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PRELIMINARY REPORT

IMMUNOFLUORESCENT STUDIES USING FRACTIONATED IMMUNOGLOBULIN OF SERA OF FOWLS WITH MAREK'S DISEASE[1]

MATAO NAITO[2], KOICHI ONO

Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

SHIZUO TANABE

Department of Bacteriology, Osaka University Medical School, Osaka

SHIRO KATO

Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

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Marek's disease (MD) of domestic chickens has recently attracted considerable attention in Japan for economic reasons. The disease also deserves our attention because it is a lymphomatosis which is considered to be caused by a virus belonging to a herpes group associated with Burkitt's lymphoma in man (Epstein et al., 1964). A simple method for serological diagnosis of the disease seems a matter of urgency.

The fluorescent-antibody (FA) test for MD antigen was first described by Kottaridis and Luginbuhl (1968). They examined chick embryo fibroblasts inoculated with the MD agent and bone marrow smears of birds with MD using sera from rabbits which had been hyperimmunized with extracts of infected bloods. They used the indirect FA test throughout their study. Purchase (1969) also reported using the indirect FA test for MD antigen in duck and chick embryo fibroblasts and kidney cells of chicks infected with MD virus. He used sera from 8 week-old chickens which had been immunized with fresh whole blood of birds with MD. The present preliminary report described the application of the direct FA test to the detection of herpes antigen in duck embryo fibroblasts (DEF) inoculated with herpes virus using immunoglobulin fractions of the sera of birds with MD.

Birds (De Karb 161 strain) showing typical clinical signs of MD were obtained from the Director, Akio Nagai of Kaibara Noh-kyo Poultry Center, Kaibara, Hyogo Prefecture. They were screened by the COFAL and anti-RAV 1 and anti-RAV 2 antibody tests, and birds giving a positive in any of these tests were excepted. Using blood samples from these birds 3 strains of herpes type virus (HTV) were isolated in DEF culture by the method of Solomon et al. (1968) (Kato et al., 1969). The biological and morphological characteris-

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2 On leave from Himeji Women's College, Himeji, Hyogo Prefecture.

tics of these 3 strains of virus in vitro were identical with those described by previous authors (Churchill and Biggs, 1967; Nazerian et al., 1968; Solomon et al., 1968; Witter et al., 1968). The code numbers of the birds from which these strains were isolated, were No. 72, 73 and 76. Histologically, these birds also showed typical MD lesions. The strains isolated from these birds were named the Biken A, B and C strain, respectively. Sera were obtained from these birds and the standard ammonium sulfate method was used for separation of globulins from these sera. After labeling with fluorescein isothiocyanate (FITC), unreacted dye was removed from the crude conjugates by gel filtration on Sephadex G-25. Conjugates were separated into two fractions by chromatography on DEAE-cellulose eluting Fraction I with 0.005 M phosphate buffer, pH 7.0, in distilled water and Fraction II with 0.005 M phosphate buffer, pH 7.0, in 0.5 M NaCl. DEF inoculated with Biken A, B and C and showing typical cytopathic effects were stained with the 2 fractions. Only Fraction II showed the selective activity of the antibody.

A more specific fraction was separated by agar zone electrophoresis. The sera of birds No. 88, 89 and 90 (diagnosed as having MD by the histo pathologic changes observed) were pooled. On electrophoresis of this, an immunoglobulin was separated which was named original IG. This immunoglobulin was applied to a column of DEAE cellulose equilibrated with 0.01 M phosphate buffer, pH 7.8. The column was eluted until the E_{280} of the effluent was below 0.01. This effluent was combined and named Fraction I. Then the column was eluted with 0.5 M sodium chloride in 0.01 M phosphate buffer until the E_{280} was again below 0.01. This effluent was pooled and named Fraction II. Original IG, Fraction I and Fraction II were each concentrated until E_{280} was about 1.1. Then these immunoglobulins were conjugated with FITC. DEF inoculated with Biken A or B stained with these 3 conjugates. Fraction II gave a bright, specific fluorescence while Fraction I showed hardly any antibody activity.

TABLE 1. *FA tests on the antigenic relationship among Biken A, B and C and two other cell lines using 3 MD sera, pooled MD serum and 4 other sera.*

Cells stained \ Labeled globulin		MD[a] No. 72	MD[a] No. 73	MD[a] No. 76	Pooled[a] MD	Anti-RAVI antibody	Anti-AAV antibody	Anti-VZV antibody	Anti-HSV antibody
DEF infected with	Biken A	+	+	+	NT	−	−	−	−
	Biken B	+	+	+	+	−	−	−	−
	Biken C	+	+	+	+	−	−	−	−
QEF infected with Biken B		NT	NT	+	NT	NT	NT	NT	NT
Uninfected DEF		−	−	−	−	−	−	−	−
Burkitt cells		−	NT	NT	−	NT	NT	NT	NT
CKC infected with AAV		−	NT	NT	−	NT	+	NT	NT

a Fraction II was used for staining.

DEF : duck embryo fibroblast
QEF : quail embryo fibroblast
CKC : chick kidney cells
AAV : avian adeno virus
VZV : varicella-zoster virus
HSV : herpes simplex virus
NT : not tested

Uninfected DEF was also allowed to react with Fraction II of each of the MD sera, Fraction II of pooled MD serum, antisera prepared in rabbits to avian adenovirus (AAV) and to varicella-zoster virus (VZV), anti-RAV 1 antibody-positive chick sera and anti-herpes simplex virus human sera (Table 1). Upon direct FA staining none of these cultures showed fluorescence. The cross reactivity of the 3 MD sera, the pooled MD serum and the 4 other sera mentioned above with DEF infected with Biken A, B and C were examined. DEF infected with Biken A, B or C gave bright fluorescence with each of the 3 MD sera, and with pooled MD serum. Japanese quail embryo fibroblasts infected with Biken B and stained with fluorescent serum of MD No. 76 also showed specific fluorescence (Onoda et al. unpublished). However the 4 sera other than MD sera did not react with any of these infected cells. Burkitt lymphoma cells, the P3 (Jijoye) strain of P3HR-1 and chick kidney cells infected with AAV were stained with MD No. 72 serum and pooled sera but no fluorescence could be found on these cells.

The appearance of the fluorescence in DEF infected with Biken A, B or C was similar to that described by Purchase (1969). At low magnification the fluorescent foci appeared identical with morphological foci staining with hematoxylin-eosin and consisting of many cells bearing intranuclear inclusions (Fig. 1). At high magnification fluorescent areas were observed in the cytoplasm and nuclei. The nucleus usually stained diffusely (Fig. 2). The cytoplasm stained in various ways, diffuse fluorescence being the most common though some cells contained small irregular-shaped flecks of fluorescence or brightly staining irregular-shaped granules (Fig. 3).

Summary: The direct fluorescent-antibody technique (FAT) was applied to the detection of antigen induced by a herpes type virus (HTV) in duck embryo fibroblasts inoculated with preparations of MD. The FITC conjugated fraction of the immunoglobulin of sera from birds with MD, obtained by DEAE-cellulose chromatography eluting with 0.5 M sodium chloride, gave bright specific fluorescence in both the cytoplasm and nuclei of infected cells. The results show that sera of the birds with MD examined had antibody activity against herpes antigen by FAT and could be used for diagnosis of samples containing HTV.

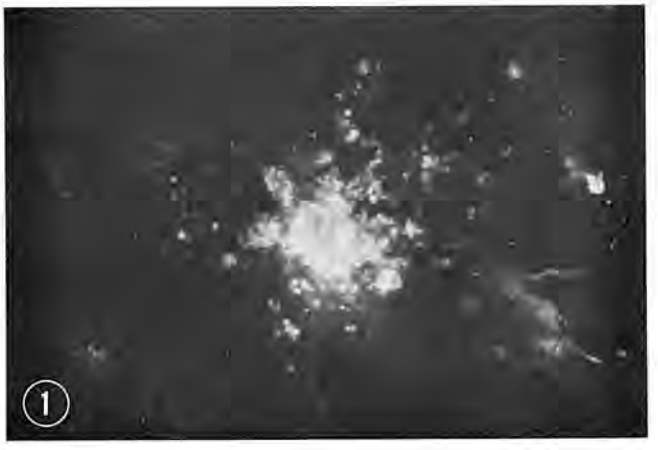

FIGURE 1. *A fluorescent focus composed of cells bearing intranuclear inclusions. DEF 5 days after infection with Biken B, stained with Fraction II of MD No. 72 serum.*

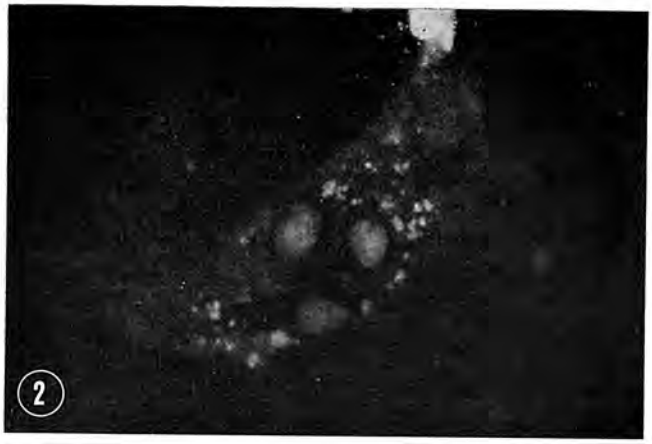

FIGURE 2. *A multinucleated giant cell with 3 diffuse nuclear antigens and irregular cytoplasmic granules. DEF 7 days after infection with Biken B, stained with Fraction II of MD No. 72 serum.*

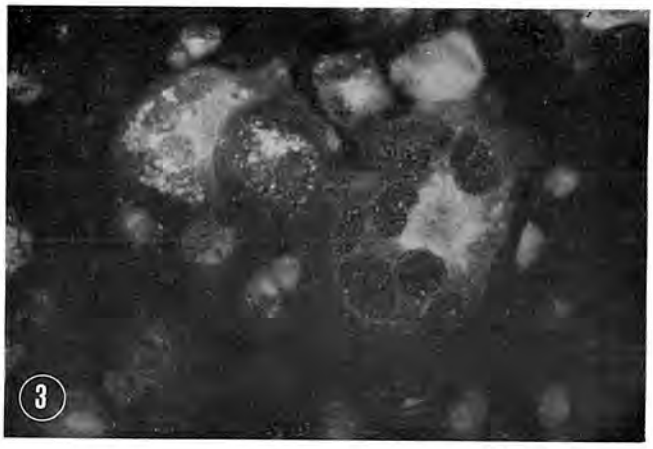

FIGURE 3. *A group of flattened cells with diffuse, flecks and granules of cytoplasmic fluorescence. DEF 7 days after infection with Biken B, stained with Fraction II of MD No. 72 serum.*

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