Available online at www.sciencedirect.com
SCIENCE DIRECT®
Developmental and Comparative Immunology 29 (2005) 153–160
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Specific antibody secreting cells from chickens can be detected by three days and memory B cells by three weeks post-infection with the avian respiratory coronavirus

Jianwu Pei, Ellen W. Collisson*

Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4467, USA

Received 20 January 2004; revised 14 June 2004; accepted 23 June 2004
Available online 12 August 2004

Abstract

Infectious bronchitis virus (IBV), the first coronavirus described, has been a continuing problem in poultry for more than 70 years. IBV, causing a highly contagious respiratory disease in chickens, resembles the recently described severe acute respiratory syndrome virus in pathogenesis and genome organization. While previous studies demonstrated that effector and memory $CD8^+$ T lymphocytes are critical in controlling acute IBV infection and disease in chickens, here chicken anti-IBV antibody (IgG) secreting cells (ASC) in both peripheral blood mononuclear cells (PBMC) and spleens collected following IBV Gray infection were evaluated using an ELISPOT assay. The ASC in peripheral blood and spleens can be detected from 3 to 7 days post-infection (p.i.), which is 3–7 days earlier than anti-IBV IgG detected in the serum. The ASC frequency reached a maximum at 7–10 days p.i., and decreased more than 90% in the spleen and 70% in PBMC by 14 days p.i. The ASC levels in the PBMC then decreased gradually to 0.5 ASC/10^6 over the next 8 weeks. The higher concentration of about 20 ASC/10^6 cells in spleens may, at least partially, account for the presence of antibody in the serum although bone marrow ASC were not determined. In vitro stimulation of PBMC and splenocytes with IBV antigen demonstrated that memory B cells can be activated to secrete antibody by 3 weeks p.i. ELISPOT detection of primary B cells could be useful in the early detection of infection following infection with respiratory coronaviruses.

Keywords: Coronavirus; Chicken; Antibody secreting cell; ELISPOT; Memory B cell

1. Introduction

It has been recently shown that the highly contagious severe acute respiratory syndrome (SARS) in humans is caused by a coronavirus (CoV) that resembles infectious bronchitis in transmission, pathogenesis and genome structure [10,19,26]. Infectious bronchitis virus (IBV) infection causes a highly contagious respiratory disease in chickens, especially in young chicks [3,5]. The disease was first described in 1931, and has since remained a major problem in the poultry industry worldwide [3,13].

* Corresponding author. Tel.: +1-979-845-4122; fax: +1-979-862-1088.
E-mail address: ecollisson@cvm.tamu.edu (E.W. Collisson).

0145-305X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dci.2004.06.009

Vaccines are available, but they are not effective long-term in controlling IBV infection, especially for variant strains. Genetic variations are common in new strains because of both point mutations and recombinants [14,15,40,41]. The many years of experience dealing with IBV should provide a valuable model for understanding SARS CoV infection in humans.

Recent studies have shown that effector $CD8^+$ T cells are critical in controlling acute IBV infection [6,9,30]. Adoptive transfer of T cells collected at 10 days post-infection (p.i.) protected syngenic chicks from clinical illness [30]. IBV specific memory T cells can be generated at 3 weeks p.i., and adoptive transfer of the memory T cells passed protection to the recipient chicks [22]. Innate immunity may also be instrumental in controlling IBV infection. Chicken interferon type I (ChIFN-I) inhibits IBV replication in vitro and in vivo [23]. Local administration of ChIFN-I inhibited IBV associated respiratory illness [23]. The importance of humoral immunity was indicated by Cook et al. (1991), who demonstrated that after IBV infection, bursectomised chicks suffered more severe and longer illness than intact chicks. The viral titers in tissues were also higher and lasted longer in bursectomised chicks than in normal chicks [7].

Individual antibody secreting cells (ASC) and activated T cells can be detected using ELISPOT assays, powerful tools for quantifying individual cell responses [1,27,28,35,42]. In the current experiments, IBV specific IgG secreting cells were detected in peripheral blood and spleens using an ELISPOT assay, while memory B cells were detected after antigen stimulation.

2. Materials and methods

2.1. Animals and virus

SPAFAS specific, pathogen-free (SPF) chickens were hatched in our laboratory and housed in an SPF environment at the Laboratory Animal Resources and Research Facility (Texas A&M University, College Station, TX). Immune chickens were generated by inoculating 1-week-old chickens with 10^7 EID_{50} of the IBV Gray strain by the eye–nasal routes. The virus, propagated by inoculating the allantoic sac of 11-day-old chicken embryos with the Gray strain of IBV and harvesting allantoic fluid 36 h p.i., was used for in vivo inoculation [34]. The IBV antigen used in the ELISA and ELISPOT assay was purified by polyethylene glycol (PEG) 8000 precipitation. Briefly, allantoic fluid collected at 36 h p.i. was centrifuged at 10,000 rpm for 25 min to remove any cells and cell debris. Sodium chloride (2.33%) and PEG 8000 (7%) were added to the supernatant and incubated overnight at 4 °C. The virus was collected by centrifuging at 12,000 rpm for 40 min and resuspended in PBS (pH 7.4).

2.2. Cell culture and antigen stimulation

Peripheral blood mononuclear cells (PBMC) and spleen cells were prepared at varying times p.i. [22]. Briefly, 0.5 ml of blood was collected from each of three chicks at each time point through a wing vein with a 1-ml syringe containing anticoagulant EDTA-K3 and mixed with an equal volume of PBS. PBMC were isolated by centrifuging the diluted blood for 20 min at 2000 rpm through Histopaque-1077 (Sigma). The cells collected from the interface were pooled for each group, washed three times and cultured in complete RPMI-1640 in six-well plates at a density of 10^7 cells/ml. Single splenocyte suspensions were prepared as described [22,23]. To detect memory responses, the PBMC and splenocytes were stimulated with UV-inactivated IBV Gray strain ($10^{7.3}$ EID_{50}/well). One hundred microliters of cell suspension were collected at 3, 6, 9, and 12 days after stimulation. The cells were washed three times with RPMI-1640 before using in an IgG ELISPOT assay. The supernatants were saved for antibody detection.

2.3. Detection of antibody by ELISA

One-week-old chicks were infected with the IBV Gray strain through the eyes and nose. Sera were collected from three chicks at various times p.i., and antibody responses were determined using ELISA [21]. Briefly, 96-well microtiter ELISA plates (Nunc Maxisorb) were coated with 100 μl/well of purified IBV antigen, diluted in bicarbonate/carbonate buffer (2.93 g $NaHCO_3$, 1.59 g Na_2CO_3, 0.203 g $MgCl_2$ in 1 l of distilled water, pH 9.6) at a concentration of 10 μg/ml. The plates were incubated overnight at 4 °C before