Fine Structure of Germinal Center Forming Cells in Chick Spleen

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ABSTRACT. Ultrastructural changes of the cells forming germinal centers were examined in the chick spleen after intravenous injection of sheep red blood cells. The germinal center was formed by a cluster of large lymphoid cells and a septum of flattened reticular cells. The large lymphoid cell was characterized by numerous polysomes and a large euchromatin nucleus with a prominent nucleolus. In the developing germinal center the large lymphoid cells proliferated and became smaller in size. After the peak of development of the center they were gradually atrophied and finally became degenerative to exhibit pyknotic nuclei, condensed cytoplasm, some vacuoles and many cytoplasmic processes. From the formation of the germinal center through its degeneration, the transformation of lymphoid cells into plasma cells did not occur within the center. Plasma cells were appeared in the red pulp prior to the germinal center formation, and then they proliferated to aggregate around a periarterial lymphatic sheath.—KEY WORDS: chick spleen, germinal center, large lymphoid cell.


The germinal center consists mainly of large lymphoid cells belonging to B-cell line [21]. They are called germinocytes or germinalblasts, and considered to be transformed finally into plasma cells by antigenic stimulation [18], in other words the germinal center seems to be the site for the precursors of plasma cells to mature [5, 18]. However, mature plasma cells are not found in the germinal center except for the human tonsil [22].

In the chick spleen the germinal center is surrounded by a connective tissue septum and has no blood vessels [12]. It is formed by large pyroninophilic cells derived from a pericapsoidal lymphoid tissue [PELT] following antigenic stimulation [11]. Clawson et al. [5] observed transformation of germinocytes into pre-plasma cells in the germinal center of the chick spleen by electron microscopy, while Edwards et al. [8] reported migration of plasma cells into the germinal center.

In the present study, primary formation of germinal centers was induced in the chick spleen by intravenous injection of sheep red blood cells (SRBC), and ultrastructural changes of the cells composing the center were examined in detail from the formation of germinal center to its involution.

MATERIALS AND METHODS

Young White Leghorn chicks used were hatched and maintained in the Laboratory of Veterinary Anatomy, University of Tokyo. At 21 days of age, they received intravenous injection of $1 \times 10^9$ SRBC sus- pended in sterile phosphate-buffered saline. Two chicks were killed by decapitation at each stage of 1, 2, 4, 6, 8, and 13 days after injection. The spleen was collected from each chick, cut into small blocks, fixed in a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) for 2 hr at 4°C, rinsed with the same buffer, and then post-fixed in 2% osmium tetroxide for 1.5 hr at 0°C. After dehydration in a series of graded ethanol, the specimens were embedded in Epon.
Ultrathin sections were stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEOL JEM-100S).

RESULTS

In the chick spleen at one day after injection, a few large lymphoid cells were scattered in a periarterial lymphatic sheath (PALS) which was a mass of small lymphocytes surrounding a central artery. On the second day, the large lymphoid cells increased in number and some of them appeared in the vicinity of the central artery. The cell was about 7.9 μm in diameter. The nucleus was large and oval in shape, and contained a prominent nucleolus and a small amount of heterochromatin forming a thin marginal layer. The abundant cytoplasm was filled with an enormous amount of polysomes showing rosette-like arrangement, but contained very few free ribosomes. Other organelles of the lymphoid cells were poorly developed, showing some mitochondria with lamellar cristae, a small amount of rough-surfaced endoplasmic reticulum (rER), and rarely small Golgi apparatus (Fig. 1). Sometimes the mitotic figures of the large lymphoid cells were observed at the periphery of the PALS.

Small lymphocytes composing the PALS were about 4.0 μm in diameter. They belonged to T-cell line and their cellular features were the same as those of blood lymphocytes. Electron dense heterochromatin formed a thick marginal layer and a few central masses in the nucleus. The cytoplasm formed a thin rim around the nucleus and contained a few mitochondria, rER and ribosomes. Occasionally small vesicles and Golgi apparatus were observed, but polysomes were very few (Fig. 1).

The basic structure of the PALS consisted of the network of stellate reticular cells extending their processes (Fig. 1). The reticular cell was electron lucent and had a
large nucleus showing a coarse chromatin deposit with one or two nucleoli. The cytoplasm contained a few mitochondria, rER and Golgi apparatus. Fine filaments were observed at the peripheral region and cytoplasmic processes of the cell. Dark reticular cells were occasionally recognized.

On the 4th day the large lymphoid cells further increased in number and accumulated in the vicinity of the central artery to form a nodule. Their cellular features were almost the same as those at the 1st and 2nd days, except for a slight increase in the number of mitochondria. Within the nodule there were a few small lymphocytes and macrophages among the large lymphoid cells. The macrophages were irregular in shape and contained lysosomal granules and phagosomes engulfing debris of lymphoid cells. Some reticular cells were present at the periphery of the nodule.

On the 6th day the nodule of lymphoid cells was completely encapsulated by 3 to 5 layers of flattened reticular cells to form a typical avian germinal center. On the 8th day they were most numerous and well-developed. In the germinal center lymphoid cells frequently showed mitotic figures. Smaller lymphoid cells, 4.5–6.0 μm in diameter, were present along with large lymphoid cells, 7.0–8.5 μm in diameter (Fig. 2). The smaller lymphoid cells contained numerous polysomes in the cytoplasm and increased amount of heterochromatin in the nucleus. With the increase in the size of germinal centers, the circumferential layers of reticular cells encapsulating the center became more tight and thick.

The germinal centers were in the process of degeneration at the 13th day. The cellular density in the center was reduced, so the contours of the lymphoid cell became apparent. Although a few large lymphoid cells still remained normal, most lymphoid cells were atrophied, showing a pyknotic nucleus, condensed cytoplasm, and elongating processes (Fig. 3). The cytoplasm was electron dense and contained some vacuoles.
Macrophages in the center frequently phagocytized degenerative lymphoid cells (Fig. 3). The circumferential layers of reticular cells were loosened and discontinuous.

From the formation of the germinal center through its involution, rER in the lymphoid cell did not increase either in size or in number, and no plasma cell was observed within the center. In the red pulp, however, plasma cells appeared at one day after injection of SRBC. They increased in number on the 4th and 6th days, and accumulated in several cells around the PALS. The plasma cell was about 5.9 μm in diameter and had an oval nucleus with a thick marginal layer of heterochromatin to show its cart-wheel arrangement. The cytoplasm was occupied by the piles of rER. Some mitochondria and vesicles containing fine granular materials were scattered among the piles of rER.

**DISCUSSION**

In the avian spleen, plasma cells and the cells composing germinal centers and PELTs belong to B-cell line originated from the bursa of Fabricius [15, 16, 23]. Although germinal centers are considered to play a role in B-cell differentiation [13, 22, 25] as the site for the precursors of plasma cells to mature [5, 18], plasma cells appear in the red pulp before the formation of germinal centers following antigenic stimulation in chicks [2, 20, 24], ducks [23], mice [1], and rats [14].

In the present study, the germinal center in the chick spleen was formed by large lymphoid cells characterized by numerous polysomes and a large euchromatic nucleus with a prominent nucleolus. Generally, B-cells of the chicken are larger than lymphocytes of T-cell line [21]. The proliferated lymphoid cells in the germinal center became as small as lymphocytes of PALS, but
they were readily distinguished from small lymphocyte by electron microscopic observations, depending on their numerous polysomes. From the formation of the center through its degeneration there was no plasma cellular reaction, that is, lymphoid cells were not transformed into plasma cells. Although Clawson et al. [5] observed pre-plasma cells in germinal center of the chick spleen, even the slight increase of rER in the lymphoid cells was not observed in the present study. Plasma cells appeared in the red pulp on the first day prior to the germinal center formation, and increased in number with the lapse of time after SRBC injection to aggregate around the PALS.

Germinal center cells proliferate, differentiate into smaller lymphocytes, and move out to form the mantle around the germinal center [25]. Many investigators confirmed that progenitors of plasma cells migrated from the white pulp to the red pulp in mice [6, 9], rabbits [7, 16, 26], and turtles [3, 4]. In the chick spleen, however, the mantle is not formed. Avian germinal centers are encapsulated completely by connective tissue septum and usually no lymphoid cells migrate outwards from the center, although Nagy and Feher [19] reported that plasma cells were derived from the germinal center in the chicken spleen. Ogata et al. [20] studied the germinal centers of the chick spleen following the primary and secondary immunization with bovine serum albumin, and presumed that the germinal centers were divided into 2 types; one managed immunological memory and the other was the source of immunoglobulin producing cells. In the secondary immune response most of pre-existing germinal centers are dissociated rapidly, then new centers proliferate markedly [2, 10]. There is a possibility that the cells of the dissociated center may disperse and transform into plasma cells. Judging from the present observations, the germinal center seems to be not always neccessary for plasma cell maturaition.

The germinal center is always formed in the vicinity of the central artery, but the reason is not clarified. The microenvironment around the artery is considered to be adequate for the blood cells to proliferate in the spleen, because granular leukocytes and lymphocytes gather around arteries to proliferate before and after birth.

The septum of the well-developed germinal center encapsulated the center so tightly that it might prevent blood cells migration into or out of the center. Small lymphocytes and macrophages were included in the center at the time of the cell aggregations. In the degenerating germinal center, the septum was loosened and discontinuous, so that another macrophages seemed to invade the center.

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REFERENCES

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ニワトリの脾臓における胚中心形成細胞の微構造：福田勝洋, 望月公子1) (農林水産省家畜衛生試験場,
1) 日本大学農幼医学部)——半赤血球をニワトリの静脈内に投与し, 脾臓における胚中心形成細胞の変化を超微形態学的に検索した。胚中心は大型リンパ細胞の集積とこれに続く扁平な細網状細胞の隅縁と考えられ, 大型リンパ細胞は無数のポリゾーム, 正円形質に富む大型明調の核, 明瞭な核小体が特徴であった。発達中の胚中心では, リンパ細胞は分裂, 增加して, 小型細胞であるが, 細胞内小器官にほとんど変化があり, 多数のポリゾームを有していた。胚中心は発達のピークに達した後, 退行過程に入りリンパ細胞は次第に萎縮し, 核, 細胞質が濃縮し, 空胞が増し, 細胞質突起が顕著になった。形成から退行に至る期間を通じて, 胚中心には形質細胞, 形質芽胞はみられず, 胚中心でのリンパ細胞から形質細胞への変化はなかった。形質細胞は, 胚中心形成よりも早く赤脾髄に出現, 増殖して, 動脈周囲リンパ組織に接して集簇した。