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<td>Author(s)</td>
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<tr>
<td>Citation</td>
<td>Japanese Journal of Veterinary Research, 19(3), 64-72</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1971-09</td>
</tr>
<tr>
<td>DOI</td>
<td>10.14943/jjvr.19.3.64</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/1980">http://hdl.handle.net/2115/1980</a></td>
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<td>Type</td>
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<td>File Information</td>
<td>KJ00002369917.pdf</td>
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### Instructions for Use

PATHOLOGICAL STUDIES OF MAREK'S DISEASE II
ELECTRON MICROSCOPIC OBSERVATION OF THE CELLULAR LESIONS IN THE PERIPHERAL NERVES

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(Received for publication, June 1, 1971)

The fine structure of the peripheral nerve lesions, especially cellular lesions, from the field cases of Marek’s disease, has been described. The morphological classification of the peripheral nerve lesions used in the previous report has been adopted.

The T1-type lesion consisted of mainly uniform small lymphoid cells.

The T2-type lesion consisted of pleomorphic cells which included small, medium and large (lymphoblastic and hemocytoblastic) lymphoid cells. The lymphoid cells (mainly large lymphoid cells) often had the nuclear pockets in the nucleus and mitoses were frequently found. The more the lymphoid cell was immature, the more it increased free ribosomes in the cytoplasm. The hemocytoblastic lymphoid cell was characterized by unusually large nucleus and numerous polysomes in the cytoplasm.

The R-type lesion consisted of infiltration of small lymphocytes and plasma cells and was accompanied by fewer reticulum cells.

Furthermore, fibroblasts, mast cells and macrophages were occasionally found in these lesions. Macrophages were active and phagocytized various kinds of materials, such as myelin debris, lipids and cellular debris. Cytoplasmic bridge between macrophage and plasma cell was occasionally found in the R-type lesion.

INTRODUCTION

Marek’s disease (MD) is a common lymphoproliferative disease affecting the peripheral and the central nervous systems, and the viscera of chickens. Considerable evidence has indicated that a group B herpesvirus is the etiological agent of MD4,17,18).

The purpose of the present study is to elucidate the fine structure of the peripheral nerve lesions, especially cellular lesions, from the field cases of MD.

This work was submitted by K. OKADA in partial fulfillment of the requirements for the Master degree at Hokkaido University.

Supported in part by a grant from the Ministry of Education.
As in the description of the previous study\(^9\), the lesions of MD were basically classified into 2 types: Tumorous proliferation (T-type) and non tumorous response (R-type), according to the types of cells which appeared and the characteristics of the lesions. Furthermore, the T-type lesions could be subdivided into 3: T\(_1\)-, T\(_2\)- and T\(_3\)-types. Except in the T\(_3\)-type lesions, we could indicate the fine structures of various cells which were composed of the lesions of all types.

There were only a small number of reports on electron microscopic studies of MD. DEUTSCH \& SILLER, (1961), NAKAGAWA (1965), and WIGHT (1969) described certain ultrastructural features of the peripheral nerve lesions. CALNEK et al. (1970) (herpesvirus), UBERTINI \& CALNEK (1970) (herpesvirus) and OKADA \& FUJIMOTO (1970) (leukosis/sarcoma group like viruses) reported virus particles associated with MD nerve lesions. SIMPSON (1969)\(^{26,27}\) also reported viral particles in the spontaneous ocular leukosis. Ultrastructures of degeneration and regeneration of the peripheral nerves will be reported in the next report of this series.

**MATERIALS AND METHODS**

Light and electron microscopic investigation was conducted on 17 cases (3 cases of T-type, 4 of T\(_+\)R type, 10 of R-type) which were considered to be the field cases of MD on the basis of clinical and pathological (histopathology) features and 4 cases of control normal birds.

Electron microscopic materials were chiefly collected from the brachial plexus, lumbosacral plexus, spinal ganglia of the cervical, thoracal and lumbal regions and vagosympathetic trunk. The materials were collected as soon as possible after the birds were depleted. Small portions of the nerve tissues collected were fixed in chilled 1\% osmium tetroxide (method of MILLONIG) without mincing. After one hour had passed, they were minced in the size of 1 mm\(^3\) and then refixed for 3 hours by the same fixative. After dehydration through a graded ethanol series, the specimens were embedded in an epoxy resin mixture (MOLLENHAUER). Thin sections were cut with glass knives using a Porter-Blum MT-1 ultramicrotome. The sections were mounted on 200-mesh nickel grids. They were stained with uranyl acetate followed by lead citrate. Thick sections of approximately 1 or 2 \(\mu\) were stained with toluidine blue for orientation by light microscopy. A JEM-7 electron microscope was used for examination under 80 KV. The tissues for light microscopy were collected as many as possible from various parts of the organs and tissues. Sections were stained with hematoxylin and eosin.

**RESULTS**

**Light Microscopy**

The morphological classification of the peripheral nerve lesions was already fully described in the previous paper\(^9\).
TI-type lesion consisted of only uniform small lymphoid cells.

TI1-type lesion consisted of pleomorphic cells which included small, medium and large (lymphoblastic and hemocytoblastic) lymphoid cells and fewer reticulum cells.

R-type lesion was characterized by infiltration of small lymphocytes and plasma cells and was accompanied by frequent edema and proliferation of Schwann cells.

Electron Microscopy

Lesions

The TI-type lesion (fig. 1) consisted of focal proliferation of tumor cells of the lymphoid series. The predominated cells in this lesion were uniform small lymphoid cells which coincided with the findings of the light microscopy. Medium lymphoid cells and reticulum cells scattered indiscriminately in small numbers. A small number of bundles of collagen fibers scattered between the nerve fibers.

The TI1-type lesion (fig. 2) consisted of proliferation of tumor cells of the lymphoid series. The cells which appeared were pleomorphic, and included lymphoid cells of various degree of maturation. In the fig. 2, the lesion consisted of small and medium lymphoid cells. Furthermore, the lesion of this type usually had large (lymphoblastic and hemocytoblastic) lymphoid cells (fig. 7 & 8), reticulum cells and fewer macrophages.

R-type lesion (fig. 3) was characterized by infiltration of small lymphocytes and plasma cells, and by proliferation of fewer reticulum cells. Plasma cells had well developed lamellar structures of the rough surfaced endoplasmic reticulum in the cytoplasm. Some or all of the rough surfaced endoplasmic reticulum were widened into cisternae and filled with a homogeneous proteinic electron opaque substance. There were bundles of collagen fibers between the nerve fibers.

Cellular Elements

Small lymphoid cell (figs. 1, 2 & 4) (T-type)

The size of the small lymphoid cell was almost the same as that of the small lymphocyte (mature lymphocyte) or a little larger. The nucleus was spherical and rich in chromatin. One or more nucleoli were present, depending on the plane of section. Contour of the cell was spherical or somewhat irregular. Frequently irregular cytoplasmic processes were found. The rim of the cytoplasm was small. As a characteristic of cells of the lymphoid series, cytoplasmic organelles were poorly developed. A small number of large mitochondria was dispersed around the nucleus. The rough surfaced endoplasmic reticulum was very rare. A small number of fine vesicles and a Gall-body were also found. The free ribosomes remained abundant. Mitoses were frequent.

Medium lymphoid cell (figs. 1, 2, 4, 5 & 6) (T-type)

The size of the medium lymphoid cell varied, but it was intermediate between the small lymphoid cell and the large lymphoid cell which will be described in the next chapter. The form of the nucleus was somewhat irregular. Sometimes deep cleft of the nuclear membranes and nuclear pockets due to the cytoplasmic invagination of the nucleus were seen. The nucleolus of this cell was larger than that of the small lymphoid cell. The rim of the cytoplasm was somewhat large. The contour of the cell was usually spherical
or often irregular. The cytoplasmic organelles were also poorly developed and a few large pale mitochondria showed a tendency to group in one pole of the cell. The free ribosomes were distributed closely in the cytoplasm. A small number of the smooth and rough surfaced endoplasmic reticula were distributed. A Golgi apparatus was more developed than that of the small lymphoid cell and sometimes multivesicular bodies were accompanied.

Lymphoblastic large lymphoid cell (fig. 7) (T-type)

The lymphoblastic large lymphoid cells (8~10 μ) were larger than the medium lymphoid cells and were smaller than the hemocytoblastic large lymphoid cells. The form of the nucleus and cell was spherical or somewhat irregular. The nucleus often had irregular indentation and frequently the nuclear pockets were found. Some of the nuclear membranes which wrapped the nuclear pockets often partially disappeared. The nucleolus was pale as in the reticulum cell. The chromatin formed dense clumps and showed a tendency to aggregate at the marginal area of the nuclear membranes. The cytoplasm was well developed and contained an abundance of free ribosomes among which only a few grouped in "rosettes." A Golgi apparatus was well developed and a pair of centrioles distinctly could be seen. In this region, compound vacuoles were often found. Large mitochondria were dispersed around nucleus or grouped in one pole of the cell. A small number of unusually long flattened rough surfaced endoplasmic reticula and lysosomes were seen.

Hemocytoblastic large lymphoid cell (fig. 8) (T-type)

The hemocytoblastic large lymphoid cell was very large and round. The nucleus was round and voluminous. Fine granular chromatin was more or less accumulated at the marginal area of the nuclear membranes. The appearance of the nucleoplasm was as in the reticulum cell. Some of the nucleus was often irregular and occasionally the nuclear pockets could be seen. The most striking feature was several unusually large nucleoli within the same nucleus. Cytoplasmic organelles were poorly developed. A Golgi apparatus was well developed. A small number of the smooth and rough surfaced endoplasmic reticula were distributed in the cytoplasm. But the most characteristic feature was the forming of numerous free ribosomes in rosettes. Mitoses were frequent. Large mitochondria were grouped in one pole of the cell or dispersed around the nucleus.

Small lymphocyte (mature lymphocyte) (figs. 3 & 9) (R-type)

The small lymphocyte was ca. 4~7 μ in size. The nucleus was almost round or kidney-form. Sometimes the nucleus had deep indentation. The nucleoplasm contained several small nucleoli. Cytoplasmic organelles were poorly developed, as the characteristics of cells of the lymphatic series. A small number of mitochondria was dispersed around the nucleus. A Golgi apparatus was small and rudimental. Compound vesicles were rare. Sometimes a small Gall body was found. Along the cytoplasmic membranes pinocytotic vesicles were seen.

Plasma cell (figs. 3, 10 & 16) (R-type)

The plasma cell was ca. 9~10 μ in size. The nucleus was spherical and usually excentrically placed. Some of the nucleus showed irregular contour. The nucleoplasm was rich in chromatin and the chromatin clumped at the margin of the nuclear membranes. The nucleus was moderate in size. The contour of the cell was round or ellipsoid and
sometimes irregular with cytoplasmic processes. The most characteristic feature was well
developed rough surfaced endoplasmic reticula which formed lamellar structure throughout
the cytoplasm. Sometimes the rough surfaced endoplasmic reticula were dilated as cisternae
and were filled with electron opaque substances which we call "Russell's body." Some plasma
cells showed mulberry-like structure. A Golgi apparatus was particularly developed and
contained a pair of centrioles. Mitochondria were scattered irregularly in the whole
cytoplasm.

Reticulum cell (Histiocytic cell) (figs. 1, 3, 10 & 11)

The size of the cell varied from 8~12 μ or larger. The form of the nucleus was
spherical or on the contrary, often showed deep clefts. The nucleoplasm was pale and
interchromatin space was enlarged. The nucleolus was small. The cytoplasm was well
developed. A small number of string-like rough surfaced endoplasmic reticula and sparsely
distributed free ribosomes were found in the cytoplasm. A Golgi apparatus was well
developed and rich in microvesicles and vacuoles. The characteristic feature of this cell
was the presence of vacuoles and phagosomes in the cytoplasm. These phagosomes were
of variable size and density. Frequently, there were a few dense and small granules near
the Golgi area. A few mitochondria were distributed in the cytoplasm.

Fibroblast (fig. 12)

The fibroblast was a spindle shaped cell with many cytoplasmic processes, some of
which were extremely long. It had a pale elongated nucleus with a nucleolus of medium
size. Sometimes the nuclear bodies were also found in the nucleus. A Golgi apparatus
was well developed. Well developed rough and smooth surfaced endoplasmic reticula were
present in considerable number. They often formed cisternae or vacuoles. Free ribosomes
were abundant and often formed rosettes. The microfibrils were found in the cytoplasm.
Some of the cytoplasmic processes wrapped the bundles of collagen fibers (fig. 12). A
few small mitochondria were distributed in the cytoplasm.

Mast cell (fig. 13)

The nucleus was pale, round or uneven and contained a nucleolus of medium size.
The cytoplasm was well developed and had electron opaque granules which were surrounded
by a limited membranes. The size and density of the granules were variable. A Golgi
apparatus was well developed and composed by accumulation of vacuoles and cisternae.
The mitochondria were small and distributed throughout the cytoplasm. Vacuoles and the
rough surfaced endoplasmic reticulum were moderately developed in the cytoplasm. The
mast cells were frequently found in normal nerve tissues and the R-type lesion.

Macrophage (figs. 14, 15 & 16)

Macrophages were usually large and their forms were variable. The nucleus was of
irregular form and often had deep indentation. The nucleoplasm had a small amount of
chromatin and a small nucleolus. The cytoplasm was well developed and its cytoplasmic
process was often extremely elongated. Most of the surface membrane showed ruffled
appearance. The mitochondria were distributed in small numbers. The rough surfaced
endoplasmic reticulum was poorly developed. The Golgi apparatus was large and consisted
of many microvesicles. The most characteristics of the macrophages was the presence of
variable-sized vacuoles of the smooth surfaced endoplasmic reticulum in the cytoplasm (digestion vacuoles). These vacuoles phagocytized various kinds of elements: myelin debris, electron opaque cellular debris, various pigments and lipids etc. Mitoses were frequently seen. In the edematous area with degeneration and loss of the nerve fibers, there were often many macrophages with numerous vacuoles. The macrophages of this type may correspond to scavenger cells in the light microscopy (fig. 15). In the R-type lesion, close connection between macrophage and plasma cell was often found (cytoplasmic bridge) (fig. 16).

DISCUSSION

The light microscopic findings of the peripheral nerve lesions of MD have been studied by many workers, since Marek's and later Papenheimer's et al. reports of the disease. Although many authors disagree as to the interpretation of the lesions and of the cell involved, the most characteristic lesions were cellular infiltration or proliferation in the peripheral nerves.

The cells involved have generally been referred to as "lymphoid cells" or "large or small mononuclear cells." Yamagiwa et al. used the term lymphocytes, light and dark cells. Sevoian & Chamberlain described that the lesions mainly consisted of proliferated cells originating from the primitive mesenchymal cells. Wight classified the peripheral nerve lesions into the following 3 types. Type I was characterized by cellular infiltrations and relatively little edema. The majority of the infiltrating cells were small lymphocytes and plasma cells. Type II showed marked edema in contrast to the type I, and the total number of infiltrating cells was small. In severe cases degeneration of the myelin sheaths and axons, and a tendency to fibrosis was marked. Type III was neoplastic and the lesions were characterized by a massive infiltration of morphologically similar lymphoblasts. Payne & Biggs classified the lesions of the nerves into 3 types, on the basis of their studies of the pathogenesis of MD. A-type was characterized by proliferation of the lymphoid cells, the presence of MD cells, and sometimes by demyelination and Schwann cell proliferation. B-type was characterized by diffuse infiltration of plasma cells and mainly small lymphocytes and edema. C-type lesion was considered as a mild form of the B-type. From the above findings, we considered that our T-type was similar to their III and A-types and our R-type was also similar to their I- and II-types and B- and C-types.

The T-type lesions consisted of the tumor cells of the lymphoid series under electron microscope; such as small, medium and large (lymphoblastic and hemocytoblastic) lymphoid cells. In addition to the above variable pleomorphic cells of the lymphoid series, some reticulum cells, degenerated cells and macrophages etc. were found. We could not encounter the cases of the TIII-type lesion which was described in our previous paper. It consisted mainly of reticulum or
primitive mesenchymal cells. As the cells of the lymphoid series, the most characteristic findings were the poorly developed cytoplasmic organelles and the presence of a small number of large mitochondria which dispersed around the nucleus or grouped in one pole of the cell. The more the lymphoid cell was immature, the more it increased free ribosomes in the cytoplasm. Especially the hemocytoblastic large lymphoid cell had several unusually large nucleoli and extensive increase of polysomes in the cytoplasm. Lymphoid cells frequently had the nuclear pockets due to the invagination of the cytoplasm into the nucleus. The similar findings were already frequently reported in cases of MD by Wight \(^3\), in bovine lymphosarcomas \(^7,13,31\), human, lymphosarcomas \(^1,6\) and leukemias \(^8,12,15\). Since this findings also have been reported in a variety of apparently normal cells from humans and animals \(^10,25,29\), they may not be regarded as characteristics of the tumors. They may indicate the active cell metabolism. The reticulum cells were uneven in the contour of the nucleus and cell. They had a pale nucleus with large interchromatin space and comparatively rich in the smooth and rough surfaced endoplasmic reticula in the cytoplasm. They also had a small number of small phagosomes and vacuoles. Although it was very difficult to draw a line between the reticulum cells and the macrophages, the macrophages were generally large and characterized by increase and enlargement of lysosomes and phagosomes. Contour of the macrophages had marked cytoplasmic processes with a ruffled appearance. As to the phagocytized materials in the phagosomes, myelin debris was often found. This informs us of participation of macrophages in the demyelination of the nerve fibers. At the edematous area of the interneurites, the macrophages had numerous fine vacuoles. There has been controversy as to the role of the Schwann cells and the macrophages, and the present authors will discuss it in the next report. Since the T-type lesion consisted of tumorous proliferation of the lymphoreticular cells, the cells involved indicated various degrees of maturation and polymorphism from the lymphoid to the reticular series.

Fibroblasts were present between the nerve fibers. They had elongated nuclei and cytoplasms, and had well developed rough surfaced endoplasmic reticulum throughout the cytoplasm. The increase of collagen fibers between the nerve fibers, was already reported by Deutsch & Siller (1961) and Wight (1969). Although the authors also found such lesion in the present cases, it could not be regarded as the typical lesion of MD. As to the lesion, we will report in the next report.

It is a well known fact that the plasma cell is usually rich in the rough surfaced endoplasmic reticulum in the cytoplasm and produce \(\beta\)-globulin. In the R-type lesion, we could often find many plasma cells with numerous widened
cisternae of the rough surfaced endoplasmic reticulum. They contained homogeneous electron opaque substances. We often found the appearance of close connection with macrophage and plasma cell. This was called "cytoplasmic bridge" as report of Schoenberg et al. This findings may suggest some cytoplasmic interaction between the macrophages and the plasma cells in antibody synthesis. From the above findings, it may be considered that the R-type lesion has some connection with immunological phenomena.

Although Ubertin & Calnek already found herpesvirus particles in the peripheral nerves, the present authors could not find any herpesvirus particles, except we26) found leukosis/sarcoma group like viruses in the peripheral nerves and the skeletal muscles of 2 cases of MD and one apparently normal chicken.

Acknowledgements

The authors are grateful to Dr. A. Shimizu, Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, and Dr. K. Matsukawa, College of Dairy Agriculture, Ebetsu, Japan, for providing us with the field cases of Marek's disease.

References

EXPLANATION OF PLATES

PLATE I

Fig. 1 T1-type lesion in the spinal ganglion
The predominant cells in the lesion are small lymphoid cells (sL).
Medium lymphoid cells (mL) are present in small numbers and
one reticulum cell (R) can be seen. Co: collagen fibers
×7,500

Fig. 2 T1-type lesion in the brachial plexus
Medium (mL) and small (sL) lymphoid cells are present. One
medium lymphoid cell in the upper right corner of the figure has
well developed Golgi apparatus (G) and the nuclear cleft (arrow)
×7,500
Fig. 3 R-type lesion in the spinal ganglion
Infiltration of small lymphocyte (l), plasma cell (P) and reticulum cell (R) can be seen. A plasma cell has well developed Golgi apparatus (G) with a pair of centrioles (C). The other plasma cell in the upper left corner of the figure has many dilated rough surfaced endoplasmic reticula which contained electron opaque substances. Co: collagen fibers $\times 7,500$

Fig. 4 Small (sL) and medium (mL) lymphoid cells are found between the unmyelinated nerve fibers in the brachial plexus. Arrow indicates outer mesaxon of the unmyelinated nerve fiber. TH-type lesion $\times 15,000$
Plate III

Fig. 5 A medium lymphoid cell in the brachial plexus Tn-type lesion
G: Golgi apparatus  C: centriole  × 15,000

Fig. 6 A medium lymphoid cell with the nuclear pocket (arrow) in the brachial plexus Tn-type lesion G: Golgi apparatus C: centriole
Ly: Lysosome  × 15,000
Plate IV

Fig. 7 A lymphoblastic lymphoid cell in the brachial plexus TII-type lesion G: Golgi apparatus C: centriole \( \times 15,000 \)

Fig. 8 A hemocytoblastic lymphoid cell. Unusually large nucleolus (Nl) and polysomes in the cytoplasm TII-type lesion Spinal ganglion G: Golgi apparatus \( \times 18,500 \)
PLATE V

Fig. 9 A small lymphocyte in the spinal ganglion R-type lesion
G: Golgi apparatus  mV: multivesicular body  × 15,000

Fig. 10 A plasma cell (P) and a reticulum cell (R) in the brachial plexus
R-type lesion  G: Golgi apparatus  × 7,500
PLATE VI

Fig. 11 A reticulum cell (R) in the spinal ganglion R-type lesion
Ax: axon  Co: collagen fibers  × 10,000

Fig. 12 A fibroblast is found between the unmyelinated nerve fibers in the brachial plexus. Arrow indicates that the bundles of the collagen fibers are wrapped by the cytoplasmic processes R-type lesion Nb: nuclear body  × 15,000
Fig. 13 A mast cell with variable density of granules (g) in the brachial plexus R-type lesion 15,000

Fig. 14 Myelin debris (Md) is phagocytized by a macrophage in the lumbosacral plexus R-type lesion x 15,000
Fig. 15 A macrophage with numerous vacuoles in the spinal ganglion
R-type lesion  $\times 7,500$

Fig. 16 A close connection between macrophage and plasma cell is seen
(cytoplasmic bridge: arrows) in the brachial plexus  R-type lesion
$\times 9,250$