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Adoptive Transfer of Infectious Bronchitis Virus Primed αβ T Cells Bearing CD8 Antigen Protects Chicks from Acute Infection

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Infectious bronchitis virus (IBV) infection and associated illness may be dramatically modified by passive transfer of immune T lymphocytes. Lymphocytes collected 10 days postinfection were transferred to naive chicks before challenge with virus. As determined by respiratory illness and viral load, transfer of syngeneic immune T lymphocytes protected chicks from challenge infection, whereas no protection was observed in the chicks receiving the MHC compatible lymphocytes from uninfected chicks. Protection following administration of T lymphocytes could be observed in chicks with three distinct MHC haplotypes: B1/1B, B1/1B12, and B4/1B19. Nearly complete elimination of viral infection and illness was observed in chicks receiving cells enriched in αβ T lymphocytes. In contrast, removal of γδ T lymphocytes had only a small effect on their potential to protect chicks. The adoptive transfer of enriched CD81 T lymphocytes indicated that protection was also a function primarily of CD8-bearing cells. These results indicated that αβ T lymphocytes bearing CD81 antigens are critical in protecting chicks from IBV infection. © 2000 Academic Press

Key Words: IBV; coronavirus; protection; immune T lymphocytes; adoptive transfer; MHC haplotype.

INTRODUCTION

Infectious bronchitis virus (IBV) is a prototype of the Coronaviridae family (Collisson et al., 1992; Siddell et al., 1983). Infection with IBV causes a highly contagious, respiratory disease in chickens with high mortality in young chicks (Alexander et al., 1978; Crinion and Hofstad, 1972; Hofstad, 1984). In addition to the upper and lower respiratory tract, IBV affects the genital and urinary systems and has been isolated from many tissues, including the lung, trachea, cecal tonsils, kidneys, Harderian (paraocular) glands, and spleen (Alexander et al., 1978; Crinion and Hofstad, 1972; Hofstad, 1984). In laying flocks, IBV infection can cause complete cessation of egg production or production of thin-shelled, rough, and misshapen eggs, and in broilers, infection can result in decreases in feed consumption.

Cytotoxic T lymphocytes (CTL) specific for IBV, associated with the control of acute infection in chicks, have been shown in vitro to be MHC restricted and mediated primarily by cells with the CD8 phenotype (Seo and Collisson, 1997, 1998). Initial elimination of virus following infection has been associated with this CTL response (Seo and Collisson, 1997). Furthermore, the nucleocapsid polypeptide, known to induce CTL activity, could protect chicks from IBV challenge, when expressed in vivo by a eukaryotic expression plasmid (Seo et al., 1997). Protection occurred in the absence of neutralizing antibody, which is induced later during a viral infection by the spike, not the nucleocapsid, protein (Ignjatovic and Galli, 1994).

Adoptive transfer of immune lymphocytes has been used to evaluate their role in protecting mice from severe viral infections (Offit and Dudzik, 1990; Cannon et al., 1987; Klavinsks et al., 1989). For example, suckling mice were protected from murine rotavirus-induced gastroenteritis after adoptive transfer of splenic lymphocytes from immunized animals. Protection against disease occurred in the absence of rotavirus-specific neutralizing antibodies in the sera of suckling mice (Offit and Dudzik, 1990).

In a study with respiratory syncytial virus (RSV), primed RSV-specific immune T cells collected between days 5 and 8 of infection cleared the lungs of nude and irradiated mice of RSV within 10 days of transfer (Cannon et al., 1987). When adoptively transferred at the time of infection, MHC Class I restricted CTL protected mice from lymphocytic choriomeningitis virus infection (Klavinskis et al., 1989).

The chicken is an especially appealing model for doing adoptive transfer studies. In addition to the ease of synchronizing hatching and maintaining these animals, a number of stocks are available with well-defined MHC haplotypes (Briles and Briles, 1982, 1987) and recombinants within the MHC chromosomal region (Miller et al., 1988; Aeed et al., 1993; Golemboski et al., 1995). In this

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RESULTS

Adoptive transfer of immune T lymphocytes protected chicks against IBV infection. Previous studies indicated that maximum CTL activity could be observed in lymphocytes prepared from spleens of chicks, 10 days postinfection (p.i.) with the Gray strain of IBV. The elimination of virus during acute infection was found to be closely correlated with CTL activity, and to a lesser extent with the presence of IgM antibody, but not with the presence of IgG antibody. The correlation of CTL with elimination of virus in vivo was further investigated through transfer of 4 × 10^5 immune (group 1) or nonimmune (group 2) lymphocytes to naïve 5-day-old B19/B19 chicks 1 day prior to infection with IBV. A third group with age-matched birds received PBS in the absence of lymphocytes. After challenge with the Gray strain, the effects of lymphocyte transfer on associated respiratory illness were examined for 5 days. The transfer of immune cells delayed the onset of clinical illness, reduced the number of chicks infected, and reduced the clinical illness observed (Table 1). On day 3, only two of six chicks displayed any signs of respiratory illness and they experienced only mild signs of occasional sneezing. In contrast, five of five chicks receiving normal lymphocytes or four of five inoculated with only buffer showed mild clinical signs of respiratory illness by day 2. On days 3, 4, and 5, all chicks inoculated with only buffer or with nonimmune lymphocytes were sneezing, in addition to experiencing more severe signs of tracheal and lung congestion as indicated by observed rales.

The effects of immune lymphocytes on viral replication were determined in lungs collected at 5 days p.i. No virus was detected in the lungs of chicks given immune lymphocytes, whereas the viral load in lungs from the chicks receiving nonimmune lymphocytes or buffer alone prior to infection was 10^5.06 and 10^5.24 EID_{50}, respectively, per 0.5 g of tissue (Fig. 1). Transfer of immune lymphocytes protected chicks against clinical illness and viral replication in the lungs. Because lymphocytes from uninfected chicks had no impact, protection was not the result of providing a more mature population of T lymphocytes.

Varying doses of immune lymphocytes were given to B19/B19 chicks 1 day prior to infection with IBV. Protection from transferred immune lymphocytes at 5 days p.i. is shown in Table 2. Protection was negligible or marginal when chicks were given 5 × 10^4 (data not shown) or 10^5 lymphocytes, respectively.

| Transferred lymphocytes | Cells given per bird | − | + | ++ |
|-------------------------|----------------------|---|---|---|
| PBS                     | 0                    | 0 | 1 | 4 |
| Immune                  | 10^5                 | 0 | 4 | 1 |
| Immune                  | 10^6                 | 4 | 1 | 0 |
| Immune                  | 10^7                 | 5 | 0 | 0 |

Note. Significant differences (P < 0.05) were determined between chicks given 0 or 10^4 and chicks given 10^5 or 10^6. The results are representative of two experiments.

Table 1

| Transferred lymphocytes | − | + | ++ |
|-------------------------|---|---|---|
| None                    | 0 | 0 | 5 | 0 |
| Nonimmune               | 0 | 0 | 5 | 0 |
| Immune, not depleted*   | 4 | 2 | 0 | 0 |

Note. Data are representative of three experiments.
*Statistically significant (P < 0.05) by analysis of variance.

FIG. 1. Effects of transferred immune cells on viral titers in the lungs of chicks 5 days after infection with IBV. Transferred lymphocytes were collected from the spleens of 38-day-old chicks 10 days p.i. or from uninfected age-matched chicks. T lymphocytes were transferred to naive chicks 1 day before challenge with IBV. The mean titer of five animals/group is shown. The standard deviation of the mean for chicks receiving no lymphocytes and that for chicks receiving nonimmune lymphocytes were both 0.17. No virus was detected in the lungs of chicks receiving immune lymphocytes. The results are representative of three experiments.

![Image](350x590 to 520x722)
CTL responses were observed with immune lymphocytes depleted of \( \gamma \delta \) T cells and with undepleted immune effector cells. Responses were dose-responsive with approximately 50% lysis at an effector:target ratio of 50:1. In contrast, lysis of IBV-infected target cells by effector cells depleted of \( \alpha \beta \) T cells or lysis of uninfected target cells by effector cells from unfraccionated lymphocyte preparations was undetectable (Fig. 2b).

Adoptive transfer of immune CD8 T cells protected chicks from acute IBV infection. Stimulated T cells depleted of CD8\(^+\) or CD4\(^+\) cells (1 \( \times 10^7 \)) were transferred to naive chicks before challenge with IBV. Clinical signs of respiratory illness were minimal in chicks receiving the immune cells depleted of CD4\(^+\) T lymphocytes. In contrast, chicks receiving immune lymphocytes depleted of CD8\(^+\) cells presented clinical signs that were similar to those of control infected chicks that did not receive immune lymphocytes (Table 3). Virus was not detected in either lungs or kidneys of chicks receiving immune T lymphocytes depleted of CD4\(^+\) cells. In contrast, viral titers in both lungs and kidneys of chicks receiving immune T cells depleted of CD8\(^+\) T cells were not significantly reduced (\( P > 0.05 \)) compared to infected control chicks (Fig. 3). CD8\(^+\) T lymphocytes are critical in protecting chicks from IBV infection.

Adoptive transfer of immune \( \alpha \beta \) T cells, not \( \gamma \delta \) T cells, protected chicks from acute IBV infection. Similarly, 10\(^7\) immune lymphocytes depleted of cells bearing TCR1 (\( \gamma \delta \)) or TCR2 (\( \alpha \beta \)) antigens were inoculated in chicks (five per group) prior to challenge with virus. Following challenge, chicks receiving immune lymphocytes depleted of \( \gamma \delta \) T cells suffered only minor respiratory illness, whereas

### TABLE 3

Phenotypes of Transferred T Lymphocytes Reducing Acute Respiratory Illness 5 Days p.i. with IBV

| Transferred lymphocytes | \(-\) | + | ++ | +++ |
|-------------------------|-----|---|----|-----|
| None                    | 0   | 0 | 2  | 3   |
| Immune CD4 depleted     | 4   | 1 | 0  | 0   |
| Immune CD8 depleted     | 0   | 3 | 2  | 2   |
| None                    | 0   | 0 | 2  | 3   |
| Immune \( \gamma \delta \) depleted | 3 | 2 | 0 | 0 |
| Immune \( \alpha \beta \) depleted | 0 | 1 | 2 | 3 |

Note. The results are representative of three experiments.

\( ^{a} \) Number of \( B^{12}/B^{12} \) chicks with observed respiratory illness, scored as: \(-\), no illness; +, sneezing or nasal discharge; ++, plus dyspnea or rales; ++++, plus lack of appetite or pale color ataxia. Five or six chicks per group.

\( ^{b} \) Statistically significant difference (\( P < 0.05 \)) from chicks not receiving transferred lymphocytes and from those receiving CD8-depleted lymphocytes.

\( ^{c} \) Statistically significant difference (\( P < 0.05 \)) from chicks not receiving transferred lymphocytes and from those receiving \( \alpha \beta \)-depleted lymphocytes.
chicks receiving immune cells depleted of αβ T cells displayed clinical signs that were similar to those in IBV-infected chicks not given immune lymphocytes (Table 3). The viral titers in lungs and kidneys of chicks receiving immune cells depleted of either αβ or γδ T lymphocytes were also examined. Viral replication in both lungs and kidneys from infected chicks having received immune cells depleted of αβ T cells was not significantly different (P > 0.05) from that in control infected chicks receiving only buffer, while no virus was detected in chicks given lymphocytes depleted of γδ cells (Fig. 4). Therefore, αβ T lymphocytes are capable of controlling IBV infection and associated disease.

DISCUSSION

The capacity for transferred syngeneic immune T lymphocytes to protect birds from challenge infection further indicated that T lymphocytes play a critical role in resolving acute IBV infection. Consistent with observations that the kinetics of MHC restricted CTL response can be associated with decreases in viral load, CD8+ lymphocytes were mostly responsible for the observed protection. In addition, cells that play a critical role in resolving early IBV infection in chicks were TCR2-bearing (αβ) lymphocytes. Thus, the transfer of immune cells depleted of CD4+ T cells or γδ T cells protected chicks from acute IBV infection.

Antibody responses do not appear to participate in the initial elimination of viral replication following infection, although their control of chronic infection is probably critical (Ignjatovic and Galli, 1994; Seo and Collisson, 1997). Cook et al. (1991) also showed that in ovo bursectomy did not appear to increase the level of mortality or early control of IBV replication in leghorn chickens although bursectomized chicks experienced longer lasting infection and illness than intact chicks. The cellular immune responses were not evaluated. However, we have found that bursectomy does not decrease, but may actually increase, the IBV-specific CTL response (Seo and Collisson, unpublished data).

In evaluating the role of T lymphocytes in murine hepatitis virus (MHV) infection in the central nervous system, the adoptive transfer of both a CD8+ T cell clone and a CD4+ T cell clone was shown to suppress viral growth and viral antigen-positive cells in the brain (Yamaguchi et al., 1988). In addition, the transfer of MHV nucleoprotein-specific CTL protected mice from a subsequent lethal MHV challenge by reducing virus replication within the central nervous system (Stohlman et al., 1995). Transfer of these CTL directly into the central nervous system was at least 10-fold more effective than peripheral transfer. Histological analysis demonstrated that CTL reduced virus replication in ependymal cells, astrocytes, and microglia. However, CD4+ T lymphocytes do not appear to play a critical role in resolving the viral load after infection with IBV in chicks. Therefore, the impact of various cellular immune responses to distinct coronaviruses differs, depending on the host and virus.

The distribution of chicken αβ and γδ T lymphocytes can be differentiated in developing chicks and chick embryos (Chen et al., 1988, 1990). The γδ T lymphocytes appear earlier and migrate more quickly from thymic cortex to medulla and to peripheral lymphoid tissues and differ in their composition of CD4- and CD8-bearing cells compared to mammalian γδ T cells (Bucy et al., 1988, 1990; George and Cooper, 1990). Greater numbers of γδ T lymphocytes in chickens are γδ cells than in mammals. Thirty percent of the splenic T lymphocytes in the
CHICKEN HAVE THE \( \gamma \delta \), CD3 PHENOTYPES (BUCY ET AL., 1988; SOWDER ET AL., 1988). AVIAN \( \gamma \delta \) T CELLS ARE FOUND PROMINENTLY IN SINUSOIDAL AREAS OF THE SPLEEN AND INTESTINAL EPITHELIUM, WHEREAS \( \alpha \beta \) T CELLS RESIDE PRIMARILY IN THE SPLENIC PERIARTERIAL SHEATH (VAINIO ET AL., 1990). THE MAMMALIAN \( \gamma \delta \) LYMPHOCYTES, DISPLAYING LESS DIVERSITY THAN \( \alpha \beta \) T CELLS, ARE PRESENT IN SMALLER NUMBERS IN THE PERIPHERAL LYMPHOID TISSUES, BUT ARE RELATIVELY ABUNDANT IN THE EPITHELIUM OF THE INTESTINES, SKIN, AND REPRODUCTIVE ORGANS, SUCH AS THE UTERUS AND VAGINA (DURUM ET AL., 1998; GUEHLER ET AL., 1999; LIVAK ET AL., 1999; SALERNO AND DIELI, 1998; ZORBAS AND SCOLLAY, 1995).

THE MAMMALIAN \( \gamma \delta \) T CELLS APPEAR TO PLAY A PROTECTIVE ROLE IN EARLY INFECTION WITH INTRACELLULAR BACTERIA, SUCH AS LISTERIA MONOCYTOGENES (HASEGAWA ET AL., 1992), MYCOBACTERIUM BOVIS (INOUÉ ET AL., 1991), AND SALMONELLA CHOLERAESUIS (EMOTO ET AL., 1992). MURINE \( \gamma \delta \) T LYMPHOCYTES WERE PREVALENT WITHIN INFLAMMATORY LESIONS LATE, RATHER THAN EARLY, IN THE COURSE OF PNEUMONIA CAUSED BY INFLUENZA VIRUS A (CARDING ET AL., 1990) OR SENDAI VIRUS (HOU ET AL., 1992) AND MIGHT, THEREFORE, BE IMPORTANT FOR THE RESOLUTION OF THE INFLAMMATORY PROCESS. IN MICE INFECTED WITH SENDAI VIRUS (OGASAWARA ET AL., 1994), THE TIME COURSE OF VIRUS PROPAGATION WAS IMMEDIATELY AND COINCIDENTLY FOLLOWED BY THE DEVELOPMENTAL COURSE OF \( \gamma \delta \) T CELLS. IN OUR STUDY, \( \gamma \delta \) T LYMPHOCYTES DO NOT APPEAR TO BE IMPORTANT IN RESOLVING IBV INFECTION IN CHICKS, ALTHOUGH WE CANNOT RULE OUT THE POSSIBILITY THAT THEY PRODUCE CYTOKINES THAT INFLUENCE \( \alpha \beta \) T CELL FUNCTION. HUMAN \( \gamma \delta \) T LYMPHOCYTES FROM PERIPHERAL BLOOD LYMPHOCYTES OF HEALTHY DONORS SECRETED IL-2 FOLLOWING EXPOSURE TO MYCOBACTERIA (MUNK ET AL., 1990). CONSISTENT WITH IBV INFECTION IN CHICKENS, THE CTL RESPONSE TO RETICULOENDOTHELIOSIS VIRUS INFECTION IN CHICKENS WAS MEDIATED BY \( \alpha \beta \), BUT NOT BY \( \gamma \delta \), T CELLS (Merkle ET AL., 1992). BECAUSE AVIAN \( \gamma \delta \) CELLS REPRESENT A LARGE PROPORTION OF T LYMPHOCYTES IN PERIPHERAL ORGANS IN THE CHICKEN, THE ACTUAL ROLE OF \( \gamma \delta \) T CELLS IN CHICKS INFECTED WITH AVIAN VIRUSES SHOULD BE FURTHER EXAMINED.

THE CHICKEN IS PROVING TO BE A REWARDING MODEL IN EXAMINING THE ROLE OF CTL IN THE PATHOGENESIS AND CONTROL OF VIRAL INFECTION. ONGOING STUDIES MAKING USE OF \( \alpha \beta \), CD8\(^+\) T LYMPHOCYTES FROM CHICKS EXPOSED TO IBV ARE CRITICAL IN DETERMINING FACTORS THAT PROTECT CHICKENS AGAINST IBV INFECTION. FUTURE EXPERIMENTS WILL UTILIZE AVAILABLE MHC RECOMBINANTS. BECAUSE CELLS WITH THE MHC RESTRICTED CAPACITY TO PROTECT AGAINST CHALLENGE VIRUS CORRELATED WITH THE CTL RESPONSE, IT MAY BE SUGGESTED THAT THE MAJOR MECHANISM FOR CONTROL OF ACUTE INFECTION IS DUE TO CD8\(^+\), MHC RESTRICTED \( \alpha \beta \) T LYMPHOCYTES. BECAUSE IBV-SPECIFIC CTL TEND TO RECOGNIZE EPITOPES CONSERVED AMONG SEROLOGICALLY DISTINCT STRAINS (SEO ET AL., 1997), MECHANISMS THAT INDUCE CTL SHOULD BE EXPLAINED WITH THE DEVELOPMENT OF EFFECTIVE VACCINES.

MATERIALS AND METHODS

**Experimental animals.** Chicks used in these studies were Hy-VAC B/1B/18 (Hy-VAC, Adel, IA) as well as B/1B/18 B/1B/19 from the Department of Biological Sciences, Northern Illinois University (DeKalb, IL) (Briles and Briles, 1982). Chicks were hatched in our laboratory and housed in a specific, pathogen-free environment at the Laboratory Animal and Resources Facility (Texas A & M University, College Station, TX).

**Viral inoculations.** The nephrotrophic Gray strain of IBV, used as the source for viral inoculation, was propagated by inoculating the allantoic sac of 11-day-old chicken embryos and harvesting the allantoic fluid 36 h p.i. (Sneed ET AL., 1989).

**Preparation of donor lymphocytes.** Chicks at the age of 28 days were infected with 10\(^2\) EID\(_{50}\) of the Gray strain of IBV. Splenocytes were collected from IBV-infected chicks at 10 days p.i. Control donor cells were prepared from age-matched uninfected chicks. Lymphocytes were separated with a Ficoll-Hypaque density gradient (Histopaque 1.077; Sigma, St. Louis, MO), adherent cells were removed by incubation on plastic petri dishes, and B lymphocytes were depleted using nylon wool columns (Seo and Collisson, 1997). T-cell-enriched preparations were transferred directly to naive chicks or were first stimulated in vitro for 10 days with antigen-presenting cells (APC) in RPMI with 10% chicken serum, 50 \( \mu g/ml \) gentamicyn, 5 \( \times \) 10\(^{-5}\) M 2-mercaptoethanol, and 2 mM L-glutamine from Gibco BRL (Grand Island, NY). IBV-infected CK cells (Seo and Collisson, 1997), irradiated with 10,000 rad, were used as APC.

**Inoculation of donor lymphocytes and challenge virus.** Each group consisted of at least five chicks. Immune or nonimmune donor lymphocytes were given at 5 days of age through a wing vein. One day after receiving donor lymphocytes, chicks were challenged with 2 to 4 \( \times \) 10\(^6\) EID\(_{50}\) of the Gray strain of IBV by the nasal–ocular route. Birds were observed twice each day for clinical signs of illness, such as coughing, sneezing, rales, or dyspnea. Statistical differences in illness were determined by analysis of variance (Ott, 1984). Viral load was evaluated in lungs and kidneys collected 5 days p.i. (Seo and Collisson, 1997).

**Depletion of T lymphocyte subtypes.** Stimulated T cell subtypes were enriched, after B cells and adherent cells were removed, with antisera specific for CD4, CD8, TCR1, or TCR2 (Seo and Collisson, 1997). Briefly, tissue culture flasks were coated with goat anti-mouse IgG. The immune T cells were labeled with mouse anti-chicken CD4, CD8, TCR1, or TCR2 monoclonal antibody (Southern Biotechnology Associates, Birmingham, AL) before being added to IgG-coated plates and being incubated at room temperature for 30 min. The suspensions were swirled gently before being incubated for an additional 30 min at room temperature. Nonadherent cells were decanted.
from the plates following gentle agitation, centrifuged for 10 min at 600 g, and resuspended in PBS before being transferred to chicks.

**CTL assay. In vitro** stimulated T lymphocytes depleted of T cell subtypes were tested for CTL activity using target CK cells infected with the Gray strain of IBV as described previously (Seo and Collisson, 1997; Seo et al., 1997). Target cells were labeled with $^{51}$Cr for 90 min before being washed and mixed with effector lymphocytes (Seo and Collisson, 1997). Varying amounts of effector cells in 100 $\mu$l of RPMI with 10% chicken sera were added to each well in 96-well round-bottom microtiter plates, and 100 $\mu$l of 2 $\times$ 10$^5$ of $^{51}$Cr-labeled target cells that were infected with IBV or uninfected was added to each well. Microtiter plates were centrifuged at 100 g for 5 min before being incubated for 4 h at 40°C. After being centrifuged at 600 g for 10 min, 100 $\mu$l of the supernatants in triplicate samples was collected for determining $^{51}$Cr release using a gamma radiation counter (Cobra Autogamma, Canberra Packard). The percentage of specific lysis was calculated as $(E-S/M-S) \times 100$, 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. The spontaneous $^{51}$Cr release of target cells was less than 15% of the maximum release.

**Determination of viral load in chicken lungs or kidneys.** Lungs and kidneys were collected and pooled from syngeneic chicks in each group (Seo and Collisson, 1997). Tissues from the chicks in each group were pooled and homogenized. A 10% w/v tissue suspension was prepared in Eagle’s MEM containing 200 U/ml of penicillin and 200 $\mu$g/ml streptomycin. After standing for 90 min at 4°C, cells were pelleted by centrifuging for 20 min at 2000 g and serial 10-fold dilutions of supernatants were prepared in Eagle’s MEM. One-tenth of a milliliter of each virus dilution was injected into the allantoic sac of six 11-day-old embryo-nated chicken eggs (Cook, 1983). The eggs were sealed with collodion before being incubated at 38°C for 5 days and then the embryos were examined for dwarfing by weighing. EID$_{50}$ was calculated by the Reed and Muench method (Burleson et al., 1992).

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**REFERENCES**

Aeed, P. A., Bries, W. E., Zsigray, R. M., and Collins, W. M. (1993). Influence of different B-complex recombinants on the outcome of Rous sarcomas in chickens. *Anim. Genet.* 24, 177–181.

Alexander, D. J., Goush, R. E., and Pattison, M. (1978). A long-term study of the pathogenesis of infection of fowls with 3 strains of avian infectious bronchitis virus. *Res. Vet. Sci.* 24, 223–227.

Bries, W. E., and Bries, R. W. (1982). Identification of haplotypes of the chicken major histocompatibility complex (B). *Immunogenetics* 15, 449–459.

Bries, W. E., and Bries, R. W. (1987). Genetics and classification of the major histocompatibility complex antigens of the chicken. *Poultry Sci.* 66, 776–781.

Bucy, R. P., Chen, C. L., Chihak, J., Losch, U., and Cooper, M. D. (1988). Avian T cells expressing gamma delta receptors localize in the splenic sinusoids and the intestinal epithelium. *J. Immunol.* 141(7), 2200–2205.

Bucy, R. P., Chen, C. H., and Cooper, M. D. (1990). Ontogeny of T cell receptors in the chicken thymus. *J. Immunol.* 144(4), 1161–1168.

Burleson, F. G., Chambers, T. M., and Weidbrauk, D. L. (1992). "Virology: A Laboratory Manual," Academic Press, San Diego, CA.

Cannon, M. J., Stott, E. J., Taylor, G., and Askonas, B. A. (1987). Clearance of persistent respiratory syncytial virus infections in immuno-deficient mice following transfer of primed T cells. *Immunology* 62, 133–136.

Carding, S. R., Allan, W., Kyes, S., Hayday, A., Bottomly, K., and Doherty, P. C. (1990). Late dominance of the inflammatory process in murine influenza by $\gamma$$\delta$ T cells. *J. Exp. Med.* 172, 1225–1231.

Chen, C. L., Chihak, J., Losch, U., and Cooper, M. D. (1988). Differential expression of two T cell receptors, TCR1 and TCR2, on chicken lymphocytes. *Eur. J. Immunol.* 18(4), 539–543.

Chen, C. L., Bucy, R. P., and Cooper, M. D. (1990). T cell differentiation in birds. *Semin. Immunol.* 2(1), 79–86.

Collisson, E. W., Parr, R. L., Wang, L., and Williams, A. K. (1992). An overview of the molecular characteristics of avian infectious bronchitis virus. *Poultry Sci. Rev.* 4, 41–55.

Cook, J. K. A. (1983). The classification of new serotypes of infectious bronchitis virus isolated from poultry flocks in Britain 1981 and 1983. *Avian Pathol.* 13, 733–741.

Cook, J. K. A., Davison, T. F., Huggins, M. B., and McLaughlin, P. (1991). Effect of in ovo bursectomy on the course of an infectious bronchitis virus infection in line C white Leghorn chickens. *Arch. Virol.* 118, 225–234.

Crinion, R. A., and Hofstad, M. S. (1972). Pathogenicity of two embryo-passage levels of avian infectious bronchitis virus for the oviducts of young chickens of various ages. *Avian Dis.* 16, 967–973.

Durum, S. K., Candeias, S., Nakajima, H., Leonard, W. J., Baird, A. M., Berg, L. J., and Muegge, K. (1998). Interleukin 7 receptor control of T cell receptor gamma gene rearrangement: Role of receptor-associated chains and locus accessibility. *J. Exp. Med.* 188, 2233–2241.

Emoto, M., Danbara, H., and Yoshikai, Y. (1992). Induction of $\gamma$$\delta$ T cells in murine salmonellosis by an avirulent but not by a virulent strain of *Salmonella choleaeus*. *J. Exp. Med.* 176, 363–372.

George, J. F., and Cooper, M. D. (1990). Gamma/delta T cells and alpha/beta T cells differ in their developmental patterns of receptor expression and modulation requirements. *Eur. J. Immunol.* 20(10), 2177–2181.

Golemboski, K. A., Taylor, R. L., Bries, W. E., Bries, R. W., and Dietert, R. R. (1995). Immuno-inflammatory function of macrophages from chickens with B-recombinant haplotypes. *Avian Pathol.* 24, 347–352.

Guehler, S. R., Bluestone, J. A., and Barrett, T. A. (1999). Activation and peripheral expansion of murine T-cell receptor gamma delta intra-epithelial lymphocytes. *Gastroenterology* 116, 327–334.

Hasegawa, T., Tanaka, T., and Yoshikai, Y. (1992). The appearance and role of $\gamma$$\delta$ T cells in the peritoneal cavity and liver during primary infection with *Listeria monocytogenes* in rats. *Int. Immunol.* 4, 1129–1136.

Hofstad, M. S. (1984). Avian infectious bronchitis. In "Diseases of Poultry" (W. C. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Eds.), 8th ed., pp. 429–443. Iowa State Univ. Press, Ames, IA.

Hou, S., Katz, J. M., Doherty, P. C., and Carding, S. R. (1992). Extent of $\alpha$$\beta$-recombinant haplotypes. *Avian Pathol.* 21, 363–372.
T cell involvement in the pneumonia caused by Sendai virus. *Cell. Immunol.* **143**, 183–193.

Ignjatovic, J., and Galli, L. (1994). The S1 glycoprotein but not the N or M proteins of avian infectious bronchitis virus induces protection in vaccinated chickens. *Arch. Virol.* **138**(1–2), 117–134.

Inoue, T., Yoshikai, Y., Matsuzaki, G., and Nomoto, K. (1991). Early appearing γδ-bearing T cells during infection with Calmette Guerin bacillus. *J. Immunol.* **146**, 2754–2762.

Klavinskis, L. S., Tishon, A., and Oldstone, M. B. (1989). Efficiency and effectiveness of cloned virus-specific cytotoxic T lymphocytes in vivo. *J. Immunol.* **143**, 2013–2016.

Livak, F., Tourigny, M., Schatz, D. G., and Petrie, H. T. (1999). Characterization of TCR gene rearrangements during adult murine T cell development. *J. Immunol.* **162**, 2575–2580.

Merkle, H., Cihak, H., and Losch, U. (1992). The cytotoxic T lymphocyte response in reticuloendotheliosis virus-infected chickens is mediated by alpha beta and not by gamma delta T cells. *Immunobiology* **186**(3–4), 292–303.

Miller, M. M., Goto, R., and Briles, W. E. (1988). Biochemical confirmation of recombination within the B-G subregion of the chicken. *Immunogenetics* **27**, 127–132.

Munk, M. E., Gatrill, A. J., and Kaufmann, S. H. (1990). Target cell lysis and IL-2 secretion by gamma/delta T lymphocytes after activation with bacteria. *J. Immunol.* **145**, 2434–2439.

Offit, P. A., and Dudzik, K. I. (1990). Rotavirus-specific cytotoxic T lymphocytes passively protect against gastroenteritis in suckling mice. *J. Virol.* **12**, 6325–6328.

Ogasawara, T., Emoto, M., Kiyotani, K., Shimokata, K., Yoshida, T., Nagai, Y., and Yoshikai, Y. (1994). Sendai virus pneumonia: Evidence for the early recruitment of γδ T cells during the disease course. *J. Virol.* **68**, 4022–4027.

Ott, L. (1984). Introduction to the analysis of variance. In *An Introduction to Statistical Methods and Data Analysis,* 2nd ed., pp. 325–336. Duxbury, Boston, MA.

Salerno, A., and Dieli, F. (1998). Role of gamma delta T lymphocytes in immune responses in humans and mice. *Crit. Rev. Immunol.* **18**, 327–357.

Seo, S. H., and Collisson, E. W. (1997). Specific cytotoxic T lymphocytes are involved in in vivo clearance of infectious bronchitis virus. *J. Virol.* **71**, 5173–5177.

Seo, S. H., and Collisson, E. W. (1998). Cytotoxic T lymphocyte response to infectious bronchitis virus infection. *Adv. Exp. Med. Biol.* **440**, 455–460.

Seo, S. H., Wang, L., Smith, R., and Collisson, E. W. (1997). The carboxyl terminal 120 residue polypeptide of infectious bronchitis virus nucleocapsid induces cytotoxic T lymphocytes and protects chicks from acute infection. *J. Virol.* **71**, 7889–7894.

Siddell, S. G., Wege, H., and Ter Meulen, V. (1983). The biology of coronaviruses. *J. Gen. Virol.* **64**, 761–776.

Sneed, L., Butcher, G., Wang, L., Parr, R., and Collisson, E. W. (1989). Comparisons of the structural proteins of avian bronchitis virus as determined by western blot analyses. *Viral Immunol.* **2**, 221–227.

Sowder, J. T., Chen, C. H., Ager, L. L., Chan, M. M., and Cooper, M. D. (1988). A large subpopulation of avian T cells express a homologue of the mammalian T γδ receptor. *J. Exp. Med.* **167**, 315–322.

Stohlman, S. A., Bergmann, C. C., Van der Veen, R. C., and Hinton, D. R. (1995). Mouse hepatitis virus-specific cytotoxic T lymphocytes protect from lethal infection without eliminating virus from the central nervous system. *J. Virol.* **69**, 684–694.

Vainio, O., Lassila, O., Cihak, J., Losch, U., and Houssaint, E. (1990). Tissue distribution and appearance in ontogeny of alpha/beta T cell receptor (TCR2) in chicken. *Cell. Immunol.* **125**, 254–260.

Yamaguchi, K., Kyuwa, S., Nakanaga, K., and Hayami, M. (1988). Establishment of cytotoxic T-cell specific for cells infected with mouse hepatitis virus. *J. Virol.* **62**, 2505–2507.

Zorbas, M. I., and Scollay, R. (1995). The gamma delta T lymphocytes of the perinatal murine thymus. *Dev. Immunol.* **4**, 93–100.