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AUTORADIOGRAPHY OF THE TISSUES OF CHICKS INFECTED WITH MAREK'S DISEASE VIRUS USING ³H-THYMIDINE¹

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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 ³H-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 μ Ci/ml and chicks were injected with 1 μ Ci per g body weight of ³Hthymidine 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.

0	Chicken							
Organ	No. 1	No. 2	No. 3	No. 4	No. 5	Noninfected		
Liver	+	+	+	÷	+			
Heart	+	+	+	+	+			
Kidney	+	+	- <u>1</u> -	+	+			
Lung	+	+	+		+			
Proventriculus	+	+	+	+	+			
Pancreas		NT			+	\mathbf{NT}		
Ovary	+	+	+	\mathbf{NT}	+	\mathbf{NT}		
Testis	NT^a	NT	\mathbf{NT}	+	NT	_		
Skin	+	+	+	+	+			
Spleen		+	_					
Bursa of Fabricius		_			_			
Thymus		NT	_		+			
Peripheral nerves								
vagus	+	+	+		+			
brachial	+	+	+	+	+			
lumbosacral plexus	+	NT	+	+	+			
sciatic	+	+	+	+	+			

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

a Not tested.

removed and fixed with Carnov'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

	Labeled nuclei (%)						
Organ	In lymphocytic foci			In normal tissues			
	No. 2	No. 3	No. 4	No. 2	No. 3	No. 4	Noninfected
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	\mathbf{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	\mathbf{NT}	0.6	0.6	NT
Ovary	26.8	45.8	NT	\mathbf{NT}	\mathbf{NT}	\mathbf{NT}	NT
Testis	\mathbf{NT}	\mathbf{NT}	40.2	\mathbf{NT}	\mathbf{NT}	1.6	1.0
Skin	26.8	29.6	\mathbf{NT}	3.6	2.8	\mathbf{NT}	2.6
Peripheral nerves	24.6	42.6	25.8	0.4	0.8	0.2	0.2
Spleen	35.2	\mathbf{NT}	\mathbf{NT}	NΤ	12.8	12.4	10.8
Bursa of Fabricius	NT	\mathbf{NT}	NT	18.4	19.6	15.6	11.6
Thymus	NT	NΤ	NT	NΤ	15.8	NΤ	12.2

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

a Not tested.

Organ	Mitotic indices						
	In lymphocytic foci		In normal tissues			λτ. ' C Ι	
	No. 2	No. 3	No. 4	No. 2	No. 3	No. 4	Noninfected
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

NT

1.2

NT

 \mathbf{NT}

1.6

NT

0.6

NT

 \mathbf{NT}

NT

0.0

0.0

 \mathbf{NT}

 \mathbf{NT}

NT

0.0

0.0

NT

0.8

NT

0.0

0.0

0.0

 \mathbf{NT}

 \mathbf{NT}

0.0

0.0

0.6

0.8

0.4

0.0

0.0

0.0

 \mathbf{NT}

0.2

 \mathbf{NT}

0.0

0.6

0.6

NΤ

0.0

0.0

NT

NT

0.6

0.0

0.0

0.4

0.6

0.6

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

Thymus a Not tested.

Lung

Pancreas

Ovary

Testis

Spleen

Skin

Proventriculus

Peripheral nerves

Bursa of Fabricius

0.6

1.0

 NT^a

0.8

NT

0.4

0.2

1.0

 \mathbf{NT}

NT

0.8

0.8

1.2

2.2

NT

0.4

1.8

 \mathbf{NT}

 \mathbf{NT}

NT



FIGURE 1. Autoradiogram of a lymphocytic lesion of liver tissue. The lymphoid cells in the lymphocytic lesion are intensely labeled with ³H-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).

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 Hitch-



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).

ner, 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).

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FIGURE 3. Autoradiogram of a lymphocytic lesion of peripheral nerve tissue. The lymphoid cells are intensely labeled with ³H-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 $(\times 1,500)$.

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