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# ELECTRON MICROSCOPIC STUDIES ON THE MATURATION PROCESS AND ORIGIN OF PLASMA CELL IN THE LYMPHNODE

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Masao IKUHASHI. *Electron Microscopic Studies on the Maturation Process and Origin of Plasma Cell in the Lymphnode.* Kobe J. Med. Sci. **14**, 155-181, September 1968 ——Experiments, pursuing after the ultrastructure of immature reticulum cells, lymphocytes and plasma cells often occured together in lymphatic tissues after antigenic stimulations, were performed and clarified their relationship and origin among them.

Immature forms of both lymphocytes and plasma cells show morphologic similarities which seems to derive from same origin. And so they both should be called by the common name of "Immunoblast".

However, they vary each to some extent. Immature plasma cells have tendencies to increased numbers of rER and to large mitochondria in accordance with elevated globulin synthesis, and its proliferation is appeared in the medullary pulp and cortical tissue close to the sinus, where antigenic content is very high.

Vigorous proliferation of plasma cells in lymphnodes depends on the cell division of plasmoblast, preplasmocytes and plasma cells.

## INTRODUCTION

A great number of investigations to elucidate the origin of immunological competent cells were attempted by many authors by means of various methods of observation or treatment. By use of improved methods the results obtained became more advanced. Since Rohr reticulum cell's origin of abtibody producing plasma cell was widely accepted by many Europian authors. Among them Fagraeus was able to catch large pyroninophilic blast cells as an origin of antibody forming cells by histological method and tissue culture stained by methyl green pyronin and concluded that plasma cell originated from reticulum cells passing through a chain of development : transitional cell → immature plasma cell → mature plasma cell. Blast cell origin of immunologically competent cells, including plasma cell and lymphocyte series was repeatedly confirmed by several means, such as microscopic autoradiography, fluorescent antibody technique, and elctron microscopy with or without tritiated thymidine radioautography, or electron microscopy on antibody producing cells detected by hemolysis plaques. However, with regard to such large pyroninophilic blast cells it was very difficult to establish their undisputed origin.

Many ingenious experiments were performed by transfer of peritoneal macrophages, or lymphnode, spleen, or bone marrow cells, by lymphocyte depletion by

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thoracic duct fistula or X-irradiation, by inducing graft reaction, or by extirpation of thymus of newborn animals. Results of these research works were distinguished. However, there are a number of disagreements between them, so it is necessary to survey and consider basically the general features of immunologic phenomena.

Electron microscopy seemed to be enough helpful to solve this difficult enigma to some extent, and such investigations by Amano et al., Andre Schwarts, Hummeler et al, and Movat et al., could establish the fairly noted results and presented an interesting general view of immunology. Present investigation was also designed to pursue electron microscopically the origin and development of three component cells of immunologically competent or associated cells, lymphocyte, plasma cell and macrophage lines, especially with regard to their stage of emergence, localization and transformation in the lymphnodes stimulated by primary or secondary administration of antigens.

## MATERIALS AND METHODS

The 1st, 2nd, 3rd and 4th groups, each consisted of 9 albino rabbits were respectively treated with 5 mg per kg of following suspensions into peritoneal cavities for the first injection and 2 weeks thereafter with 0.5 mg of the same suspension into foot pad for the second injection; The suspension for the first group contained 7 gr % egg albumin, for the 2nd group contained 1 mg % died tubercle bacilli (H37Rv), for the 3rd group contained horse serum (Wako-Jun Yaku, containing 7 gr % protein), and for the 4th group 1 gr % Soya lecithin. In the 5th group, ten albino rabbits were sensitized with 0.5 ml bovine serum albumin emulsified with Freund's incomplete Adjuvant into peritoneal cavities once a week for three weeks and 4 weeks thereafter injected with 0.5 ml of the same suspension into foot pad. The popliteal lymph node of each group were investigated with electron microscopy at time intervals of 6, 12, 24, and 48 hours and 3, 7, 10 and 40 days after the last injection.

Small pieces from these lymph nodes were fixed for 90 minutes in a 2 percent solution of $OsO_4$ buffered with veronal-acetate (at a PH 7). Therafter they were transferred for successive dehydration into 60 percent, 70 percent, 80 percent, 99 percent ethanol for each one hour and, twice, 100 percent ethanol for 30 minutes.

They were embeded in metacrylate or Epon and were cut with Leiz ultramicrotome, and were placed on "formvar" coated wire meshes. The sectioned material were observed with a Hitachi HU-11 type electron microscope.

In a case of embeding with Epon, the sectioned materials were stained by Uran and lead.

## RESULTS OF OBSERVATIONS

Electron microscopic observation was made mostly on the cell of lymphocyte-, reticulum cell-and plasma cell series which were noticed in the proliferation nests

of lymph nodes after various antigenic stimulation, especially on the originating cell, immature forms of each cell series and their maturation process. Each cell series was classified mostly by morphological criteria of the ultrastructure. The reticulum cell series (macrophage series) was divided into young free reticulum cell (young macrophage), ingesting free reticulum cell (ingesting macrophage) and fixed reticulum cell. The lymphocyte series was classified into primitive lymphoid blast (lymphogonia), lymphoblast, young lymphocyte (medium or large lymphocyte) and small lymphocyte.

The plasma cell series was also classified correspondingly into primitive lymphoidblast originating from the plasma cell series, plasmoblast, preplasmocyte and mature plasma cell.

*1. Reticulum cell series*

Shortly after the stimulation free reticulum cells (mature macrophages) ingested vigorously the administrated substance, and many ingesting macrophages appeared abundantly in the medullary and cortical sinus but in small amount in the cortical tissue.

They underwent degeneration gradually after about a week or more when plasma cell series in maximal development became to be sheded.

The young free reticulum cells (young macrophages) proliferated at 24 hours to 3 or 4 days after the stimulation in the medullary or cortical sinus, but a small amount in the outer portion of the cortical tissue and around the postcapillary venules.

*a) Fixed reticulum cell*

The size of the cell was about 10$\mu$ in long diameter, and the margin showed irregular with frequent small process.

The nucleus was circuloid, with deep irregular indentations and located eccentrically. The cytoplasm was diffusely distributed and its density was far more coarse than small lymphocytes. No nucleoli were noted in many instances but occasionally 1-2 small nucleoli could be found. Within the cytoplasm, RNP granules (Ribosomes) were rather scarce and diffusely distributed, while many small vesicular smooth surfaced endoplasmic reticula were scattered.

Rough surfaced endoplasmic reticula were usually fine and short, with scattering distribution.

In the centrosphere golgi apparatus was relatively well developed, consisting mainly of small vacuolar system and inconsiderably of the membranous system. The mitochondria were oval or short rod shaped and frequently found on the opposite side of the eccentrically located nucleus, being scattered around the nucleus. Besides, small or medium sized pinocytic vesicles were found within the cytoplasm and lysosome-like corpuscles containing more or less high electron-dense substances were observed near the golgi area.

*b) Ingesting free retuculum (ingesting macrophage)*

The size of the cell measured 10-15$\mu$ or occasionally larger and showed

irregular margin, with frequent occurrence of small processes. The nucleus was usually kidney-shaped, long oval, or irregularly circular. In the nucleoplasm chromatin was fine and diffusely distributed. The density might be somewhat higher around the nuclear membrane. The cytoplasm contained many ingested materials and many small vesicular smooth surfaced endoplasmic reticula appeared aligning between the ectoplasm and the endoplasm and assumed rosary-like arrangement. The development of golgi apparatus was pronounced, representing well developed both vesicular and membranous systems and 2 or 3 wide areas were noted. The development of rough surfaced endoplasmic reticula was varied. Fibrilar formation was occasionally noticed within the cytoplasm.

*c) Young free reticulum cell (Young macrophage)*

The size of the cell was about 10$\mu$, frequently with minute cytoplasmic processes. The cytoplasm was remarkably narrower than that of the ingesting type. The nucleus was located almost in the center. The nucleoplasm was distributed diffusely, being slightly more coarse than the small lymphoblasts. One or two nucleoli were frequently found. Within the cytoplasm many polysomes were distributed diffusely, and a few rough surfaced endoplasmic reticula and smooth surfaced endoplasmic reticula were scattered. Around the nucleus, oval or short rod shaped mitochondria were noticed. The size of mitochondria was frequently smaller than those in the lymphoblasts and plasmoblasts. The development of golgi apparatus was rather poor.

*2. Lymphocyte series*

The immature cell of lymphocyte series proliferated rather in the later stage of about 10 days after the stimulation, especially in the middle or periphery of secondary follicles.

*a) Small lymphocyte*

The size of the cell was mostly 5–8$\mu$ in diameter. Nucleus was usually oval or kidney-shaped, and showed occasionally deep indentations. In the peripheral part adjacent to the nuclear membrane, the nucleoplasm was distributed rather diffusely, while in the central part it was usually distributed diffusely.

Golgi area in the centrosphere was small, and consisted mainly of many small vesicles and few membranous system. The central body was generally distinct.

Mitochondria, circuloid or oval in shape were a few in number and seen near the centrosphere. The cytoplasm was extremely poor and relatively coarse, containing a small number of smooth surfaced endoplamic reticula and a very small number of rough surfaced endoplasmic reticula.

*b) Young lymphocyte (medium or large lymphocyte)*

These cells resembled so closely to young free reticulum cell that the differentiation was rather difficult. The long diameter of the cell was 8–12$\mu$. The nucleoplasm distributed somewhat densely and the nucleus occasionally showed deep indentations.

The developments of golgi area and smooth surfaced endoplasmic reticula were poorer than those of young free reticulum cells.

*c) Lymphoblast*

The size of the cell was 10-15$\mu$ in diameter. The nucleus was circuloid or kidney-shaped, showing occasionally deep indentation and located almost in the center of the cell.

The nucleoplasm distributed more diffusely than that of small and young lymphocytes, with a little tendency to condensation in the periphery, and represented 1-2 medium-sized nucleoli, which showed a complicated and fine structure but were not as developed as those of the primitive lymphoidblast (lymphogonia). Within the cytoplasm a marked increase in polysomes was found with a small number of scattered smooth surfaced endoplasmic reticula. A few fine and short rough surfaced endoplasmic reticula were irregularly distributed near the mitochondria.

Golgi apparatus was present in the centrosphere, and showed well developed vesicles and scanty membranous structures. Mitochondria, circuloid or short rod in shape were approximately 0.5$\mu$ in long diameter, and scattered mostly around the nucleus.

*d) Primitive lymphoid blast (lymphogonia)*

The ultrastructure of the primitive lymphoidblast was similar to the lymphogonia described by Amano and Tanaka.

These cells were still more undifferentiated than lymphoblast, assuming ultrastructures similar to those of primitive plasmoblast and reticuloblast. The size of the cell was about 15$\mu$ in diameter. The nucleus was located nearly in the center of the cell and usually oval in shape. In the nucleoplasm chromatin was fine and distributed diffusely without tendency of aggregation, and contained one or two large nucleoli with complicated fibrilar structure.

Polysomes were distributed densely within the cell and a few large mitochondria with diameter as large as 1$\mu$ scattered aound the nucleus. Golgi apparatus was scarcely seen by the ultramicroscopy despite of repeated investigations.

Rough surfaced endoplasmic reticula were thin and short, and could be absent or observed fewer, if any, than in lymphoblasts.

*3. Plasma cell series*

The plasma cell series proliferated very early 24 hours to 7 days after the stimulation of protein antigens, such as egg albumin and horse serum or serum albumin emulsified with incomplete Freund's adjuvant, mainly in the medullary cord, in the outer part of cortex along the sinuses, or in the cortical tissue surrounding the arterioles and more inconspicuously surrounding or within follicles.

*a) Mature plasma cell*

The size of the cell was mostly about 10$\mu$ in diameter. The nucleus, oval in shape, located eccentrically in the cell and contained almost none of a small

concentrated nucleolus or very rare, if any. The nucleoplasm distributed rather densely, but a little more diffusely than in mature small lymphocytes, showing slightly higher condensation around the nuclear membrane which could be noticed as a clock faced appearance, when sectioned thicker.

Rough surfaced endoplasmic reticula developed markedly and occupied almost the whole area of the cytoplasm, showing lamellar arrangement and occasionally containing amorphous substance. In cells undergoing degeneration, some rough surfaced endoplasmic reticula showed saccular enlargement. Among the rough surfaced endoplasmic reticula several mitochondria with diameter of around 0.6$\mu$ were noticed.

Mitochondria in plasma cell series were generally larger in size than those in any cells of lymphocyte and reticulum cell series, and indicated one of the characteristics of plasma cell series. Golgi apparatus which consisted mainly of many membranous systems and few vesicular systems occupied rather a wide area in the centrosphere of the cell. Concerning Russel bodies, only those derived from rough surfaced endoplasmic reticula with adherance of granules were observed.

*b) Preplasmocyte*

The size was almost similar to the plasma cell. The nucleus was located eccentrically, the nucleoplasm distributed diffusely with rather fine chromatin and in which a small nucleolus was frequently seen. Rough surfaced endoplasmic reticula developed moderately, undergoing some distension, but scarcely developed the saccular dilatation and did not represent regularly lamellar arrangement.

Mitochondria were circuloid or round and relatively large. They are frequently scattered around Golgi area in the centrosphere. The Golgi apparatus consisted mostly of well developed membranous system and few of vesicular system and occupied rather wide area. Russel bodies were occasionally seen.

*c) Plasmoblast*

The plasmoblast was mostly 10–15$\mu$ in long diameter. The nucleus was eccentrically located on one side, assuming frequently oval shape. Mild concavity was also occasionally noticed. Nucleoplasm was fine and distributed more diffusely without tendency to aggregation and contained a larger nucleolus. The rough surfaced endoplasmic reticula were noticed few in number with a slight tendency to lamellar arrangement, or without bending and long connected figures. Mitochondria were oval or circuloid in shape and measured around 0.6$\mu$ or generally much larger. Many mitochodria were distributed around the centrosphere but could be seen on the opposite side of the nucleus.

Golgi area was wide but poor in the development of both membranous and vacuolar system. A marked increase in polysomes was seen in the cytoplasm.

*d) Primitive lymphoidblast of plasma cell series*

These primitive lymphoidblasts represented more primitive structures than

Table 1. Reticulum cell series.

| | ingesting type free Rc | mature Rc | young free Rc | Reticuloblast | Primitive Reticuloblast |
|---|---|---|---|---|---|
| Cell···Size<br>Form | 10–15$\mu$<br>round or oval | Ca 10$\mu$<br>irregular | Ca 10$\mu$<br>round or oval | 8–15$\mu$<br>round or oval | 15$\mu\leqq$<br>round or oval |
| Nucleus<br>1. shape<br>2. location<br>3. nucleoplasm | <br>1. irregular<br>2. eccentric<br>3. diffuse | <br>1. irregular<br>2. eccentric<br>3. diffuse | <br>1. irregular<br>2. eccentric<br>3. diffuse | <br>1. irregular<br>2. in the center<br>3. diffuse, fine | <br>1. round or oval<br>2. in the center<br>3. diffuse, fine |
| Nucleolus | (+) | (+) small occasionally appeared | (+) usually small | (+) prominent, medium sized 1 or 2 | 1 or 2<br>(+) large and complicated |
| Cytoplasmic Processes | (++) | (++) | (+) | (+) | (+) |
| RER | (++) | (+) fine and short | (+) fine and short | (+) fine and short | (+) poorly developed sparse |
| SER | (++) | (+)-(+++) | (+) | (+) Rb Lb | (+) sparse |
| Mitochondria | short-rod shaped, in centrosphere | short-rod shaped, in centrosphere | circuloid, relative small, in centrosph, | circuloid, large, arround nucleus | circuloid, large, arroun nucleus |
| Golgi-apparatus | (+++) 2 or 3 groups, vacuolar and<br>(++) membranous systems | (+++) 2 or 3 groups, vacuolar and<br>(++) membranous systems | (++) well developed | (+) poorly developed | (±) rarely developed |
| Ribosomes | (+) | (+) | (+) | (++) polysomes | (+++) polysomes |
| Phagosomes | (+++) | (++) | (±) | (−) | (−) |
| Lysosomes | (+) | (±)-(++) | (±) | (−) | (−) |

Table 2. Lymphocytic series.

| | small Lymphocyte | young Lymph | Lymphoblast | prim, Lymphoidblast (Lymphogonia) |
|---|---|---|---|---|
| Cell···Size<br>From | 5–8$\mu$<br>round or oval | 8–12$\mu$<br>round or oval | 10–15$\mu$<br>round or oval | 15$\mu$≦<br>round or oval |
| Nucleus<br>1. shape<br>2. location<br>3. nucleoplasm | <br>1. kidney form<br>2. in the center (almost)<br>3. condensation | <br>1. kidney-like or oval<br>2. diffuse,<br>3. tendency to condensation | <br>1. oval<br>2. in the center<br>3. fine and diffuse | <br>1. round or oval<br>2. in the center<br>3. fine and diffuse |
| Nucleolus | (−) | (±) small | (+) 1–2, medium siged | (++) large, complicated |
| Cytoplasmic processes | (−) | (±) | (−) | (+) |
| RER | most sparse (±)<br>poorly developed | sparse (+)<br>poorly developed | sparse (+)<br>poorly developed | mostly sparse (+)<br>poorly developed |
| SER | (±) most sparse | (±) most sparse | (+) sparse | (±) most sparse |
| Mitochondria | round or oval | round or oval | round, large | circuloid, large |
| Golgi-apparatus | (+) poorly developed small<br>central-body appeared | (+) poorly developed small<br>central-body appeared | rarely developed (±) | rarely developed (±) |
| Ribosome | (±) | (+) moderate number | (+) polysomes numerous | (++) polysomes most numerous |
| Phagosome | (−) | (−) | (−) | (−) |
| Lysosome | (−) | (−) | (−) | (−) |

Table 3. Plasma cell series.

| | mature Plasmocyte | Preplasmocyte | Plasmoblast | Prim, Lymphoidblast |
|---|---|---|---|---|
| Cell···Size<br>Form | Ca 10$\mu$<br>oval | 10–15$\mu$<br>oval | 10–15$\mu$<br>oval | 15$\mu\leqq$<br>round or oval |
| Nucleus<br>1. shape<br>2. location<br>3. nucleoplasm | <br>1. oval<br>2. eccentric<br>3. condensation arround the periphery | <br>1. oval<br>2. eccentric<br>3. tendency to condensation in the periphery | <br>1. oval<br>2. eccentric<br>3. diffuse, fine | <br>1. round or oval<br>2. in the center<br>3. diffuse, fine |
| Nucleolus | (±) small<br>rarely appeared | (+) small<br>constantly appeared | (++) 1, large<br>fairly prominent | (+++) 1–2, large,<br>complicatedly developed |
| Cytoplasmic Processes | (−) | (−) | (−) | (+) |
| RER | (+++) distinct lamellar arrengement<br>(±) Russel body | (++) lamellar arrengement | (+) undistinct lamellar arrengement | (+) sparse and poorly developed |
| SER | (−) | (−) | (−) | (±) |
| Mitochondria | circuioid, large,<br>in centrosphere | circuloid, large,<br>in centrosphere | circuloid, large,<br>Rb<Lb<Pb | circuloid, large<br>arround nucleus |
| Golgi area | (+++) wide area<br>membranous system | (+++) wide area<br>membranous system | (+) wide area<br>poorly developed | (±) wide area<br>poorly developed |
| Ribosomes | (+) | (+) | (++) polysomes | (+++) polysomes |
| Phagosomes | (−) | (−) | (−) | (−) |
| Lysosomes | (+) | (±) | (−) | (−) |

the plasmoblast and showed almost similar structures to the primitive lymphoidblast of the lymphocyte series in spite of the difference of the location, the time of appearance and the origin of the cell between them.

The size of the cell was about 15$\mu$ in diameter or larger. They appeared frequently to be surrounded by plasma cells or plasmoblasts. The nucleus was usually oval, located slightly eccentrically in the cell and contained conspicuously large nucleolus.

The nucleoplasm was distributed diffusely with fine chronatin. Mitochondria were large, circular or short rod shaped, and scattered around the nucleus. The rough surfaced endoplasmic reticula developed very scantily and they were usually very thin and slender.

The development of Golgi apparatus was scarcely observed, while the cytoplasm was distributed densely with polysomes. Mitotic figures of the cell were observed very rarely.

These cells were often found among proliferated cells of plasma cell series, adjacent to plasmoblats and preplasmocytes.

## DISCUSSION

The fact that the proliferation of plasma cells occurred at the adventitia of the small blood vessel was observed by Amano, Hayami, and Kikuchi by means of the light microscopic investigation on the subcutaneous connective tissue, and by Sundberg and Messerschmitt (1954) in the investigation of expansive peritoneal preparation in rats. Moreover, Amano developed the theory of adventitia cell origin of plasma cell on the base of the observation mentioned-above, and Tanaka succeeded it by the electron microscopic evidence.

In our laboratory it was possible to observe many plasma cells at the surroundings of the blood vessel, but impossible to catch the dramatic scene that adventita cells transformed into plasma cells in the subcutaneous tissue of rabbits by means of electron microscopy. Nakagawa, a member of our laboratory, found adventitial pericytes which carried lysosme-like dense body. At the same time proliferation of the plasma cell in the localization of the pericyte at the surrounding of tuberculous focus was produced experimentally. From these evidences Yamori postulated that adventitia pericytes should have not only the properties producing basal-membrane, but also phagocytosis activity or maturating ability toward antibody-producing cells. He also observed that in the inflammatory foci produced experimentally it was impossible to find a predominant proliferation of plasma cells in the localization where pericytes were encompassed constantly by the basal membrane around the vessls were found.

From these results he concluded that the pericyte was not regarded as a source of vigorous multiplication of the plasma cell. The same result was obtained by present studies in the lymph nodes.

It was also confirmed that among proliferated plasma cell line in the lymph node mitotic division of plasmoblasts could be observed most abundantly but those of adventitia pericytes were never observed. Lamellar arrangement of rough

surfaced endoplasmic reticula which was interpreted generally to be a morphological representation of active synthesis and accumulation of immunoglobulin was noted by many authors and considered as a characteristics of the plasma cell. By the present investigation it was also observed that mature and immature types of the plasma cell line were located frequently a little apart from the blood vessls. Besides, they contained well developed Golgi apparatus and a number of mitochondria comparatively larger than that in lymphocyte and reticulum cell lines. Accordingly it must be added that mitochondrial enlargement in plasam cell line might appear to be parallel to the elevated intraplasmic synthesis and should be interpreted as a representation of another morphological characteristics. Since Rohr many Europian authors ascribed the origin of plasma cell to the reticulum cell.

Marchall and White concurred with the famous Fagraeu's observation that proliferation of plasma cells was proceeded by the advent of large transitional cells which originated from reticulum cells. The potential of the reticulum cell to transform into the plasma cell was construed by Fresen by supposing that the cell of reticuloendotherial system remained hematopoietic potencies to produce lymphocytes, or monocytes and to transform into plasma cells as a postnatal remainder of fetal mesoderm. On the basis of mitosis index rate of transfer cells it was demonstrated by Saint-Marie and Coons that a direct transformation of lymphocytes into plasma cells might be highly improbable and reticulum cells might surely be plausible sources of plasma cells, supposing that reticulum cells could transform to activated basophilic cells and then could enter into mitotic cycles.

Lennert noticed proliferation of the large basophilic cell (Stammzellhyperplasie) simultaneous with appearrance of large reticulum cells in inflammed lymph nodes of Rubeolae, infectious mononucleosis and toxoplasmosis and considered them to be precursor of plasma cells.

Recently Ioachim observed a continuous formation of plasma cells in long term culture of spleen cells and attributed the origin of antibody producing cells to reticulum cells. It was remarkable that the same assummption of reticulum cell origin was already obtained by means of tissue culture of the spleen by Fagraeus.

It was also suggested by McGregor et al. that plasma cells might be originated from primitive mesenchymal cells at Arthus lesions of animals whose lymphocytes were depleted by X-irradiation. However, it is still doubtful whether the fixed reticulum cell can easily return to the primitive mesenchymal cell. Rieke et al. investigated the rate of tritiated thymidine labeled cells in the lymph node and concluded that the most primitive cells of lymphocyte and plasma cell lines were the hemocytoblast, or the plasmoblast, and that the reticulum cell could probably not arise to a stem cell. The fixed reticulum cell was also excluded from the stem cell by marked research work of Masshoff and Frosch. They pursued mice lymph nodes after administration of human serum and concluded that small reticulum cells showed a high proliferative activity to transform throgh transitional cells to plasma cells and through large lymphocytes to small lymphocytes, but large reticulum cells could not show an activity of stem cells. However, it is difficult to identify the small reticulum cell obtained by light microscopy with the

cell type by electron microscopy.

Many experimental data demonstrating the lymphocyte origin of antibody-forming cells have been reported since Hungerfold and others' discovery of the mitotic activity of cultured human blood cells after adding phytohemoagglutinin. Hasting et al. could exclude mononuclear cells which are active in phagocytosis from human blood to be cultured by adding very fine steril iron powder to the blood, and then they could recognize that small lymphocytes were responsible for the reactive mitosis in blood culture.

Moreover, Hirschorn et al. noticed the transformation of small lymphocytes into large cells 72 hours after cultivation and found synthesis of protain of gamma-globuin region in culture medium. They could also show that lymphocytes in cultured blood of sensitized individuals were able to demonstrate mitosis when added purified protein derivative, diphtheria toxoid, pertusis vaccine, penicillin etc. By the same method many investigators such as Evans et al. and Senda et al. proved "blast" transformation of lymphocytes in cultured blood of previously immunized donors by adding corresponding antigens. Concerning the lymphocyte origin of antibody-forming cells there were remarkable studies of Gowans' school. They obtained 99.4–99.5% small lymphocytes after 24 hours incubation of rat's thoracic duct lymphocytes in a conventional tissue culture medium at 37°C and by administrating these small lymphocytes into lethaly irradiated mice, they could noticed large pyroninophilic cells of rat's origin in the white pulp of mice's spleen. The evidence was that the origin of these pyroninophilic cells from rat's small lymophocytes was confirmed by means of autoradiography or chromosome analysis. They verified the same evidence of small lymphocytes transformation toward large pyroninophilic cells in the experiment of homograft reaction.

On the other hand, Nossal and Maekelae noticed labelling of large and medium sized lymphcytes after a single pulse injection of tritiated thymidine to rats immunized with salmonella flagella, and suggested that the cells that could be possible to transform to plasma cells at the secondary stimulation might be large lymphocytes, the progeny cells possessive of D. N. A. synthetic power at resting lymph nodes. They made further researches on the primary immune response against salmonella fragella by means of autoradiographic and single cells micro-manipulatory techniques, and concluded that plasmoblasts might be derived from the transformation of primitive lymphocytes (large lymphocytes), and not from that of small lymphocytes.

Swartzendrüber and Hanna recognized positive labelling of tritiated thymidine in large lymphocytes carrying a great number of ribosomes and negative in reticulum cells in the germinal center of mice's spleen by electron microscopic autoradiography. Movat and Fernando investigated precisely electron microscopic features of the normal lymph node and confirmed that there could not be found any primitive reticulum cell which is possessed of the power to transform to plasma cells and there were only fibroblast-like reticulum cells which participated only in formation of reticular framework and in it's maintainance, but might hardly have a relation to transformation to plasma cells, and that there could be observed intermingling of lymphocytes, blast cells and intermediate cells in the lymph nodes. The last cell

type showed a resemblance to lymphocytes and was characterized by a number of ribosomes in the cytoplasm.

By the transfer experiments of lymphnode cells or peritoneal macrophages of sensitized rabbits to X-irradiated animals, Robert, Dixon and Wergle confirmed an occurrence of typical secondary response in recipient animals by antigen stimulation. The view that lymphocytes or macrophages could be a source of antibody-forming cells was admitted by Dameshck and by Berman, and proposals for the nomenclature of immunological competent cells, raising both lymphocytes and reticulum cells as origin of immunoblasts, were made by them.

However, many marked studies by Gowans et al. or Nossal et al. induced to suppose that immunological competent blast cells might be derived from small lymphocytes or large lymphocytes by stimulation with antigen (or antigen-RNA complex as will be cited later) and not from reticulum cell line.

Recently the significance of macrophage RNA in antibody production was elucidated by Fishman, Fishman et al., Friedman et al., Askonas et al., Cohen, and Gottlieb et al. They showed the evidence that a portion of RNA from antigen-exposed macrophages could initiate antibody formation when added to spleen or lymph node cells in tissue culture, and that antigen or antigen fragment was associated with this immunogenic RNA. It became also probable that this immunogenic RNA-protein complex might be the essential means by which the information-eliciting specific antibody production was processed, even though the RNA itself would not be specific.

It the meanwhile, Han and Johnson observed the rapid uptake of antigen into lymphocytes by means of autoradiographic electron microscopy. From these results it can be postulated that the surface membrane of lymphocytes might allow the entrance of antigen when influenced by contact with antigen-RNA complex, and that stimulated lymphocytes might be then induced to transform to blast cells, to arise to their multiplication and to form antibody.

In order to clarify the origin of proliferative macrophages it must be necessary to search a plausible primitive cell possessed of strong power of multiplication among lymphocyte and reticulum cell lines. Adult macrophages demonstrating active phagocytosis and inactive fixed reticulum cells must be excluded from candidate for probable stem cells of macrophage proliferation.

Rebuck and others were able to observe the transition from lymphocytes to macrophages in the course of inflammation on subcutaneous cover slips by means of "skin window" technique. However, it remained still obscure whether lymphocytes in Rebuck's observation should be identified as proper lymphocytes or young cells from young reticulum cell.

Recently Volkman and Gowans used the same method and acquired the results indicating that the macrophages were derived from cells which migrated from the blood, and that the precursor of these blood cells were dividing continuously at rapid rate in tissues other than in the area of inflammation. By experiments using lymphocyte-depletion by either chronic drainage from the thoratic duct or 400 rads of X-irradiation, or by transfer experiments of using radioactively-labelled cell suspensions obtained from thoracic duct lymph, lymph nodes, thymus, spleen

and bone marrow it was concluded by them that, in the rat, bone marrow, and to a lesser extent spleen were major sources of the macrophages which emigrate into foci of acute, nonbacterial inflammation.

Judging from various reports, it seemed most improbable that small lymphocytes represented a major source of either free or fixed macrophages under physiological conditions.

Nevertheless, it is interesting to notice that Howard et al. could indicate the occurrence of lymphocyte into liver macrophage transformation during states of graft-versus-host reaction.

In our laboratory the formation and origin of tubercle epitheloid cell in various tissues and organs has been studied by supravital observation with phase-contrast microscopy and electron microscopy. In these studies it was represented that the occurrence of typical epithelioid cells was preceded by proliferation of young or immature mononuclear cells in the course of experimental tuberculous inflammtion. According to the report by Yamori and Mori these young or inmature mononuclear cells belonged to line of macrophage (histiocyte) or free reticulum cell. They composed of poor cytoplasm; a few mitochondria and poor development of endoplasmic reticula and represented in some extent development of Golgi apparatus and small vesicles. They were also characterized by distribution of polysomes in the cytoplasm. Their fine structure was very much similar to large or medium sized lymphocytes, but a little coarseness of their nucleoplasm containing large nucleolus might be considered to be more close to the macrophage than to the lymphocyte.

In the recent study on the fine structure of reticuloendothelial system in the spleen, Yamori and Mori calculated the rate of cell types reognized in the red pulp; there were 50 small lymphocytes and lymphoreticulm cells, 10 fixed reticulum cells, 10 macrophages (ingesting free reticulum cells) and 5 plasma cells. They supposed the transformation of lymphoreticulum cell (young free reticulum cell resembling large lymphocytes) to macrophages (free ingesting reticulum cells).

Harui and Yamori also observed proliferation of blast cells characterized by higher content of intraplasmic polysomes in the red pulp of rabbit's spleen 5 days after stimulation by typhoid vaccine and found transformation of blast cells to free reticulum cell (macrophages) 10 days after stimulation.

Moreover, Ito and Yamori investigated recently electromicroscopic features of responsive proliferation of macrophages (free reticulum cells) in the cortical tissue of the popliteal lymph nodes of rabbits caused by inoculation of autoserum into the footpad. They noticed blasts cells characterized by high content of polysomes in cytoplasm. Blast cells produced by stimulation of autoserum might be most probably reticuloblasts, precursor cells of free reticulum cells, because it seems to be sure that active proliferation of antibody forming cells of lymphocyte or plasma cell lines can hardly be occurred by stimulation with autoserum. On the base of results obtained by the presnent study, it is obvious that the origin, characteristcs and behavior of macrophages are independent and somewhat different from that of the fixed reticulum cells or immunocytes. Since morphological distinction of young macrophages from large lymphnocytes is very difficult even with electron microscopy, both young macrophages and large lymphocytes were called by the name of young

lymphoreticulum cell (by Yamori and Mori). They were to some extent more mature than reticuloblasts or lymphoblasts, and demonstrated a fewer cytoplasmic polysomes or smaller and lesser obvious nucleolus. In comparison with large lymphocytes, young macrophages, however, showed a little better development of Golgi apparatus and small vesicles which indicated some low grade potentiality for ingestion or digestion.

The evidence that new small lymphocytes were produced in lymphoid tissue during antibody formation was obtained by Nossal and Maekelae and the prolonged retention of labelled small lymphocytes in the regional lymph nodes when tritiated thymidin was given to rats was described by Miller. By using several techniques of X-irradiation, tritiated thymidine autoradiography, and chromosome analysis Gowans et al. established the evidences that, in a graft-versus-host reaction, small lymphocytes enlarged into pyroninophilic cells which could divide, and that the progenies of large pyroninophilic cells were identified autoradiographically in histological sections of spleens as a population of lymphocytes of progressively decreasing size. It was also observed by the present investigation with electron microscopy that pyroninophilic blast cells could develope to lymphoblasts and large or small lymphocytes frequently at later stage after stimulation and in the middle of cortical tissue a little distant from the lymphnode sinus.

The difficulty of molphological distinction of plasmoblasts from lymphoblast even by electron microscopy in the present investigation may induce to support the view that both plasmoblsts and lymphoblast originate from same precursor, probably from small or large lymphocytes stimulated antigenically and both have respectively different destination, localization and time of occurrence.

It was observed by the present investigation that cells of plasma cell series were located mostly in the medullary cord or cortical tissue closed to the sinus where antigen is concentratedly retained by the sinus macrophages and the cell of lymphocyte series in the neighbor of follicle tissue, where content of retained antigen in the macrophages seemed to be very low and germinal center were to be produced in the course of lymphoblast proliferation.

Observations of the present study and recent studies in our laboratory are summarized as follows in table 4 and 5, by modifying slightly the proposal issued by Yamori and Sashikata at the 7th Jap. Soc. R. E. S. (1967).

Table 4

| | | | | |
|---|---|---|---|---|
| primitive immunoblast (lymphogonia, Amano) (primitive lymphoidblast | ↔ | plasmoblast (immunoblast) | → preplasmocyte (plasmocytoid cell) (lymphocytoid cell) | → (mature) plasma cell |
| | | lymphoblast (immunoblast) | → young lymphocyte (large lymphocyte) (young lymphoreticulum cell) | → mature lymphocyte (small lymphocyte) |
| primitive reticuloblast | ↔ | (free) reticuloblast | → young macrophage (immature free reticulum cell) (young lympho-reticulum cell) | → mature or ingesting macrophage (free reticulum cell) |
| | | (fixed) reticuloblast | → young fixed reticulum cell | → mature fixed reticulum cell |

Table 5

| | | |
|---|---|---|
| small lymphocyte<br>large lymphocyte | stimulation by antigen-RNA complex | immunoblast<br>primitive immunoblast |
| young macrophage (young lympho-reticulum cell)<br>large lymphocyte?<br>small lymphocyte? | stimulation by autologous protein? | free reticuloblast<br>primitive reticuloblast |


## SUMMARY

Proliferative responses of the lymphocyte, plasma cell and macrophage lines in the primed or non-primed lymph nodes stimulated by primary or secondary antigen challenge were investigated by means of electron microscopy.

1) The immature types of the lymphocyte and plasma cell lines, the lymphoblast and plasmoblast show almost same fine structure, characterized by high content of polysomes in cytoplasm and by large and distinct nucleolus in nucleoplasm and they both are worth being called by the common name of "immunoblast", and most probably derived from same origin, may be from large or small lymphocytes.

The most primitive cell of plasma cell and lymphocyte lines show entirely simillar fine structure, and are called as primitive immunoblast.

2) However, they vary each other to some extent. The cell of plasma cell line, especially plasmoblast and preplasmoblast have tendencies to an increase in numbers of rough surfaced endoplasmic reticula and to large mitochondria in accordance with elevated globulin synthesis, and their proliferations are located mostly in the medullary pulp and cortical tissue close to the sinus, where antigenic content is very high, or occurs at an earlier stage, when antigen has high content and not yet disintegrated. Proliferation of the lymphocytic line occurs generally in the later stage and in the middle of cortical tissue, in which antigenic content is low, dilute and gradually disintegrated.

3) In the stimulated lymph nodes young macrophages (young free reticulum cells) are noticed as an origin of proliferation of mature or stimulated macrophages. The young macrophage show almost similar fine structure to large lymphocytes, however represent some disperseness of nucleoplasm and slight inclease in the width of Golgi area and numbers of small vesicles.

Intermingled with proliferated young macrophages the most immature cells of macrophage line, free reticuloblasts are observed occasionally. They are characterized by increased content of polysomes in cytoplasm and by large and distinct nucleolus in nucleoplasm, however, represent slight tendency to develope Golgi apparatus, small vesicles and processus formation.



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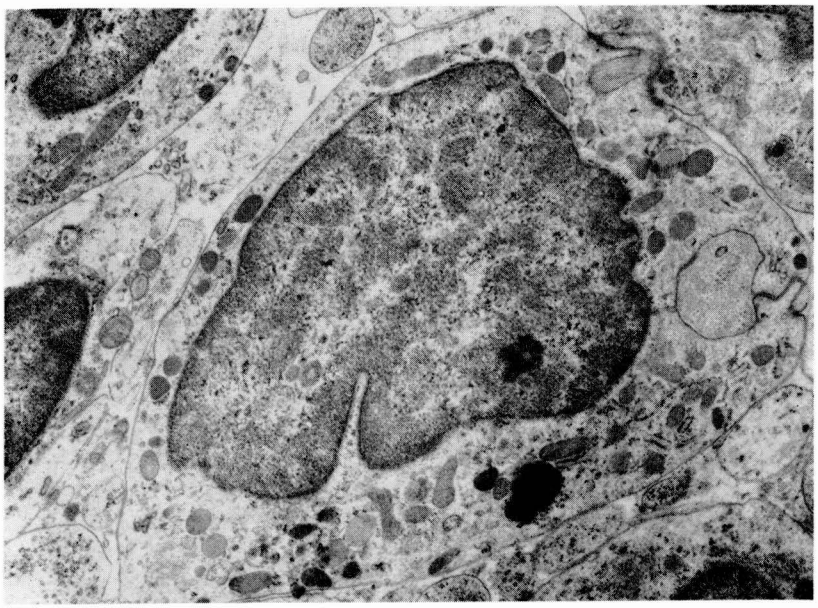

Fig. 1. Fixed reticulum cell ; Reticular fiber encompassed by the cytoplasm is showed on the figure. ×11500

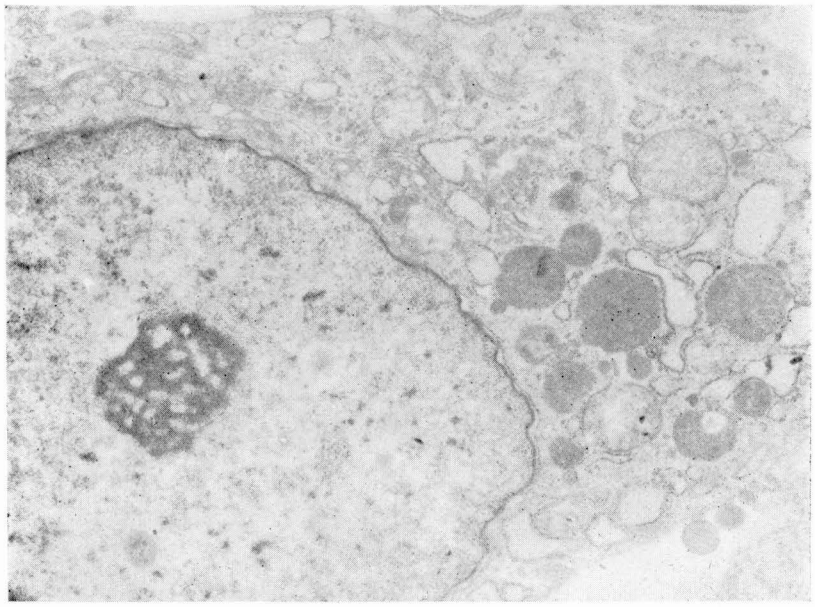

Fig. 2. Ingesting free reticulum cell ; Ingested dense bodies surrounded by a single layer of limiting membrane are distributed in the cytoplasm. ×11500

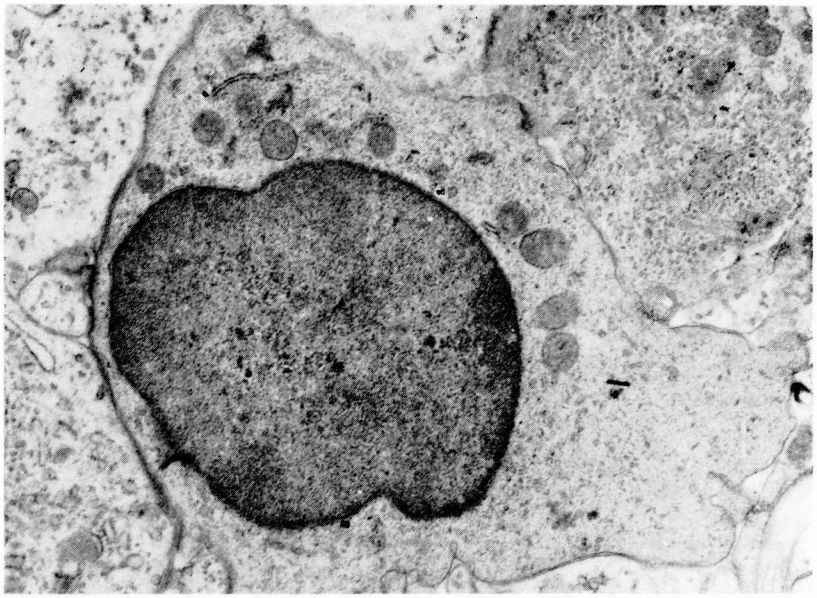

Fig. 3. Young free reticulum cell ; Reticular fibers are not seen. Cell-organellae are poorer than that of mature type. Poorly developed Golgi-apparatus, a few medium sized mitochondria and sometimes ingested dense bodies are showed. ×11500

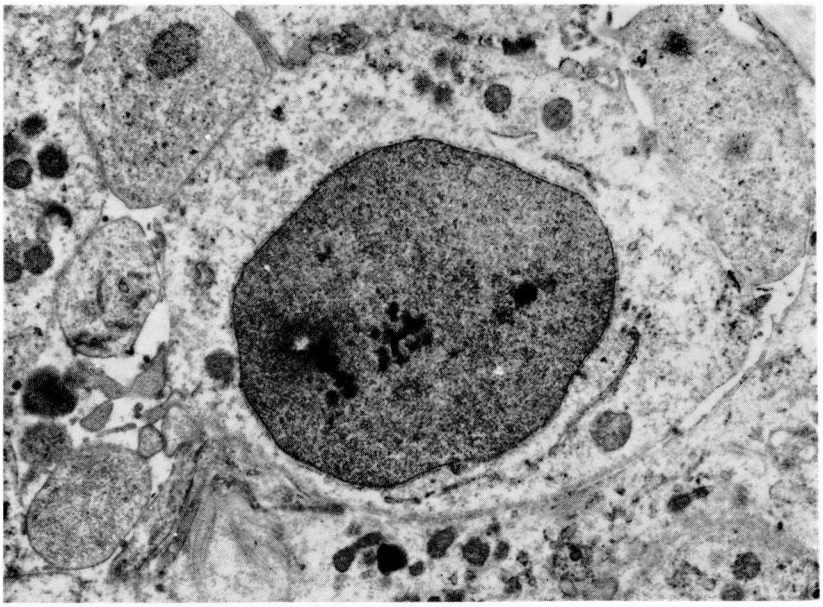

Fig. 4. Young fixed reticulum cell ; Poorly developed cell-organellae and reticular fibers come in contact with cell membrane are seen. Polysomes are increased in the cytoplasm. ×11500

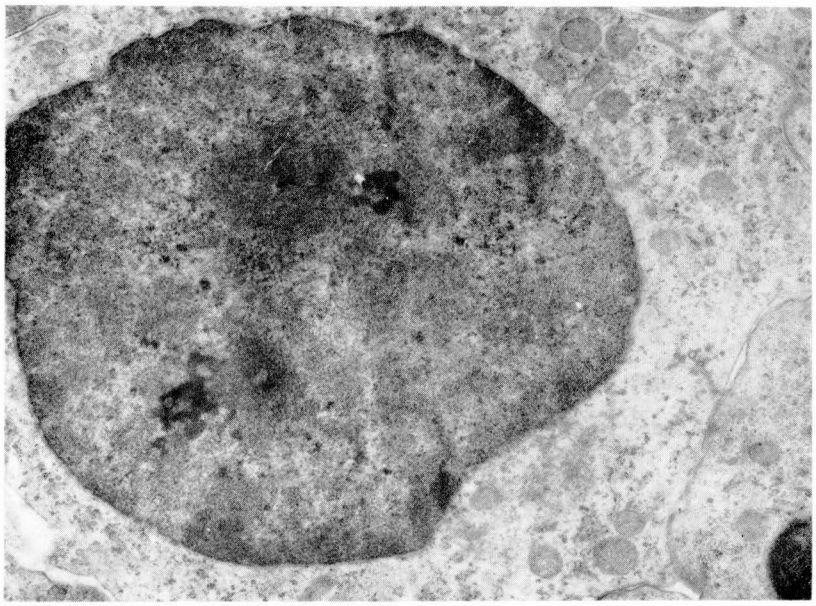

Fig. 5. Reticuloblast ; Numerous polysomes, sER, a few rER and medium sized mitochondria are characteristic. ×11500

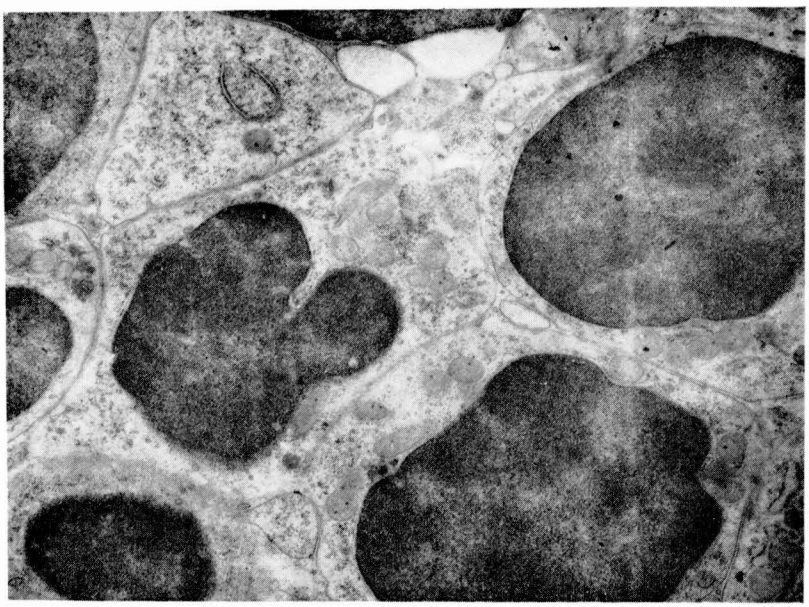

Fig. 6. Small lymphocyte ; Kidney-shaped nucleus with deep indentation is characteristic. Cell-organellae are represented in the centrosphere. ×11500

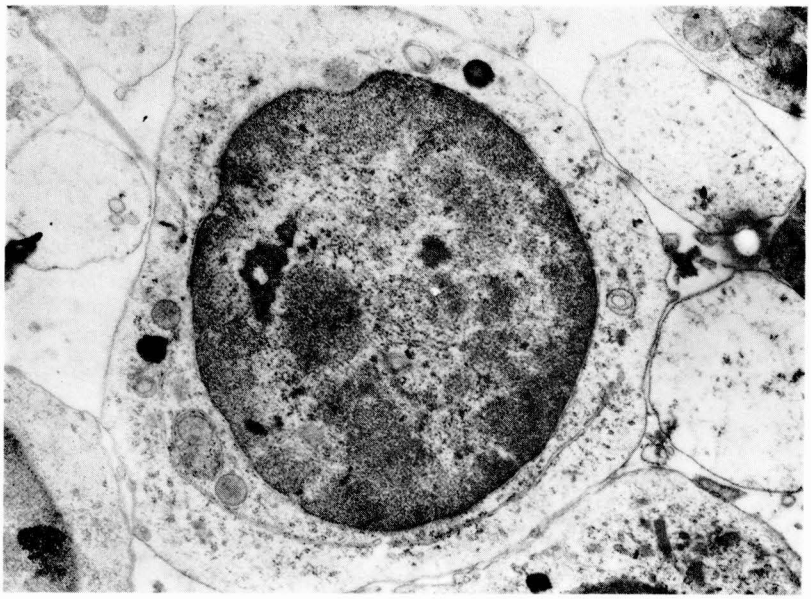

Fig. 7. Young lymphocyte (medium and large lymphocyte) ; Small nucleolus is distinctly noticed in the nucleoplasm. A few rER and polysomes are seen. ×11500

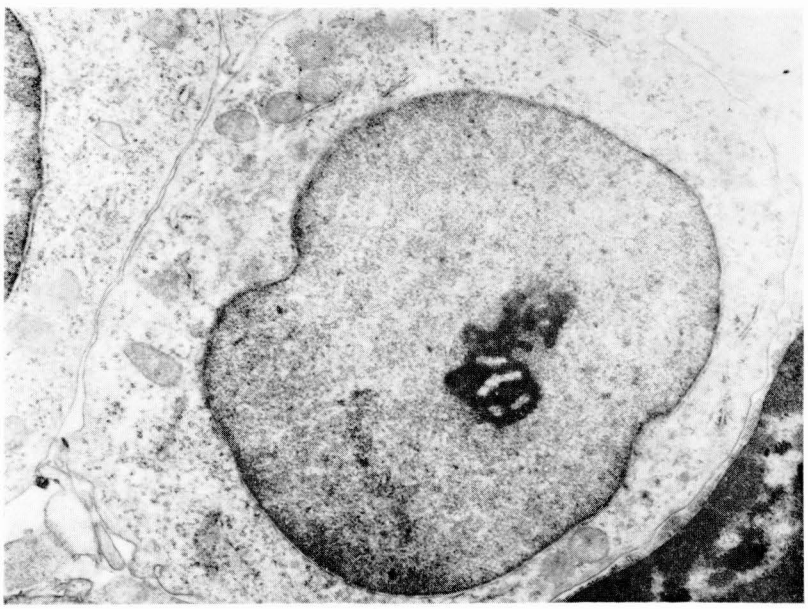

Fig. 8. Lymphoblast ; Increased polysome, a few fine rER in the cytoplasm and 1 or 2 medium sized nucleoli in the nucleoplasm are distinctly observed. ×11500

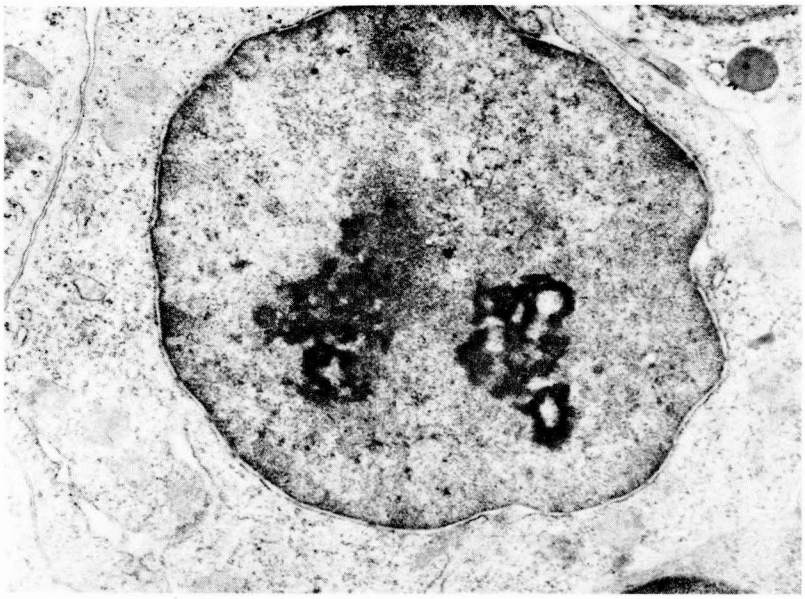

Fig. 9. Primitive lymphoid-blast (originating from lymphocyte series), found in the nest of prominent lymphocytic proliferation. ×11500

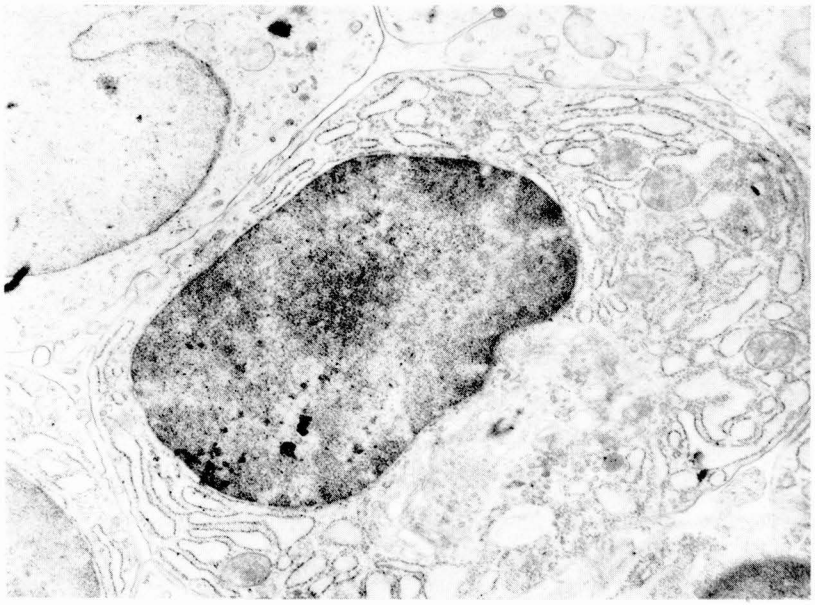

Fig. 10. Mature plasma-cell ; Specific lamellar arrangements of rER, a wide Golgi-area and large sized mitochondria are noticed in the cytoplasm. ×11500

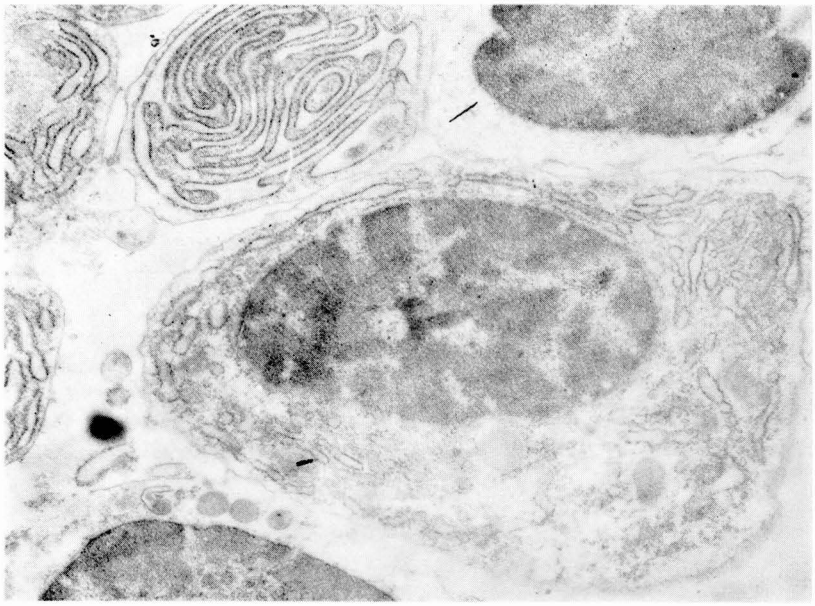

Fig. 11. Preplasmocyte, showing a small nuclolus in the nucleoplasm and moderately developed rER. ×11500

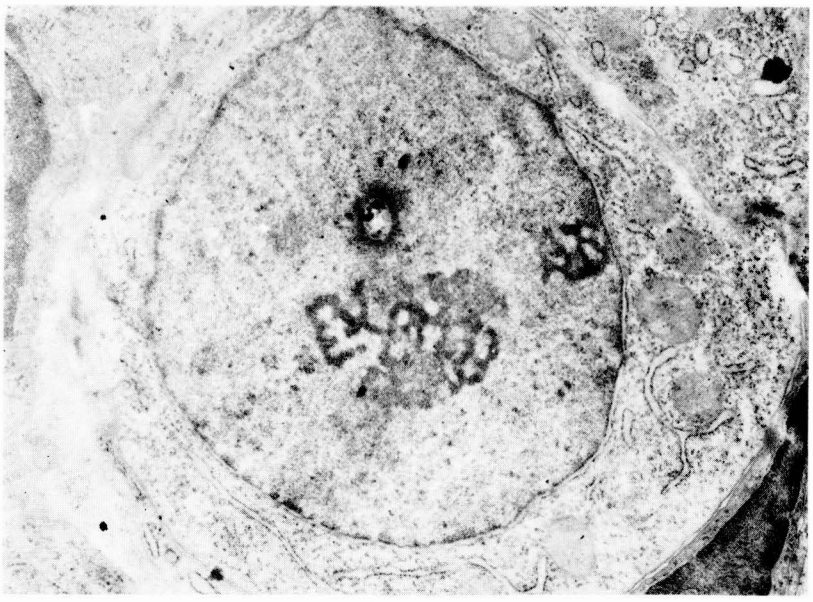

Fig. 12. Plasmoblast ; Numerous polysomes in the cytoplasm and large nucleoli in the nucleoplasm are showed. Development of rER is observed. ×11500

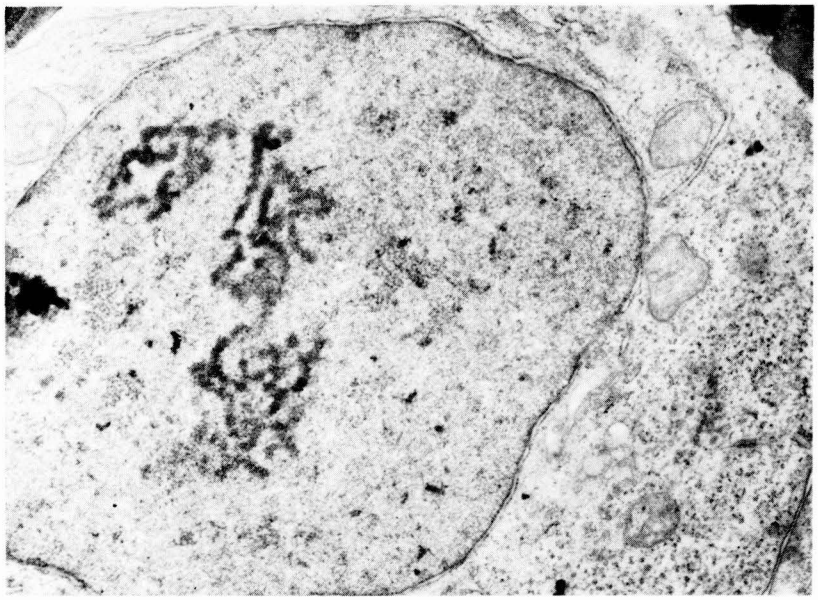

Fig. 13. Primitive lymphoid-blast (originating from plasma cell series), found in the nest of prominent plasmocytic proliferation. Large sized mitochondria and fine rER are observed. ×11500