

# Specific Cytotoxic T Lymphocytes Are Involved in In Vivo Clearance of Infectious Bronchitis Virus

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Received 13 December 1996/Accepted 28 March 1997

**Cytotoxic T-lymphocyte (CTL) responses to infectious bronchitis virus (IBV) were determined at regular intervals between 3 and 30 days postinfection (p.i.). The maximum response with 82% lysis of labeled target cells was detected at 10 days p.i. The specific CTL response did not begin to decline until the amount of virus, which peaked at day 8 p.i. in both the kidneys and lungs, started to decrease. Clinical respiratory signs of illness also correlated with amount of virus. CTL activity was shown to be major histocompatibility complex (MHC) class restricted because the lysis of MHC-mismatched targets was negligible, and lysis was mediated by CD8<sup>+</sup> CD4<sup>-</sup> T cells, as the CTL response could be abolished with removal of CD8<sup>+</sup> CD4<sup>-</sup> but not CD4<sup>+</sup> CD8<sup>-</sup> lymphocytes. In contrast, immunoglobulin M (IgM) antibody was not detected until day 10 p.i., and levels peaked at day 12 p.i.; IgG antibody levels were minimal until day 15 p.i. but continued to increase exponentially until day 30 p.i., the last day examined. In summary, CTL responses correlated with initial decreases in infection and illness.**

Cytotoxic T lymphocytes (CTL) provide a critical arm of the immune system in eliminating autologous cells expressing foreign antigen. Unlike humoral immunity, the specificity of CTL activation depends on membrane receptors rather than secreted molecules, and antigen receptors of CTL interact with peptide determinants only in association with matched major histocompatibility complex (MHC) molecules (11, 29). Virus-specific CTL have been shown to be important, if not critical, for resolution of infection and elimination of viral shedding (17, 21, 23).

Most virus-specific CTL activity identified is MHC class I restricted and mediated by CD8<sup>+</sup> CD4<sup>-</sup> T lymphocytes (1, 22) and is targeted toward cells that endogenously express viral determinants. Although most of our current understanding of cellular immunity comes from human and mouse studies, molecular counterparts to mammals tend to be conserved in the bird (6, 10, 24). Limited CTL studies have indicated that avian CTL functions parallel those for mammals (18, 32). For example, MHC-restricted, CD8<sup>+</sup> T-cell-mediated lysis of avian leukosis retrovirus-infected target cells has been found in chicks (28).

Infectious bronchitis virus (IBV), a prototype of the *Coronaviridae* family, causes a highly contagious respiratory disease of chickens that is particularly severe for very young chicks, in which signs of illness include tracheal rales, coughing, sneezing, and nasal discharge. Some strains also have tropism for the urinary tract, causing kidney damage (5, 7, 9, 15) and serious production losses in laying hens (8). Outbreaks of disease associated with IBV infection can occur in vaccinated flocks. These vaccine failures probably result from antigenic variants of IBV emerging from wild-type or vaccine viruses by point mutations or genome RNA-RNA recombination (31). Whereas current vaccines have targeted induction of viral neutralizing antibody, development of effective prophylactic control of IBV should include antigen preparations that induce CTL as well as humoral immunity. IBV-specific CTL have not been demonstrated, although murine CD4 T-cell epitopes were identified on the nucleocapsid protein of IBV (3). How-

ever, mice are not natural hosts for IBV. CTL from murine hepatitis virus-infected mice that lysed target cells expressing whole virus and the nucleocapsid alone have been identified (26, 33). In the present study, IBV-specific CTL responses were examined in chicks. MHC class I-restricted and CD8<sup>+</sup> T-cell-mediated lysis of target cells infected with IBV were identified, and maximal activity was found to occur on day 10 postinfection (p.i.). The virus control of replication correlated with CTL activity.

## MATERIALS AND METHODS

**Source of virus.** The nephropathogenic Gray strain of IBV was propagated by inoculating the allantoic sacs of 11-day-old chicken embryos, and allantoic fluid harvested 36 h p.i. was collected as the source for viral inoculation (25). Tissue culture-adapted virus was propagated in primary chicken kidney (CK) cells and in an established CK cell line before harvesting at 48 h p.i.

**Experimental animals.** Embryonated eggs of inbred (B<sup>12</sup>/B<sup>12</sup>) and outbred (R68C) chickens were obtained from Hy-VAC (Adel, Iowa), and SPAFAS Incorporated (Preston, Conn.), respectively. Eggs were hatched in our laboratory, and chicks were housed in a specific-pathogen-free environment at the Laboratory Animal and Resources Facility, Texas A&M University, College Station. Chicks were inoculated at 4 weeks by the nasal-oral routine with 10<sup>6</sup> egg infectious doses (EID<sub>50</sub>) of the Gray strain. At 5-day intervals, chicks were recorded as experiencing clinical illness when they displayed signs of coughing, sneezing, or dyspnea.

**Cell culture.** A CK cell line was established from 10-day-old chicks (B<sup>12</sup>/B<sup>12</sup>). Briefly, CK cells were dispersed with trypsin digestion and cultured in a 75-cm<sup>2</sup> tissue culture flask which was coated overnight with 1 mg of calf collagen (Sigma Chemical Co., St. Louis, Mo.) per ml in water at room temperature. After air drying, tissue culture flasks were sterilized with UV light for 6 h (20). The CK adherent cells were continuously cultured by passing approximately every 4 days in MEM (minimal essential medium) supplemented with 10% chicken serum (Sigma), 1.4% sodium pyruvate, and 1.4% MEM nonessential amino acids. After 50 passages, the established CK cell line was used for target cells.

**Effector cell preparation.** Splenocytes from infected or uninfected chicks were used as the source of effector cells. Spleens from chicks were collected at various days p.i. before the single-cell suspension was minced and passed through a 150-mesh stainless-steel sieve to remove the connective tissue fragments. Spleens from six chicks were pooled to obtain a sufficient quantity of T lymphocytes. Lymphocytes were isolated by centrifuging 5 ml of the diluted spleen cells for 20 min at 1,500 rpm through 5 ml of Ficoll-Hypaque gradient (Histopaque; Sigma). Viable cells were collected from the interface, washed three times with phosphate-buffered saline (PBS; pH 7.2), and washed once with RPMI 1640. Macrophages were removed by incubating 5 × 10<sup>7</sup> cells in 75-cm<sup>2</sup> tissue culture flasks with RPMI 1640 supplemented with 10% chicken serum (Sigma), 2 mM L-glutamine, 5 × 10<sup>-5</sup> M 2-mercaptoethanol, 0.01 mM sodium pyruvate, and 0.1 mM MEM nonessential amino acids for 4 h at 41°C. B lymphocytes were re-

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moved by passing the cell suspension through a nylon wool fiber column previously equilibrated with complete medium (16).

**Depletion of CD4<sup>+</sup> and CD8<sup>+</sup> T cells.** The CD4<sup>+</sup> and CD8<sup>+</sup> antigen-bearing lymphocytes were separated by a modification of the panning method of Mage et al. (19). Briefly, 75-cm<sup>2</sup> tissue culture flasks were incubated overnight at room temperature with goat anti-mouse immunoglobulin G (IgG; 60 mg/ml in PBS [pH 7.2]). T cells purified as described above were labeled with a 1:500 dilution of either mouse anti-chicken CD4 or anti-chicken CD8 monoclonal antibody (Southern Biotechnology Associates, Birmingham, Ala.) in PBS. After the cells were washed three times with ice-cold PBS (pH 7.2) and the concentration was adjusted to 10<sup>7</sup> cells per ml in PBS, the antibody-labeled cells were added to goat anti-mouse IgG-coated plates and incubated at room temperature for 30 min. The suspensions were swirled gently before incubation for an additional 30 min at room temperature. Nonadherent cells were decanted from the plates following gentle agitation.

**Flow cytometry.** T lymphocytes were incubated on ice for 30 min with 1:50 dilution in PBS of mouse monoclonal antibodies specific for chicken CD4 or CD8 antigen before being washed three times with PBS (pH 7.2) and incubated on ice with 1:50 dilution of fluorescein isothiocyanate-labeled goat anti-mouse IgG (1:50) in PBS for 30 min. After five washes with PBS, the reaction samples were fixed with 2% formaldehyde in PBS and counted with a FACSCALIBUR (Becton Dickinson, San Jose, Calif.).

**Cytotoxic T-cell assay.** Splenic lymphocytes were tested for cytotoxic activity against syngeneic and heterologous target cells by using a modification of the procedures described by McGuire et al. (20). Target cells (2 × 10<sup>4</sup> per well) were incubated for 24 h at 41°C with 6% CO<sub>2</sub> in a 96-well plate. Target cells were inoculated with 10<sup>6</sup> EID<sub>50</sub> of CK cell-adapted Gray strain per well for 5 h before labeling with <sup>51</sup>Cr. Cells were labeled for 90 min with 5 μCi of <sup>51</sup>Cr per well in 100 μl of MEM supplemented with 1% sodium pyruvate, 1% MEM nonessential amino acid, and 5% syngeneic chicken serum. Various ratios of effector and target cells (E/T ratios) were incubated for 24 h in 200 μl of complete RPMI 1640 at 41°C. After removal of 100 μl of supernatant, the amount of <sup>51</sup>Cr release was determined. Percent specific lysis equaled  $(E - S/M - S) \times 100$ , where *E* is the mean of three test wells, *S* is the mean of spontaneous release from the three target cells without effector cells, and *M* is the mean maximal release from the three target cells with 3% Triton X-100.

**ELISA.** Gray strain viral antigen was coated onto 96-well plates by adding 100 μl of whole virus diluted in carbonate-bicarbonate coating buffer (pH 9.6) at the concentration of 50 ng per well, and the plates were incubated overnight at 4°C before being washed three times with PBS-0.05% Tween 20 (4). Each well was blocked with 100 μl of 1% bovine serum albumin for 2 h at room temperature. After three washes with PBS-0.05% Tween 20, 100 μl of serum diluted in PBS (pH 7.2) was added to each well. The plates were incubated for 1 h at room temperature and washed three times with PBS-0.05% Tween, 100 μl of horseradish peroxidase-conjugated rabbit anti-chicken IgG diluted in PBS (1:2,000) was added, and the plates were incubated for 1 h at room temperature and again washed three times with PBS-0.05% Tween. After 100 μl of 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) (ABTS) peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) was added to each well, the plates were incubated for 20 min in the dark, and the optical density was determined at 405 nm on an enzyme-linked immunosorbent assay (ELISA) spectrophotometer.

**Indirect immunofluorescence assay.** CK cells were cultured overnight in six-well plates at 41°C before being infected with Gray strain IBV for 48 h. Cells were collected by trypsinization and fixed with 1.8% formaldehyde for 20 min on ice. The fixed CK cells were resuspended to 10<sup>5</sup> cells per ml in PBS and spotted on the wells of microscope slides (Celtline Associates, Inc., Newfield, N.J.) before air drying. The dried CK cells were dipped in PBS for 30 min, incubated on ice with Gray anti-mouse polyclonal antibody for 30 min, and then washed three times with cold PBS-0.05% Tween 20. CK cells were incubated on ice with fluorescein isothiocyanate-labeled goat anti-mouse IgG for 30 min, washed five times with cold PBS-0.05% Tween 20, and then evaluated under a UV microscope (4).

**EID<sub>50</sub>.** Lungs and kidneys were collected from 28-day-old syngeneic chickens at various days after infection with Gray strain IBV. Each organ was weighed and homogenized, and then a 10% (wt/vol) of dispersed cell suspension was prepared in Eagle's MEM containing penicillin (200 U/ml) and streptomycin (200 mg/ml). After standing for 90 min at 4°C, cells were pelleted by centrifugation for 20 min at 2,000 × *g*, and serial 10-fold dilutions were prepared in Eagle's MEM. One-tenth milliliter of each virus dilution was injected into each of six 11-day-old embryonated chicken eggs (8a). The eggs were sealed with collodion and incubated at 40°C for 5 days, and then the embryos were examined for dwarfing. The EID<sub>50</sub> was calculated by method of Reed and Muench as described by Burleson et al. (4):  $(\log \text{dilution above } 50\%) + (\text{proportional distance} \times \log \text{dilution factor}) = \log \text{EID}_{50}$ .

## RESULTS

**Detection of IBV-specific CTL.** CK cells established from 10-day-old syngeneic chickens (B<sup>12</sup>/B<sup>12</sup>) were used as CTL targets. The expression of IBV Gray strain antigens in infected cells was evaluated by indirect immunofluorescence following

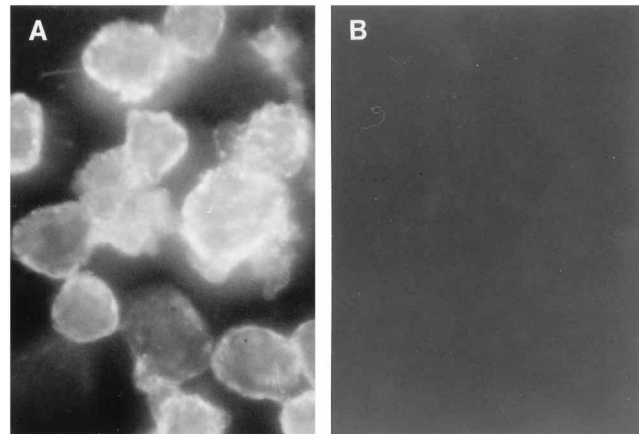


FIG. 1. Detection of IBV antigens in IBV-infected (A) and uninfected (B) CK cells, using indirect immunofluorescence assay.

infection of 5 × 10<sup>6</sup> cells with 10<sup>6</sup> EID<sub>50</sub> of IBV (Fig. 1). Positive fluorescence in about 90% of infected cells was detected only in the cytoplasm (Fig. 1A), whereas no fluorescence was detected in the uninfected control CK cells (Fig. 1B).

To optimize conditions for detection of CTL, unstimulated splenic T cells were collected from IBV-infected and uninfected syngeneic chicks at 5 day p.i. and their IBV-specific cytolytic activity evaluated at various incubation times at an E/T ratio of 100:1. The 15- and 24-h incubations resulted in 22.5 and 36% lysis, respectively, of infected target cells (Fig. 2). Incubation periods extending past 24 h resulted in an unacceptable spontaneous release of more than 30%. Since the IBV-specific CTL lysis was maximal at 24 h of incubation, with effector and target cells exhibiting less than 30% spontaneous release, this incubation time was used in the subsequent assays. The unstimulated T cells from spleens of IBV-uninfected chicks caused negligible lysis of less than 1%.

**CTL activity in IBV-infected chicks was greatest at 10 days p.i.** The cytolytic activity of splenic T cells from syngeneic infected chicks was evaluated between 3 and 30 days p.i. at an E/T ratio of 100:1 (Fig. 3A). IBV-specific CTL activity was detected at day 3 p.i., with 22.9% release of radioactivity. The maximum specific lysis was observed at day 10 p.i., with 82.8% lysis. The CTL response decreased on days 15 and 20, with release of radioactivity of 46.30 and 38%, respectively. The

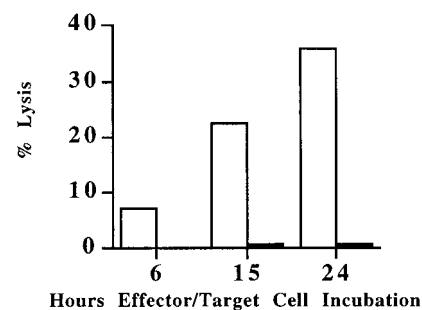


FIG. 2. Incubation time for optimal CTL lysis. Splenic T cells were collected from six chicks at day 5 p.i., pooled, and incubated for various times with infected syngeneic CK target cells at an E/T ratio of 100:1. Percent lysis of syngeneic target cells by splenic CTL of IBV-infected chicks is represented by open bars; percent lysis of syngeneic target cells by splenic CTL of uninfected chicks is represented by dark bars.

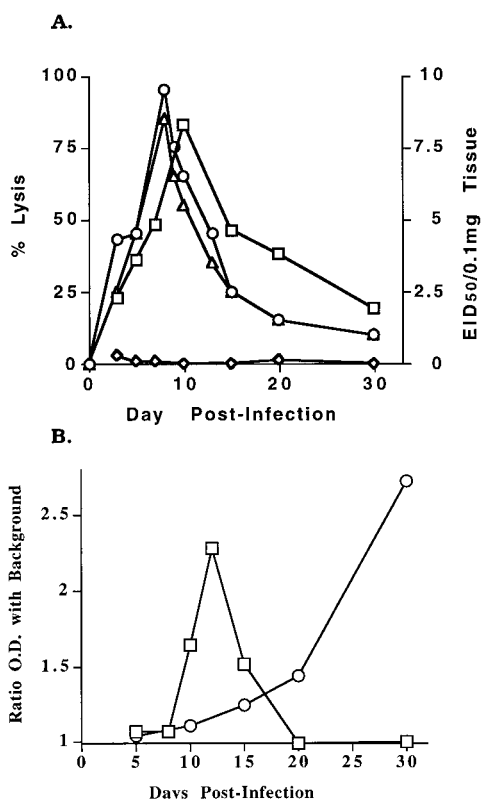


FIG. 3. (A) Time course of the specific CTL response and viral replication in chicks infected with IBV. Effector T cells were collected from spleens of six chicks infected with  $10^6$  EID<sub>50</sub> of Gray strain IBV, pooled, and incubated for 24 h with  $^{51}\text{Cr}$ -labeled syngeneic target cells at an E/T ratio of 100:1. Squares, lysis of target cells by effector T cells from spleens of IBV infected chicks; diamonds, lysis of target cells by control effector T cells from spleens of IBV uninfected chicks; circles, EID<sub>50</sub> in lungs; triangles, EID<sub>50</sub> in kidneys. (B) Time course of antibody response in IBV-infected chicks. At various days p.i., anti-IBV sera were collected from six chicks infected with Gray strain IBV and pooled before quantitation by ELISA, using Gray strain whole viral antigen. Squares, IgM; circles, IgG. O.D., optical density.

CTL activity continued to decrease to 19.23% on day 30 p.i. The CTL activity of spleen cells from uninfected chicks was negligible.

**Amount of virus isolated from lungs and kidneys was greatest at 8 days p.i.** To determine the viral titer in two target organs of IBV, lungs and kidneys were collected from Gray strain IBV-infected chicks at various days p.i., and the viral titer (EID<sub>50</sub>) was determined. Virus was detected as early as day 3 p.i. in lungs ( $10^{4.3}$  EID<sub>50</sub>) and kidneys ( $10^{2.5}$  EID<sub>50</sub>). The viral titer increased continuously until day 8 p.i., with a maximum of  $10^{9.5}$  EID<sub>50</sub> in lungs and  $10^{8.5}$  EID<sub>50</sub> in kidneys. However, the viral titers started to decrease from day 9 until day 30 p.i. in both lungs and kidneys (Fig. 3A). Clinical signs of disease in chicks infected with Gray strain IBV were also observed (Table 1). Respiratory symptoms appeared between 5 and 10 days p.i., and most chicks recovered before day 15 p.i. The correlation of viral replication and clinical signs suggests that IBV-specific CTL played an important role in resolving the illness.

**IgG antibody titer increased exponentially in contrast to CTL activity.** The chicken sera were collected at various days p.i., and the antibody titer was evaluated by ELISA. The IgG antibody titer increased exponentially from day 10 p.i., when CTL activity was maximum, whereas the highest titer of IgG

TABLE 1. Number of IBV-infected chicks showing clinical signs of illness

Clinical sign <sup>a</sup>	No. of affected chicks				
	5 days p.i.	10 days p.i.	15 days p.i.	20 days p.i.	30 days p.i.
Respiratory illness	7	15	1	1	1
Death	0	0	2	0	0

<sup>a</sup> Twenty chicks in either infected or uninfected groups were examined. Only illness from the infected chicks is described, as none of the uninfected control chicks showed clinical signs of illness. Fifteen of 20 infected showed respiratory illness.

antibody was observed at day 30 p.i., the last day examined, when CTL activity dropped to the lowest level. IgM antibodies were detectable at day 10 p.i. and peaked at day 12 p.i. (Fig. 3B).

**CTL activity was MHC class I restricted, mediated by CD8<sup>+</sup>.** A dose-responsive increase in specific lysis of syngeneic target cells infected with  $10^6$  EID<sub>50</sub> of Gray strain IBV by unstimulated splenic T cells from chickens at day 10 p.i., was observed, with maximum lysis of 82% at an E/T ratio of 100:1. Specific lysis of the IBV-infected heterologous target cell line was observed, with maximum lysis of only 11.67% at an E/T ratio of 100:1. Therefore, the IBV-specific lysis of target cells by chicken CTL was mostly MHC restricted. The unstimulated splenic T cells from uninfected chicks did not indicate any detectable lysis of the syngeneic CK cells infected with Gray strain IBV (Fig. 4).

The CD8<sup>+</sup> T cells or CD4<sup>+</sup> T cells were removed by the panning method from the unstimulated splenic T cells of chicks at day 10 p.i. to determine which subpopulations of T cells mediate lysis of IBV-infected CK target cells. Lysis of target cells by T-cell populations depleted of CD8<sup>+</sup> T cells was minimal, with maximum lysis of only 6.7% at an E/T ratio of 40:1. Meanwhile, the unstimulated splenic T cells depleted of CD4<sup>+</sup> T cells mediated the specific lysis in a dose-responsive manner, with the maximum cytolysis of 38.3% at an E/T ratio of 40:1. Control T cells from uninfected chicks did not mediate any detectable lysis of IBV-infected target cells (Fig. 5).

The phenotypes of depleted cell populations ( $10^6$  T cells per ml) were evaluated by flow cytometry (Fig. 6). All cells examined were CD3<sup>+</sup> whether they were treated with anti-CD4 or

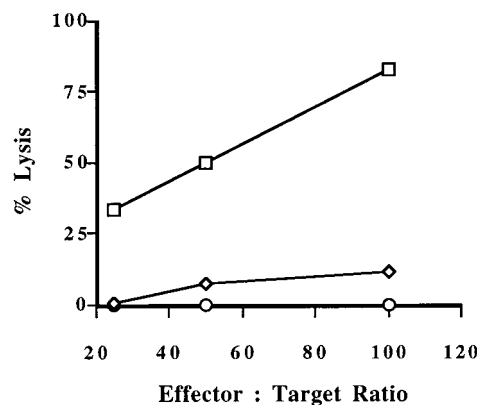


FIG. 4. Demonstration of MHC class I restriction of IBV-specific CTL. At 10 days p.i., effector splenic T cells were collected from six IBV-infected chicks and pooled. Splenic effector cells were incubated for 24 h at an E/T ratio of 100:1 with CK target cells. Squares, IBV-infected syngeneic CK target cells; diamonds, heterologous IBV infected target cells; circles, uninfected syngeneic target cells.

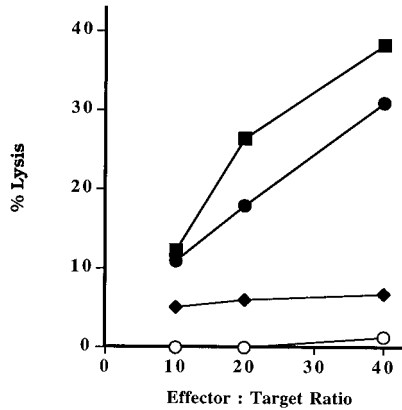


FIG. 5. CD8<sup>+</sup> T-cell-mediated lysis of IBV infected target cells. Splenic T lymphocytes from chicks infected with IBV were collected at day 10 p.i. and depleted of CD8<sup>+</sup> T cells or CD4<sup>+</sup> T cells before evaluation of the CTL responses. Symbols represent lysis of infected targets with splenic effector T cells depleted of CD4<sup>+</sup> cells (squares), with those depleted of CD8<sup>+</sup> cells (diamonds), and with undepleted cells (closed circles). Lysis of uninfected CK target cells with effector cells depleted of CD4<sup>+</sup> T cells is shown by open circles.

anti-CD8. After depletion with CD4 antibody, all remaining cells were characterized as CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>+</sup> (Fig. 6A to C), and depletion with CD8 antibody removed all detectable cells with CD8, leaving only CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>-</sup> T cells (Fig. 6D to F). Therefore, removal of CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>+</sup> T cells correlated with depletion of the IBV-specific CTL response, whereas the CTL response was not affected by removal of cells with the CD3<sup>+</sup> CD4<sup>+</sup> CD8<sup>-</sup> phenotype.

## DISCUSSION

The role of CTL in inhibiting coronavirus replication has not been previously examined. The CTL response of chickens to avian leukosis virus and the response of mice to murine hep-

atitis virus have been described but not correlated with acute viral infection. In this report, we have shown that during acute infection, IBV induces vigorous specific CTL activity. This cellular response can be correlated more closely to the initial decline in viral load than the subsequent specific humoral responses.

As in mammals, chicken CTL lysis of virus-infected target cells is mostly MHC restricted. CTL lysis of syngeneic CK target cells was dose responsive; the CTL lysis of heterologous CK cells was also dose responsive, but the maximum was less than 14% of the cytolytic response. The lysis of heterologous target cells by splenic T cells may be due to natural killer cells, which do not require MHC restriction to kill virus-infected target cells (14, 30). In addition, the depletion of T-lymphocyte subtypes and phenotypic determination of the remaining cells indicated that specific lysis by splenic T cells from IBV-infected chickens was mediated by CD8<sup>+</sup> CD4<sup>-</sup> T cells, not by CD4<sup>+</sup> CD8<sup>-</sup> T cells.

The kinetics of the IBV-specific CTL response is similar to the CTL response described for avian leukosis virus in chickens (28). In addition, a maximum murine CTL response to Sendai virus was observed at about 12 p.i. (27). In contrast, maximum direct CTL responses in horses against equine herpesvirus were detected by 2 and 3 weeks p.i. (1). The impact of animal species and viral pathogenesis on the nature of the CTL response can be elucidated only with the characterization of CTL responses following infection of greater varieties of animals with additional viruses.

Viral infection in both lungs and kidneys shown as log titrations in embryonated chicken eggs was biphasic, with maximum levels observed at day 8 p.i., and the clinical signs of respiratory distress correlated with viral infection. The decline of viral amount at 15 days p.i. was followed by a decline in the specific CTL activity. However, the transient presence of specific IgM antibody, although somewhat delayed in comparison, could also be roughly correlated with viral infection. In contrast, the IgG antibody levels were relatively low until after day 15 p.i., when viral amounts in IBV target organs were 10<sup>2.5</sup>

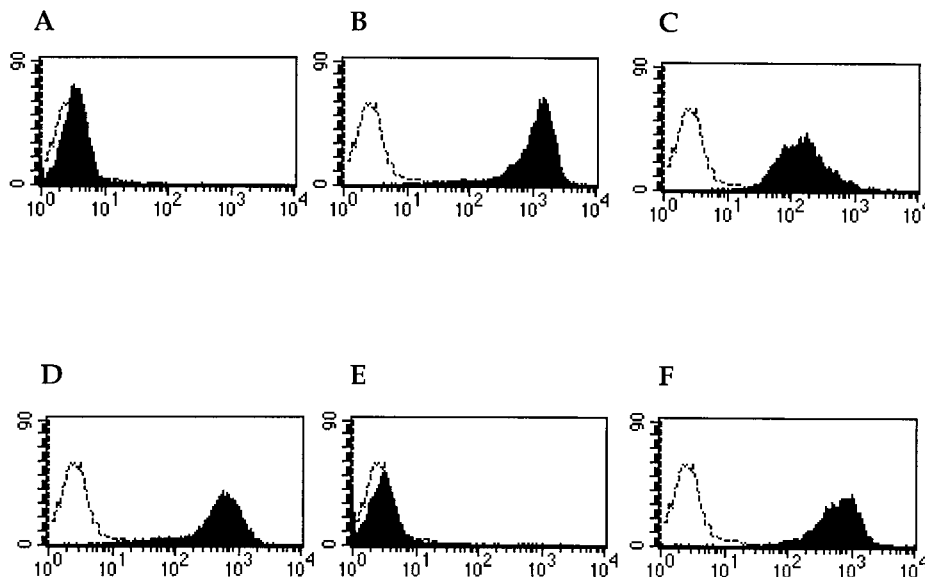


FIG. 6. Identification by flow cytometry of the T-lymphocyte phenotype of splenic cells depleted of CD4 or CD8. Control splenic T cells were stained with only the secondary antibody. Splenic T lymphocytes depleted of CD4<sup>+</sup> T cells were stained with mouse anti-chicken CD4 monoclonal antibody (A), with CD8 monoclonal antibody (B), and with CD3 monoclonal antibody (C). Splenic T lymphocytes depleted of CD8<sup>+</sup> T cells were stained with CD4 monoclonal antibody (D), with CD8 monoclonal antibody (E), and with CD3 monoclonal antibody (F).

EID<sub>50</sub>, compared to 10<sup>8</sup> EID<sub>50</sub> on day 8. Chicks infected with IBV began showing respiratory clinical signs between days 5 and 10 p.i., and most recovered from illness before day 15. The resolution of clinical illness also suggests that IBV-specific CTL played a major role in pathogenesis of acute viral infection.

The CTL response declined with the appearance of humoral immune response, especially IgG, which is described as critical in viral neutralization. The mechanism which the chicken immune system uses to switch from cellular immunity to humoral immunity after IBV infection remains to be elucidated. Although helper T-cell subtypes have not been characterized in chicks, it seems likely that until day 15 p.i., the Th1 subtype of CD4<sup>+</sup> T cells producing lymphokines, such as interleukin-2 (IL-2), gamma interferon, and tumor necrosis factor beta, involved in cellular immunity may be the predominant helper T cells, but after day 15 p.i., the Th2 subtype of CD4<sup>+</sup> T cells, involved in induction of humoral immunity and producing IL-4, IL-5, IL-10, and IL-13, may replace Th1 cells.

At the later stage of IBV infection, the chicken immune system may depend on humoral immunity to control viral infection. However, these results overall indicate that the chicken immune system may utilize specific CTL to eliminate IBV at the early stage of acute infection. The early CTL responses to individual structural polypeptides are now being determined. Although IBV-specific CTL activity correlated with clearance of virus from target organs, the absolute role of CTL can be determined only in the absence of antibody. Humoral immunity can be virtually eliminated following bursectomy of late-stage chicken embryos (12, 13). Chickens, in general, will prove to be a unique animal model for determining the role of immunity in controlling viral pathogenesis. Unlike the case for mammals, immune cells can be easily manipulated in the embryo; moreover, inbred strains are readily available, and the pathogenesis of and immune responses to viral infections are similar to those in mammals. Adoptive transfer studies and identification of T-cell epitopes on IBV structural proteins are in progress.

#### ACKNOWLEDGMENTS

This research was supported by Southeast Poultry & Egg Association no. 142, USDA Animal Health (section 1433) no. TEXO-6824, and USDA Cooperative State Research Service no. 93-37207-9296.

We thank Roger Smith for help with flow cytometry and Betty Rosenbaum and Li Wang for technical advice. We also thank Ian R. Tizard for critical review of manuscript.

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