Pathogenicity of Fowl Adenovirus Isolated from Gizzard Erosions to Immuno-Suppressed Chickens

Norihiko MUROGA1), Satoshi TAHARAGUCHI1), Hideyuki OHTA2), Ken-ichi YAMAZAKI2) and Kozo TAKASE1)*

1)Laboratory of Veterinary Microbiology, Department of Veterinary Medicine, Faculty of Agriculture, Kagoshima University, 1–21–24 Kohrimoto, Kagoshima 890–0065, Japan. 2)The Chemo-Sero-Therapeutic Research Institute, 668 Okubo, Kumamoto 860–8568, Japan.

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ABSTRACT. Pathogenicity of a fowl adenovirus (FAV), JM1/1 strain of serotype 1 derived from gizzard erosions of a broiler chicken, was examined to specific pathogen-free (SPF) chickens pre-treated with infectious bursal disease viruses (IBDVs) or cyclophosphamide (CY). Virulent IBDVs, classical type, were inoculated orally at 3 days of age of SPF chickens. CY was treated subcutaneously for 3 days after hatch. FAV was given orally at 30 days of age. At 40 days of age, all chickens were bled and autopsied for serology and gross observation. Gizzard lesions were ranked by the scores depending on their severities. IBDV- or CY-treated chickens showed significantly higher gizzard lesion scores than non treated birds. There were no gross lesions in any other organs except for bursal atrophy. Serologically, antibody production against FAV was highly suppressed by IBDV infection or CY treatment.

KEY WORDS: fowl adenovirus, gizzard erosion, immuno-suppression.

Recently, epizootic outbreaks of gizzard erosion in broiler flocks caused by fowl adenovirus infection have been reported in Japan [1, 9, 15]. Most of the FAV isolates from the gizzard lesions were serotype 1, but a few were serotype 8 [8, 15]. The gizzard lesions were reproduced in broiler and specific pathogen-free (SPF) chickens by oral infection with the isolated FAVs [6–8, 15]. However, some of the experimentally infected birds failed to show the gizzard lesions [5, 15]. On the other hand, FAV has been well known as an agent of inclusion body hepatitis, which was rather facilitated in immuno-deficient birds caused by infectious bursal disease virus (IBDV) infection [2, 5]. In this study, the pathogenicity of FAV strain isolated from gizzard erosion to immuno-deficient chickens was investigated using IBDV-infected or cyclophosphamide (CY)-treated chickens. IBDV infects and destroys B-cells inducing suppression of antibody production. CY, which has been used as one of immunosuppressive reagents or anticancer drugs, destroys both B- and T-cells, and also suppresses macrophage-function.

FAV, strain JM1/1 isolated from a slaughtered broiler chicken affected with gizzard erosion and identified as serotype 1 [15], was prepared by chicken kidney cell culture (CKC) at the 6th passage level. IBDVs, strains G691 and Ku-52, both were classical type of virulent virus [12], were of homogenate of bursa of Fabricius (BF) of SPF chickens infected with each virus. SPF chickens infected from the Chemo-Sero-Therapeutic Research Institute (Kumamoto) were used and reared in isolated rooms. Three experiments were conducted as shown in Table 1. In Expts. 1 and 2, chickens were treated orally with 10⁵ TCID₅₀/bird of each IBDV strain at 3 days of age. In Expt. 3, CY (Wako Pure Chemical Ind. Ltd.; Osaka) was injected subcutaneously at 1, 2 and 3 days of age with 40 mg/bird/day [4]. In each experiment, inoculation with JM1/1 strain of FAV was done by oral route at 30 days of age with 10⁶ TCID₅₀/bird. In each experiment, non-treated group was contained. On the last day of each experiment, 40 days of age, all chickens were bled, weighed and sacrificed by anesthesia with chloroform for dissection and main organs were observed grossly. Bursa of Fabricius (BF) was removed and weighed to calculate the ratio to body weight [(weight of BF / body weight) × 100]. Gross gizzard lesions were ranked by the scores as shown in Fig. 1 [–(0): no change, +(1): small lesions, ++(2): moderate lesions, +++(3): severe lesions]. In Expts. 1 and 3, virus recovery was tried from 10% homogenate using CKC employing round-type cytopathogenic effect for indication of FAV. For statistical analysis of BF-BW ratios and mean lesion scores, Student’s t-test was applied and significance was assumed at the 0.05 level of probability.

For serology, an agar-gel-precipitation (AGP) test was done to IBDV and FAV infection using FAV-antigen prepared from chorioallantoic membrane [13] and IBDV-antigen from BF-s [12]. A neutralization test for FAV was also carried out using CKC by the serum-dilution method in 24-well-plates, as usual.

Results of three experiments are shown in Table 2. In Expts. 1 and 2, the infection with IBDV in two groups (B and E) was confirmed by the sero-conversion in the AGP test, but not in any other groups. BF/BW ratios of IBDV-infected (B and E) or CY-treated (H) group were lower significantly than those of any other groups.

Serological response to FAV was observed in FAV-treated groups (A, B, D, E, G and H). However, in IBDV-infected (B and E) and CY-treated (H) group, neutralizing antibody titers and AGP antibody-positive ratio were lower than non-treated groups (A, D and G).
FAV was re-isolated from many gizzard homogenates of inoculated groups (A, B, G and H), but not at all from non-inoculated groups (C and I).

Gizzard lesions were observed in FAV-infected groups, although the lesion scores were varied. Especially, in groups B, E and H, infected with IBDV or treated with CY at early age, the gizzard lesions were severer than non-treated groups, showing significantly higher lesion scores. No gross lesions were observed in any other organs at post-mortem.

From these results, it is suggested that the gizzard erosion by FAV infection could be facilitated in immuno-suppressed chickens as like inclusion body hepatitis [2]. In the field, chickens may happen to be immuno-suppressed by various known or unknown factors, and the gizzard erosion by FAV may be produced more easily in these birds than non-immuno-deficient birds.

All FAVs, which have been isolated so far from gizzard erosion, were restricted to serotypes 1 or 8 only [6, 15], but the FAVs from IBH belong to many serotypes including serotype 1 and 8 [5]. In addition, adenoviral gizzard erosion had not been recognized until the first report in 1993 [14]. In our present study, even in experimentally immuno-suppressed birds, gross hepatic lesions were not observed. From these points, it will be interesting to clarify if the FAV strains from the gizzard erosion have an organ-tropism to gizzard, or not.

Histopathological examinations have shown that FAV has an ability to cause ventriculitis or pancreatitis accompanying with intranuclear inclusion bodies [3, 10, 11, 14], except for hepatitis. Our observation in this study was done grossly. For further discussion, histopathological examination will be required.

REFERENCES

Table 2. Results of serology and gross pathology

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>Group</th>
<th>BF/BW Ratio</th>
<th>Antibodies</th>
<th>Virus recovery</th>
<th>Lesion score of gizzard</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FAV-N</td>
<td>FAV-AGP</td>
<td>IBD-AGP</td>
<td>from gizzard</td>
</tr>
<tr>
<td>1 A</td>
<td>0.47b</td>
<td>1 : 161.7</td>
<td>7/8</td>
<td>0/8</td>
<td>3/8</td>
<td>5</td>
</tr>
<tr>
<td>1 B</td>
<td>0.07b</td>
<td>1 : &lt;10</td>
<td>2/9</td>
<td>9/9</td>
<td>7/9</td>
<td>1</td>
</tr>
<tr>
<td>1 C</td>
<td>0.62b</td>
<td>1 : &lt;10</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>3</td>
</tr>
<tr>
<td>2 D</td>
<td>0.43b</td>
<td>1 : 149.3</td>
<td>6/10</td>
<td>0/10</td>
<td>nt</td>
<td>3</td>
</tr>
<tr>
<td>2 E</td>
<td>0.14b</td>
<td>1 : 11.4</td>
<td>5/10</td>
<td>10/10</td>
<td>nt</td>
<td>1</td>
</tr>
<tr>
<td>2 F</td>
<td>0.52b</td>
<td>1 : &lt;10</td>
<td>0/3</td>
<td>0/3</td>
<td>nt</td>
<td>3</td>
</tr>
<tr>
<td>3 G</td>
<td>0.50b</td>
<td>1 : 260.0</td>
<td>7/10</td>
<td>0/10</td>
<td>5/10</td>
<td>3</td>
</tr>
<tr>
<td>3 H</td>
<td>0.10b</td>
<td>1 : &lt;10</td>
<td>1/10</td>
<td>0/10</td>
<td>10/10</td>
<td>–</td>
</tr>
<tr>
<td>3 I</td>
<td>0.62b</td>
<td>1 : &lt;10</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>3</td>
</tr>
</tbody>
</table>

a) Different letters indicate significant differences. \( p<0.05 \).
b) Geometric mean neutralizing titer against FAV.
c) No. of AGP-positive / No. of tested.
d) No. of FAV-positive / No. of tested.
e) Not tested.