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IMMUNOHISTOCHEMICAL DETECTION OF INFECTIOUS BURSAL DISEASE VIRUS ANTIGEN IN THE BURSA OF FABRICIUS OF EXPERIMENTALLY INFECTED CHICKENS

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The indirect immunoperoxidase method (IP) with polyclonal antibodies was used for detecting infectious bursal disease (IBD) virus antigen in the bursa of chickens experimentally infected with a highly virulent virus designated as KA. Detection of IBD virus by the IP method on methanol fixed, paraffin embedded tissue samples is possible ten days after infection (PI). Three to seven days PI an intensive expression of IBDV antigen can be observed on lymphocytes in the cortex and medulla of bursal follicles, as well as on macrophages in the follicles and interstitium. Ten days PI the immunohistochemical reaction is very discreet, but IBD virus detection is still possible. The IP method, used in our work, is a sensitive and specific way of demonstrating IBDV in the bursa of Fabricius of experimentally infected chickens.

Key words: chicken, Infectious bursal disease, virus, immunohistochemistry.

INTRODUCTION

Infectious bursal disease (IBD) is an acute, highly contagious viral infection of young chickens that has lymphoid tissue as its primary target with a special predilection for the bursa of Fabricius (BF). The IBD virus which is classified into the fammily Birnaviridae, attacks the BF causing depletion of B lymphocytes which is followed by atrophy of this very important immunological organ (Lasher and Shane 1994; Rosenberger 1989). In recent years, highly virulent serotype 1 isolates of IBDV have been reported from several laboratories in different countries. These highly virulent isolates can cause up to 90-100% mortality of young chickens.

A large quantity of viral deposit in the bursa of infected chickens during the first 72 hours after infection enables the virus to be detected by the double gel immunodiffusion test (Rao and Kumar 1994). It takes several days to isolate the

virus on a culture of chicken fibroblasts and on ebryonated eggs. Some strains cannot adapt themselves and someothers do not show characteristic changes on embryos, which makes diagnosis of the disease difficult (Jonson and Engstrom 1986). Immunoperoxidase methods are used for the detection of antigens in diagnostic tests. Results for the immunoperoxidase procedure using polyclonal antibodies correlate with the results for immunofluorescence assays for the detection of IBDV (Aleksić-Kovačević 1997; Hiraga et al. 1994).

This paper reports rapid detection of infectious bursal disease virus (IBDV) in the bursa of Fabricius by an indirect immunoperoxidase method using polyclonal antibodies, within 10 days after experimental infection with a highly virulent strain.

MATERIALS AND METHODS

Virus. The initial material for these investigations was the IBD virus, which caused 50% mortality in a chicken flock of the Orpington breed at the age of 10 weeks. The IBDV demontrated in bursae of these chickens by the agar gel precipitation test (AGPT) with a positive monovalent hyperimmune serum and was designated as KA.

Experimental design. Two biological experiments were performed: one aiming to investigate the virulence and the other to detect antigens of the IBD virus in bursae of infected chickens. For both experiments chickens of the Harco provenience were used.They were raised in isolated units of the Institute according to rountine technological procedures. At the moment of infection chickens did not have detectable IBDV antibodies, as confirmed by the ELISA method (IDEXX, Portland, ME). For testing virus virulence, 10 chickens were infected at the age of 6 weeks by intracloacal application of 0,2 ml of bursal homogenates in PBS (1:10) obtained from chickens of the Orpington breed which were previously tested for bacterial contamination. The observation period lasted for 14 days. In the second experiment, 40 chickens at the age of 12 weeks infected in the same way. Three, five, seven and ten days after infection groups of 6 chickens were euthanased by servical dislocation. The bursae were removed, fixed for 48 hours in 99,9% methanol and processed for paraffin embedding. Two sections were cut from each bursa; one for immunoperoxidase and the other for hematoxylin and eosin staining. The control group included 15 chickens, whose bursae were sampled at the same intervals.

Immunohistochemistry. The indirect immunoperoxidase method (IP) was performed as follows: methanol-fixed and paraffin embedded bursal sections were deparaffinised and hydrated through xylene and 100% and 96% ethanol. Hydrated samples were reacted with 3% Hydrogen peroxide (H_2O_2) in methanol for 30 minutes to quench endogenous peroxidase activity. Following a 10 minute wash in Tris-buffered saline (TBS, 0,1M Tris-HCl, 0,9%NaCl, pH7,8) samples were blocked with 20% nonimmune rabbit serum for 10 minutes. The serum was blotted and the slides were incubated with primary polyclonal chicken antiserum to IBDV, (1:100, Vet. Inst. N. Sad), for 30 minutes in a humidified chamber. After 10 minutes in TBS the samples were incubated with peroxidase-labelled rabbit

anti-chicken IgG (1:100, ICN). After 10 minutes wash in TBS the slides were incubated for 10 minutes in peroxidase substrate-0,05% diaminobenzidine tetrahydrochloride (DAB, Serva) in 0,1M buffered imidazole/HCl (pH 7,1) solution. The slides were washed in TBS for 10 minutes, then in distilled water for 2 minutes, dehydrated through graded alcohol and xylene, and counterstained with hematoxylin. They were then mounted for micorscopic evalution. Control procedures involved the use of nonimmune chicken serum and TBS instead of antiserum to IBDV. Furthermore, a negative control experiment was carried out, i. e. application of antiserum to IBDV on noninfected sections of bursa of Fabricius.

RESULTS

The IBD virus which was isolated from chickens of the Orpington breed, caused 80% mortality in the biological experiment on 6 week old chickens of the Harco provenience. Out of 10 infected chickens 8 died within the first 96 hours. The same virus designated as KA caused 30% mortality during the first 72 hours in chickens of the same provenience, which were inoculated at the age of 12 weeks.

A close relationship between the presence of the virus and histological changes was noticed. Viral antigen was seen in each group from 24 hours until ten days post infection (PI). From three to ten days many antigen positive lymphoid cells were detected in lymphoid follicles, but also antigen could be found in macrophages within follicles and the interstitium. The intensity of reaction

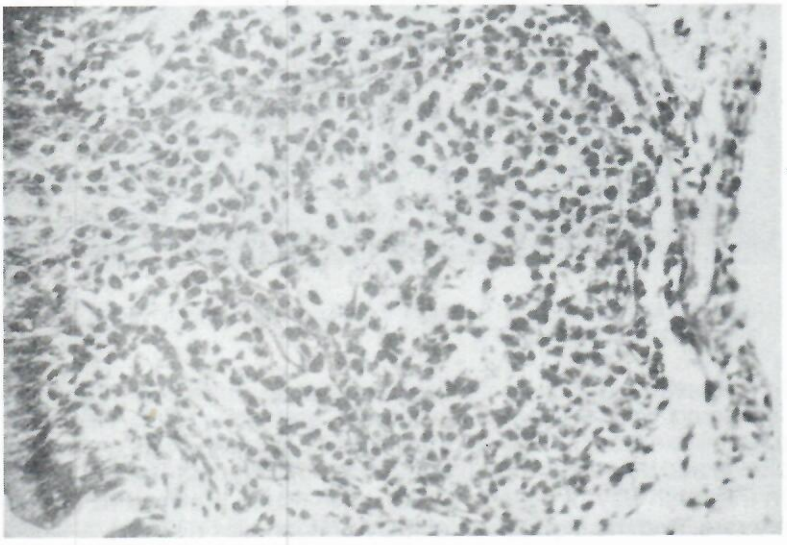

Figure 1. Bursa of Fabricius, sampled 5 days PI. Cystic cavities in bursal follicles. HE, x 100.

and pathohistological changes were different during the third, fifth, seventh and tenth day after infection.

During the third day after infection a great number of lymphocytes from the cortex and medulla of bursal follicles, together with some macrophages expressed IBDV antigens. At the same time, the main pathohistological alterations were degenerative and multifocal necrotic changes in the lymphocytes belonging to the medullar zone of bursal follicles, with the accumulation of cellular debris.

On the fifth day after infection, in parallel with depletion and atrophy of follicles, a positive anti-IBDV reaction could be seen on several lymphocytes in the medulla, but IBDV antigens were mainly expressed on cortical lymphocytes of bursal follicles. Cystic cavities were found in the medulla of some follicles. At the same time, proliferation of connective tissue was observed. (Figure 1)

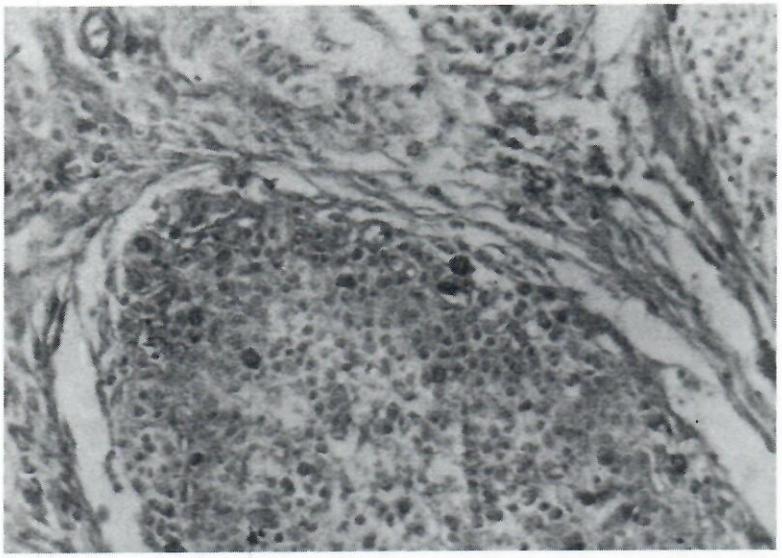

Figure 2. Bursa of Fabricius, sampled 7 days PI. Expression of IBDV angigens on diffuse scattered lymphocytes. IP, x 160.

On the seventh day after infection there was expression of virus antigens on diffuse scattered lymphocytes in the follicles and interstitium. Necroses in the medulla were increased, as well as phagocytosis. Depletion was observed in most of the follicles and the rest of the lymphocytes showed apoptosis. (Figure 2)

On the tenth day of infection connective tissue proliferated, lymphocytes were very rare, and the expression of IBDV antigen was discreet.

Control bursae showed a negative immunohistochemical reaction.

DISSCUSION

The mortality that was caused in the flock of Orpington breed chickens as well as in both experimental groups of Harco chickens by the KA virus indicates high virulence. Highly virulent IBDV infection may be characterised by severe lesions, high mortality and severe depletion of lymphoid cells in the bursae of Fabricius (Cosgrove 1962; Chettle et al. 1989; Nunoja et al. 1992).

This study showed a relationship between the extent of microscopic lesions in the bursae and the level of intensity of staining by the indirect immunoperoxidase method. The immunoperoxidase test allowed the observer to correlate the location, number and intensity of immunoperoxidase-stained cells with microscopic pathology. The pathohistological findings in bursae which were sampled at different periods after infection showed inflammatory changes followed by atrophy and the formation of cystic cavities in the medullae of bursal follicles, which was also described by other authors (Helmboldt and Garner 1964; Sharma et al. 1989). However, similar histological lesions in the bursa of Fabricius also occur in Marek's disease, chicken anemia as well as mycotoxin poisoning from the trichotecene group. The indirect immunoperoxidase method is sufficiently specific and sensitive to determine these lesions (Etteradosi et al. 1992; Fermandes et al. 1989). Detection of the IBD virus by the IP method was possible during the first 10 days after infection. From day 3 to 7 PI many antigen positive lymphoid cells were detected in the cortex and medulla of lymphoid follicles. On days 5 and 7 PI antigen could be found mainly in macrophages within all follicles and the interstitium. On day 10 PI the intensity of the immunohistochemical reaction was very low, but detection of viral antigen was still possible. Sometimes IBD virus was demonstrated 13 days after infection with highly virulent viruses isolated in Europe and Japan by complex formation with avidin-biotin-peroxidase (ABC). IBDV antigen localisation corresponds with the results obtained in our investigation (Tanimura et al. 1994).The diagnostical value and sensitivity of the indirect peroxidase method was investigated and it was similar to the antibody sandwich ELISA test. Using monoclonal antibodies, antigens were found to several vaccinal and pathogenic strains of the IBD virus by the ABC method. Monoclonal antibodies are currently more often used for immunocytochemical reactions (Allan et al. 1984; Cho et al. 1987; Crus et al., 1993). However, the protocol used in this work allows high sensitivity of the IP test to be attained with a polyclonal serum, without previous tretment of the tissue with enzymes, along with minimal time necessary for reaction.

The indirect immunoperoxidase method eliminates conventional isolation and identification of the virus and, at the same time, enables the specific diagnosis of IBDV during the first 10 days after infection of chickens with highly virulent virus.

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IMUNOHISTOHEMIJSKO DOKAZIVANJE ANTIGENA VIRUSA ZARAZNE BOLESTI BURZE U FABRICIJEVOJ BURZI EKSPERIMENTALNO INFICIRANIH PILIĆA

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SADRŽAJ

Metoda indirektna peroksidaza (IP) upotrebljena je za dokazivanje antigena virusa zarazne bolesti burze (IBD) u Fabricijevoj burzi pilića eksperimentalno inficiranih visoko virulentnim virusom označenim K. A. Na parafinskim isečcima tkiva fiksiranih u metanolu, metodom IP, moguće je dokazivati IBD virus deset dana posle infekcije. Trećeg do sedmog dana posle infekcije intenzivna ekspresija IBDV antigena zapaža se na limfocitima u korteksu i meduli burzalnih folikula i na makrofagama u folikulima i intersticijumu. Desetog dana posle infekcije imunohistohemijska reakcija je veoma diskretna, mada je detekcija IBD virusa još uvek moguća. Metoda IP, predstavlja senzitivan i specifičan put dokazivanja IBD virusa u Fabricijevoj burzi eksperimentalno inficirane živine.