of TCV antigens in the intestines of turkey poult not treated with CsA or CY at 9 and 14 days PI. In addition, the present study showed that suppression of humoral immunity by CY enhanced the severity of TCV infection in the intestines of turkey poult infected with TCV. In summary, significantly decreased body weight gain along with increased severity of TCV infection in turkey poult treated with CY and infected with TCV suggest that humoral immunity may provide protective immunity for turkey poult against infection with TCV.

Acknowledgements

This research was supported by the Commission of Agriculture, State of Indiana, Pfizer Animal Health, North Carolina Turkey Spiking Mortality Task Force, and United States Department of Agriculture. The authors thank Dr David Hermes from Perdue Farm for providing the turkey poult for the study.

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