ELECTRON MICROSCOPY OF NEUTROPHILS IN PERIPHERAL BLOOD IN EQUINE INFECTIOUS ANEMIA

Mitsuo SONODA

*Department of Veterinary Internal Medicine, Faculty of Veterinary Medicine,
Hokkaido University, Sapporo*

(Received for Publication November 30, 1970)

In his previous paper¹⁰⁾, the author reported the fine structure of neutrophils in the peripheral blood of clinically healthy horses.

In this paper, the fine structure of neutrophils in the peripheral blood of a horse infected with equine infectious anemia will be described.

MATERIALS AND METHODS

The horse

A seven-year-old mongrel Percheron mare infected naturally with equine infectious anemia was provided for the experiment. She had been suffering from a severe high fever of 40.5~41.2°C since 4 days before, distinctly manifesting anorexia, cardiac weakness, edema in the lower part of the abdomen, icterus and anemia of the mucous membrane.

Hematological observation was conducted daily for three consecutive days at the time of pyrexia. The following findings were obtained: erythrocyte count, 3.40~3.65 millions; leukocyte count, 4,700~5,600; hemoglobin, 6.4~6.8 g/dl; differential leukocyte count, neutrophils 49.0~63.0%, lymphocytes 31.5~45.0%, monocytes 4.0~7.5%, eosinophils 0~0.5% and basophils 0%; sideroleukocytes, 42~104 per 10 thousand leukocytes.

Leukocytes

Leukocytes were collected by centrifugation from the plasma of blood drawn into vials containing EDTA-2Na by the jugular-vein puncture.

Making blocks

The method used for making blocks was just the same as that described in the previous paper¹⁰⁾.

Cutting and observation

Ultrathin sections were cut with glass knives on a Porter-Blum MT-1 ultra-microtome. After mounted on copper grids, the sections were double-stained with uranyl acetate and lead citrate. They were examined under an electron microscope, JEM 7 type, at magnifications varying from 3,000~60,000.

OBSERVATION

Nucleus

The macular appearance of the nucleus with two parts more or less dense depending upon the amount of chromatin condensation was very prominent. The denser parts

were usually attached to the periphery of the nuclear lobes along the nuclear membrane. On the other hand, the less dense parts were located in the central areas of the lobes. However, they were extended and attached to the nuclear membrane at smaller frontages than those of the parts of dark chromatin nodes.

In the less dense areas, round dense solitary nodes with or without a hole were sometimes observed. On the margins of the nuclear lobes of some cells, round half sharp concave was noticed. From the one edge of the concave, the nuclear membrane was projected and made a whirlpool-like structure in the cytoplasm. Furthermore, from some parts of the nuclear lobes, the nuclear membranes were projected like a ring and made circles in the cytoplasm. They contained substances similar to the cytoplasmic substances.

Cytoplasm

In the cytoplasm, there were many specific granules of round or rod-like form. When cut longitudinally, some granules showed a structure consisting of parallel lines like fibers along the long axis. There were a number of dense granules throughout the cytoplasm. In particularly magnified micrographs, they were slightly irregular in outline and approximately 120~240 Å in diameter.

In some cells, these granules were aggregated in some areas of the cytoplasm. In general, specific granules were few or absent in these areas of the cytoplasm. Not rarely, these granules made clusters surrounded by double-layered membranes.

In some cells, granules were arranged into a braid, which was dged by fine membranes, on the cut surface of the cytoplasm.

In the micrographs of sections stained only with uranyl acetate, the areas supposed to coincide with the sites of these clusters of dense granules were clear and showed no collections of granules.

Sometimes, erythrophagia was observed in the cells. Phagocytized erythrocytes were delineated with a fine membrane. They were digested or degenerated and changed into granular or flocculent substances of moderate or high density. Among these substances, myelin-like structures were seen. There were also very fine granular substances of high density. Some of them were supposed to be hemosiderin and ferritin particles. Furthermore, in some cells, the cytoplasm contained substances of very variable shape, size and structure. These substances were mostly round in shape and considerably complex in structure and density. Some of them were circle-forming highly dense bodies with a delineated fine membrane.

DISCUSSION

There have been a number of publications on hematological observations by light microscopy of the peripheral blood of horses infected with equine infectious anemia. However, there is only one report with a short description on the electron microscopic observation of neutrophils in equine infectious anemia.⁶⁾

In the present observation, it was revealed that the neutrophils of the horse infected with equine infectious anemia had generally a number of dense granular particles distributed sparsely or collectively in the cytoplasm. These granules were thought to coincide with glycogen granules of β -type from their shape, size, and character of staining for uranyl acetate and lead citrate.²⁾

It was a very interesting finding in the infected horse that glycogen granules were aggregated markedly, and that they made clusters surrounded by double-layered membranes in some cells. In the neutrophils of the clinically healthy horses used as controls¹⁰⁾, fewer glycogen granules were distributed more sparsely in the entire cytoplasm;

that is, they were not aggregated in such manner as those in the infected horse.

KOMIYA⁷⁾ described PAS-positive inclusion bodies encircled by a membranous structure in the human leukemic cell. He considered that these bodies might have been made by the accumulation of polysaccharide or glycoprotein in the cytoplasm under pathological conditions. The presence of clusters of glycogen granules in some neutrophils observed in the present observation may also have a similar significance.

Furthermore, erythrocytes were observed not rarely inside the cytoplasm of neutrophils. Almost phagocytized erythrocytes were digested, degenerated, and changed into variably irregular substances in the cytoplasm. Dense bodies were contained in the cytoplasm of some cells. They were assumed to be fragments of degenerated erythrocytes from their shape and structure. Judging from the fine structures of the phagocytized erythrocyte and dense body, some of the degenerated erythrocytes might have already been changed into hemosiderin and ferritin⁸⁾.

It has been well known that in the peripheral blood, so-called sideroleukocytes appear in high percentage of horses infected with equine infectious anemia³⁻⁵⁾. On the nature of the sideroleukocytes in the peripheral blood in equine infectious anemia, the following opinions are proposed at present: These cells may be histiocytes containing hemosiderin, which is derived from the reticulo-endothelial system³⁾, or may be neutrophils, monocytes and histocytes, all of which contain hemosiderin and/or ferritin^{9,11-13)}.

On the basis of the results obtained from the present observation, the author considers that erythrophagia observed not infrequently in neutrophils may be one of the important mechanism of formation of sideroleukocytes in equine infectious anemia.

SUMMARY

The fine structure of neutrophils in the peripheral blood of a horse infected naturally with equine infectious anemia was observed. Among the results obtained from the observation, the following findings are interesting.

1. The nuclei of some cells showed a half-round sharp concave on their margins and projections of nuclear membranes in a circular or whirlpool form.
2. A number of glycogen granules were aggregated and made clusters in the cytoplasm, which were surrounded by double-layered membranes in some cells.
3. Erythrophagia was observed not infrequently in the cells.

REFERENCES

- 1) BOCCIARELLI, D. S., TENTORI, L. and VIVALDI, G. (1959): *Rc. Ist sup. Sanità*, **22**, 1059~1067.
- 2) FAWCETT, D. W. (1966): *The Cell*, pp. 293~306. Philadelphia and London, W. B. Saunders Company.
- 3) ISHII, S. (1942): *Jueki-Chosajo Kenkyū-hōkoku*, No. 19, 294~317 (in Japanese).
- 4) ISHII, S., NOBUTŌ, K. and TANAKA, K. (1942): *Jueki-Chosajo Kenkyū-hōkoku*, No. 19, 318~338 (in Japanese).
- 5) ISHII, S. (1963): *Advance in Vet. Sci.*, **8**, 263~298.
- 6) ITŌ, Y. (1964): *Nōrinshō Kachiku Eisei Shikenjō Nempō*, **4**, 386~388 (in Japanese).
- 7) KOMIYA, E. (1964): *Clinical Hematology*, 11th Ed., p. 792, Tokyo, Nanzando (in Japanese).
- 8) RICHTER, G. W. (1957): *J. exp. Med.*, **106**, 203~218.
- 9) SAKAMOTO, T. (1960): *Jap. J. vet. Res.*, **8**, 12~28, 173~190.
- 10) SONODA, M. and KOBAYASHI, K. (1966): *Jap. J. vet. Res.*, **14**, 71~78.
- 11) TABUCHI, E. (1968): Report on Research of Equine Infectious Anemia, No. 3, 62~68 (in Japanese).

- 12) TABUCHI, E. (1968): Exp. Reports of Equine Health Lab., No. 5, 27~34 (in Japanese with English summary).
 13) YAMADA, E. (1959): *Acta hemat. jap.*, **22**, 872~888 (in Japanese with English summary).

馬の伝染性貧血の末梢血好中球の電子顕微鏡的観察

其 田 三 夫

北海道大学獣医学部家畜内科学教室

(昭和 45 年 11 月 30 日受付)

熱発作中の 1 例の自然感染伝貧馬から得た末梢血中の好中球について、電子顕微鏡的観察を行ない、その微細構造の詳細を記載した。そのうち、特に興味ある所見は、次のとおりである。

1. 核の周辺部に鋭い半円形の陥凹部を有するもの、および核膜の一部が細胞質内に突出して小

円形あるいは渦巻状を呈するものが見られた。

2. 細胞質内には、一般にグリコーゲン顆粒が多い。若干の細胞質内では、それらが集合して明瞭な集塊を形成し、その一部は膜状物で圍繞されていた。

3. 稀ならず erythrophagia が認められた。

EXPLANATION OF PLATES

PLATE I

- Fig. 1. A neutrophil with aggregation of glycogen granules in the cytoplasm. $\times 15,000$.
 Fig. 2. Glycogen granules have a circular demarcation by a fine double-layered membrane (an arrow). A large round aggregation of glycogen granules has been formed. A round dense aggregation of chromatin granules, resembling nuclear bodies, is located at the lower left corner. $\times 30,000$.
 Fig. 3. Glycogen granules are arranged to form a braid. They are edged by fine membranes. $\times 30,000$.

PLATE II

- Fig. 4. A half-round sharp concave on the margin of a nucleus. $\times 20,000$.
 Fig. 5. A nuclear membrane projected into the cytoplasm from the edge of a nuclear concave. It makes a whirlpool. $\times 20,000$.
 Fig. 6. A body resembling the nuclear body. It has a round hole at its center. $\times 25,000$.
 Figs. 7 & 8. Erythrophagia observed in two neutrophils. Phagocytized erythrocytes are degenerated and contain very irregular materials. A number of fine dense particles supposed to be hemosiderin and ferritin are seen among them. Dense materials with a myelin-like structure (arrows) are present in Fig. 8. Fig. 7: $\times 15,000$. Fig. 8: $\times 30,000$.
 Figs. 9 & 10. Dense bodies in the cytoplasm. They are very variable in size, electron density, and fine structure, and may be fragments of degenerated erythrocytes. $\times 20,000$.