The role of mucosal antibody in immunity to infectious laryngotracheitis virus in chickens

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The role of mucosal antibody in recovery from a primary infection and resistance to reinfection with infectious laryngotracheitis (ILT) herpesvirus was studied in bursectomized chickens, which were unable to synthesize specific antibodies. Viral antigen in the infected trachea was assessed by indirect immunofluorescence on tissue sections and by ELISA. The ability of bursectomized chickens to resolve primary infections as effectively as intact chickens and of vaccinated–bursectomized chickens to prevent the replication of challenge virus without the participation of mucosal antibody, is evidence for the importance of local cell-mediated rather than humoral immune mechanisms in the outcome of infection with ILT virus.

Introduction

Infectious laryngotracheitis (ILT) virus is an alphaherpesvirus which causes a highly contagious respiratory disease in chickens (Jordan, 1981). The virus causes sporadic outbreaks of severe disease, presumably due to its ability to persist in a latent state in previously infected birds. Pathogenesis studies indicate that the virus causes a highly localized infection in the respiratory tract (Hanson, 1984). Viraemia and infection of visceral tissues occurs infrequently (Purcell & McFerran, 1969) and then usually in young chickens (Bagust et al., 1986).

Recent studies on the role of local immunity in the recovery of chickens from ILT found a correlation between the disappearance of viral antigen from the trachea and the appearance of mucosal antibody (York et al., 1989). This finding contrasted with previous studies which showed that bursectomized chickens, which were unable to synthesize specific antibodies, could be protected against clinical disease by vaccination (Robertson, 1977; Fahey et al., 1983). Although cellular immunity may prevent clinical ILT and mortalities in vaccinated chickens (Fahey et al., 1984) the question of whether mucosal antibody assists in the clearance of a primary infection from the trachea, or plays a role in preventing the establishment of a secondary infection remains unanswered.

This study records the rate of recovery from a primary infection with ILT virus in bursectomized chickens unable to synthesize mucosal antibody and also the ability of challenge virus to establish an infection in the tracheal epithelial cells of vaccinated chickens lacking mucosal antibody. The insights gained into the role of mucosal antibody in ILT are discussed.

Methods

Chickens. Specified pathogen-free (SPF) White Leghorn chickens of the CSIRO-W line produced by the CSIRO SPF Poultry Unit, Maribyrnong, Victoria were used throughout. The chickens were housed in flexible plastic isolators and fed fumigated feed and acidified water ad libitum.

Bursectomy. A combination of hormonal treatment in ovo and cyclophosphamide injections after hatching was used for bursectomy (Kowalski et al., 1978; Rose & Hesketh, 1979). Twelve-day-old embryonated eggs were injected via the allantoic sac with 3.5 mg of testosterone propionate (Sigma) in 0.1 ml sterile corn oil. Each chick was injected intraperitoneally with 2 mg cyclophosphamide (Sigma) at days 1 and 2 after hatching and with 3 mg on day 3 after hatching.

Viruses. SA-2, the vaccine strain of ILT virus used in Australia (Arthur Webster Pty Ltd, Castle Hill, Australia) and CSW-1, a virulent isolate of ILT virus, were propagated and assayed in primary chicken kidney (CK) cells as described previously (Fahey et al., 1983).

Detection of antibody by ELISA. The ELISAs for the detection of ILT antibody were performed essentially as described by York et al. (1989) except that the antigen used was the SA-2 strain of ILT virus in tissue culture supernatant harvested 20 h after infection of CK cells. The medium was centrifuged at 1000 g for 10 min and the supernatant stored at −70 °C.

Polyvinyl chloride microtitre plates (Dynatech Laboratories) were coated overnight with rabbit IgG antibody to ILT virus. Antigen was added at a dilution of 1:5 followed by serum or tracheal washings at initial dilutions of 1:20 and 1:5 respectively. Affinity-purified goat anti-chicken IgG (heavy and light chains) conjugated to horseradish peroxidase (HRP; Kirkegaard and Perry Laboratories) was used at a dilution of 1:1000 to detect total antibody. Hybridoma culture supernatant containing monoclonal antibody to chicken IgA and...
affinity-purified goat anti-mouse IgG conjugated to HRP (Tago) were
used at dilutions of 1 : 100 and 1 : 1000 respectively to detect IgA
antibody. The substrate was recrystallized 5-aminosalicylic acid (5-AS;
Merck). The titre was taken as the highest dilution that gave an
absorbance reading more than three standard deviations above the
mean reading of samples obtained from uninfected chickens.

Detection of antigen by ELISA. A monoclonal antibody against ILT
virus was used to detect viral antigen as described by York & Fahey
(1988). Briefly, 150 µl volumes of undiluted tracheal washings were
added in duplicate to polyvinyl chloride microtitre plates coated with
rabbit antibody to ILT virus. Hybridoma culture supernatant
containing monoclonal antibody against ILT virus (designated 131-24)
was diluted 1 : 30 and added to the plates. The monoclonal antibody was
followed by affinity-purified goat anti-mouse IgG conjugated to HRP
(Tago) at a dilution of 1 : 1000 and then 5-AS. An absorbance reading,
corrected for background, of greater than 0.1 was considered positive.

Histology. Sections prepared from formalin-fixed tissues were
stained with haematoxylin and eosin. Frozen sections were fixed in
acetone for 5 min after cutting and stored at -70 °C until examined.

Immunofluorescence. IgA- and IgG-synthesizing cells present in
frozen tracheal sections were quantified by incubating the sections with
monoclonal antibodies against IgA (Southern Biotechnology Associ-
ates) and IgG (Australian Monoclonal Development) as described by
York et al. (1989). Individual fluorescing cells in the tracheal
epithelium and connective tissue of the whole section were counted;
two sections from each chicken were counted.

Viral antigen in frozen tracheal sections was detected in a similar
manner, using undiluted monoclonal antibody culture supernatant
(131-24) followed by sheep anti-mouse Ig conjugated to fluorescein at a
dilution of 1 : 25 (Silenus Laboratories). The level of replication in each
section was quantified by giving a score from 0 to 10 to the proportion
of the area of the section that contained fluorescing cells. The score was
based on the area affected, not the intensity of fluorescence in any
region of the section.

Statistical analysis. The ELISA absorbance readings for viral antigen
were analysed using a one-way analysis of variance.

Experimental design. A total of 99 4-week-old bursectomized and
intact chickens were vaccinated intratracheally with 1.5 x 10^8 p.f.u. of
SA-2 ILT vaccine in a 100 µl volume. At days 0, 3, 5, 6, 7 and 8 post-
infection (p.i.), groups of five chickens were killed by asphyxiation
with CO2. The thymuses and the bursal remnants in the bursectomized
chickens were fixed in formalin. The tracheas were removed
aseptically and the ends of the trachea were closed with surgical suture,
which also secured a syringe and needle containing 1 ml sterile
phosphate-buffered saline (PBS) into the bottom opening of the
trachea. The trachea was washed by squirting the PBS in and out of the
trachea several times and by manually massaging the trachea. The
trachea was opened longitudinally and scraped with a scalpel. The
tracheal scrapings were added to the washings which were then
sonicated using an ultrasonic processor (Sonic and Materials). PMSF
was added to a final concentration of 1 mM. The washings were
centrifuged at 12000 r.p.m. for 2 min and stored at -70 °C until
assayed.

At day 28 p.i. blood and tracheal washings were collected from three
chickens from each of four groups: vaccinated-intact and vaccinat-
bursectomized and age-matched unvaccinated-intact and
unvaccinated-bursectomized chickens. Both vaccinated and unvaccin-
ated groups of chickens were challenged by intratracheal inoculation of
5 x 10^8 p.f.u. of CSW-1 in a 100 µl volume. Serum and tracheal
washings were collected from eight to 10 chickens from each of the four
groups at day 3 post-challenge, the time of maximum viral replication
detectable by immunofluorescence (J. J. York, unpublished data) and
the time after which viral antigen detectable by ELISA decreases (York
et al., 1989). Bursal remnants collected from the bursectomized
chickens at post mortem were fixed in formalin. Frozen tracheal
sections were also prepared from the upper 0.5 cm of the trachea of
three to five birds from each group at day 1 and day 3 post-challenge.

Results

The effect of bursectomy on a primary infection with ILT
vaccine virus

Following vaccination with SA-2 ILT vaccine more than a
third of the intact chickens showed signs of respiratory
distress (gasping) on days 5 and 6 p.i., but none of the
bursectomized chickens showed clinical signs. In total,
six of the 44 (13-6%) intact chickens died following
intratracheal vaccination, compared to two of the 70
(2-9%) bursectomized chickens.

The mean ELISA reading for ILT viral antigen in the
tracheal washings of the five bursectomized chickens
examined post mortem at days 3, 5, 6 and 7 p.i. did not
differ significantly from that detected in the five intact
chickens at each of these time points (Fig. 1). In both
groups the amount of viral antigen present in the tracheal
mucosa declined from day 3 p.i. to reach undetectable
levels by day 6 p.i. in the bursectomized chickens and by
day 7 p.i. in the majority of intact chickens. Thus
bursectomized chickens cleared the vaccine virus as
effectively as the intact chickens.

When the tracheal washings from these chickens were
assessed for the presence of total antiviral antibody by
ELISA, none of the bursectomized chickens had
detectable levels of humoral or mucosal antibody, even at
day 8 p.i. (data not shown).

Histological examination of the thymuses and bursal
remnants from each of the hormonally bursectomized
chickens showed that the follicular structure of the
bursae had totally collapsed, but the thymuses appeared
unaffected. Over 50% of the treated chickens had no
lymphocytes in the subepithelial regions. The remainder
showed some repopulation, with from one to several
small aggregations of lymphoid cells which showed no
cortico-medullary differentiation.

The effect of bursectomy on the ability of vaccinated
chickens to resist infection with virulent ILT virus

Immediately before challenge, both the vaccinated-
bursectomized and vaccinated-intact chickens were
negative for ILT viral antigen as measured by ELISA. At
this time only the vaccinated-intact chickens had
detectable levels of humoral and tracheal antibody to
ILT virus (Table 1).

Three days after intratracheal challenge with virulent
ILT virus neither the vaccinated-bursectomized chickens nor the vaccinated-intact chickens had detectable levels of viral antigen in their tracheal washings, whereas unvaccinated-bursectomized and unvaccinated-intact chickens had readily detectable levels of viral antigen (Table 2). When frozen sections of the tracheae obtained from these chickens at 3 days post-challenge were examined by immunofluorescence, no cells infected with ILT virus were detected in the sections from either group of vaccinated chickens, both groups having a score of 0. Extensive viral replication was evident in the tracheal sections from both groups of unvaccinated chickens; the unvaccinated-bursectomized chickens had a mean score of 6.4, while the unvaccinated-intact chickens had a score of 6.7.

Only the vaccinated-intact chickens had detectable levels of humoral or mucosal anti-ILT virus antibody, a minor portion of which was IgA (Table 1). Frozen sections from the trachea of all four groups of chickens were also examined for the presence of IgA- and IgG-producing cells. Three days after intratracheal challenge with virulent ILT virus, immunoglobulin-synthesizing cells were detected only in the epithelial layer and lamina propria of the intact chickens (Table 3). In unvaccinated chickens IgA-positive cells predominated; in vaccinated chickens IgG-positive cells were most abundant.

To investigate the presence of a transient infection in the vaccinated-bursectomized and intact chickens, frozen tracheal sections were examined by immunofluorescence at 24 h after challenge with virulent ILT virus. No ILT viral antigen was detected in sections collected from a group of four vaccinated-bursectomized chickens, or three vaccinated-intact chickens, whereas four of five unvaccinated-bursectomized chickens showed low grade
infections at that time, with less than 10% of the section containing infected cells.

Histological examination of the bursal remnants from hormonally bursectomized chickens showed that the subepithelial region of the majority of bursae contained lymphoid cells in small aggregations or follicles. These follicles showed no cortico-medullary differentiation.

Discussion

The role of cell-mediated immunity in recovery from herpesvirus infections and resistance to reinfection is well documented (Nash et al., 1985; Zarling, 1986), whereas the role of humoral or mucosal antibody is less clearly established (Kapoor et al., 1982b; Simmons & Nash, 1987). The concurrence of the disappearance of ILT virus from the trachea of infected chickens and the appearance of mucosal antibody (York et al., 1989) raised the possibility that mucosal antibody may play a role in recovery from infection. An extension of this finding would be that mucosal antibody plays a role in preventing reinfection of vaccinated chickens. The readily detectable levels of mucosal antibody found in the trachea following vaccination in both this and a previous study (York et al., 1989) provides support for such a hypothesis, particularly as virus-neutralizing activity has been detected in the tracheal washings of vaccinated chickens, albeit at low titre (Bagust et al., 1986; York et al., 1989).

Testosterone treatment in ovo and cyclophosphamide treatment post-hatching ablates the bursa of Fabricius and destroys the chicken’s ability to synthesize specific antibody in response to antigenic challenge (Rose & Hesketh, 1979). In this study the hormonally bursectomized chickens failed to produce either humoral or mucosal antibodies to ILT virus following vaccination or challenge, even though there was some evidence of lymphoid repopulation of the treated bursae, particularly at 8 weeks of age. Similarly, bursectomized chickens had neither IgA- nor IgG-producing cells in the trachea after vaccination or challenge with ILT virus. Yet the bursectomized chickens recovered from a primary infection with ILT virus at a rate similar to that of the intact chickens. In fact there was an indication that antibody may contribute to the pathogenesis of disease since the bursectomized chickens appeared to have a less severe clinical reaction to the vaccine virus. The appearance of antibody in the trachea may affect the viscosity of the exudate which accompanies viral infection, thereby contributing to the onset of gasping and mortality due to asphyxiation.

More interesting, however, was the finding that the absence of mucosal antibody did not impair the ability of vaccinated-bursectomized chickens to resist totally a challenge infection with virulent ILT virus. As measured by immunofluorescence there was no evidence of a transient infection in these chickens at 24 h post-challenge. Furthermore there was no ILT viral antigen present at 3 days post-challenge, the time of optimal antigen production in unvaccinated-intact chickens (York et al., 1989). It would appear that in the case of ILT virus cellular, rather than humoral, immune mechanisms are able to protect the tracheal epithelial cells of vaccinated chickens from reinfection.

Evidence to date suggests that secretory IgA, and presumably other mucosal antibodies, protect the host by reacting with bacteria or viruses and preventing them attaching to or colonizing mucosal surfaces as was initially proposed by Freter (1969). In the absence of mucosal antibody therefore, pathogens may be able to establish infections before other specific and non-specific protective mechanisms are invoked to limit the disease. In this study the failure to detect any replication of the challenge virus in vaccinated-bursectomized chickens does not support a role for mucosal antibody in vaccinal immunity to ILT virus.

It is not possible to conclude from the literature whether mucosal antibody plays a role in immunity to other herpesviruses, although Martin & Wardley (1987) reported that there was no correlation between mucosal antibody levels and protection of pigs against Aujeszky’s disease. In humans there is no correlation between the presence of maternal antibodies to herpes simplex virus (HSV) and neonatal infection with HSV (Whitley et al., 1980). Similarly it has been reported that maternal IgG antibody does not confer resistance to infection with ILT virus (Cover et al., 1960; Jordan, 1981). However, immunization of female mice and oral feeding of newborn mice with hyperimmune human IgG antibodies has been shown to confer protection against an intraperitoneal infection with HSV (Hayashi et al., 1983). Experiments involving the passive transfer of monoclonal antibodies in mice also suggest that antibody may limit the spread of virus (Dix et al., 1981, Kapoor et al., 1982a; Simmons & Nash, 1985). Conversely, B cell-suppressed mice unable to make antibody cleared HSV as effectively as conventional mice (Kapoor et al., 1982b; Simmons & Nash, 1987).

Although much remains to be learnt of the immune mechanisms that resolve infections with ILT virus in chickens and those which enable vaccinated chickens to resist reinfection, it is apparent from this study that mucosal antibody does not have a central or critical role in recovery or vaccinal immunity. This may be explained by the intracellular nature of herpesvirus infections and the ability of the virus to spread from cell to cell, but our inability to detect even a transient low grade or focal...
challenge infection in the tracheal epithelial cells of antibody-negative vaccinated chickens points to the efficacy of these antibody-independent mechanisms. Whether protection of the epithelium is mediated directly by cytotoxic lymphocytes, or by soluble factors (lymphokines) released by activated T cells, or both, remains to be answered.

The authors wish to thank Ms Jackie Pallister who established the hormonal bursectomy procedure employed in the study and acknowledge the financial support of the Chicken Meat Research Council.

References


(Received 27 April 1990; Accepted 3 July 1990)