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<td>Author(s)</td>
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<tr>
<td>Citation</td>
<td>Japanese Journal of Veterinary Research, 13(3): 96-103</td>
</tr>
<tr>
<td>Issue Date</td>
<td>1965-09</td>
</tr>
<tr>
<td>DOI</td>
<td>10.14943/jjvr.13.3.96</td>
</tr>
<tr>
<td>Doc URL</td>
<td><a href="http://hdl.handle.net/2115/1827">http://hdl.handle.net/2115/1827</a></td>
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<td>File Information</td>
<td>KJ00002369158.pdf</td>
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*HOKKAIDO UNIVERSITY*
PECULIAR NUCLEAR INCLUSION, NUCLEOLOID BODY, IN LYMPHOCYTES

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(Received for publication, July 28, 1965)

INTRODUCTION

Recently David (1964) using the electron microscope reported the physiological and pathological changes in nuclear structures. He found various types of nuclear inclusions, but could not determine the genesis of the inclusions in many cases.

In a previous paper 27), the writers, examining mouse lymph nodes, also found a peculiar nuclear inclusion in nuclei of lymphocytes and endothelial cells of post-capillary venules. In the present study, the same nuclear inclusions were found in several types of cells, particularly in small lymphocytes in lymph nodes of mice and hemal nodes of sheep. The writers call them "nucleoloid bodies", as they appear to derive from the true nucleolus. In this paper the genesis of the nucleoloid body, the cell types containing the body, and the ultrastructure of the body are described in an effort to gain some insight into the function of lymphocytes.

MATERIALS AND METHODS

Mandibular, subiliac and mesenteric lymph nodes of 6 healthy dd strain mice were used. The mice were of both sexes, weighing 21.5-37.5 g and from 60 to 150 days old. In addition hemal nodes of two sheep were used for comparison with the mouse lymph nodes. The sheep were a female and a male, 15 and 17 months in age, and weighing 18 and 23 kg respectively.

Node specimens were fixed with osmium tetroxide (Palade's or Millonig's 17) methods) for 1-1.5 hours. After fixation, the specimens were dehydrated with graded ethanols and then embedded with Epon 812 (Luft). Sections were cut on a Hitachi UM-3 microtome using glass knives. After mounting on copper grids, the sections were stained with uranyl acetate (Watson) 20, lead tartrate (Millonig) 18 and/or lead citrate (Raynold), and then examined with JEM-4 CHD and JEM-6AS electron microscopes at magnifications varying from 2,000 to 30,000.

* Electron Microscope Laboratory

JAP. J. VET. RES., VOL. 13, No. 3, 1965
RESULTS

1 Ultrastructure of the nucleoloid body

The nucleoloid bodies are always spherical in shape, ranging from 200 to 900 μm in diameter, and morphologically independent of the nucleoli and the chromatin (fig. 3). When the bodies are observed in sections they are usually separate (fig. 1) but they are also found in groups (fig. 2). There is no difference in the occurrence of the bodies between the sexes. The bodies are invariably composed of two layers, inner and outer.

In mice the outer layer consists of numerous filaments in a concentric (figs. 1~8) or spiral (figs. 9 & 10) arrangement which encircles perfectly the inner layer. At high magnification the filaments seem to be fibrils oriented longitudinally, usually in pairs showing cross-banding, or coiled helically (fig. 11-a). From these findings it is suggested that each filament is formed by the coiling of a thin fibril, as shown in schema.

SCHEMA A diagrammatic illustration showing the ultrastructure of the outer and inner layers of the nucleoloid body (ref. fig. 11-a)

The filaments are approximately 70~80 Å in width. The diameter of the fibril coiled into the filament can be estimated to be about 20~30 Å. The same helical fibrils are also found in the nucleoplasm around the nucleoloid bodies (figs. 11-a & b). The inner layer is usually composed of a homogeneous substance of lower electron density with a variable number of dense granules (figs. 1~10). The dense granules are similar to the ribosomes in the cytoplasm and to the dense granules in the true nucleolus (fig. 3). At high magnification, however, the granules seem to be twisted threads formed by coiled fibrils (fig. 11-a). The twisted threads are 200~250 Å in width and the diameter of the fibril coiled in the thread is about 50 Å, as shown in schema.

The nucleoloid bodies may be separated into two types according to the appearance of their inner layers and their size. The first type is small, about 200~300 μm in size, and has no or a few dense granular elements in the inner layer (figs. 1 & 5~7). The second type
is larger, over 400 μm in size, whose inner layer has numerous dense granules (figs. 3, 4 & 9–11-a).

In sheep the nucleoloid bodies are similar to those of mice, and are also found in lymphocytes (fig. 17-a), but the inner layer, which consists of dense granules or threads, often appears to be hexagonal in shape (fig. 17-b).

2 Cell types containing the nucleoloid body

The nucleoloid bodies are found in the nuclei of small lymphocytes (figs. 1–4), plasma cells (figs. 8 & 9), macrophages (fig. 7), littoral cells of sinus (fig. 6) and endothelial cells of blood vessels (fig. 5). No evidence is found that the bodies are specific to a cell type. But, they are most frequently found in small lymphocytes, particularly the large nucleoloid bodies (second type) which are almost only found in the small lymphocytes (figs. 3, 4, 10 & 11-a), though rarely in the plasma cells as well (fig. 9). Even if nucleoloid bodies are found in cells other than small lymphocytes, it is a rare finding and usually involves the small bodies (first type). The bodies are not observed in the nuclei of immature lymphoid cells.

3 Genesis of the nucleoloid body

It is difficult to determine the origin of the nucleoloid bodies, but several observations were made which might explain their genesis (figs. 12–15). In lymphocytes a filamentous structure, which is similar to that in the outer layer of the nucleoloid body, is often found in contact with the true nucleolus (fig. 12). The structure is cap-like in shape and is situated on one side of the nucleolus (figs. 12 & 13). This finding suggests that the outer layer originates from the true nucleolus. In figure 13, which may be an advanced stage, the filamentous structure seems to be separating from the nucleolus. It is unquestionable that the outer layer of the nucleoloid body is formed through intimate contact with the true nucleoli, but it is not clear whether the dense ribosome-like granules of the inner layer are derived from the true nucleolus or are synthesized in the body after completion of the outer layer.

In a mitotic cell, nucleoloid bodies were observed in the chromosomes without disappearing (fig. 16).

DISCUSSIONS

At present most authors agree that the nuclei of interphase cells are composed of the nuclear envelopes, nucleoli and nucleoplasm including chromatins observed in electron microscopic studies (DE ROBERTIS & DE IRALDI, and WISCHNITZER). Some investigators, however, found nuclear inclusions in the nuclei of liver cells of snakes (KUROSUMI), as well as in the nuclei of various cells under physiological and pathological conditions (DAVID) and also in the nuclei of rat liver cells (WATSON). The writers found a different type of nuclear inclusion in the cells of mouse lymph nodes, which was morphologically independent of the nucleoli and
Peculiar nuclear inclusion in lymphocytes

Some authors reported that osmic acid was not suitable for fixation of nucleoplasm, but the writers' observations on lymph node cells revealed that the highly organized structure in the interphase nuclei was fixed by osmic acid. The nuclear inclusions, ranging from 200 to 900 μm in size, are spherical in shape and composed of outer and inner layers. The outer layer consists of numerous filaments arranged concentrically or spirally. At high magnification the filaments seem to be helical fibrils, approximately 70~80 Å in width. The diameter of the fibrils coiled into the helices is estimated to be about 20~30 Å. This corresponds to one or two DNA double helices with associated protein chains, since the thinnest microfibrils of the chromosomes which represent single nucleoprotein molecules are considered to be of the order of 30 Å (DE ROBERTIS & DE IRALDI). As the helical fibrils are also found in chromatin of the nucleoplasm around the nucleoloid body, so the fibrils of the outer layer appear to be mainly made from DNA protein. At low magnification the inner layer is usually composed of a homogeneous material of low electron density and of a variable number of dense granules which are similar in size and appearance to ribosomes and dense granules in the nucleoli. But at high magnification the granules seem to be twisted threads which are formed by coiled fibrils. The threads are 200~250 Å in width and the coiled fibrils measure about 50 Å in diameter. The basic structure of the nucleoli is either that of granules (MACRAE and MARINOZZI) or helical threads (KUROSUMI and YASUZUMI et al.). The fine structure of the threads of the inner layer seems to be very similar to that of true nucleoli which consists mainly of RNA protein. It seems reasonable to assume that the outer layer and the thread-like dense material of the inner layer may be mainly composed of DNA and RNA proteins respectively.

The nucleoloid bodies were found in the nuclei of small lymphocytes, plasma cells, macrophages, littoral cells and endothelial cells of blood vessels so that the occurrence of the bodies is not specific to a particular type of cell. Actually, nuclear inclusions corresponding to nucleoloid bodies are described in the acinus cells of rat mandibular glands ("reticular nucleolus" by KURTZ), in the PANETH cells of rat small intestines (BEHNKE & MOE), in the endothelial cells of the rat pulmonary arteries (WEIBLE & PALADE) and in macrophages of human lymph nodes ("whorl-like inclusions" by BERNIARD & LEPLUS).

It is true that nucleoloid bodies are most often observed in small lymphocytes, in particular the large bodies (second type) over 400 μm in size are almost exclusively found there in mice. In addition nucleoloid bodies were also often found in small lymphocytes of sheep hemal nodes, so that the large bodies may almost be limited to the small lymphocytes of animals other than mice. Even if nucleoloid bodies are found in cells other than small lymphocytes, it is a rare finding and usually
involves the small bodies only.

The genesis of the described nuclear inclusions was unknown up to this time. In this study the writers made several observations which may explain origin of the nucleoloid bodies. It is suggested that the outer layer of the nucleoloid bodies originated from the true nucleoli, while the thread-like dense materials of the inner layer was derived from either the nucleoli or was newly synthesized in the layer after completion of the outer layer.

The chemical components of the nucleoloid bodies have not been resolved. From light microscopic studies and from histochemical studies, however, it is well known that the nuclei of small lymphocytes of mouse lymph nodes have DNA but no pyroninophilic nucleoli (SUGIMURA et al.33). From observations made using the electron microscope it is possible that the outer layer of the nucleoloid body consists mainly of DNA protein, and that the dense thread-like materials of the inner layer are the same as those of the nucleoli. Significant are the birefringent bodies in the nuclei of small lymphocytes, the so-called atypical nuclei which were detected by TOMPKINS, because the type and occurrence of the cells with the atypical nuclei are very similar to the cells with nucleoloid bodies. Further studies are necessary to clarify this similarity.

Recently a new theory supported by many investigators was presented suggesting that small lymphocytes have an important function in immunological reactions under control of the thymus (MILLER13-15 and WAKSMAN et al. and many others17). Though most observers believed the lymphocytes were an end-stage cell no longer capable of mitosis and with no pluripotential capacity, recent reports of GOWANS, PORTER & COOPER, and RIEKE & SCHWARZ seem to show that small lymphocytes transform into large pyroninophilic cells. According to MURRAY et al.18-19 and YOFFEY & COURTICE, the lymphocytes develop into other cells in response to stimuli, for example into plasma cells by antigenic stimuli.

At the present time the writers have no experimental data in this regard. It is noteworthy that the nucleoloid bodies do not disappear in the mitosis of a lymphoid cell, though it is well known that the true nucleolus disappears in mitosis as does the nuclear envelope (ROBBINS & GONTAS and YASUZUMI). If the finding is substantiated, the nucleoloid body may be a specialized nuclear protein, probably genetic, different from the chromosomes. Moreover, the nucleoloid bodies are most frequently found in the lymphocytes, and particularly, the large bodies (second type) are practically limited to small lymphocytes. The nucleoloid bodies may be a key to the function of small lymphocytes, but further studies are necessary.
SUMMARY

Peculiar nuclear inclusions, "nucleoloid bodies", were found in nuclei of several types of cells in mouse lymph nodes and in sheep hemal nodes.

The nucleoloid bodies were morphologically independent of the nucleolus and the chromatin. They were spherical in shape, ranging from 200 to 900\(\mu\) in size, with outer and inner layers that could be differentiated. The outer layer consisted of numerous filaments arranged concentrically or spirally which encircle completely the inner layer. The filaments were helical coils, measuring 70\(\sim\)80 Å in width. The diameter of the fibril coiled into the helix was approximately 20\(\sim\)30 Å. The same helical fibrils were also found in the chromatin of nucleoplasm around the nucleoloid bodies. The inner layer was composed of a homogeneous substance of lower electron density and of a variable number of dense granules which were similar to ribosomes in appearance. At high magnification, however, the granules seemed to be twisted threads in which thinner fibrils were coiled. The threads were 200\(\sim\)250 Å in width and the helical fibrils measured about 50 Å in diameter.

The nucleoloid bodies were separated into two types. The first type was small, about 200\(\sim\)300 \(\mu\) in size, and had no or a few dense granular elements in the inner layer. The second type was larger, over 400 \(\mu\) in size, whose inner layer had numerous granular elements. The nucleoloid bodies were most often observed in small lymphocytes, in particular the large bodies (second type) were almost limited to the small lymphocytes, though rarely in plasma cells as well. Even if nucleoloid bodies were found in cells other than small lymphocytes, it was rare finding and usually involved the small bodies (first type) only.

The nucleoloid bodies seemed to derive from the true nucleoli and were observed in the chromosomes throughout mitosis.

From the ultrastructure of the nucleoloid bodies, it was suggested that the bodies consist of a specialized nuclear protein unlike the chromosomes.

ACKNOWLEDGMENT

The writers would like to thank to Prof. T. Nei and Dr. Y. Kojima, members of the staff of the Institute of Low Temperature Science, Hokkaido University, for their kind permission and aid in using the JEM-6AS electron microscope belonging to the Institute.

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EXPLANATION OF PLATES

All figures are electron microscope photographs of sections embedded in Epon 812 and stained with uranyl acetate (Uran) and/or lead citrate (Lead).

Abbreviations to plates

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<tr>
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<tr>
<td>Bm</td>
<td>Basement membrane</td>
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<tr>
<td>Ch</td>
<td>Chromosome</td>
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<tr>
<td>Ce</td>
<td>Centriole</td>
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<td>D</td>
<td>Degenerated cell</td>
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<td>Endothelial cell</td>
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<td>Eosinophil leukocyte</td>
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<td>Rf</td>
<td>Reticular fiber</td>
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<tr>
<td>S</td>
<td>Sinus space</td>
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<tr>
<td>Sm</td>
<td>Smooth muscle</td>
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PLATE I

Fig. 1  Cortex of mandibular node
In lymphocytes illustrated, nuclei of two lymphocytes contain the nucleoloid bodies (arrows). Note the filamentous structure of the outer layer which encircles the inner layer. Dense granules are observed in the inner layer of two bodies, but not in the other.
Uran $\times$ 16,000

Fig. 2  Mandibular node
Nucleoloid bodies (arrows) occur solitarily (right), but less often in groups (center).
Uran $\times$ 20,000
Fig. 3  Mandibular node
A nucleoloid body (arrow) and a nucleolus (N) are observed in the nucleus of a small lymphocyte (L). Note the similarity in dense granules of the inner layer with these at the margin of the true nucleolus (N) and with the ribosomes in the cytoplasms of lymphocytes (L) and reticular cell (R). The nucleoloid body consists of inner and outer layers. Lead × 20,000

Fig. 4  Subiliac node
A nucleoloid body (arrow), with inner and outer layers, is visible in the nucleus of lymphocyte (L). Note numerous dense granules in the inner layer and filaments arranged concentrically in the outer layer. Uran × 20,000
Fig. 5 Arteriole of mandibular node
The nucleus of an endothelial cell (En) contains a small nucleoloid body (arrow) by the nucleolus (N). The endothelial cell is surrounded by a basement membrane (Bm) and discontinuous smooth muscles (Sm).
Uran × 16,000

Fig. 6 Sinus littoral cell of a mandibular node
The medullary cord is separated from the sinus (S) by a single layer of littoral cells (Li), which are not surrounded by a basement membrane. Note a small nucleoloid body (arrow) and a nucleolus (N) in the nucleus of the littoral cell.
Uran × 16,000
PLATE IV

Fig. 7 Mandibular node
A fixed macrophage (M) is shown whose cytoplasm contains numerous vesicles and lysosome-like inclusions. Reticular fibrils (Rf) are encircled by the cytoplasm. In the nucleus, a small nucleoloid body (arrow) is visible.
Uran  × 16,000

Fig. 8 Mandibular node
Two plasma cells (P) are shown which contain well developed Golgi complex (G) and rough-surfaced endoplasmic reticulum. Note three nucleoloid bodies (arrows) in the nucleus of a plasma cell.
Uran  × 16,000
PLATE V

Fig. 9 Mandibular node
Plasma cell (P) and lymphocyte (L) contain a nucleoloid body in each nucleus. The outer layer of the body (arrow) of the plasma cell consists of numerous filaments showing spiral arrangement.
Uran+Lead × 24,000

Fig. 10 Lymphocyte in mesenteric node
A nucleoloid body at high magnification is shown. Note the dense granules of the inner layer and filaments of the outer layer showing spiral arrangement.
Uran+Lead × 90,000

Fig. 11-a Lymphocyte in mesenteric node
A large nucleoloid body at high magnification Dense granules of the inner layer seem to be twisted threads showing the cross-banding or helical feature (arrows). Filaments of the outer layer show the cross-banding (upper left circle), threads in pairs (lower right circle) and helical fibril (lower left circle). The helix-like fibrils are also visible in the karyoplasm around the outer layer.
Uran+Lead × 100,000

Fig. 11-b Shown is the karyoplasm of another lymphocyte of the same node with a helix sectioned longitudinally (upper arrow) and a helix cross-sectioned (lower arrow). The structure is truly the same as the filaments of the outer layer of the nucleoloid body. A helix measures about 75 Å in diameter and an original fibril of helix is about 20 to 30 Å in diameter.
Uran+Lead × 200,000
Figures 12-15 show the nucleoloid body deriving from the true nucleolus.

**Fig. 12** Lymphocyte in mandibular node
Two diffuse dense nucleoli (N) are visible in the nucleus of lymphocyte. Filamentous structures, which are quite similar to the outer layer of the nucleoloid body, are arranged in cap-like shape to cover on a top of a hillock-like nucleolus (arrow).
Uran + Lead × 16,000

**Fig. 13** Reticular cell in mesenteric node
Filamentous structure (arrow) arranged in cap-like shape cover on the hilltop of true nucleolus (N).
Uran × 30,000

**Fig. 14** Lymphocyte in mesenteric node
Nucleolus (N) with filaments at high magnification. The filaments are numerous fibrils which tend to occur in pair and exhibit cross-banding or helical pattern. The same fibrils are visible in the nucleolus.
Uran + Lead × 90,000

**Fig. 15** Lymphocyte in mesenteric node
Cross section of a nucleolus (N) encircled by filaments (arrow)
Uran + Lead × 22,000
PLATE VII

Fig. 16 Lymphoid cell in mitosis in mesenteric node
A nucleoloid body (arrow) are visible in chromosomes (Ch).
Uran × 16,000

Fig. 17-a Hemal node of sheep
Erythrocytes, Lymphocytes (L), eosinophil leukocyte (Eo) and degenerated cell (D) are visible. Note two nucleoloid bodies in nucleus of lymphocyte (arrows).
Uran × 15,000

Fig. 17-b Lymphocyte from another hemal node
Note the inner layer of the nucleoloid body showing a hexagon in shape.
Uran × 24,000