CYTOLOGICAL STUDIES ON LYMPH NODE IN EQUINE INFECTIOUS ANEMIA III.

SO-CALLED LYMPHOID CELL

Kan-ichi Ohshima

Department of Veterinary Pathology,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan

(Received for publication, April 26, 1958)

Preface

In 1934, Dobberstein published his critical views on the pathogenesis of equine infectious anemia (EIA). In that publication he pointed out that the virus of EIA attacked both undifferentiated and differentiated mesenchyme causing histiocytic reaction, lymphoid-cellular reaction and fibrillar reaction in order. Subsequent to this report, many other investigations supporting his opinion were reported by Dobberstein and his co-workers. Among these reports, Piering, in 1935, conducted a minute pathological survey on lymph node and pointed out the occurrence of extensive histiocyte formation in the early stage of EIA and the appearance of lymphoid cells with the chronic course and of new formation of primitive collagenous fiber. With regard to the pathogenesis of EIA, studies made by Dobberstein and his co-workers have been supported by many other investigators ever since the original publication.

Although, in 1954, Yamagiwa et al. conducted extensive clinico-pathological studies on natural and experimental cases of EIA, and confirmed that the lesions existed mainly on lympho-reticular tissue and reticulo-endothelial system, they also classified EIA into four types as acute, subacute, chronic and relapsed from the patho-morphological point of view. The lesions of acute type showed parenchymatous degeneration and dynamic activity of reticulo-endothelial system; the sub-acute type manifested reactions by large lymphoid cells in the lympho-reticular tissue which were provisionally named as “L-cells” having a large nucleus with poor chromatin, being rich in basophilic protoplasm, and being accompanied by both additional parenchymatous degeneration and dynamic activity of reticulo-endothelial system. These two types of EIA were known as almost certain to lead to death. In the chronic type, the lesions mainly showed reactions by small lymphoid-cells in the lympho-reticular tissue and clinically this type of cases took chronic course. The relapsed type EIA is one in which lesions of acute or sub-
acute type occurred in company with the chronic type lesions. YAMAGIWA et al. explained that the cases of chronic type showed chronic type lesions from the beginning of affection and a principal change was the reaction by small lymphoid-cells. This explanation differed from DOBBERSTEIN's opinion that the lymphoid-cellular reaction occurred with the development of the chronic course of the histiocytic reaction.

The author has participated in pathological studies on lymph node in EIA, as a co-worker with YAMAGIWA, in making every possible effort to clarify the pathogenesis of EIA. Granting that lympho-reticular tissue plays a principal role in the changes of EIA and that emphasis is laid on the lymphoid-cellular reaction, the obscurity of cellular characteristics should naturally be subjected to further study for clarification of the problem. From this point of view, the author published, in 1954, “Cytological studies on lymph node in EIA I. Characters of ‘L-cell’ and its relations to visceral lesions,” and a second report, “Occurrence of the ‘L-cell’ in the peripheral blood and its relations to visceral lesions” made in the next year. This “L-cell” is recognized as a large lymphoid cell. In this study, the author concluded his reports on the characteristics of the small lymphoid cells by describing detailed cytological investigations employing phase contrast microscope. He has confirmed, as a result, that the cells were obviously distinguished from the lymphocyte, contributing to a clarification of the nature of EIA. General descriptions on the lymphoid cells are herein consolidated with supplementary interpretations lately developed concerning the “L-cell.”

MATERIALS AND METHOD

Materials examined were 58 cases of chronic type (Z 127, 128, 156, 161～214, 218) obtained principally from the Sapporo slaughter house in 1957 and two experimental cases, of which one case (Z 222) was of relapsed type and the other (Z 224) of chronic type. In addition, 44 cases, formerly reported as natural cases in the first report (4 subacute, 27 chronic type and 13 non-EIA cases) as well as 6 experimental cases (1 relapsed, 3 chronic type and 2 non-EIA cases) were also subjected to examination.

In classifying the types of EIA use was made of lesions obtained from liver, spleen, lymph nodes and other organs which were fixed with CARNOX's and formalin solutions and stained with hematoxylin-eosin. For the cytological studies, splenic lymph node was mainly used. To the paraffin sections, UNNA-PAPPENHEIM's pyronin-methylgreen staining, digesting examination by ribonuclease, MCMANUS's periodic acid SCHIFF reaction, and MASSON's staining GOLDBERG's modification were applied; MAY-GIEMSA staining and ARMITAGE's peroxidase reaction were applied to the touch preparations of lymph nodes. For the fresh materials, supravital staining with neutral red and Janus green, examination of phagocytic affinity to carbon particles were adopted, and observations were made with the warm phase microscope. The phase microscopic findings obtained were most valuable.
A phase contrast microscope of dark, medium and immersion, made by Olympus Co., was used. In preparing materials, the cell suspension of lymph node was prepared as follows: one drop of 0.2% neutral red in physiological saline solution and two drops of 0.02% Janus green in physiological saline solution were worked up on a clean-wiped slide with small specimen obtained from the cut surface of splenic lymph node chopped fine with a sharp knife. As most of cases examined belonged to chronic type showing slight dilatation of the lymph vessels, the above described method was intended to make easy the selection of the portions for examination. For microscopical investigation, the suspension of supravital stained cells was smeared thin on a coverslip without drying; coverslip was put upon the liquid paraffin filled into the hollow of a hollow ground slide, and sealed with melted solid paraffin on the periphery. This preparation is better than one obtained by holding a material between slide and coverslip in respect to the points that the cells extend suitably for observation and that there is no pressure on the cells.

In conducting examination on the white blood cells in peripheral blood, MAY-GIEMSA staining and peroxidase reaction were employed for the blood smears used as material for phase contrast microscopic observation of the fresh materials. In the phase microscopic observation, an expected result could not be obtained by the direct observation on the blood, as the preparation had a plenty of red blood cells showing a stronger resistance than the white blood cells. After testing preparations by various methods the author has concluded to adopt the following method on a trial basis. Citrated blood kept in a still-standing test tube showed swift deposition of the red blood cells after about ten or fifteen minutes. The supernatant fluid was centrifuged 600~800 rpm. for about 5 minutes. The sediment suitably diluted with physiological saline solution was used for phase microscopic observation as well as supravital staining. Attention must be paid in employing the method described above, because prolonged centrifugation or increased rotation may cause damage or distortion of the cells and weaken the affinity of supravital staining.

RESULTS

1. Cytology of Lymphoid Cells

It is needless to say that the study was carried out for the purpose of cytological examination on the small lymphoid cells. However, for the sake of a general description regarding "so-called lymphoid cells," it may be good also to explain in this paper about the large lymphoid cell (L-cell) reported in the past, although there may seem to be some overlapping.

Large lymphoid cell (L-cell) (Fig. 4): Comparatively large sometimes as large as 30μ in diameter (Figs. 16 & 17). The protoplasm is basophilic and shows a syrupy condition under the phase microscope (Figs. 24, 25 & 38). The protoplasm and nucleoli are pyroninophilic; the nucleus accompanied by a thick nuclear membrane contains two or three nucleoli. After supravital staining, Janus green granules and few neutral red vacuoles are seen scattered about in the protoplasm, and no phagocytosis of carbon particles is manifested. Occasionally vacuole
formation in protoplasm and sometimes mitosis are recognized. The forgoing observations have already been reported in the author's first report. Later he found that the pyroninophilic substance in the protoplasm and nucleoli is digested by ribonuclease and loses its staining property. Polysaccharide by periodic acid SCHIFF reaction is proven absent.

Small lymphoid cells: The small lymphoid cells remarkably increase their number in medullary cord on lymph node in EIA, and the existence of the cells has been reported but not clearly by many investigators of EIA. The author classified the cells for the time being into three sub-types from the view point of the findings based on hematoxylin-eosin-staining preparation. The first one is the most typical and resembles the lymphocyte (Fig. 1), the second resembles the "L-cell" and is small in size (Fig. 2) and the last resembles the plasma cell (Fig. 3).

These cells exist in the medullary cord and frequently quite fill up the tissue in EIA cases, whereas such cells slightly distributed are recognized in normal horses. In some cases of EIA the cells severely increase their number in the medial layer of the follicles, and in other cases the increased lymphoid cells closely pack the lymph sinus. Cytological descriptions of the three types of lymphoid cells are given below especially pertaining to the differences between them and the lymphocyte. These cells in three types, show no periodic acid SCHIFF nor peroxidase reaction but more or less pyroninophilic characteristic digestible by ribonuclease reaction. The pyroninophilic nature will be discussed together with the electron-microscopic findings of lymphoid cells by MATSUWA, a member of the staff of this laboratory. The author, accordingly, will simply refer to this problem in the present paper.

Typical small lymphoid cell In the lymph node of chronic type EIA, a great quantity of the small lymphoid cells is recognized, but not so conspicuous for the number owing to its characters. Generally speaking, preparations formalin-fixed are not suitable for cytological observation, because not only the nucleus, but also protoplasm is indistinctly stained by this method as compared with CARNOY's fixation. The protoplasm of the lymphocyte hardly appears in the hematoxylin-eosin-staining preparations with formalin fixation. The nucleus stains in blue tone colour and its nuclear nets show rather fine. On the other hand, with regard to the typical small lymphoid cell, the protoplasm is poor in area and hardly distinguished from the lymphocyte, and the contour of the nucleus resembles that of the lymphocyte. However, detailed observation enables one to discover differences from the lymphocyte in that the nucleus of the lymphoid cell stains more purplish tone and its chromatin network presents more coarse and lumpy appearance than is the case in the lymphocyte. In the same tissue fixed
with Carnoy's solution, the differences between the two are more distinct, viz., the protoplasm in typical small lymphoid cell is recognizable and the fine chromatin network of the lymphocyte is more obvious.

The pyroninophilia of the protoplasm resulting from Unna-Pappenheim's staining is slightly positive. It is seen to contain a nucleus deeply stained in methylgreen in the lymphoid cell; on the contrary, the lymphocyte shows a negative result of the pyroninophilia.

The lymphocyte in touch preparations with May-Grünwald staining contains a protoplasm either very poor in area or slightly stained, even if it is at all distinct. On the contrary, the typical small lymphoid cell (Figs. 5-7 & 18) has much more distinct and deeply stained protoplasm. The nucleus of the lymphocyte shows purplish tone in colour and an indistinct water-colour-like appearance. The majority of these lymphoid cells have a nucleus showing in blue colour with coarse chromatin and the nucleus sometimes contains distinct nucleoli and often shows pyknosis and mitosis (Figs. 14 & 15).

**L-cell-like small lymphoid cell**  The size of the cell is similar to that of the lymphocyte or barely larger, and the contour resembles that of the "L-cell" (Fig. 2); the protoplasm is basophilic and rather large in area holding the nucleus in its central portion. Nuclear membrane is thick, chromatin network is coarse, and one or two nucleoli are sometimes recognized in the nucleus. These characters are more distinctly observed in Carnoy's fixation than in formalin fixation, as in the former typical small lymphoid cell. The protoplasm, accordingly, becomes more reddish in colour.

The pyroninophilia by Unna-Pappenheim's staining is slightly greater as compared to the typical small lymphoid cell and granular red substances are clearly observed in the protoplasm when the cell is in the midst of mitosis.

By May-Grünwald staining (Figs. 8 & 9), the protoplasm becomes strongly stained basophilic, sometimes has perinuclear bright area and in some cases shows many spongy degenerative figures. The nucleus is observed, accompanied with purplish blue or purplish reddish coarse and scattered chromatins. Some of these cells have distinct nucleoli and are generally larger than the lymphocyte in size. The cells are occasionally recognized in the preparation with pyknotic nucleus and are seen to be smaller than the lymphocyte.

At any rate, such cells are not so many in quantity, but they show a conspicuous character from their contour. It is interpreted that the appearance of L-cell-like small lymphoid cell is an aspect of lymphoid cells with nucleoli in an early stage and that hyperchromasia of the nuclear wall, karyopyknosis or spongy changes in a degenerative stage mixedly exist.

**Plasma-cell-like small lymphoid cell**  Plasma cells have been under discussion...
in the cytological investigations in EIA; certainly the plasma-cell-like small lymphoid cell seems to be entitled to be regarded as a sort of plasma cell. With regard to this problem, the author intends to discuss the matter in more detail in the latter part of this paper and now would merely like to point out that it is reasonable to consider the lymphoid cell as being different from the plasma cell.

In the preparation stained with hematoxylin-eosin fixed with formalin solution, the cells are recognized in the medullary cord forming foci or distributed cells and being small in number. The protoplasm is very large in area, its basophilic character is rather severe and the appearance is occasionally indistinct. The nucleus holds a peripheral position showing robust nuclear membrane with adherent coarse chromatin blocks, but never shows typical Marschalko-type nucleus. With Carnoy's fixation, the protoplasm is stained reddish tone in colour and becomes confused with the plasma cell (Fig. 3). The protoplasm has pyroninophilic character as strong as that of the "L-cell," and it often has granular structure.

By May-Giemsa staining (Figs. 10~13), the cell shows that it has basophilic protoplasm in deep colour which is large in area, many of the nuclei are located in a peripheral portion with bright juxtanuclear area, and occasionally show spongy protoplasm. The nucleus shows almost pyknotic, with robust chromatin blue tone in colour. However, the cell, occasionally, shows some resemblance to the plasma cell, and it is not very difficult to distinguish the lymphoid cell from the plasma cell. Under detailed observation, the author recognizes, in some cases, the plasma-cell-like small lymphoid cell which shows similar appearance to the L-cell-like small lymphoid cell that is located in a central nuclear position with distinct nucleoli and basophilic protoplasm slightly stained. In contrast with this matter, the author hardly recognizes cases of increased Marschalko-type plasma cell.

The above mentioned findings outline the general morphology of the small lymphoid cells. The following are the results of survey conducted on these cells under the phase microscope applying supravital staining.

**Findings under the phase microscope** It is recognized that the cells from lymph node in EIA consist of two major sorts under the phase microscope applying supravital staining. It is needless to say that one is the lymphocyte and the other is the lymphoid cell. Besides, an insignificant number of reticular cells (Figs. 21~23 & 37), plasma cells (Fig. 20), white blood cells (Figs. 19, 39 & 45) etc. are recognized. In this paper, however, it is proposed to describe the difference between the lymphocyte and lymphoid cells, omitting any description of the other cells or of the "L-cell" already reported in the first report.

Usually, the small lymphoid cells are slightly larger than the lymphocyte; they measure at 7~9 μ in diameter. In some cases, the lymphoid cells are smaller
than the lymphocyte, but almost all of them are considerably larger than the lymphocyte. Regarding this variation, the author would like to lay emphasis on the individuality of each case of EIA rather than on the variations in one case. This fact also applies to the cytological appearances of the lymphoid cell, and presents an important problem of the histogenesis of the lymphoid cells. The small lymphoid cell (Figs. 19, 24, 26~33 & 39~42) has slightly more extensive and rather deficiently viscous protoplasm compared with that of lymphocyte showing serrate margin. The nucleus is disk-like and flat in appearance; in some cases it has a sharp notch. The nucleus has robust membrane with a distinct boundary as if drawn artificially. The chromatin network takes the form of coarse blocks, the structure is sparse and fairly bright in appearance. Some of these nuclei have one, two or three distinct nucleoli (Figs. 26 & 27). On the other hand, the nucleus of the lymphocyte is spherical and solid, sometimes has a dented portion. No lymphocyte, however, is recognized as accompanied with a sharp notch like the one in the lymphoid cell. The nuclear membrane is described with thin line and often present fine unevenness. The chromatin is fine and homogeneous. The nucleoli are usually indistinct but occasionally seen in the center of the nucleus as dark spots (Figs. 20~22, 24~26, 28~31, 34~36, 43 & 44).

When the lymphoid cells are stained supravitally with neutral red and Janus green, they appear at a glance not different from the lymphocyte. The difference between them, however, can be recognized by detailed observation as follows. In the lymphoid cells, several Janus green granules are scattered about in their protoplasm, with a tendency to gather on one side of nucleus, while they are rather scattered on the perinuclear area of the lymphocyte. The size of the granules of the lymphoid cells is rather slightly larger than that of lymphocyte. These findings, to the end, show a mere tendency and it is a fact that there are a considerable number of cases which show opposite findings. The fine neutral red granules coloured deep purplish reddish are recognized, one or two to several, in the protoplasm of the small lymphoid cell. Contrary to that several granules are occasionally observed in the protoplasm of the lymphocyte. There seems to be no phagocytosis in small lymphoid cells in the event carbon particles are given at the same time with supravital staining. The author, however, recognized the presence of one or two carbon particles when they were supplied independently. This fact should be stressed as a matter different from the case of the lymphocyte. No distinct mobility is recognized in both sorts of cells.
2. The Occurrence of the Lymphoid Cells in the Peripheral Blood

Large lymphoid cell (L-cell): On the basis of the viewpoint of the relationship with the visceral lesion, the author assumed that "so-called lymphoid cells" greatly increased on the lympho-reticular tissue should have been carried away to the other visceral organs by the blood circulation. So he examined the occurrence of the "L-cell" in the jugular vein (Fig. 38). The results obtained from this examination as already published in the second report of the writer are re-stated as follows.

The examination was carried out on 58 slaughtered EIA cases and 21 non-EIA cases. The occurrence of the "L-cell" in the peripheral blood was quite in parallel with that in visceral lesions; in subacute case as many as 3.8% of "L-cells" were observed among white blood cells. The percentage of the occurrence of the "L-cell" decreased in the order acute, chronic (still active), relapsed and chronic type cases. In non-EIA cases the "L-cells" were recognized at 0.4% among white blood cells (Table 1).

<table>
<thead>
<tr>
<th>TYPE</th>
<th>NUMBER OF EXAMINED CASES</th>
<th>RATE OF APPEARANCE OF L-CELL AMONG WHITE BLOOD CELL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subacute</td>
<td>1</td>
<td>3.8</td>
</tr>
<tr>
<td>Acute</td>
<td>6</td>
<td>1.8 (0.6~2.6)</td>
</tr>
<tr>
<td>EIA Chronic (still active)</td>
<td>9</td>
<td>1.4 (0.4~2.6)</td>
</tr>
<tr>
<td>Relapsed</td>
<td>1</td>
<td>0.8</td>
</tr>
<tr>
<td>Chronic</td>
<td>41</td>
<td>0.6 (0~1.8)</td>
</tr>
<tr>
<td>Non-EIA</td>
<td>21</td>
<td>0.4 (0~1.6)</td>
</tr>
</tbody>
</table>

In four cases of chronic type EIA, the "L-cell" in the peripheral blood was examined for between 30 or 74 days; the number was counted to be more than one hundred the highest being 364 in 1 mm³. On the other hand, the observation was carried out for one month in one each case of 2 non-EIA. The "L-cell" was rarely found with the highest count at 76 in 1 mm³.

Small lymphoid cell: With regard to the small lymphoid cell, the same result was obtained. It should not be denied that the appearance of the lymphoid cells dominate the lymphocytosis which has been taken up in the clinical hematology of EIA. The author, in this report, will limit the discussion to the presentation of the fact only that the small lymphoid cells are recognized in considerable numbers in the jugular vein of EIA cases. He wishes to go on further to clarify the pathogenesis of EIA leaving the quantitative examination of lymphoid cells in the peripheral blood to the clinical hematologist.

DISCUSSION

The author, presenting the results of examination, has described the lymphoid
cells of lymph node in EIA cases. A discussion in reference to the literature and the writer's findings will be presented, with some explanation of the writer's opinions.

1. Histogenesis of Lymphoid Cells

Small lymphoid cells: These cells are classified into three types in the hematoxylin-eosin preparation. The author, however, would place them under one category for the reasons that every sample of protoplasm is pyroninophilic, also that the distinction of the three types occasionally becomes difficult under the May-Giemsa staining and almost unable to differentiate under the phase microscope. If the view that various kinds of management in staining do not always manifest the true figures of each cell is granted, that should also be added to one of the above reasons. Furthermore, the lymphoid cells possess slight phagocytosis to carbon particles and show thick nuclear membrane, disk-like flat and bright nucleus; they are comparatively rich in protoplasm and have reticular-cell-like appearance. That is, the small lymphoid cells are clearly distinguishable from the lymphocyte. Moreover, these cells are mainly recognized in the medullary cord of the lymph node. From above mentioned facts, it is not difficult to consider that the small lymphoid cell may originate in the lympho-reticular tissue.

Naturally the differentiation among the stages of cell development becomes a matter to be discussed. Concretely speaking, whether the small lymphoid cells explained before show only some stages of cell development or only some degenerative changes of the lymphocyte is the matter of question. The author, in this study, carefully examined the small lymphoid cells not only qualitatively, but also quantitatively with comparisons with the tissue preparations. As a result it becomes evident that the small lymphoid cells are never recognizable as a changed figure of the lymphocytes under the phase microscope in this study. No essential morphological difference between each small lymphoid cells of the three types can be observed in spite of the polymorphous appearances with spongy protoplasm, and with or without nucleoli, etc. The morphology of the process of development of the lymphocyte was reported by Tanaka as observed under the phase microscope. It is his opinion that it is difficult to recognized a difference among the figures of the development process of the lymphocyte. Based on this fact, the author's small lymphoid cells observed under the phase microscope are undoubtedly the same thing as the small lymphoid cells in the tissue preparation.

There are some questions with regard to the discrepancies between the small lymphoid cells and the plasma cell. The cell, called plasma-cell-like small lymphoid cell, is resemblant to the plasma cell in respect to its appearance on the tissue.
preparation and its extensive pyroninophilia. In recent years, Ichikawa et al. conducted cytological observations on the liver biopsy materials in EIA, and classified the appeared cells as “RNA-rich cell” and “RNA-poor cell.” The former consisted of “large nuclear cell (lymphoid cell)” and “small nuclear cell (plasma cell);” they assumed that the “large nuclear cell” coincided with the plasma-cellar-recticular cell named by Rohr.

It, however, should be indicated that the following fact exists without necessity of referring to any complicated cytological theories. That is, neither the Marschalko-type plasma cell nor the Russell body cell considered to be in the degenerative stage of the plasma cells are observed under the phase microscope with supravital staining. The lymphoid cells can be observed in the peripheral blood, but not the Marschalko-type plasma cell. Such facts are also proved clearly by many investigators of clinical hematology in EIA, but no plasma cell is detected in the peripheral blood except in the Türk’s irritated form reported by Wirth.

It is necessary to reconsider the pathogenesis of EIA recalling the above mentioned findings. Dobberstein assumed that the EIA lesions represent a systematic change, and that the monocytosis and the relative lymphocytosis of the circulatory blood might have the same character as the visceral changes. This opinion is still right nowadays. He regarded that the lymphoid cell was differentiated from the stimulated mesenchyme of the blood vessels by the EIA virus. The present author, however, regards it as having appeared mainly as a result of the lympho-recticular tissue hyperplasia, but would not deny Dobberstein’s mesenchyme transformation theory completely. The present author’s such opinions were discussed in the first report pertaining to the “L-cell”; the same opinion also applies to the small lymphoid cells which are demonstrated in this observation under the phase microscope.

From the viewpoint of the pathogenesis, in regard to the conception described by Ichikawa et al., the “so-called lymphoid cells” that the present author explained, correspond to the “large and small nuclear RNA-rich cell” of Ichikawa et al. It may cause confusion to follow the nomenclature in Rohr’s classification based on results obtained from only Giemsa stained preparation. Detailed cytological observation has recently been reported to afford ground for denying the theory that the recticular cell transforms into the plasma cell as explained by Rohr and Fagraeus. In addition to this, it should not be granted to say that the “small nuclear RNA-rich cell” is a plasma cell. Then, the present author wants to stress that the lymphoid cell is not the same as the plasma cell, but “so-called lymphoid cell” itself increased characteristically in EIA lesion.

Large lymphoid cell (L-cell): The writer wishes to discuss the relationship
between the small lymphoid cells and the "L-cell" reported in the first paper. He assumed that the "L-cell" was the same as the lymphogonia described by AMANO et al.\textsuperscript{1,2} from its morphological figure, staining appearance and findings under the phase microscope, and that it was differentiated from the reticular cell. Nowadays, it must be pointed out that the "L-cell" resembles the small lymphoid cell extremely from the morphological appearances.

As mentioned above, the author gave a cytological definition making it clear that the cells appeared in the lymph node of EIA. The lymph nodes in EIA were therefore reexamined in view of the new facts obtained at that time, and it was concluded that the enlargement of lymph nodes in EIA which had been pointed out by many investigators\textsuperscript{11,12,23,26,12,33}, was traceable not only to the increasing of the lymphoid cells, but also to the increase of the lymphocytes. Recently, AMANO et al.\textsuperscript{1,2} and a co-worker\textsuperscript{27} of his again made public the lymphogonia theory saying that it differentiates to the lymphocyte through the stage of the lymphoblast. However, there is no fundamental fact to decide whether or not the "L-cell" described by the author can be the mature lymphocyte. It is commonly assumed that the "L-cell" plays a role in the increase in lymphocytes. Furthermore, it is assumed that the "L-cell" can be discussed as well in the same category as the small lymphoid cells, because the "L-cell" shows frequent cell division, immature characters and occasionally degenerative processes. Also, if TANAKA'S\textsuperscript{28} opinion that the lymphocyte differentiates almost at the lymph follicles is correct, it is naturally considered that the "L-cell" which appears in the medullary cord of lymph node in EIA appears with some influences to repeat the cell division and increase rather than to differentiate into the lymphocyte. In respect to such considerations, it is anticipated that clues will be found by electron microscopic observation now being conducted in this laboratory.

2. Pathogenesis of EIA from the Point of View of the Cytology of the Lymph Node

As for the opinion regarding the pathogenesis of EIA, aspects of the tissue and cell reaction to the EIA virus were recognized by YAMAGIWA et al., as showing some qualitative appearances from the beginning of the affection. The EIA lesion was classified into acute, subacute, chronic and relapsed types responding to the character of the reactions. It was already explained that YAMAGIWA and collaborator have disagreed with DOBBERSTIN'S opinion that the EIA lesion originated in histiocytic reaction and changed into fibrillar reaction through lymphoid-cellular reaction.

As previously stated in this paper, the author's conclusions based on cytological studies on lymph node support the views on EIA expressed by YAMAGIWA et al.
That is, with respect to the character of appearance of small lymphoid cells in lymph node of EIA, the morphological differentiation of lymphoid cell was recognized as occurring case by case rather than the variation in one case, and the manner of appearance of "L-cell" in blood and other visceral lesions has close mutual relation. The above described facts have supplied a fundamental basis upon which the author may comment that the affection of the virus under various conditions stimulates the lympho-reticular tissue causing metaplastic increase of the "L-cell" or some sort of small lymphoid cells depending on the conditions. And this is nothing but the true appearance of EIA itself.

With regard to the knowledges obtained on the "L-cell" and the small lymphoid cells in peripheral blood, it should be understood that the greater part of the monocytosis or lymphocytosis in peripheral blood, pointed out by many investigators of EIA in the past, should be regarded as "lymphoid-cytosis." While it is needless to say that there have been a few investigators who observed the changes of blood figure with relation to the visceral lesions, it is presumed that they often seemed to be slaves to the appeared changes of blood figure. One should be reminded that "lymphoid-cytosis" is the appearance which indicates the EIA lesion.

From such reasons, it was correct for DOBBERSTEIN to assume that the EIA resembled the lymphadenosis so far as appearances of lesions is concerned. The author reported a bovine case considered as lymphogonioma, in which he recognized that the tumor cell was extremely like to the "L-cell" and, histologically, showing figures differentiating it from the reticular cells. However, by reference to the literature, the author wishes to seek for analogy between EIA and the infectious mononucleosis observed by WERNER (1954). In the study of WERNER, he recognized nest-like interstitial and perivascular increases of lymphocyte and lymphoid cell in the general visceral tissues including the circulation blood, following the preliminary occurrence of hyperplasia of the lympho-reticular tissue. At the same time, many observations have reported that a cell group with basophilic character was recognized in peripheral blood of patient in this disease.

**Summary and Conclusion**

Cytological studies on the lymphoid cells in lymph node of EIA were carried out based upon patho-morphology employing phase microscope. In these studies, the "so-called lymphoid cells" were examined cytologically, dividing them into large and small lymphoid cells, and further classifying the small lymphoid cells provisionally into three sub-types as typical, L-cell-like and plasma-cell-like lymphoid cell from the appearances of the hematoxylin-eosin-stained tissue.
Cytological Studies on Lymph Node in Equine Infectious Anemia III

As to the results, no essential differences among those small lymphoid cells were recognized under the phase microscope, and it was realized that the lymphoid cells should be distinguished from the lymphocyte. It was impossible to consider that the plasma-cell-like small lymphoid cell resembling the plasma cell on the tissue preparation stained with hematoxylin-eosin was some form of the plasma cell, and that the plasma-cell-like lymphoid cell was a "lymphoid cell."

In regard to the "L-cell" described in the first report, it was found that the "L-cell" was akin to the small lymphoid cell, and that it played some role in the increase of lymphocytes from the fact that the enlargement of lymph node in EIA is due not only the hyperplasia of the lymphoid cells but of the lymphocytes.

The appearance of the "L-cell" and the small lymphoid cell in peripheral blood under phase microscopic observation indicates that these sorts of cells play a great part in monocytes and lymphocytes as pointed out by hematological investigators in the past. The fact mentioned above will arouse some interesting problems in comparative pathology.

In conclusion, the author clarified the cytological characteristics of obscurely known "so-called lymphoid cells," and found some characteristics of occurrence of these kinds of cells in visceral organs and blood. This contributes to clarifying the essential natures of EIA.

In conclusion, the author wishes to express his gratitude to Prof. YAMAGIWA for his kind direction and review of this study.

REFERENCES

12) JAPANESE COMMITTEE (1914): Report on the Results Obtained by the Special
Committee for the Investigation of Equine Infectious Anemia. The Horse Administration Bureau, Tokyo, Japan (in Japanese).


**EXPLANATION OF PLATES**

**PLATE I**

Lymphoid cells increased in the medullary cord of lymph node, hematoxylin-eosin staining. × 520.

Fig. 1 Hyperplasia of typical small lymphoid cell similar to lymphocyte accompanied with poor protoplasm (Z 201).

Fig. 2 Hyperplasia of L-cell-like small lymphoid cell showing coarse and brightly apparent nuclear chromatin network (Z 203).

Fig. 3 Hyperplasia of plasma-cell-like small lymphoid cell showing considerably distinct protoplasm which is rich in area. No relationship to the blood capillary observed (Z 206).

Fig. 4 Hyperplasia of large lymphoid cell [L-cell] in high degree. Scattered mitosis (E 988).

**PLATE II**

Touch preparation obtained from the cut surface of lymph node, MAY-GIEMSA staining. × 2,000.
Cytological Studies on Lymph Node in Equine Infectious Anemia III

Fig. 5 Typical small lymphoid cell (Z 166).

Fig. 6 Typical small lymphoid cell with nucleus containing a sharp notch from the bottom to the center (Z 203).

Fig. 7 Small lymphoid cell showing karyopyknosis; upper cell is a lymphocyte (Z 203).

Fig. 8 L-cell-like small lymphoid cell in which three nucleoli are distinct; two lymphocytes located on upper left (Z 201).

Fig. 9 L-cell-like small lymphoid cell with slightly broadened protoplasm (Z 197).

Fig. 10 Plasma-cell-like small lymphoid cell of which nucleolus is distinct (Z 206).

Fig. 11 Plasma-cell-like small lymphoid cell (Z 206).

Fig. 12 Plasma-cell-like small lymphoid cell with juxtanuclear bright area; attention to be given to the chromatin network different from the plasma cell (Z 201).

Fig. 13 Degenerative plasma-cell-like small lymphoid cell; protoplasm carries fully unstained vacuoles showing spongy appearance and karyopyknosis; the lower left shows a lymphocyte (Z 197).

Fig. 14 Mitosis of a small lymphoid cell (Z 206).

Fig. 15 Amitosis of a lymphoid cell (Z 206).

Fig. 16 The upper is a reticular cell, and the lower is a large lymphoid cell (L-cell) with a distinct nucleolus (Z 206).

Fig. 17 Particularly enlarged large lymphoid cell (L-cell) showing two clear nucleoli (Z 201).

Fig. 18 Cellular foci consisting of small lymphoid cells (Z 191).

PLATE III

Cell suspension from lymph node, supravit staining under the phase microscope, × 2,000.

Fig. 19 The upper is a small lymphoid cell, and the lower is a monocyte; the nucleus exists in the upper portion; the rosette of neutral red in the concave portion of the nucleus shows reddish orange colour (Z 164).

Fig. 20 Plasma cell; protoplasm extensive in area and comparatively small nucleus showing axial structure; distinctly recognizable stained by neutral red. The upper two are lymphocytes (Z 161).

Fig. 21 Reticular cell; lobular nucleus and plump protoplasm filled with severely moving fine granules; others are lymphocytes (Z 164).

Fig. 22 Reticular cell; protoplasm is plump and severe movement is seen of fine granules in the protoplasm; others are lymphocytes (Z 164).

Fig. 23 Two reticular cells. The upper cell contains two large liquid vacuoles stained by neutral red; one unstained vacuole and many corporeal substances in the protoplasm. The lower contains substances stained by neutral red in the protoplasm; slight phagocytic ability; it appears morphologically to transform into the large lymphoid cell (Z 164).

Fig. 24 The lower is a large lymphoid cell (L-cell) with thick nuclear membrane and distinct nucleoli. Upper is a small lymphoid cell resembling the L-cell in its appearance (Z 164).

Fig. 25 Large lymphoid cell (L-cell) with the characteristic syrupy protoplasm.
The upper left is a lymphocyte (Z 164).

Fig. 26 Two small lymphoid cells; the lower cell has a nucleolus; the lower right is a lymphocyte (Z 161).

Fig. 27 Small lymphoid cell with two nucleoli (Z 161).

Plate IV

Cell suspension from lymph node, supravital staining under the phase microscope, x 2,000.

Fig. 28 Small lymphoid cells (upper two) and lymphocytes (lower two). The former has flat disk-like nucleus showing robust chromatin and brightness; the protoplasm is rather deficiently viscous, and has two to several distinct granules stained by neutral red and several by Janus green (Z 164).

Fig. 29 Small lymphoid cell located in the center; the lower one is a lymphocyte (Z 164).

Fig. 30 Small lymphoid cell at the bottom of the figure; the upper two cells are lymphocytes; the difference of the structure of nucleus is distinctly recognized at a glance (Z 164).

Fig. 31 Small lymphoid cell at the bottom and a lymphocyte at the top (Z 164).

Fig. 32 Small lymphoid cells; gathered distinct mitochondria in the under left cell (Z 164).

Fig. 33 Plasma-cell-like small lymphoid cells; nuclear structure is robust and resembles that of the plasma cell; No gathered portions of neutral red granules are seen (Z 164).

Fig. 34 Large lymphocyte (Z 161).

Fig. 35 Lymphocyte; a mitochondria recognized at the left side of the cell (Z 164).

Fig. 36 Lymphocyte; several mitochondria observable in the perinuclear portion (Z 164).

Plate V

White blood cells in peripheral blood, supravital staining under the phase microscope, × 2,000.

Fig. 37 Large reticular cell rarely seen in the peripheral blood (Z 222).

Fig. 38 Large lymphoid cell (L-cell) (Z 222).

Fig. 39 Small lymphoid cell; several granules stained by Janus green and one or two granules by neutral red. The upper is a neutrophile leukocyte (Z 222).

Fig. 40 Small lymphoid cell; distinct nucleoli (Z 224).

Fig. 41 Small lymphoid cell (Z 222).

Fig. 42 Small lymphoid cell (Z 224).

Fig. 43 A lymphocyte at the center; upper left is neutrophile leukocyte and the others are red blood cells (Z 222).

Fig. 44 Large lymphocyte; the spherical nucleus be brought to attention (Z 222).

Fig. 45 Neutrophile leukocyte; recognizable indistinct nucleus behind the neutrophilic granules (Z 222).