BIKEN JOURNAL Vol. 16, 85-90, 1973

SHORT COMMUNICATION

AUTORADIOGRAPHY OF THE TISSUES OF CHICKS INFECTED WITH MAREK'S DISEASE VIRUS USING ^{3}H-THYMIDINE[1]

NOBUZO IWA

Osaka Institute, The Research Foundation for Microbial Diseases of Osaka University, Yamada;kami, Suita, Osaka

KOICHI ONO, MATAO NAITO and SHIRO KATO

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

(Received January 22, 1973)

Marek's disease (MD) is a widespread, readily transmissible disease causing lymphocytic lesions in chicks. Studies have revealed a cell-associated herpesvirus to be the etiological agent (Churchill and Biggs, 1967; Nazerian et al., 1968). It is unknown whether the lymphocytic lesions in MD should be regarded as true neoplasms or as lymphogranulomas. The present paper is mainly concerned with the proliferating nature of lymphoid cells in lymphocytic lesions of chicks with MD, studied by autoradiography of ^{3}H-thymidine.

A virulent MD virus, strain JM, kindly supplied by Dr. H. Sazawa (National Veterinary Assay Laboratory) was used. Virus has been propagated in chickens by serial passage of whole blood. Six white Leghorn chickens were used. They were confirmed to be free from known avian leukosis virus infection and were bred in vinyl isolators. One day old chicks were inoculated intraperitoneally with 0.5 ml of infected blood. Chickens showing severe paralysis were sacrificed. The ages and body weights of these chicks at the time of autopsy are shown in Table 1. Tritiated thymidine purchased from the Daiichi Chemical Co., Tokyo (specific activity 5 Ci/mM) was used at a level of 100 μCi/ml and chicks were injected with 1 μCi per g body weight of ^{3}H-thymidine solution. The total amounts of isotope inoculated per chick are also shown in Table 1. Chicks were sacrificed 1 hr after injection of the isotope and various tissues were

TABLE 1. *Conditions of chickens examined*

Chicken	Age (days)	Body weight (g)	^{3}H-Tdr injected (μCi)	COFAL[a]
MD No. 1	29	240	240	—
MD No. 2	72	250	250	—
MD No. 3	79	290	290	—
MD No. 4	93	220	220	—
MD No. 5	96	270	270	—
Noninfected	72	290	290	—

[a] Complement fixation of avian leukosis.

1 A part of this work was presented at the 73rd Annual Meeting of the Japanese Society of Veterinary Science at Tokyo on April 3, 1972.

TABLE 2. *Lymphocytic foci in various organs in chickens with MD*

Organ	Chicken					
	No. 1	No. 2	No. 3	No. 4	No. 5	Noninfected
Liver	+	+	+	+	+	−
Heart	+	+	+	+	+	−
Kidney	+	+	+	+	+	−
Lung	+	+	+	−	+	−
Proventriculus	+	+	+	+	+	−
Pancreas	−	NT	+	−	+	NT
Ovary	+	+	+	NT	+	NT
Testis	NT[a]	NT	NT	+	NT	−
Skin	+	+	+	+	+	−
Spleen	−	+	−	−	−	−
Bursa of Fabricius	−	−	−	−	−	−
Thymus	−	NT	−	−	+	−
Peripheral nerves						
vagus	+	+	+	−	+	−
brachial	+	+	+	+	+	−
lumbosacral plexus	+	NT	+	+	+	−
sciatic	+	+	+	+	+	−

a Not tested.

removed and fixed with Carnoy's fixative for 48 hr. Autoradiography was carried out by the techniques of Kato et al. (1963). After exposure for 60 days, the samples were developed, fixed and stained with hematoxylin and eosin. Peripheral blood smears of a control noninfected chick were made and fixed with methanol for 5 min and subjected to autoradiography. Blood smears were stained with Giemsa solution. The percentages of labeled cells and mitotic cells in autoradiograms were calculated on 500 randomly selected cells. Nuclei with more than 3 silver grains were regarded as grain-bearing cells. A Neopak microscope, Model N (Olympus Co.) was used for observation as reported by Rogers (1967).

As shown in Table 2, lymphocytic lesions were found in almost all organs examined. Autoradiography showed that many lymphoid cells in the lymphocytic lesions were well labeled, as shown in Fig. 1–4. The percentages of labeled cells in these lymphocytic lesions, in normal tissues surrounding the lesions and in normal tissues obtained from the noninfected normal chick, were calculated and are shown in Table 3. In lymphocytic lesions 24.6–57.8% of the nuclei were labeled while in normal parts of tissues 0.25–5.4% were. On the other hand only 0.2 and 2.6% of the nuclei were labeled in normal tissues other than lymphatic tissues of the noninfected chick. In normal lymphatic tissues 10.8–12.2% of the nuclei were labeled. Scarcely any labeled nuclei were seen in peripheral lymphocytes of the noninfected chick. The mitotic indices of cells in lymphocytic lesions, in normal parts of tissues and in normal control tissues were calculated on the preparations, used for autoradiography and are shown in Table 4. Mitotic figures were frequently observed in lymphoid cells in lymphocytic lesions, but rarely in normal tissues other than proliferative tissues, such as testis and lymphatic tissues (spleen, thymus and bursa of Fabricius). The mitotic indices of lymphoid

TABLE 3. *Percentages of labeled nuclei in lymphocytic foci of chickens with MD*

Organ	Labeled nuclei (%)						
	In lymphocytic foci			In normal tissues			Noninfected
	No. 2	No. 3	No. 4	No. 2	No. 3	No. 4	
Liver	57.8	52.3	41.4	0.8	0.8	0.4	0.6
Heart	53.0	41.6	46.6	0.4	1.2	0.6	0.4
Kidney	31.2	27.8	32.4	0.6	1.6	0.6	0.2
Lung	32.6	32.6	NT	1.2	5.4	1.8	0.4
Proventriculus	30.8	28.4	37.4	1.6	5.2	1.2	2.2
Pancreas	NT[a]	31.6	NT	NT	0.6	0.6	NT
Ovary	26.8	45.8	NT	NT	NT	NT	NT
Testis	NT	NT	40.2	NT	NT	1.6	1.0
Skin	26.8	29.6	NT	3.6	2.8	NT	2.6
Peripheral nerves	24.6	42.6	25.8	0.4	0.8	0.2	0.2
Spleen	35.2	NT	NT	NT	12.8	12.4	10.8
Bursa of Fabricius	NT	NT	NT	18.4	19.6	15.6	11.6
Thymus	NT	NT	NT	NT	15.8	NT	12.2

a Not tested.

TABLE 4. *Percentages of mitotic cells (mitotic indices) in lymphocytic foci of chickens with MD*

Organ	Mitotic indices						
	In lymphocytic foci			In normal tissues			Noninfected
	No. 2	No. 3	No. 4	No. 2	No. 3	No. 4	
Liver	1.8	1.8	1.4	0.0	0.0	0.0	0.0
Heart	1.0	1.2	2.0	0.0	0.0	0.0	0.0
Kidney	1.0	0.8	0.8	0.0	0.0	0.0	0.0
Lung	0.6	0.8	NT	0.0	0.0	0.0	0.0
Proventriculus	1.0	0.8	1.2	0.0	0.0	0.0	0.0
Pancreas	NT[a]	1.2	NT	NT	0.0	0.0	NT
Ovary	0.8	2.2	NT	NT	NT	NT	NT
Testis	NT	NT	1.6	NT	NT	0.2	0.6
Skin	0.4	0.4	NT	0.0	0.0	NT	0.0
Peripheral nerves	0.2	1.8	0.6	0.0	0.0	0.0	0.0
Spleen	1.0	NT	NT	NT	0.6	0.6	0.4
Bursa of Fabricius	NT	NT	NT	0.8	0.8	0.6	0.6
Thymus	NT	NT	NT	NT	0.4	NT	0.6

a Not tested.

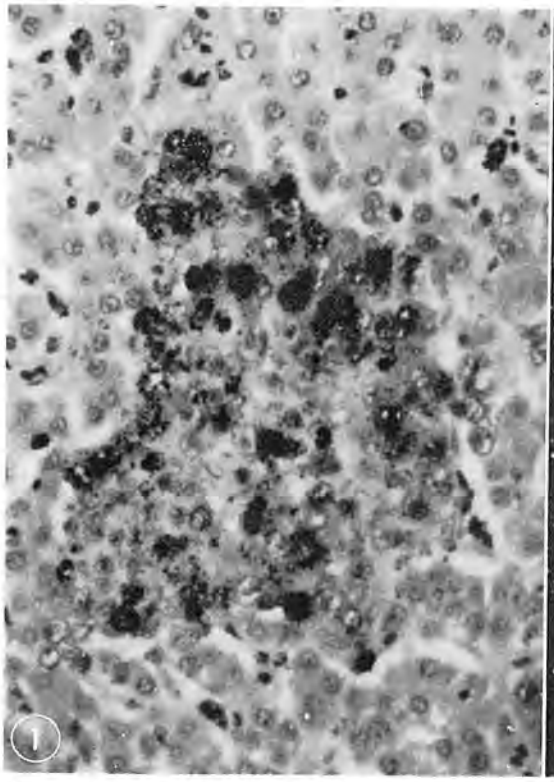


FIGURE 1. *Autoradiogram of a lymphocytic lesion of liver tissue. The lymphoid cells in the lymphocytic lesion are intensely labeled with ^{3}H-thymidine, while surrounding normal parenchymal liver cells are almost free from silver grain. Fixed with Carnoy's fixative and stained with H-E* ($\times 700$).

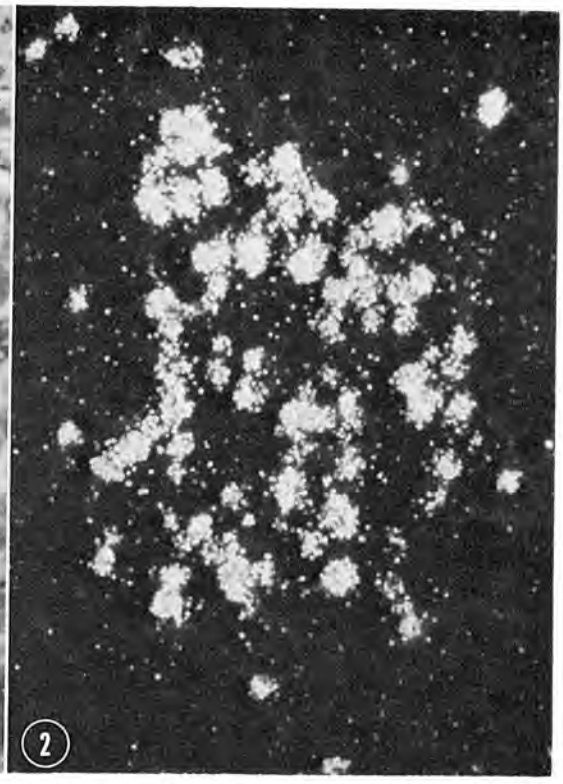
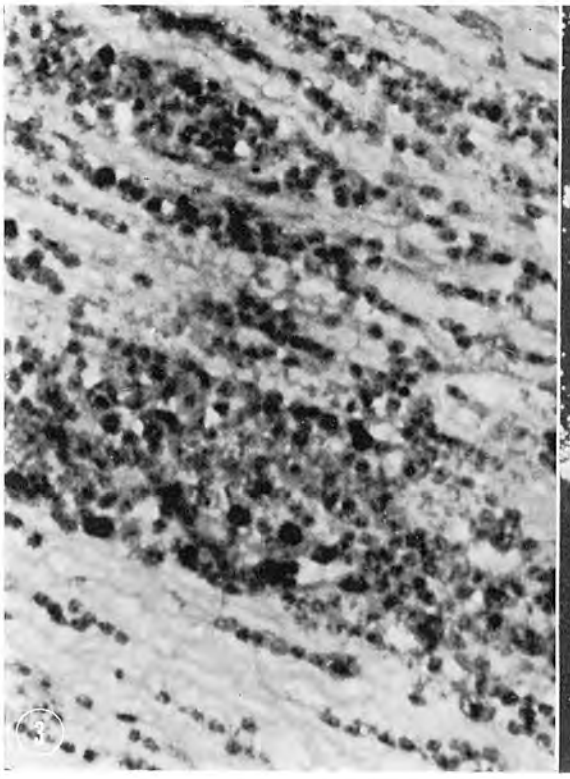


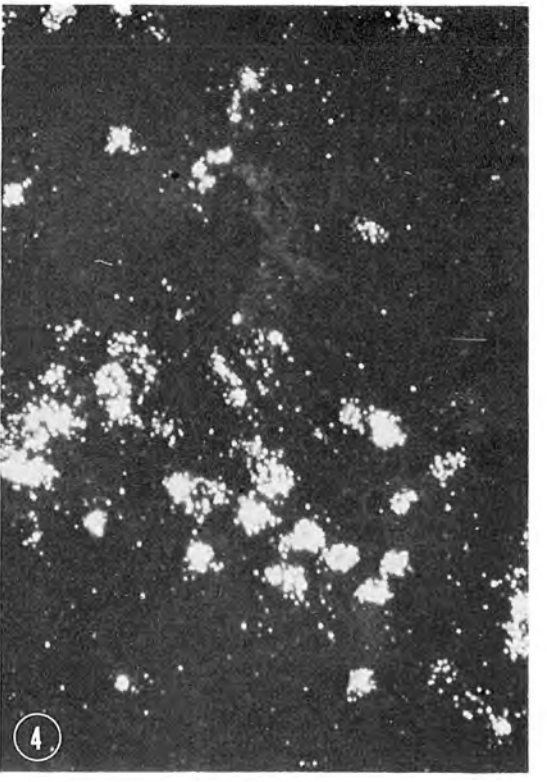
FIGURE 2. *Microphotographed with a Neopak microscopy (the same field as Fig. 1). Accumulation of silver grains in a lymphocytic lesion in liver tissue is well demonstrated* ($\times 700$).

cells in lymphocytic lesions were generally higher than those in the proliferative tissues mentioned above. All these results show that the lymphocytic lesions of chicks with MD consist of highly proliferative cells. This suggests that the lymphocytic lesions in MD should be regarded as neoplasmas with progressive extension, rather than as lymphoid infiltration. However, it is not known whether these lymphoid cells are malignant.

Feather follicle epithelium is a major site of MDV replication, as shown by detection of viral antigen in fluorescent antibody tests, of intranuclear inclusions, of mature virions and of filtrable infectious virus (Calnek and Hitchner, 1969; Nazerian and Witter, 1970; Purchase, 1970). In sections of feather follicle epithelium of the skin, of chicks with MD, many typical intranuclear inclusions with a clear halo were observed while no inclusions were seen in sections of lymphocytic lesions. Autoradiograms revealed that these intranuclear inclusions are identical with the areas where silver grains are localized, suggesting that active viral DNA synthesis takes place there (Fig. 5), as already reported in cultured duck embryo cells infected with MDV (Ono et al. 1970).

This work was supported in part by a Scientific Research Grant from the Ministry of Education.





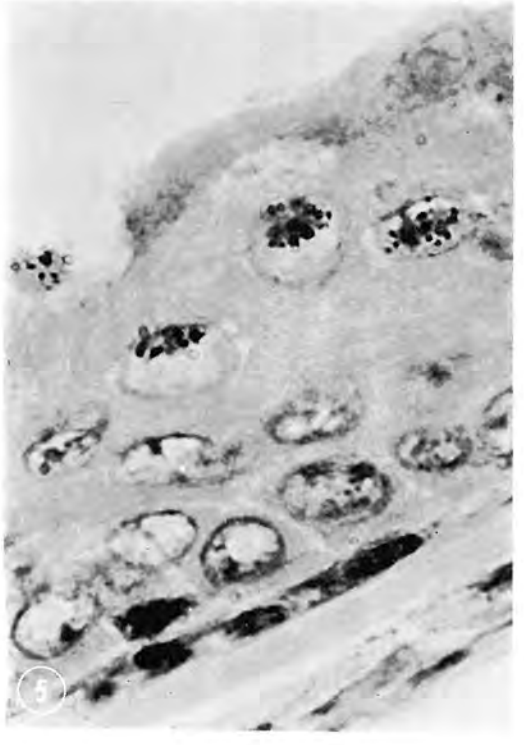


FIGURE 3. *Autoradiogram of a lymphocytic lesion of peripheral nerve tissue. The lymphoid cells are intensely labeled with* ^{3}H*-thymidine. Fixed with Carnoy's fixative and stained with H-E* ($\times 700$).

FIGURE 4. *Microphotographed with a Neopak microscopy (the same field as Fig. 3). Silver grains of the lymphocytic lesion of peripheral nerve tissue are well demonstrated* ($\times 700$).

FIGURE 5. *Autoradiogram of feather follicle epithelium infected with the JM strain of MD virus. Note many intranuclear inclusions and localized silver grains of inclusions in the superficial layers of epidermis. Fixed with Carnoy's fixative and stained with H-E* ($\times 1,500$).

REFERENCES

Calnek, B. W., and S. B. Hitchner. 1969. Localization of viral antigen in chickens infected with Marek's disease herpesvirus. J. Nat. Cancer Inst. 43: 935–945.

Churchill, A. E., and P. M. Biggs. 1967. Agent of Marek's disease in tissue culture. Nature 215: 528–530.

Kato, S., Y. Aoyama, and J. Kamahora. 1963. Autoradiography of the tissues of mice infected with ectromelia virus using ^{3}H-thymidine. Biken J. 6: 9–16.

Nazerian, K., J. J. Solomon, R. L. Witter, and B. R. Burmester. 1968. Studies on the etiology of Marek's disease. II. Finding of a herpes virus in cell culture. Proc. Soc. Exp. Biol. Med. 127: 177–182.

Nazerian, K., and R. L. Witter. 1970. Cell-free transmission and in vivo replication of Marek's disease. J. Virol. 5: 388–397.

Ono, K., S. Kato, N. Iwa, and T. Doi. 1970. Autoradiographic and cytochemical studies on nuclear and cytoplasmic inclusions of duck embryo fibroblasts infected with herpes type virus isolated from chickens with Marek's disease. Biken J. 13: 53–57.

Purchase, H. G. 1970. Virus-specific immunofluorescent and precipitin antigen and cell-free virus in the tissue of birds infected with Marek's disease. Cancer Res. 30: 1898–1908.

Rogers, A. W. 1967. Technique of autoradiography. Elsevier Publ. Co., Amsterdam/London/New York.