Hemocytic Differentiation in Hemopoietic Organ of Bombyx mori Larvae

Authors: Han, Sung-Sik, Lee, Min-Ho, Kim, Woo-Kap, Wago, Haruhisa, and Yoe, Sung-Moon

Source: Zoological Science, 15(3) : 371-379

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.15.371

ZOOLOGICAL SCIENCE **15**: 371–379 (1998)

Hemocytic Differentiation in Hemopoietic Organ of *Bombyx mori* Larvae

Sung-Sik Han[1]*, Min-Ho Lee[1], Woo-Kap Kim[2], Haruhisa Wago[3] and Sung-Moon Yoe[4]

[1]*Graduate School of Biotechnology, and* [2]*Department of Biology, Korea University, Seoul 136-701, Korea*
[3]*Department of Medical Technology, Saitama Medical School, Junior College, Moroyama, Iruma-gun, Saitama 350-04, Japan*
[4]*Department of Biology, Dankook University, Chunan 330-714, Korea*

ABSTRACT—*Bombyx mori* L. (Lepidoptera: Bombycidae) larva was investigated with a transmission electron microscopy to determine hemocytic differentiation in the hemopoietic organ located in the prothorax. Three and/or four types of stem cells in compact islets of the organ were observed. Immatured hemocytes in loose islets of the organ were more differentiated and developed than in compact islets. Four types of hemocytes such as prohemocyte, plasmatocyte, granulocyte and oenocytoid were observed in loose islets. Each type of hemocyte was differentiated from each type of stem cell. However, none of spherulocyte was observed. Each type of hemocytes matured in loose islets was discharged into hemolymph by the tearing of acellular membrane covering the islets. These observation strongly suggests that the four kinds of hemocytes except for spherulocytes first appeared in islets and then moved to the region of loose islets in matured form. The more detailed pathway of hemocytic differentiation in *B. mori* was represented here.

INTRODUCTION

Normal cells circulating in hemolymph (blood) of animals, including insects, are relatively short-lived and need to be replaced constantly throughout life (Pimentel, 1994). A constant supply of matured hemocytes (blood cells) is required to replace those lost througout damage or senescence (Pimentel, 1994). At the beginning of the study on the process of blood cell formation (haemocytopoiesis, hematopoiesis or hemopoiesis) in insect, Gulliver (1846) reported that insects possessed no spleen, thymus, or lymphocytic organs, so that hemocytes in these animals might be entirely fabricated in the hemocoele. However, it was revealed that hemocytes in insects were produced by mitotic division of hemocytes in hemolymph or by the hemopoiesis in the hemopoietic organ (Jones, 1970; Arnold and Hinks, 1976). Since that time, considerable amount of information has accumulated on hemopoiesis among insects.

After Cuenot first stated the function of the hemopoietic organ as a phagocytic organ in 1896 (Jones, 1970), this organ has been discovered in Orthoptera (Hoffmann, 1970), Coleoptera (Brehélin, 1973), Diptera (Jones, 1962), Lepidoptera (Arvy, 1952, 1953), Hymenoptera (Jones, 1964), and Thysanura (Francois, 1975). Most studies made on the hemopoietic organ were about its location and structure or hemopoiesis. In 1980's, development of hemocytes in the hemopoietic organ was studied using the electron microscopy (Sylvia *et al*., 1983; Stoltz and Guzo, 1986; Guzo and Stoltz, 1987). Recently, Chang *et al*. (1990, 1994) reported the ultrastructure and cellular immune responses of hemopoietic organ in *Euprepocnemis shirakii* (Orthoptera: Locustidae). Huh *et al*. (1994) and Kwon *et al*. (1995) studied the ultrastructure of hemopoietic organs and the hemocytic differentiation in *Sericinus montela*. However, most of the problems of the hemogram in this organ still remain unsolved. Moreover, experimental studies reported for Lepidoptera are often contradictory and the function of hemopoietic organs has sometimes been discredited (Hinks and Arnold, 1977).

In spite of some reports on hemopoiesis in the hemopoietic organ of insects, the mechanism and pathway of hemocytic differentiation has not been clear in insects. Many researchers suggested that plasmatocytes, one type of insect hemocytes, gave rise to granulocytes, another type of hemocytes, from which all other types developed (Lai-Fook, 1973; Monpeyssin and Beaulaton, 1977; Gupta, 1979; Beeman *et al*., 1983). It is not yet clear whether plasmatocytes give rise to other types of hemocytes. It is important to determine the origin of granulocytes and plasmatocytes in order to know what type of hemocytes divide when the hemocyte population increase in hemolymph (Lackie, 1988). The number and

* Corresponding author: Tel. +82-2-3290-3424;
FAX. +82-2-928-8647.

rate of each type of hemocytes in hemolymph are changed rapidly in relation to various immune reactions in insects (Han and Gupta, 1989). This may indicate that the pathway of hemocytic differentiation needs to be efficient and rapid in relation to immune reactions and short life history of insects. The hemogram in insects, therefore, need to be investigated thoroughly.

Thus, the present study was undertaken to study the differentiation pathway of hemocytes in hemopoietic organ of *Bombyx mori* using the transmission electron microscopy.

MATERIALS AND METHODS

Insects

The larvae of silkworm, *Bombyx mori* (Baekokjam, Jam 133 X Jam 124), were obtained from the National Sericulture & Entomology Research Institute at Rural Development Administration, Suwon, Korea. These insects were maintained on leaves of the mulberry at 25 ± 1°C, 70% relative humidity and under a photoperiod of 16 : 8 (L : D).

Ultrastructure of hemopoietic organ

For the study of the ultrastructure of the hemocytes in hemopoietic organs, these organs from the 3rd, 4th, and 5th instar of *B. mori*

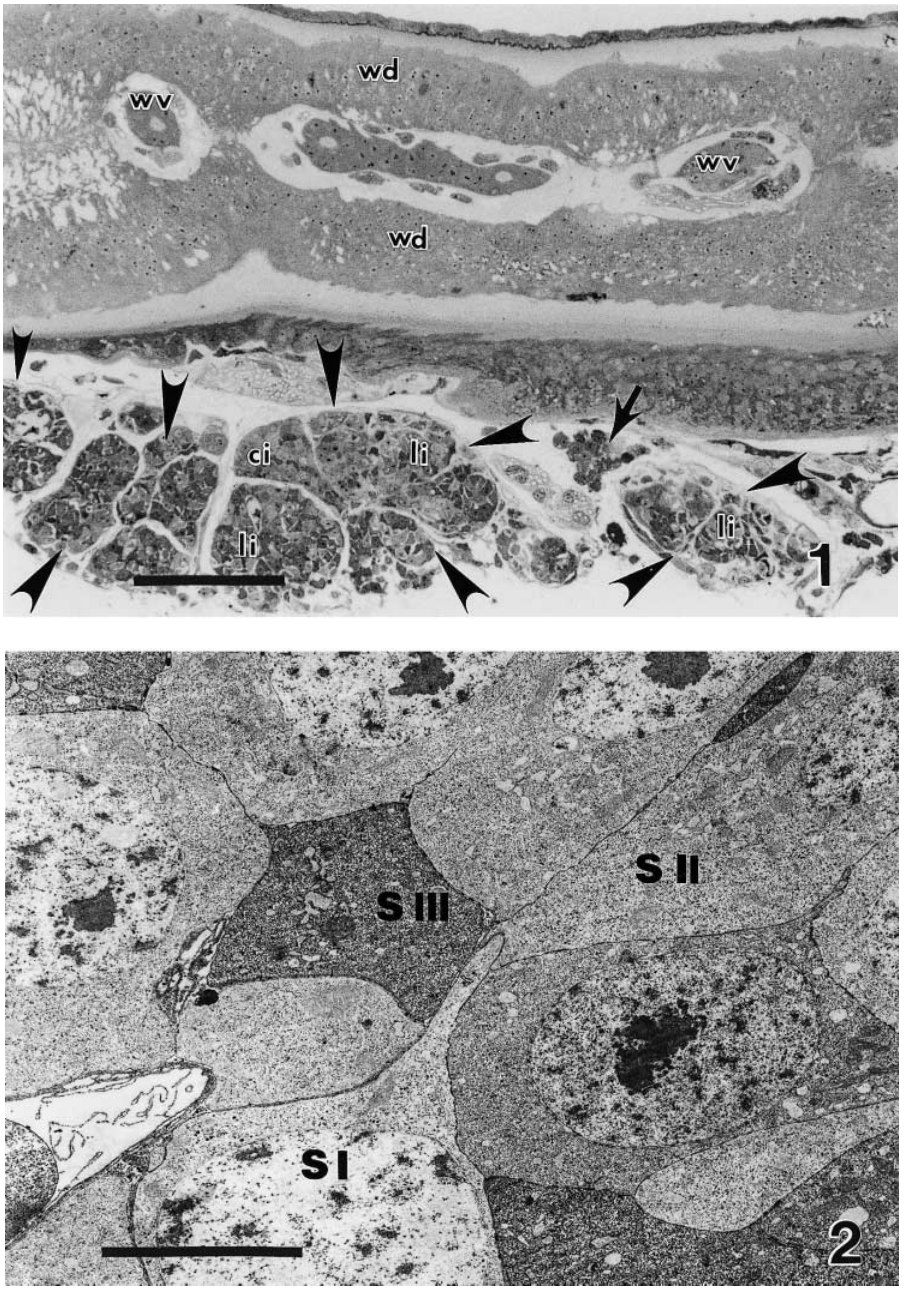
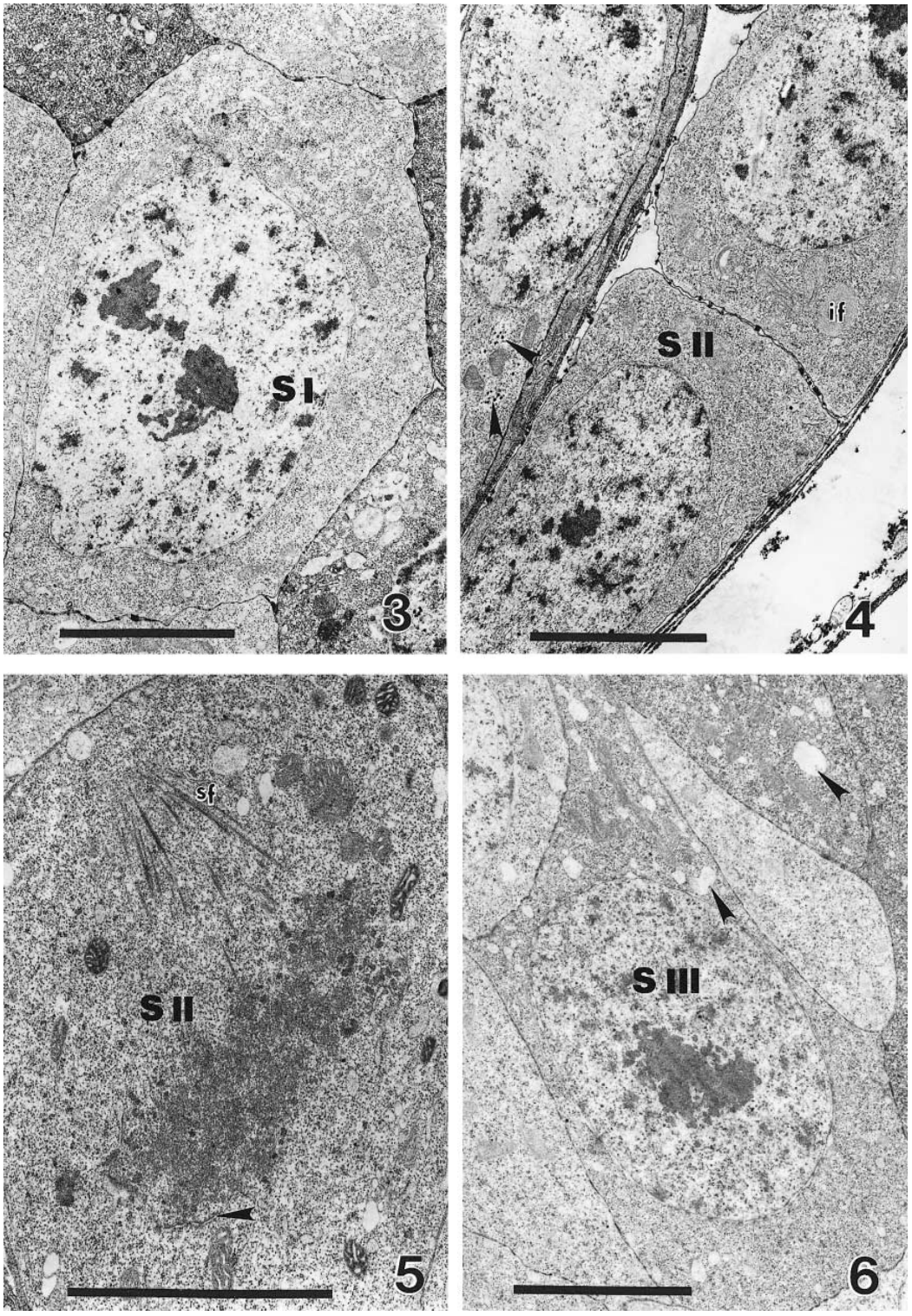


Fig. 1. Thick section of the whole hemopoietic organ (arrow-aheads) of *Bombyx mori* near the wing disc (wd). The hemopoietic organ consists of compact islets (ci), loose islets (li), and free hemocytes (arrow). These structures are surrounded by basal lamina. wv: wing vein. Scale bar = 35 μm.



Figs. 2-6. Three types of stem cells in compact islet of hemopoietic organ. Note type I stem cell (SI), type II stem cell (SII), and type III stem cell (SIII). Scale bar = 5 μm.

Fig. 2. Stem cells contact tightly each other in compact islet of hemopoietic organ. SI, type I stem cell; S II, type II stem cell; S III, type III stem cell.

Fig. 3. Type I stem cell (SI).

Fig. 4. Type I stem cell (SI) possessing glycogen particles (arrowheads) and type II stem cell (SII) possessing the arrangement of intracytoplasmic fibriles (if).

Fig. 5. Cell division of type II stem cell (SII). Microtubules of the spindle (sf) are observed and nuclear envelope was dispersed (arrow).

Fig. 6. Type III stem cell has many vacuoles (arrowheads). Scale bar = 5 μm.

were dissected under a binocular microscope and prefixed with 2.5% glutaraldehyde and 1% tannic acid (1 : 1 mixed just before use) in 0.1 M cacodylate buffer, pH 6.8, at 4°C. After prefixation for 1 hr, the organs were rinsed for 45 min at room temperature with 3 changes of the same buffer. And they were then postfixed in 1% osmium tetroxide in cacodylate buffer for 1 hr, and rinsed 3 times in the same buffer at a 15-min interval. The organs were dehydrated in a graded series of ethanol. And then, the organs were stained *en bloc* with 2% uranyl acetate in absolute ethanol for 1 hr at 60°C, rinsed twice with ethanol, and then substituted with propylene oxide. Then, the organs were embedded in Spurr kit (Electron Microscopy Sciences, USA). These organs were sectioned with glass knife on an SORVALL MT2-B Ultramicrotome (DUPONT Instruments). Ultrathin sections were collected on 200-mesh copper grids and stained in 2% aqueous uranyl acetate for 12 min and lead citrate for 8 min. Ultrathin sections were observed and photographed with a JEM 100CX-II transmission electron microscope.

Morphological identification of hemocyte

The hemocyte terminology used in this paper cited the classification of hemocytes by H. Wago (1991).

RESULTS

Hemocytes developed and differentiated in the hemopoietic organ were examined using a transmission electron microscopy. Hemopoietic organ was comparted with acellular membrane, and subdivided into either the compact (Figs. 1-6) or the loose islets (Figs. 7-12). Compact islets were lined with tracheal cells and the wing disc, while loose islets were located on compact islets (Fig. 1). This observation was similar to the report by Akai and Sato (1971).

Stem cells gathered tightly in compact islets, which may be developed into loose islets through the division of stem cells and the forking, extension, and thinning down of acellular membrane covering the hemopoietic organ (Fig. 1). Compact islets were observed in all of the larval stages (Fig. 2). However, loose islets were more often detected in the organ of 5th larval stage than in any other stage. Three or four different kinds of stem cells, different electron density and intracellular organelles, were observed in compact islets and these cells were divided actively in this islets (Table 1). We classified stem cells into 3 kinds as follow: (1) type I stem cell (Figs. 2, 3): containing glycogen particles, (2) type II stem cell (Figs. 2, 4, 5): containing the intracytoplasmic fibriles in oenocytoid of *Bombyx mori* (Wago, 1991) (Figs. 2, 5), and (3) type III stem cell (Fig. 6): containing many vacuoles which exist in developed granulocytes (Figs. 2, 6). As compact islets were developed into loose islets, stem cells in compact islets were more separated (Figs. 7, 8). A certain taking-up phenomena such as phagocytosis of autolysed cell debris by developing hemocytes was observed in developing loose islets (Fig. 8).

In loose islets, stem cells were observed to differentiate into hemocytes. For example, immature hemocytes (probably precursors) in loose islets were ultrastructurelly identified as prohemocyte, plasmatocyte, granulocyte and oenocytoid (Figs. 9-12). Coexistence of such immature hemocytes among mature hemocytes in loose islets indicates that immature hemocytes have already differentiated in compact islets respectively. Many granulocytes possessed some granules and dilated endoplasmic reticulum as shown in Figs. 11, 12, although cytoplasmic granules was amorphous electron-dense ones. Immature oenocytoid possessed the eccentric oval shaped nucleus, narrow endoplasmic reticulum and electron-dense mitochondria (Fig. 13). Basal lamina of loose islets was forked, extended, thinned down and finally disappeared when hemocytes developed and matured in loose islets (Fig. 14). Mature hemocytes discharged from loose islet were freely floated in the hemolymph. None of spherulocytes were observed here.

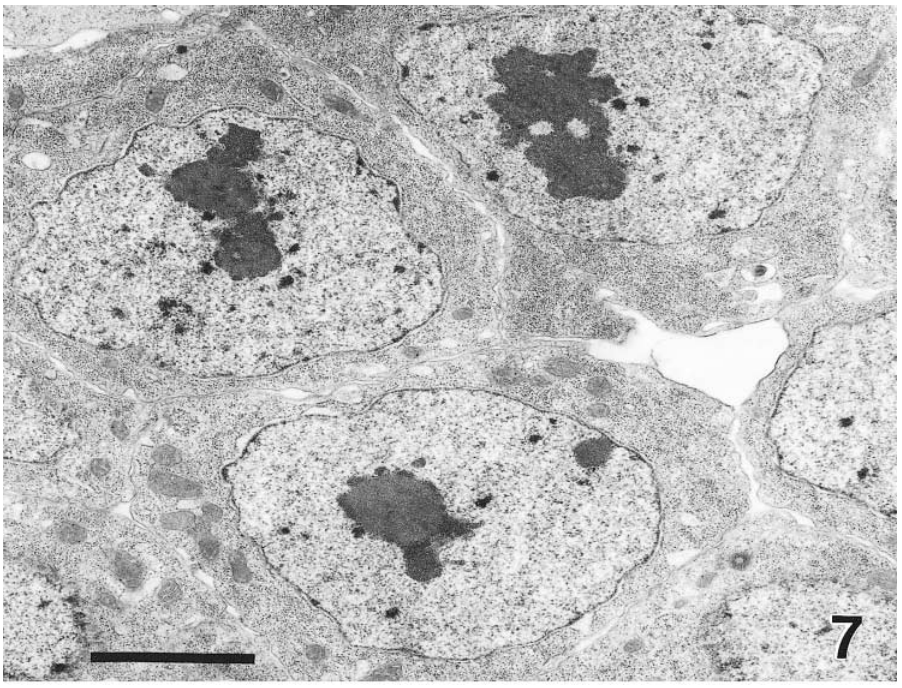
Only pluripotent stem cells were observed in this study.

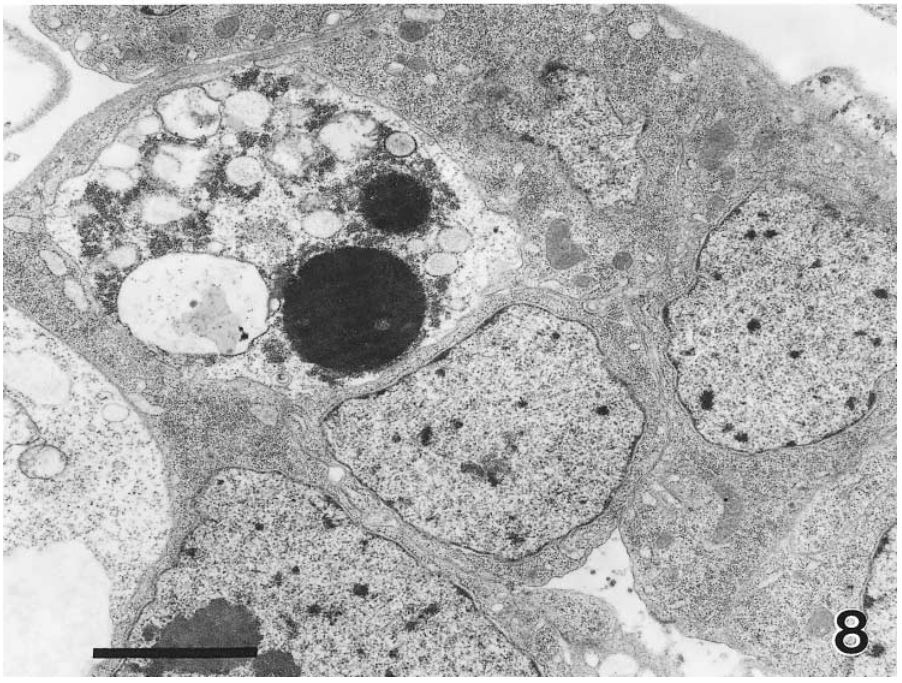
Table 1. Three types of pluripotent stem cells in the compact islets of *Bombyx mori* larva

	Intracellular Organelles[1]								
Cell Type	Mt	N	n	ER	FR	V	GP	IF	ED
Type I	typical or extended	oval (N>C)	++	± tubular dilated	±	–	–/±	–	–
Type II	two type	oval (N=C) metaphase	±	+ electron-dense more dilated	++	+	–	–/±	++
Type III	very rare localized	oval (N>C)	+	+ tubular narrow	+	+	–	–	+

These types of stem cells can also be divided into subtypes. For example, type I stem cell can divided into two sub types by the existence of glycogen particles.

[1] Mt, mitochondria; N, nucleus; C, cytoplasm; n, nucleolus; ER, endoplasmic reticulum; FR, free ribosome; V, vacuoles; GP, glycogen particles; IF, intracytoplasmic fibriles; ED, electron density; ++, very developed or many; +, developed or some; ±, poorly developed or infrequently; –, not observed.





Figs. 7-8. Stem cells developing in the early loose islet.
Fig. 7. Stem cells are loosely aggregated.
Fig. 8. Intracellular particle taken up by phagocytosis was seen in this islet. Scale bar = 2.5 μm.

By suggesting that these pluripotent stem cells must be differentiated from totipotent ones, totipotent stem cells will be expected to be observed in compact islets. Unlike finding from the others (Akai and Sato, 1971; Monpeyssin and Beaulaton, 1978), 3 or 4 types of pluripotent stem cells were observed in compact islets. Each type of pluripotent stem cells developed into different hemocyte types in loose islets. This finding indicates that cell lineage of each hemocyte, found in the hemolymph, can be formed already in compact islets. The diagramatic summary of hemocytic differentiation pathway in *B. mori* was presented in Fig. 15.

DISCUSSION

Hoffmann (1973) irradiated X-ray selectively to the hemopoietic organs of 4th and 5th larva and adult stage of

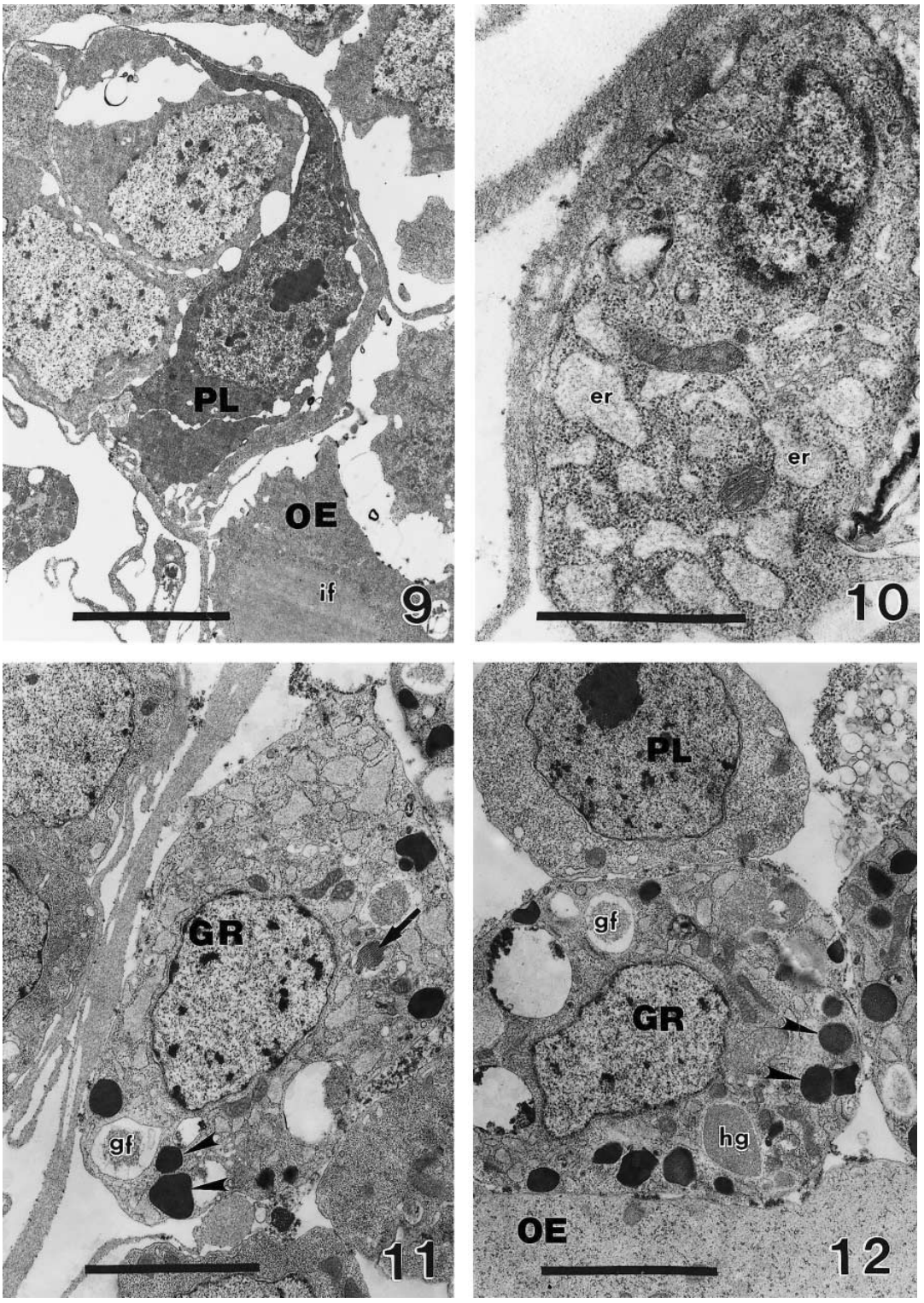
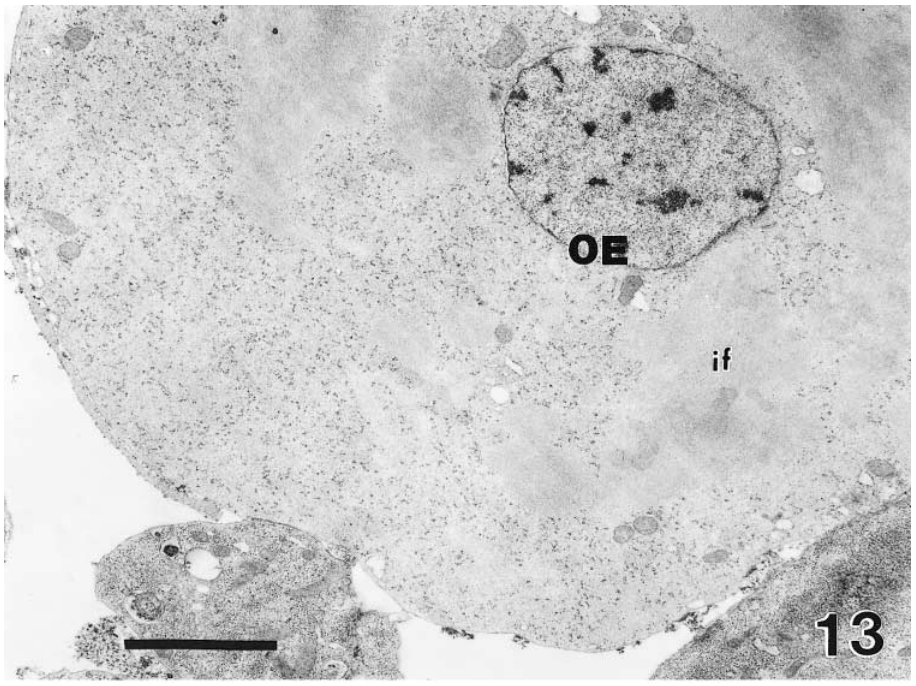
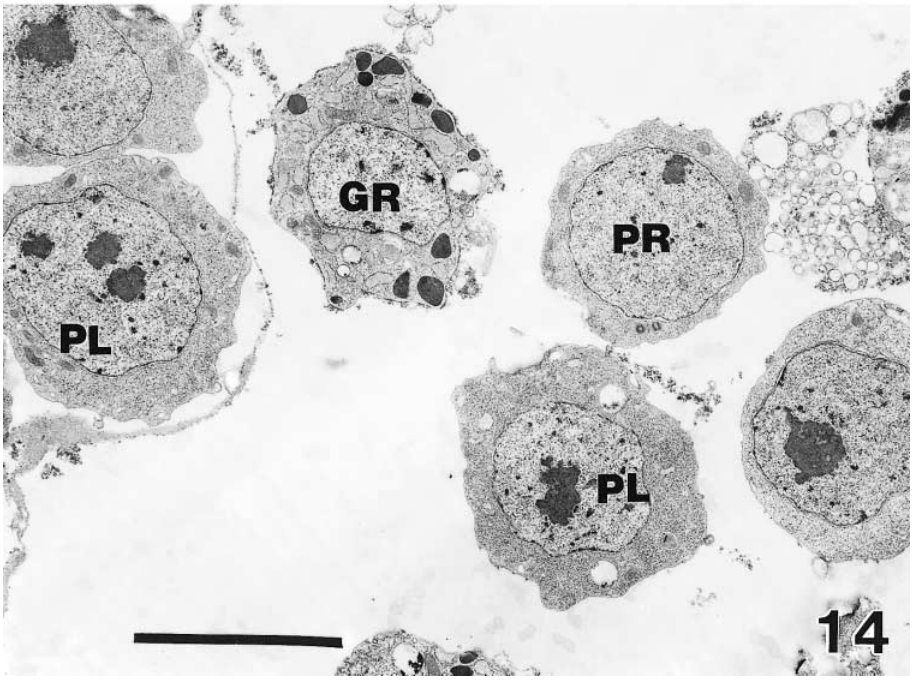


Fig. 9. Each type of immature hemocytes, plasmatocyte (PL), granulocyte, oenocytoid (OE), is observed at the same time in loose islets. Immature oenocytoid possesses the concentric arrangement of intracytoplasmic fibriles (if). Scale bar = 5 μm.
Figs. 10-12. Immature and mature granulocyte.
Fig. 10. Immature granulocyte possessing eccentric nucleus and dilated endoplasmic reticula (er). Scale bar = 2.5 μm.
Figs. 11, 12. Mature granulocytes (GR) containing four types of granules: Structureless and electron-dense granule (arrowheads), membrane-bounded granule of concentric arrangement of internal microtubules (arrow), granule-including flocculent materials (gf), and granule-including homogeneous materials (hg). PL, plasmatocyte; OE, oenocytoid. Scale bar = 5 μm.





Figs. 13-14. Matured hemocytes freely floating in loose islets before discharging (left part of this figure) and in the hemolymph (right part of this figure).
Fig. 13. Mature oenocytoid possessing many concentric arrangement of intracytoplasmic fibriles (if).
Fig. 14. Mature hemocytes discharged when acellular membrane covering loose islets were brocken down. PL, plasmatocyte; PR, prohemocyte; GR, granulocyte. Scale bar = 5 μm.

Locusta. After 24 hr of the single X-ray irradiation, the number of hemocytes decreased by about 50%. Thus, they proved that hemopoietic organ plays a primary role in the production of differentiated hemocytes during the life history of this insect. Understanding the rate and mechanism of production of hemocytes (hemopoiesis) is a very basic step in understanding the process of "immunization" (Lackie, 1988). However, the mechanism of hemopoiesis is not clear and the pathways of differentiation of hemocytes have been suggested variously from species to species in relation to investigators (Hinks and Arnold, 1977; Gupta, 1979; Beeman *et al*., 1983). This is due (1) to the different pathways which depend on morphological resemblances between each type of hemocytes in hemolymph, and due (2) to the presence and sites of hemopoietic organ

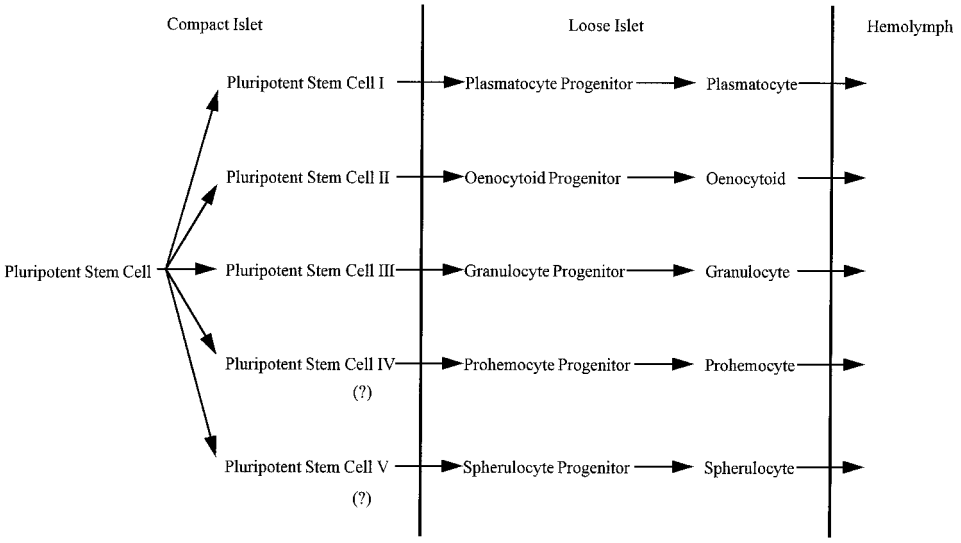


Fig. 15. A diagramatic summary of the differentiation pathway of hemocytes in *Bombyx mori*.

which differ with insect's species and developmental stage. Gupta (1979) suggested that plasmatocytes gave rise to granulocytes, from which all other types developed. The derivation of lepidopteran granulocytes from plasmatocytes is also supported by Lai-Fook (1973), Monpeyssin and Beaulaton (1978) and Beeman *et al*. (1983). Nor has been it clear whether dividing plasmatocytes give rise to other plasmatocytes as well as other cell types (Lackie, 1988). Beeman *et al*. (1983) suggested that lepidopteran oenocytes may also have an origin separated from the prohemocytes-plasmatocytes lineage.

The number and rate of each type of hemocytes in hemolymph are changed rapidly in relation to various immune reactions in insects (Han and Gupta, 1989). Han and Gupta (1989) observed 3 populations of granulocytes in hemopoietic organs of *Blattella germanica*, located on either side of the heart between abdominal first to fourth segments of this cockroach. One of these cell subpopulations consists entirely of intensely electron-dense granulocytes, and they observed an increasing number of these cells in hemopoietic organs and in their vicinity soon after implanting the surgical suture.

This may indicate that the pathway of differentiation of hemocytes has to be efficient and rapid in relation to immune reactions and short life history of insects. The hemogram in insects, therefore, needs to be investigated thoroughly. In view of this aspect, there are similarity between the differentiation pathway of hemocytes in *B. mori* and human. In human, it is well known that all types of blood cells (erythrocytes, granulocytes, monocytes, lymphocytes, macrophage and megakaryocytes) originate from common stem cells present in the bone-marrow (Pimentel, 1994; Varmus and Lowell, 1994).

Akai and Sato (1971) reported the presence of the small stem cell (cyst cell) in compact islets of the hemopoietic organ of *B. mori*. Hinks and Arnold (1977) regarded the smallest cell among all types of hemocytes in hemopoietic organ of *Euxoa declarata* as the stem cell. However, their investigations about stem cells are very lacking for the understanding for the differentiation of each type of hemocytes during postembryonic development. Our results obtained in this study suggest that three types of stem cells in compact islets may be "pluripotent", which can actively give rise to more than one type of differentiated cell. This finding and the coexistence of each type of immatured hemocytes in one loose islet indicate cell lineage of each hemocytes found in hemolymph.

ACKNOWLEDGMENTS

We wish to thank to Dr. Kwang Jun Park of the National Sericulture & Entomology Research Institute for providing all of bulberry leaves and silkworms used in this study. This study was supported (1992-1995) by the Ministry of Education, Republic of Korea.

REFERENCES

Akai H, Sato S (1971) An ultrastructural study of the haemopoietic organs of the silkworm, *Bombyx mori*. J Insect Physiol 17: 1665–1676

Arnold JW, Hinks CF (1976) Haemopoiesis in Lepidoptera. I. The multiplication of circulating haemocytes. Can J Zool 54: 1003–1012

Arvy L (1952) Particularités histologiques des centre leucopoiétiques thoraciques chez quelques Lépidoptères. C R Acad Sci 235: 1539–1541

Arvy L (1953) Données histologiques sur la leucopoiése chez quelques Lépidoptères. Bull Soc Zool Fr 78: 45–59

Beeman SC, Wilson ME, Bulla LA, Consigli RA (1983) Structural characterisation of the hemocytes of *Plodia interpunctella*. J Morph 175: 1–16

Brehélin M (1973) Présence d'un tissu hématopoiétique chez le coléoptere *Melolontha melolontha* (L.). Experientia 29: 1539–1540

Chang BS, Moon MJ, Han SS, Yoe SM (1990) Ultrastructure of the haemopoietic organ in *Euprepocnemis shirakii* Bolivar (Orthoptera: Locustidae). Korean J Electron Microscopy 20: 46–56

Chang BS, Han SS, Yoe SM (1994) Cellular Immune Reaction of Haemopoietic Organ in *Euprepocnemis shirakii*. Korean J Zool 24: 335–342

Cuenot L (1896) Etudes physiologiques sur les Orthoptères. Arch Biol Liege 14: 293–341

Francois J (1975) Hemocytes et organe hematopoietique de *Thermobia domestica* (packard) (Thysanura: Lepismatidae). Int J Insect Morphol Embryol 4: 477–494

Gulliver G (1846) The Works of William Hewson, F. R. S. London, Sydenham Society

Gupta AP (1979) Hemocyte types: their structures, synonymies, interrelationships and taxonomic significance. In "Insect Hemocytes" Ed by Gupta AP, Cambridge University Press, Cambridge, pp 86–127

Guzo D, Stoltz DB (1987) Observation on cellular immunity and parasitism in the tussock moth. J Insect Physiol 33: 19–31

Han SS, Gupta AP (1989) Arthropod immune system. II. Encapsulation of implanted nerve cord and "plain gut" surgical suture by granulocytes of *Blattela germanica* (L.) (Dictyoptera: Blattelidae). Zool Sci 6: 303

Hinks CF, Arnold JW (1977) Haemopoiesis in lepidoptera. II. the role of the haemopoietic organs. Can J Zool 55: 1740–1755

Hoffmann JA (1970) Les organes hematopoietiques de deux insectes orthopteres: *Locusta migratoria* et *Gryllus bimaculatus*. Zellforsch 106: 451–472

Hoffmann JA (1973) Blood-forming tissues in orthopteran insects: an analogue to vertebrate hemopoietic organs. Experientia 29: 50–51

Huh YH, Kwon SB, Yang HY (1994) Ultrastructure of the hemopoietic organs in *Sericinus montela* Gray (Lepidoptera: Papilionidae). Korean J Zool 37: 514–523

Jones JC (1962) Current concepts concerning insect hemocytes. Amer Zool 2: 209–246

Jones JC (1964) The circulatory system of insects. In "The Physiology of Insecta Vol 3" Ed by M Rockstein, Academic Press, New York, pp 1–109

Jones JC (1970) Haemocytopoiesis in Insects. In "Regulation of Haemocytopoiesis" Ed by Gordon AS, Appleton-Century-Crofts, NewYork, pp 7–64

Kwon SB, Huh YH, Yang HY (1995) Hemocytic Differentiation in *Sericinus montela* Gray (Lepidoptera: Papilionidae). Korean J Zool 38: 313–323

Lackie AM (1988) Haemocyte Behavior in Advanced. In "Insect Physiology Vol 21" Academic Press, pp 85–178

Lai-Fook J (1973) The structure of the hemocytes of *Calpodes ethlius* (Lepidoptera). J Morph 139: 79–104

Monpeyssin M, Beaulaton J (1977) Donnes sur la localisation ultrastructurale d'une activite phenoloxydasique dans les hemocytes circulants d' *Antheraea pernyi* au dernier age larvaire. J Insect Physiol 23: 939–943

Monpeyssin M, Beaulaton J (1978) Hemocytopoiesis in the oak silkworm *Antheraea pernyi* and some other Lepidoptera. I. Ultrastructural study of normal processes. J Ultrastruct Res 139: 79–104

Pimentel E (1994) Hematopoietic growth factors and cytokines. In "Handbook of Growth Factors Vol III" CRC Press Inc, Boca Raton, pp 1–34

Stoltz DB, Guzo D (1986) Apparent haemocytic transformations associated with parasitoid-induced inhibition of immunity in *Malacosoma disstria* larvae. J Insect Physiol 32: 377–388

Sylvia CB, Marljo EW, Lee AB Jr, Richard AC (1983) Structural characterization of the hemocytes of *Plodia interpunctella*. J Morphol 175: 1–16

Varmus HE, Lowell CA (1994) Cancer genes and hematopoiesis. Blood 83: 5–9

Wago H (1991) Phagocytic recognition in *Bombyx mori*. In "Immunology of Insects and Other Arthropods" Ed by Gupta AP, CRC Press Inc, Florida, pp 215–235

(Received November 22, 1996 / Accepted February 23, 1998)