

DYNAMICS OF CELL POPULATIONS IN LYMPH NODE DURING PRIMARY IMMUNE RESPONSE

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ABSTRACT

Based on the concept that the lymph node is composed of four functionally distinct compartments, popliteal lymph nodes of three week-old rats were studied histologically after a single primary antigenic stimulation. A variety of antigens, *i.e.*, SRBC, pertussis vaccine, bovine γ -globulin, crystalline bacterial amylase, egg albumin, diphtheria toxoid with or without the adjuvant always produced a consistent pattern of development and involution sequentially in each of four compartments: the paracortical areas were always the first in making response, followed primary follicles, medullary cords and germinal centers in this order. The mitotic indices in the paracortical areas reached the maximum on the third day whereas the peak activity of the germinal centers was observed between the 10th to 24th day.

The development of germinal centers which had not been observed until the late stage might reflect its probable role in the development of immunological memory. The role of paracortical areas in the initiation of "true" primary response was discussed in the light of current theories on the cell-to-cell interaction as well as the relationship between humoral and cell-mediated immune response. This led to postulation that the paracortical hyperplasia was essential for the induction of primary immune response, especially in "true" primary response.

INTRODUCTION

The morphological responses to various antigenic stimuli have been extensively studied in lymph nodes of various experimental animals¹⁾⁻⁷⁾. These works established that the germinal center development in the cortex and plasma cell proliferation in medullary cords were the main features of humoral antibody production. However most of these studies were undertaken on the basis of classical concept of structure of lymph node⁸⁾⁻¹⁰⁾.

Now it is well known that the lymph nodes of neonatally thymectomized mice¹¹⁾ and rat¹²⁾ are characterized by a marked depletion of lymphocytes which is primarily restricted to the mid and deep cortical areas (paracortical areas)¹³⁾ without significantly affecting the cortical follicles and medullary areas. Moreover, autoradiographic studies demonstrated that thymic lympho-

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cytes localized almost exclusively in certain areas corresponding to the paracortical areas (thymus-dependent areas)¹¹⁾ of lymph nodes. Recently it was demonstrated that the bone marrow derived cells, in contrast, homed selectively to the follicles of primary type¹⁴⁾¹⁵⁾. On the other hand, plasma cells which are located mainly in the medullary cords are also well known as descendants of bone marrow cells¹⁶⁾. It is a interesting fact that mobile lymphoid cells of indistinguishable morphology, which differ in their origins, migratory patterns, life spans, and possible roles in immune response, have the ability to recognize and select the different compartments of lymph nodes¹⁷⁾. The mobile lymphoid cell populations within lymph nodes might be divided into at least four subgroups according to the structural compartments they occupy, *i.e.*, primary follicles, germinal centers, paracortical areas and medullary cords.

From the above point of view, a new interpretation might be possible for the sequential histological events in lymph nodes stimulated with various antigenic materials. The aim of the present investigation is to follow strictly the dynamic change of four different cell populations within lymph nodes after primary antigenic stimuli. In analyzing a kinetic behavior of these compartments, an experimental system wherein *de novo* formation and growth of each compartment can be followed is desirable. Accordingly, the popliteal lymph nodes of rats aged twenty-one days were employed expecting that they might offer a place for the "true" primary immune response¹⁸⁾ or its equivalent.

METHODS AND MATERIALS

Animals. A total of 310 laboratory-bred, male and female Wistar rats was divided into antigen groups and control groups. The antigen groups were injected with various antigens twenty-one days after birth when they weighed between 25 to 40 g. All of the antigenic materials were injected subcutaneously in a volume of 0.08 ml into the right hind foot pad. A group of three to four animals was killed at intervals ranging from one to thirty-one days after injection.

Antigens and adjuvants. 1) 2.1×10^6 sheep red blood cells (SRBC); 2) 1:10 physiological saline dilution of original pertussis vaccine (PV) (Takeda Institute, Osaka, Japan) containing 400×10^9 organisms/ml suspended in physiological saline with 1:10,000 thimerosal; 3) soluble protein antigens, *i.e.*, crystalline bacterial amylase (AMYL) (Katayama Chemical, Osaka, Japan), bovine γ -globulin (BGG) (Sigma), egg albumin (OVAL) (Katayama Chemical, Osaka, Japan) and diphtheria toxoid (DT) (Kitasato Institute, Tokyo, Japan). All these antigenic materials were used either in alum-precipitated form (AL) (0.6 mg of AMYL or OVAL, and 4:5 physiological saline dilution of original DT vaccine) or in the form of emulsion with Freund's complete adjuvant (FCA) or incomplete one (FIA) (0.04 mg of AMYL or BGG); 4) To serve as

the control, some animals were injected with Freund's complete or incomplete adjuvant alone emulsified in equal volume of saline: 5) Another group of animals were left untreated and served as the absolute control.

Weight and histological techniques. The unstimulated popliteal lymph nodes were examined as a monitor of a systemic infection. Popliteal lymph nodes from untreated control animals of the same age served as the control. All lymph nodes were fixed for twenty-four hours in buffered formalin at pH 7.2. Paraffin sections were cut at 4 μ and twenty-four semiserial sections from each lymph node were routinely stained with methyl-green pyronin. Hematoxylin and eosin or reticulum-staining was supplemented when necessary.

Definition of histological compartments. In this paper, four histological compartments are defined as follows.

Primary follicles: Round areas of densely packed small lymphocytes situated immediately beneath the marginal sinus. These structures usually produce a slight convexity on the surface of the node.

Germinal centers: Round collections of large or medium-sized, pyroninophilic lymphoid cells which are easily distinguishable from the surrounding pyroninophobic lymphoid cortex. They also contain various numbers of tingible body macrophages or reticular cells. Mitotic figures are most frequent in this compartment.

Paracortical areas: The diffuse areas of the cortex which are composed of less densely packed lymphocytes, occasional large pyroninophilic blastic cells, variable numbers of clear reticular cells and several post-capillary venules lined with characteristic cuboidal endothelium.

Medullary cords: The interdigitating structures between the medullary sinuses, consisting mainly of cells of plasmocytic series located around the centrally-situated blood vessels.

Immunofluorescent technique¹⁹⁾. Pieces of lymph nodes stimulated with BGG-FCA were frozen in Dry Ice-acetone bath. Fresh frozen sections were cut at 6 μ , fixed for thirty minutes in 95% ethanol, and stained with BGG conjugated with fluorescein isothiocyanate by the direct method.

Counting of mitotic cells. The mitotic figures were counted under oil immersion in different sections and areas as far as possible. In the lymph nodes composed of well-developed individual compartments, the total number of dividing cells in twenty microscopic fields were obtained and converted into relative figures per 10^4 nucleated cells based on the average population of nucleated cells per one field of each compartment. The above mentioned counting method was not applicable in poorly-developed, small histological compartments. In such cases, mitotic figures among a total of 1,000 nucleated cells were counted and were multiplied tenfold.

Antibody titration. The sera were obtained from the external iliac artery of the animals under anesthesia and were stored at -20°C . Antibody titer was determined by a microtitration method. Tanned cell hemagglutination²⁰⁾ was performed for anti-OVAL sera using formalinized sheep erythrocytes²¹⁾ and for anti-BGG sera with untreated erythrocytes. Hemolysin titer²²⁾ was estimated for the sera from the animals treated with SRBC.

RESULTS

Weight. The popliteal lymph nodes in all the antigen groups were already markedly swollen on the third day and continued to enlarge reaching five to ten times the weight of untreated control (Fig. 1). The generally rising curve, however, tended to be biphasic in the groups treated with the antigens emulsified in Freund's complete or incomplete adjuvant. The control group injected with the saline-adjuvant alone showed even deeper fall between the second and third week and the later increment was attributable to the development of granuloma. Straight rising curves were obtained in the groups given the alum-precipitated antigens.

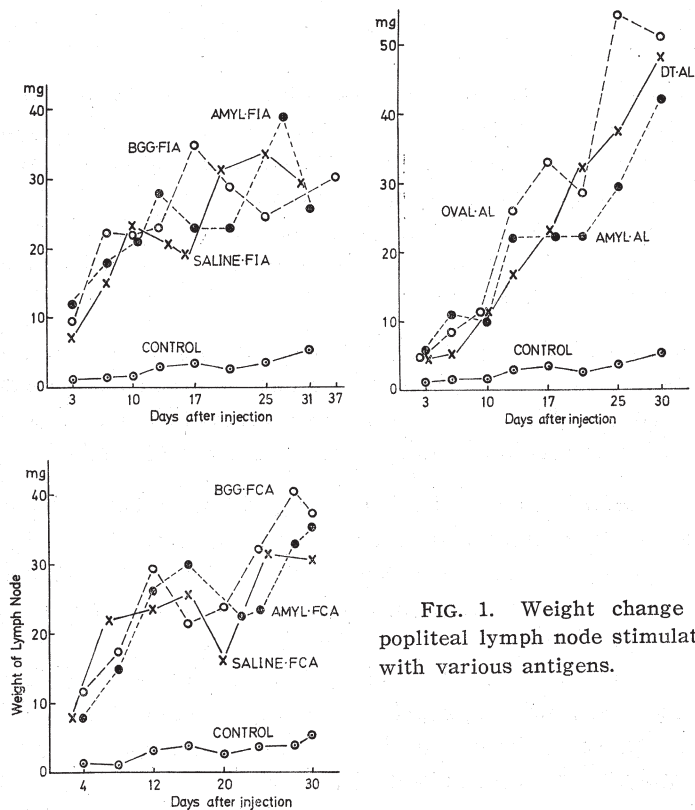


FIG. 1. Weight change of popliteal lymph node stimulated with various antigens.

Histology of the untreated control lymph nodes. The popliteal lymph nodes in the control animals aged twenty-one days already showed cortico-medullary differentiation (Fig. 2-a). The cortex was composed of a thin rim of the subcapsular mesenchymal zone as well as nodular structures which located

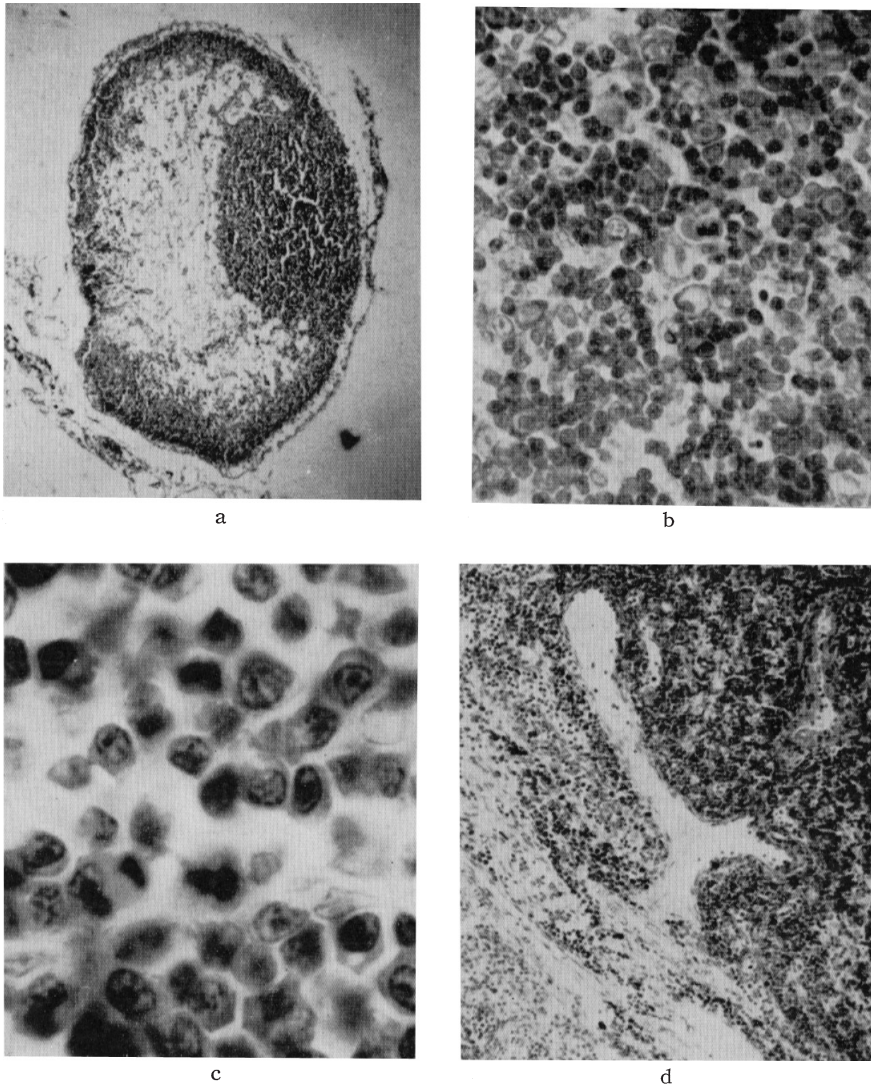


FIG. 2. (a) Immunologically virgin popliteal lymph node of young rat of 21 days of age, methyl green-pyronine, $\times 40$. (b) Activated paracortical area, 3 days after pertussis vaccine treatment, methyl green-pyronine, $\times 400$. (c) Immature plasmocytic proliferation in medullary cords, 7 days after BGG-FCA treatment, methyl green-pyronine, $\times 1,000$. (d) Transitioning blood vessels in junctional zone, 10 days after BGG-FIA treatment, methyl green-pyronine, $\times 40$.

deeper in the cortex. The subcapsular mesenchymal zone consisted of loose meshes of mesenchymal cells intermingled with a small number of lymphocytes. The deeper cortical nodules, *i.e.*, paracortical nodules were the most distinct structural element. They consisted of a single or more commonly two, small and large nodular structures which were composed of stromal reticular cell population and the less prominent small lymphocyte population although the latter varied considerably within the same nodule. A special type of venule called the post-capillary venule was consistently recognized in this compartment. In the unstimulated control animals these venules were hypoplastic and the small lymphocytes were hardly found either in the lumina or within the endothelial cells. The medullary cords consisted of poorly-developed fibrous strands composed mainly of vascular components such as capillaries, arterioles, veins or lymph vessels. Neither the germinal center nor the medullary plasmocytosis were recognized in this group of animals aged three weeks. In contrast, early primary follicles composed of spherical aggregates of small lymphocytes which were somewhat denser than in the surrounding cortex were occasionally observed. The hypoplastic appearance as described above had been retained for several weeks thereafter, although there was considerable increase in the small lymphocyte population in the deeper cortex as well as gradual development of distinct primary follicles and medullary cords. The mitotic figures were rare and essentially negligible in this group throughout the experimental period.

Histologic findings common to all of the stimulated lymph nodes. On the first day, the lymph nodes were moderately enlarged due mainly to distension of the medullary sinuses. The paracortical nodules enlarged slightly; the lymphocyte population was somewhat increased and the post capillary venules became somewhat more prominent. The subcapsular mesenchymal zone also manifested increased lymphocyte population. Accordingly, the distinction between this zone and the paracortical nodules got indistinct and the cortex as a whole became a more prominent structure.

On the second day, the lymphoid cell collections at the subcapsular mesenchymal zone bulged out from the cortical surface into the overlying sinuses. The paracortical areas continued to enlarge. The medullary cords showed edematous swelling as well as appearance of scattered pyroninophilic blastic cells and small lymphocytes.

By the third day, the paracortical areas reached a considerable size occupying a large part of the lymph node and encroached on the medulla. They were composed of many large blastic cells, increased numbers of small lymphocytes, occasional unidentified dividing cells (Fig. 2-b) and background reticular cells as well as very prominent post-capillary venules. The blastic cells had larger round nucleus containing fine networks of chromatin and a large nucleus

as well as abundant, intensely pyroninophilic cytoplasm. The cells which were seemingly at various transitional stages from these blastic cells either to small lymphocytes or to reticular cells were also recognized. The dividing cells usually showed large amount of pyroninophilic cytoplasm, but occasionally manifested vacuolated cytoplasm which stained poorly with pyronin. The paracortical areas, therefore, could be subdivided into two areas, clear and dark. The mitotic figures were more frequent in the clear area than the dark area. The post-capillary venules displayed enlarged lumina with cuboidal to low-columnar endothelium with rare but recognizable mitotic figures. Small lymphocytes were frequently observed both in the lumen and within the endothelial cells. Several dense lymphoid follicles of the primary type were observed in the outer portion of the cortex, but the germinal center was not noted as yet. The medullary cords became more prominent and were composed of medium-sized lymphocytes as well as a small number of pyroninophilic blastic cells located around centrally-situated blood vessels. The medullary sinuses contained many macrophages with abundant, vesicular cytoplasm.

On the 6th to 7th day, a collection of several large pyroninophilic cells resembling young germinal centers were recognized in some of the primary follicles particularly at their deeper portion. These structures resembling young germinal centers were much larger in the SRBC group than in any other groups. The medullary cords, especially their upper portion displayed some aggregates of plasma cells predominated by immature ones (Fig. 2-c). These plasma cell precursors were also occasionally found in the medullary sinuses or within the spaces of dilated lymphatics near the hilus. The blastic cells were found more often in the vicinity of post-capillary venules, especially in the pertussis vaccine group. The paracortical areas continued to enlarge and were characterized by abundance of small lymphocytes and pyroninophilic blastic cells as well as relative paucity of reticular cells.

By the 10th to 11th day, the medullary region had occupied the greater part of lymph node due mainly to explosive proliferation of plasma cell series. The medullary cords were enlarged and were engorged with immature plasma cells with frequent mitotic figures. Dilated blood vessels were frequently recognized at the center of the cords. The plasmocytic cells located at the deeper portion of the cords, adjacent to the hilus, tended to be smaller and rounded approximating the mature type. The cortico-medullary junction frequently showed small patent venules lined with high endothelium on their cortical side and with flattened endothelium on their medullary side (Fig. 2-d). The medullary side of these venules was immediately surrounded by layers of the most immature form of plasma cell series (Fig. 3-a). The paracortical areas maintained their large size but showed moderate decrease in

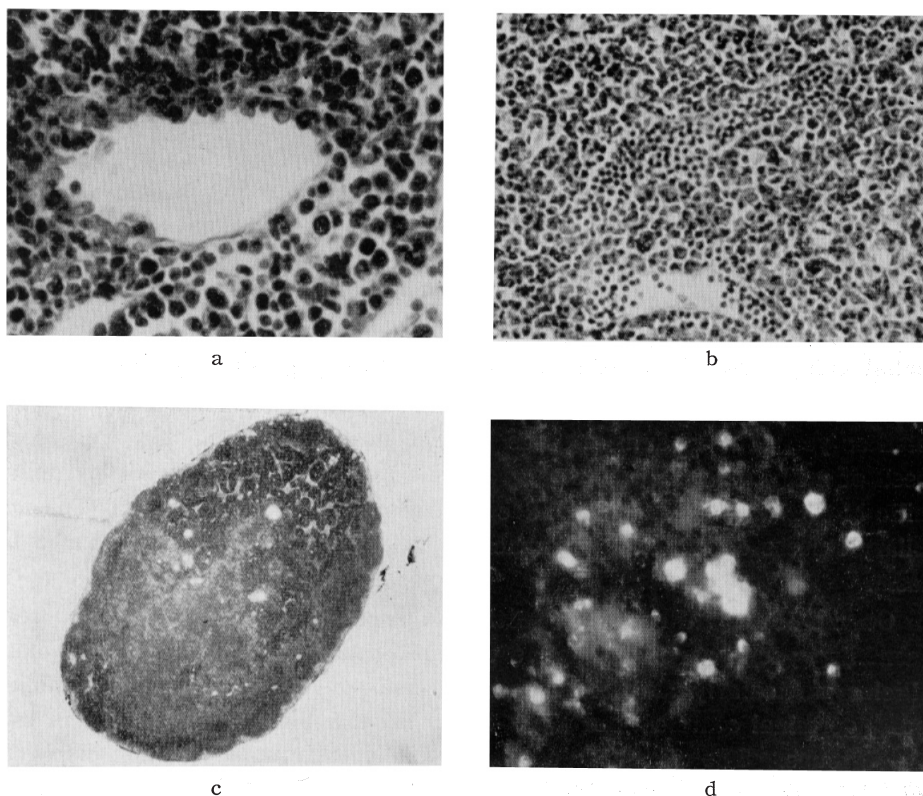


FIG. 3. (a) Abrupt occurrence of immature plasma cells along the venous aspect of transitioning blood vessel, 10 days after BGG • FIA treatment, methyl green-pyronine, $\times 400$. (b) Lymphocyte packing in paracortex, 11 days after AMYL • FCA treatment, methyl green-pyronine, $\times 200$. (c) Fully developed lymph node, showing many germinal centers in outer cortex, 18 days after BGG • FIA treatment, methyl green-pyronine, $\times 12$. (d) Fluorescent positive cells in medulla, 5 days after BGG • FCA, frozen section stained with FITC-conjugated BGG, $\times 400$.

lymphocytes and pyroninophilic blastic cells associated with relative increase of reticular cells. Densely packed small lymphocytes were located within the small lumina lined by very thin membranous connective tissue located within the paracortical areas (Fig. 3-b). Pictures suggesting streaming of these lymphocyte collections into the medullary sinuses were observed although rarely at the cortico-medullary junction. The germinal centers continued to increase in size and number. The outline of the centers became sharply demarcated from the surrounding aggregation of small lymphocytes. The bulging of the primary follicles were more prominent due to enlargement of germinal centers.

By the 13th to 14th day, plasmocytic proliferation in the medulla was maximal. Within the area of plasma cell proliferation were noted the tingible

body macrophages which were usually seen in the growing germinal centers. The greatly widened medullary cords compressed the interdigitated medullary sinuses. Multiple, very small granulomas were observed in the cortico-medullary junction of the lymph nodes stimulated with alum-precipitated antigens. The granulomatous foci were composed solely of several or more, large, elongated epithelioid cells with abundant vesicular cytoplasm. The germinal centers continued to grow in size. The paracortical areas manifested further decrease in the pyroninophilic blastic cells.

By the 16th to 18th day, the germinal centers reached a peak in size, number and activity which was reflected by numerous mitotic figures and numerous tingible body macrophages (Fig. 3-c). The medullary plasmocytosis became less prominent while mature plasma cells were increased in number.

On the 21st to 25th day, the enlarged germinal centers began to show a regressive change; they were predominated by reticular cells and nuclear fragments within or without the macrophages. Sharp boundary of the center from the surrounding lymphocytic cuff was lost. The paracortical areas were considerably varied in the population of small lymphocytes.

At the end of the experiment, about one month after the antigenic stimulation, each compartment of the lymph nodes was less distinct due mainly to regression of the germinal centers in addition to granulomatous lesions induced by the adjuvant. Now the germinal centers were apparently divided into two zones; the outer superficial portion was composed of a mass of uniform lymphoid cells with small dense nuclei and moderate amount of light cytoplasm, resembling the primary follicle. The deeper portion, on the other hand, consisted of large lymphoid cells intermingled with many tingible body macrophages, reticular cells or dividing cells of unknown origin. Plasma cell population in the medulla was decreased and was predominated by mature ones.

Antigen difference. BGG in conjunction with Freund's adjuvant of both types provoked massive proliferation of immature plasma cells as early as Day 6 to 7. The germinal centers were rarely encountered or very small in size and number at this stage. The lymph nodes stimulated with pertussis vaccine showed higher proportions of pyroninophilic blastic cell population as well as that of the dividing cells in the developing paracortical areas on the third day. This strong activation of the paracortical areas was followed with development of extremely large germinal centers by the 7th day. Coalescence of germinal centers was occasionally noted from the 10th day onward exclusively in this group. Moreover, the packed lymphocytes within the small lumina in the paracortical areas were recognized as early as the fourth day after stimulation. The animals treated with SRBC and those of pertussis vaccine group demonstrated stronger activation of the paracortical areas,

followed by accelerated proliferation of plasma cells and germinal center cells. Contrary to this, the lymph nodes given DT·AL displayed less prominent growth of the paracortical areas at the early stage as well as some delay in plasmocytosis and germinal center formation.

Adjuvant difference. When the adjuvant effects on paracortical activation were compared in the animals treated with bacterial amylase, Freund's complete adjuvant appeared to be most effective, followed by incomplete one and alum in that order. Freund's complete adjuvant was more effective than the incomplete one in BGG-treated animals as well. The reticular cell hyperplasia in the paracortical areas were marked in the animals treated with Freund's type of adjuvant both in bacterial amylase and in BGG groups. In the animal stimulated with alum-precipitated antigen many small granulomas composed of epithelioid cells were observed first at the deeper portion of the paracortical areas and later localized at the cortico-medullary junction. Freund's incomplete adjuvant emulsified with saline produced enlargement of the paracortical areas which was nearly comparable to that of the antigen groups. The post-capillary venules were well-developed and the accumulation of small lymphocytes was also noted to some extent while pyroninophilic blastic cells and

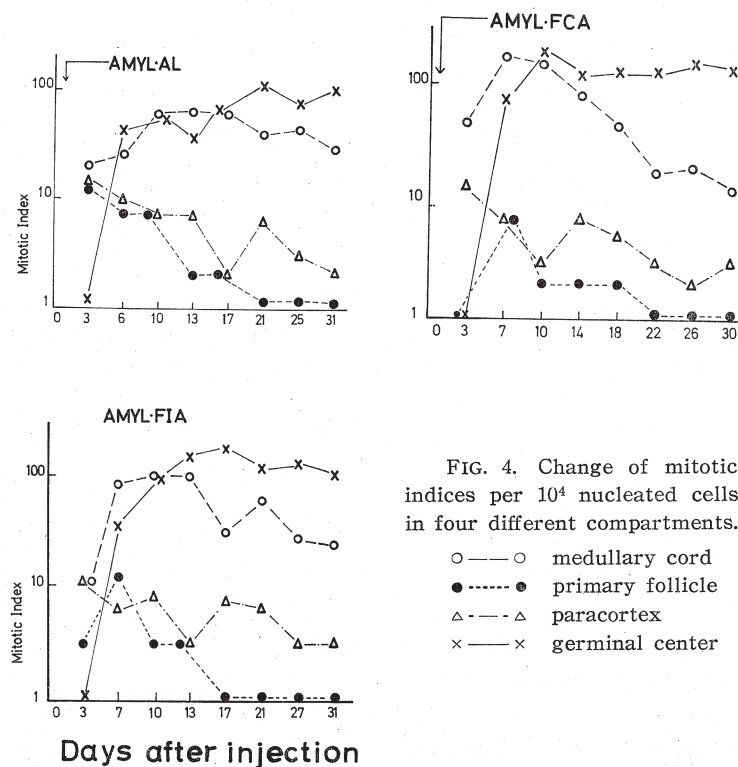


FIG. 4. Change of mitotic indices per 10^4 nucleated cells in four different compartments.

unidentified dividing cells were not significantly increased. Furthermore, no germinal centers were recognized throughout the entire period of experiment. The plasmocytosis was seen in medullary cords but was less prominent than that in the animals injected with the antigens. All the animals treated with saline-Freund's complete adjuvant developed the germinal centers although to a lesser degree by the 12th day.

Change of mitotic index. The mitotic indices in each histological compartment were followed throughout the experimental period (Fig. 4, 5 and 6). Regardless of the antigenic difference, mitotic indices were less than twenty in both primary follicles and paracortical areas, while they mostly fell into the range of twenty to two hundred in both medullary cords and germinal centers. After reaching the maximum on the third day, the number of mitotic cells in the paracortical areas decreased slowly and maintained a relatively constant value thereafter. Those in the primary follicles showed a peak on Day 6 to 7, then declined gradually and reached zero by the third week. The medullary cords showed a peak value sometime during the second week

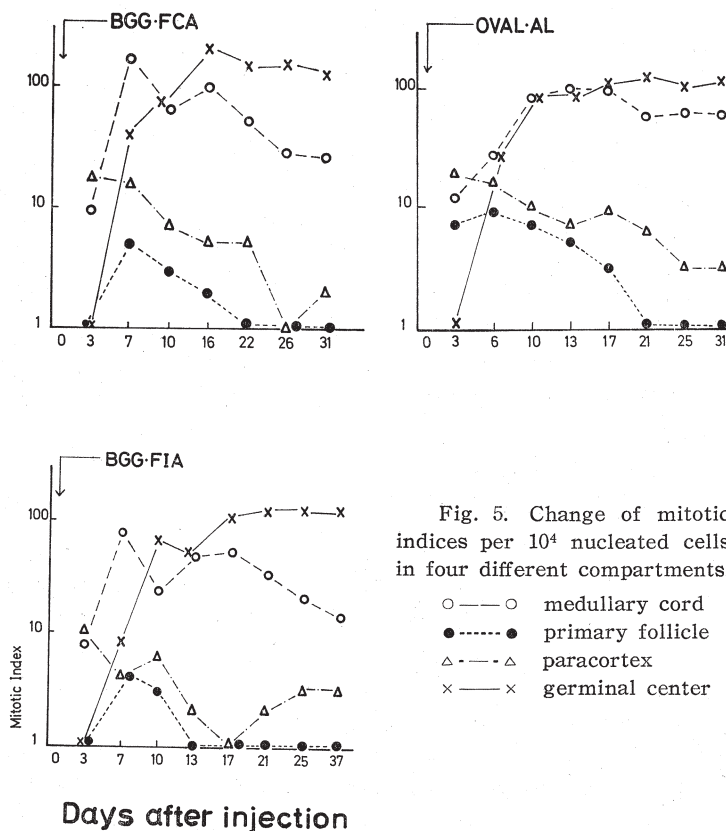


Fig. 5. Change of mitotic indices per 10^4 nucleated cells in four different compartments.

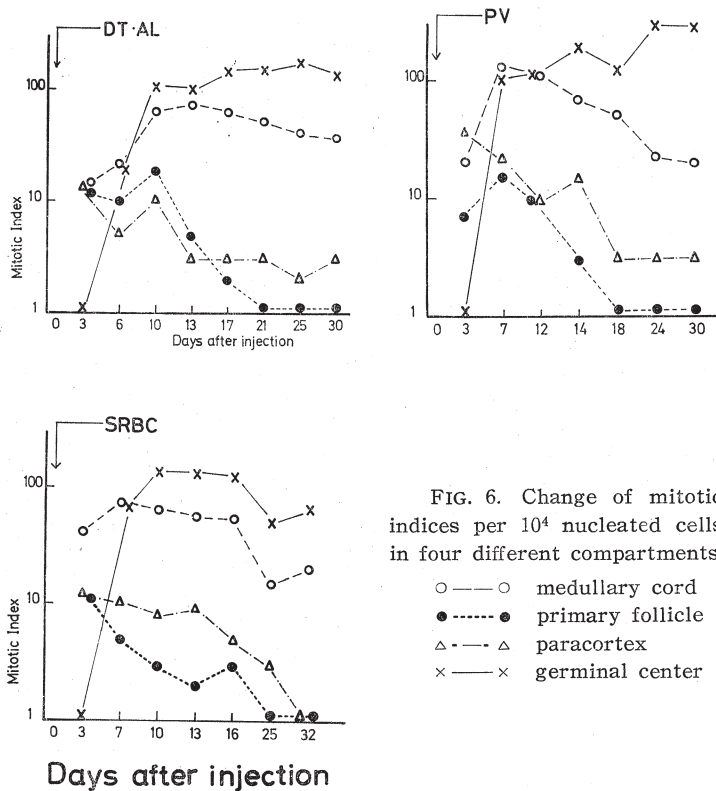


FIG. 6. Change of mitotic indices per 10^4 nucleated cells in four different compartments.

○—○ medullary cord
 ●—● primary follicle
 △—△ paracortex
 ×—× germinal center

followed by gradually decreasing curve. The mitotic indices in the germinal centers reached the maximum between the 10th to 24th day and maintained the high value until the end of experiments.

Immunofluorescent study. Cells containing specific antibody were first detected on the 5th day in the lymph nodes given BGG·FCA (Fig. 3-d). They were large cells with thin rim of fluorescent cytoplasm and were scattered mainly at or around the cortico-medullary junction. Mature plasma cells with an eccentric nucleus and fluorescent cytoplasm increased in numbers when examined on Day 10 to 12.

Antibody titer. All of three animals given SRBC were shown to have hemolysin titer on the third day. Anti-BGG antibody appeared on 6th to 7th day. The animals injected with egg albumin precipitated with alum produced the antibody by the 10th day. The curves of antibody titer in each group seemed to resemble the pattern of primary immune response (Fig. 7). The curves in each group tended to be parallel with those of the mitotic indices in the medullary cords, although the former seemed to lag slightly than the latter.

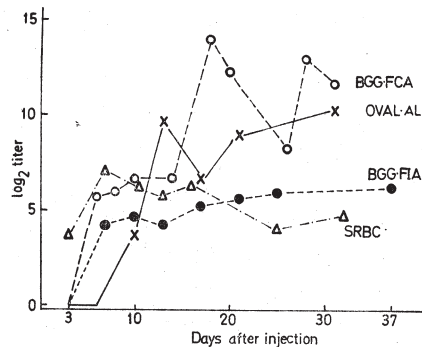


FIG. 7. Antibody titer after single injection of antigens.

DISCUSSION

A single primary injection of various antigens in this experiment always produced a consistent pattern of the development and involution in four compartments on the lymph node. This result is summarized in Fig. 8.

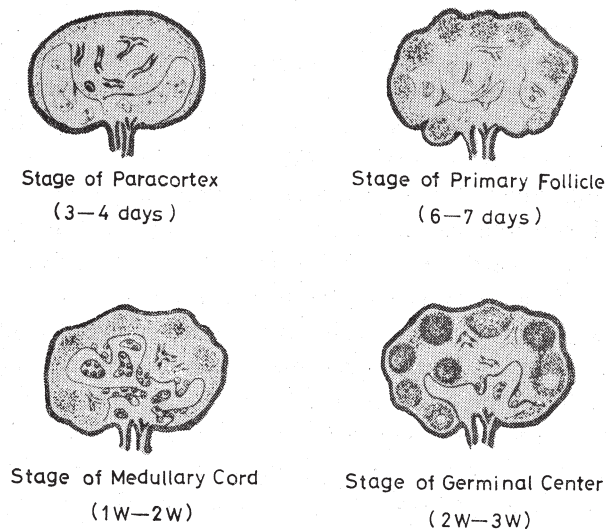


FIG. 8. Diagrammatic representation of a chain of morphological events on four histological compartments in primary immune response.

The above conclusion generally agrees with the result of the classical histopathological studies of antigen stimulated lymph node^{1)~3)23)} and compatibles with the findings obtained from the ontogenetic²⁴⁾²⁵⁾ or phylogenetic approaches^{26)~28)}. The contention that the paracortical areas were the first whereas the germinal centers were the last in reacting to the antigens has been substantiated more clearly by this experiment which took the advantage

of the immunological cleanness of the popliteal lymph nodes in very young rats.

The exact role of the germinal centers in the primary immune response has not yet been established despite a number of the works due mainly to the fact that some germinal centers had already been present even before the antigen stimulation in the conventional experimental animals. Cottier *et al.*,²⁹⁾ and Kim and Watson¹⁵⁾ demonstrated, however, that in the "true" primary immune response, the circulating specific antibody could be detected at the stage when any germinal centers had not yet been recognized. This finding seemed to deny an essential role of the center in antibody production at least at a certain early phase of the primary immune reaction. This postulation seems to be supported by the finding in the BGG group in this experiment that the appearance of the circulating antibody as well as the intense reaction of plasma cells could be observed on Day 7 or 8 when the germinal centers were essentially absent or only a few and very small if present.

On the other hand, degeneration or dissociation of germinal centers was regarded as the initial evidence of the immune response by Hanna³⁰⁾ and Kelly³¹⁾ in their experiments using the spleen of mice injected with SRBC and popliteal lymph nodes of rabbits stimulated with bovine RBC, respectively. The animals employed in these experiments, however, seem to be remote from the state of immunological cleanness and, therefore, the changes recognized earliest in the germinal centers were better interpreted as the reaction of the preexistent germinal centers against the injected antigens. Meanwhile, Thorbeck and colleagues^{32) 33)} pointed out an important role of the germinal centers in the immunological memory. The findings in this experiment that the germinal centers were the last in initiating the response and was longest in maintaining their activity seem to be in accordance with the interpretation that the germinal centers play an immunological role only at a relatively late phase of the primary immune response such as the development of the immunological memory or antibody production dependent on the memory. It is conceivable that a single primary injection of the antigen as in this experiment might not only trigger the pure primary immune response but also the secondary immune response which might start at the end of the primary immune response or even somewhat earlier than that stage³⁴⁾.

The role of the paracortical areas in the immune reaction has also been a subject of study by a number of investigators^{35) 13)}. Turk and colleagues^{36) 37)} noted proliferation of blastic cells circumscribed within the paracortical areas of the regional lymph nodes of the guinea pigs which had been painted with oxazolone, a selective inducer of cell-mediated immune reaction. They noted, conversely, that enlargement of paracortical areas was absent and, instead, germinal centers developed in the cortex together with plasma cell proliferation in the medulla and cortico-medullary region in the animals injected with

polysaccharide antigen which was supposed to produce pure humoral immune response uncoupled with cell-mediated immune response. Furthermore, a mixed type of response, *i.e.*, distension of paracortical areas as well as germinal center formation associated with plasma cell proliferation in the medulla was observed in the animals injected with bacterial or protein antigens in the form of solution, alum precipitant or emulsion with Freund's adjuvant, which produced both cell-mediated immune response and humoral antibody production. It is more reasonable to expect that the various antigens used in the present experiment produced mixed type of response rather than pure humoral antibody response. The earliest prominent change in the paracortical areas, therefore, might be only a reflection of cell-mediated component of the immune response.

There seems to remain, however, some contradiction in the postulation that the paracortical hyperplasia always reflects cell-mediated immune response. According to Micklem and Brown³⁸⁾, blastic cell proliferation within the paracortical areas was essentially absent in the mice which had received the second set skin grafting while it was prominent in the first set reaction. Furthermore, many observations^{13) 16) 35) ~39)} agreed in that the paracortical hyperplasia was most prominent at the early stage of the immune response induced by various antigens. Recently Kim and Waston¹⁸⁾, working the unstimulated "virgin" lymphoid tissues of germfree colostrum-deprived piglets, confirmed that the first morphological change was appearance of large pyroninophilic cells both in the periarteriolar lymphatic sheaths of spleen as well as the cortex and primary follicles of lymph nodes. The blastic cell proliferation attained a peak on Day 3 to 4 after injection of antigens whereas the development of germinal centers was first noted on Day 14. It might be postulated from all these results that the early appearance of paracortical hyperplasia may be a morphological reflection of the state of "true" primary immune response regardless of cell-mediated or humoral type of response.

Meanwhile, it seems established that immune response to many antigens requires a cooperation between thymus-derived and bone marrow-derived lymphocytes (a recent review of this subject is given by Miller *et al.*⁴⁰⁾). The paracortical areas were shown to be thymus-dependent¹¹⁾ and the post-capillary venules within this compartment were main entrance into lymphoid tissue for recirculating thymus-derived lymphocytes⁴¹⁾. On the other hand, Davies *et al.*¹⁶⁾ observed the mitosis of bone marrow-derived cells as well as the thymus-derived ones within the paracortical areas and suggested that the paracortical areas might be the site where two cell lines might react synergistically. Close relationship between the cell-mediated immune response and the humoral antibody production has been well known from different lines of studies^{42) 43)}. Salvin and Smith⁴⁴⁾ postulated that the delayed hypersensitivity state might be one phase of a sequence of immune reaction leading to the production of

the circulating antibody.

The present experiment demonstrated the paracortical areas were more complex than any other compartments in terms of heterogeneity of cellular components. This study also proved that the paracortical enlargement resulted from prominence of post-capillary venules, the inflow of small lymphocytes through extravasation from the venules and the proliferation of both small lymphocytes and reticular cells.

On the basis of all these data, the role of the paracortical areas in the primary immune response might be postulated as follows: the paracortical areas are essential for the induction of the primary immune response, especially in the "true" primary immune response. In the "true" primary response, the paracortical areas may provide the site either for the initial interaction between the thymus-derived and bone marrow-derived lymphocytes, or for the cell-mediated immune reaction which may be a prerequisite to the subsequent phase of the humoral antibody production. This interpretation is not contradicted with a widely believed view that the immune response of adult conventional animals commonly observed in various experiments is dominated by medullary plasmocytosis and follicular hyperplasia with germinal centers whereas the paracortical hyperplasia is not apparent. Many of these apparently primary immune response might be in fact the secondary immune response in which the paracortical hyperplasia is not an essential step.

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