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Full Length Research Paper

Reexamination of the role of hematopoietic organs on the hematopoiesis in the silkworm, *Bombyx mori*

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Larval hematopoietic organs (HPO) are thought as the only source of circulating hemocytes in most insects. In this paper, we re-checked the importance of hematopoietic organs to hematopoiesis in the silkworm through surgical operation to remove the organs from silkworm larvae at 12 h after 5th ecdysis. We observed that there was no significant decrease of hemocyte density but higher ratio of cell division in the HPO-removed wandering larvae. We checked and compared the total hemocytes in circulation and in 4 hematopoietic organs of each larva and found that even we suppose all hemocytes could be released from 4 organs at one time, it could not meet the circulating hemocytes increase *in vivo* due to huge difference. In order to monitor hemocytes movement in the hematopoietic organs to get information on hemocytes releasing *in vivo*, we labeled the dividing hemocytes with 5-bromo-2'-deoxy-uridine (BrdU) at 12 h after 5th ecdysis and observed BrdU-positive cells in the organs for several days. Our results show that the BrdU-labeled hemocytes were not released as quickly as we thought because there were still many BrdU-positive cells in the wandering organs and some cells even had almost no changed BrdU labeling. Therefore, the silkworm larvae have a novel hematopoiesis because circulating hemocyte division might contribute huge part to the hematopoiesis.

Key words: Hematopoietic organ, wing disc, hemocytes, surgical operation, silkworm, cell division.

INTRODUCTION

Insects and other invertebrate animals have no adaptive immunity like vertebrates. Therefore, they have to depend on the innate immunity to defend themselves against infectious organisms (Lavine and Strand, 2002; Cerenius and Soderhall, 2004; Kanost et al., 2004; Meister, 2004; Jiravanichpaisal et al., 2006; Strand , 2008; Cerenius et al., 2008). The innate immunity consists of humoral and cellular immunity. Humoral defenses refer to antimicrobial peptides, complement-like proteins and proteins that regulate melanization and clotting (Cerenius et al., 2004; Kanost et al., 2004; Jiravanichpaisal et al., 2006; Cerenius et al., 2008). Cellular immunity refers to the cellular immune responses such as phagocytosis and encapsulation that are mainly induced by circulating hemocytes (Lavine et al., 2002; Cerenius et al., 2004; Kanost et al., 2004; Meister, 2004; Jiravanichpaisal et al., 2006; Strand, 2008; Cerenius et al., 2008). Therefore, circulating hemocytes take a very important part in the innate immunity in insects since they not only produce many humoral immunity proteins but also do most of the cellular immunity works.

Just like *Drosophila* and other insects (Lavine et al., 2002; Cerenius et al., 2004; Jiravanichpaisal et al., 2006; Strand, 2008; Cerenius et al., 2008), the hemato-poietic organs are very important to hematopoiesis in the silkworm in which there are four hematopoietic organs (HPOs) in the thoracic segments (Akai and Sato, 1971;

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Beaulaton, 1979; Han et al., 1998). Under the electric microscope, the compact and loose islets, full of differentiated and undifferentiated hemocytes, are observed in the hematopoietic organs that are enclosed by an acellular membrane (Akai and Sato, 1971). The role of hematopoietic organs in maintaining hemocyte population was supported by artificial destruction e.g. X-irradiation (Hoffmann, 1972; Hoffmann, 1973) and ablation (Beaulaton, 1979; Tiwari et al., 2002) on these organs. Heavy ion beams, acting like surgical knife, were used to irradiate the larval hematopoietic organs of the silkworm with no influence on neighboring organs for the first time (Tu et al., 1999; Tu et al., 1999). Beyond prediction, the circulating hemocytes density increased in both irradiated and naïve wandering larvae. But the damage to hematopoietic organs caused by heavy ion beams was critical as it led to the loss of wings in the moths. To further prove this phenomenon, larval hematopoietic organs were surgically removed and no significant decrease of hemocyte density was observed (Ling et al., 2003a). In the HPO-removed silkworms, circulating hemocyte division was as high as almost 40% at wandering stage. Even in the wandering naïve larvae, there were almost 18% circulating hemocytes capable of dividing. It seems that the hematopoietic organs are not so important to hematopoiesis *in vivo*.

In this paper we carefully re-checked the hematopoiesis in the silkworm larvae in which all 4 hematopoietic organs were removed through surgical operation at 12 h after 5th ecdysis. We found that when the hematopoietic organs were removed, there were no significant differences of hemocytes density and of total circulating hemocytes between the naïve and HPO-removed larvae. But circulating hemocytes division in the HPO-removed wandering larvae was still higher than in the naïve larvae. Hemocytes movement in the hematopoietic organs was also monitored after being labeled with BrdU. We found that hemocytes in the organs might not be able to release in great amount daily. After comparing hemocytes in circulation and in the hematopoietic organs, we also found that even all hemocytes were supposed to be released from the hematopoietic organs at one time *in vivo*, it can not support the increasing of hematopoiesis during the whole 5th instar. Therefore, in addition to hemocytes produced from the hematopoietic organs, the silkworm also depends on circulating hemocytes division to balance its hemocytes level.

MATERIALS AND METHODS

Experimental animals

The *pnd pS* strain of the silkworm, *Bombyx mori*, was used for this experiment. They were reared on artificial diet at 25°C under a 16 h light and 8 h dark photocycle. Larval age is given in days described by Kiguchi and Agui (1981) for larval molting stage and Kiguchi et al. (1985) for the 5th instar and metamorphic stages. Day 0 indicates the day when larval ecdysis occurs (V-0). Most of 5th instar larvae begin to wander after day 4.

Morphology of hematopoietic organ under light and fluorescent microscope

Hematopoietic organ with the attached wing disc was dissected first, and then the fat bodies were carefully removed. The hematopoietic organs (with the attached wing discs) were dyed with 0.01% neutral red for less than 1 min and viewed and imaged under a light microscope connected to a CCD camera. To show a dissociated hematopoietic organ, the wing disc was carefully removed and then the separated hematopoietic organ was stained using acridine orange and propidium iodide and observed under a confocal microscope (Ling et al., 2003b).

Hemocyte counting in circulation and in hematopoietic organs

Total circulating hemocytes per microliter (hemocyte density) were counted on blood cell counter (Thoma, Tokyo) as described by Tu et al. (1999).

For hemocyte counting in the organs, the anterior or posterior hematopoietic organs with attached wing discs and fat bodies from the whole 5th larval stage were placed in 100 µl Grace's medium first. The wing disc and fat bodies were carefully separated but not removed from the media to avoid loss of culture medium in an unventilated clean bench. The dissociated hematopoietic organ was then thoroughly split open with forceps to release cells into culture medium. Some cells were still in the debris according to the observation under microscope, but it was not possible to release all cells even in fresh medium. Dissociated hemocytes were counted using blood cell counter (Thoma, Tokyo). In preliminary experiments, trypsin and chymotrypsin were used to digest the hematopoietic organ acellular membrane in order to release hemocytes. However, we found that free hemocytes obtained from the digestion method were fewer than mechanical dissociation (data not shown). Thus, in this paper, we mechanically split the hematopoietic organs open to release hemocytes for cell counting.

Surgical operation to remove hematopoietic organs

In previous paper, hematopoietic organs in larvae on the last day of 4th instar were surgically removed (Ling et al., 2003a). However, the operation at this time is not convenient because of the small size of larvae. As a comparison, in this paper larvae at 12 h after the 5th ecdysis (V-0) were anesthetized with diethyl ether and sterilized with 75% alcohol for operation. Using sharpened forceps, the hematopoietic organs were removed with the attached wing discs under binocular microscope in a clean bench. All removed hematopoietic organs were checked for their integrity under light microscope. If there was any loss to the hematopoietic organs or wing discs, the silkworm larvae would be discarded. The larvae received hurts on legs served as the sham-operation. The wound sites were not sealed because no cases of fatal blood leakage occurred. Larvae with the hematopoietic organs removed are called the HPO-removed in this paper.

BrdU labeling and detecting

Silkworm larvae (at 12 h after the 5th ecdysis: V-0: 12 h) were weighed and injected with 5-bromo-2'-deoxy-uridine (BrdU, Amersham) 0.5 mg/g body weight after being anesthetized. Then they were dissected for hematopoietic organs 1, 3 and 5 days after BrdU injection.

The hematopoietic organs that had incorporated BrdU were fixed in Carnoy's solution for 20 min and embedded in paraffin and sectioned as usual. Samples (5 µm in thickness) were fixed on slides coated with Biobond Tissue Section Adhesive (BBInterna-

tional). A Cell Proliferation Kit (Amersham) was used to probe BrdU-labeled cells in the organs. In order to minimize the influence by endogenous peroxidase, slides were incubated in 1% hydrogen peroxide solution for 20 min at room temperature and washed 3 times (5 min each) in phosphate-buffered saline (PBS) (NaCl 137 mmol/L, KCl 2.7 mmol/L, Na_2HPO_4 4.3 mmol/L, KH_2PO_4 1.4 mmol/L, pH 7.0). Finally, the slides were immersed in PBS and heated in a microwave oven (1 kW) for 1 min to expose the antigen (Dover and Patel, 1994). The detection of BrdU-labeled hemocytes was according to the protocol by the manufacturer.

To study circulating hemocytes proliferation, hemolymph from the HPO-removed, sham-operated and naive wandering larvae was pooled from at least six individuals and centrifuged at 3,000 rpm for 5 min. The precipitated cells were fixed and embedded in paraffin and sectioned as above. 5-Bromo-2'-deoxy-uridine Labeling and Detection Kit I (Roche) was used to probe BrdU-labeled hemocytes. Hoechst 33342 (2 μg/ml, 10 min) was used for counter-staining.

To observe the morphology of single hemocyte after BrdU labeling, hemocytes were temporal cultured on glass slide for 10 min in Grace's medium. Hemocytes were washed for 3 times with PBS buffer. Then hemocytes were fixed in 4% paraformaldehyde solution for 30 min. Hemocytes were washed with PBS buffer for another 3 times and then fluorescently labeled as above. A fluorescence microscope (Nikon Eclipse E600 System) was used for observation of BrdU-labeled hemocytes.

In a word, in this paper we prove again that the hematopoietic organs are not so important to the hematopoiesis through operation to remove all hematopoietic organs from larvae in the early 5th larval stage but not before 4th molting stage. We found that the circulating hemocytes division still takes an important role of keeping the hematopoiesis. It makes a great supplement to the previous work through comparing the total circulating hemocytes between the operated and naïve larvae and through monitoring cells movement in the organs.

RESULTS

Loss of hematopoietic organs could not affect hematopoiesis on the wandering stage

In the silkworm, *B. mori,* there are 2 pairs of hematopoietic organs in the thorax section. The hematopoietic organ sits between the wing disc and fat body (Figure 1A). After staining the dissociated hematopoietic organ in a mixture of acridine orange and propidium iodide, we observed that it is enclosed by acellular sheath as the observation under electric microscope (Akai and Sato, 1971). The organ has many acridine orange positively stained green cells and propidium iodide positively stained red cells inside (Figure 1B). In the removed hematopoietic organ, there are some fiber-like materials connecting to the wing disc. The spiracles extended and dispersed inside the hematopoietic organ (arrow in Figure 1B). The compact morphology of hematopoietic organ and wing disc makes them easily to be removed by surgical operation. When the hematopoietic organs were surgically removed from larvae at 12 h after 5th ecdysis, the larvae could still develop into pupae and moths except that they had no wings (Figure 1C and 1D). On the surface where the wings should grow out, there were melanized materials left even though the hurts were made on the larval stage (over 10 days ago for the pupa and 15 days ago for the moth). When the hematopoietic organs are lost, will the larvae lose the normal hematopoiesis as Beaulaton observed before (Beaulaton, 1979)? We then checked larvae whose hematopoietic organs were surgically removed and found that the hemocyte density in the HPO-removed, sham-operated and naïve larvae had no significant difference around the wandering stage when the naïve larvae are in need of huge hemocytes for metamorphosis (Figure 1E). We also observed the same results before wandering stage (Data not shown). As to the total circulating hemocytes, there were almost the same between the naïve and HPO-removed larvae on the whole 5th instar (Figure 5B).

Types of circulating hemocytes division *in vivo*

The HPO-removed silkworm larvae had almost the same hematopoiesis as the naïve and sham-operated larvae, which suggests that circulating hemocytes had to be induced into division in order to meet the increasing hemocyte level since there are no other organs that can produce hemocytes. To check circulating hemocytes division, BrdU was injected to label dividing hemocytes. We found that some prohemocytes (Figure 2A, 2B and 2C), granulocytes (Figure 2D, 2E and 2F) and plasmatocyte (Figure 2G, 2H and 2I) could incorporate BrdU and would divide in the future. Many circulating oenocytoids were also observed to have double nuclei (Ling et al., 2003b) and they can obviously divide. Therefore, in the silkworm, there are at least 4 types of circulating hemocytes that can divide.

Circulating hemocytes division is an important source of hematopoiesis

Circulating hemocytes in the naïve, sham-operated and HPO-removed larvae were injected with BrdU to label dividing hemocytes as above. Circulating hemocytes on wandering stage were collected by centrifuge for tissue section. Sections were fluorescently labeled with Hoechst 33342 for counting (Figure 3A, 3B). Comparing with the naïve and sham-operated larvae, the silkworm larvae with hematopoietic organs removed had significant higher ratio of BrdU-positive hemocytes (almost 40%) on wandering stage (Figure 3C). Even in the wandering naïve larvae, there are about 18% circulating hemocytes that can incorporate BrdU. The results are almost the same as previous work (Ling et al., 2003a). Therefore, in the future, in order to prepare silkworm larvae without hematopoietic organs, we can select larvae at V-0 or so for operation if taking the convenience of operation into consideration. Before wandering stage, circulating hemocyte division in the HPO-removed larvae was also the same as the previous work (data not shown). Obviously the removal of hematopoietic organs incites circulating

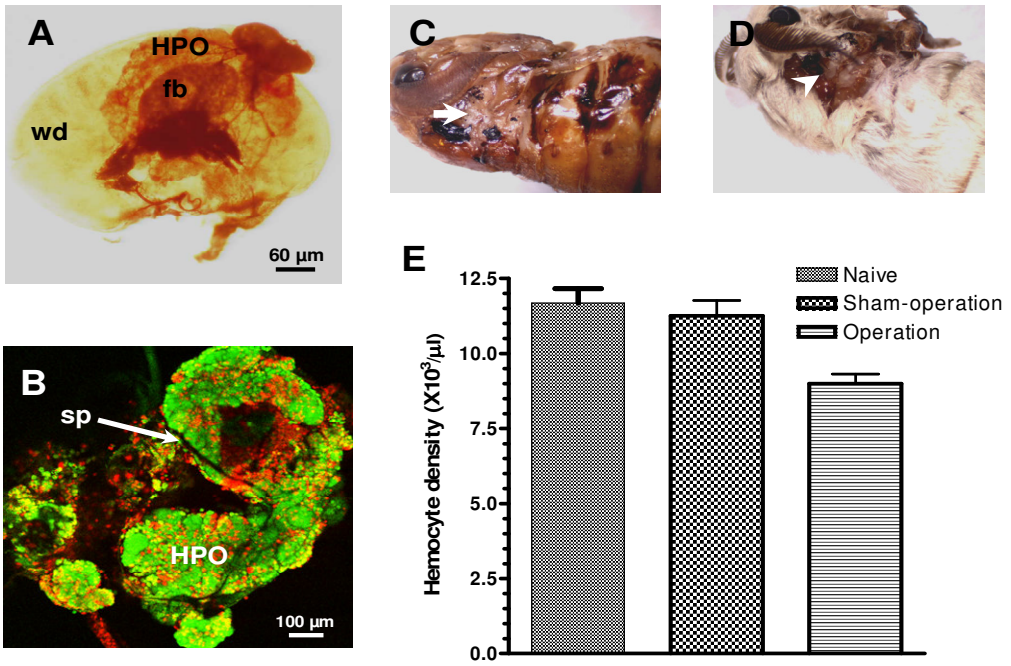

Figure 1. Loss of larval hematopoietic organs by surgical operation did not significantly affect hematopoiesis. (A) Hematopoietic organs sit between the wing discs and fat bodies. Neutral red was used to stain the dissociated hematopoietic organ. (B) A separated hematopoietic organ was stained with acridine orange and propidium iodide. Spiracle extends and disperses inside the hematopoietic organ, which might also help to compact the organ. (C) A pupa developed from the HPO-removed larva and the melanized materials were left on the place where the wing is supposed to grow out (arrow). (D) Pupa as shown in (C) developed into a moth that could also lay eggs later. Around the supposed wings (arrowhead), there were no scales but melanized material left. (E) When larvae at 12 h after 5th ecdysis were operated to remove hematopoietic organs, the hemocytes density among three treatments had no significant difference. There were 5 - 7 larvae for each treatment and this experiment was repeated 3 times with similar results. Note: wd: wing disc; HPO: hematopoietic organ; fb: fat bodies; sp: spiracle.

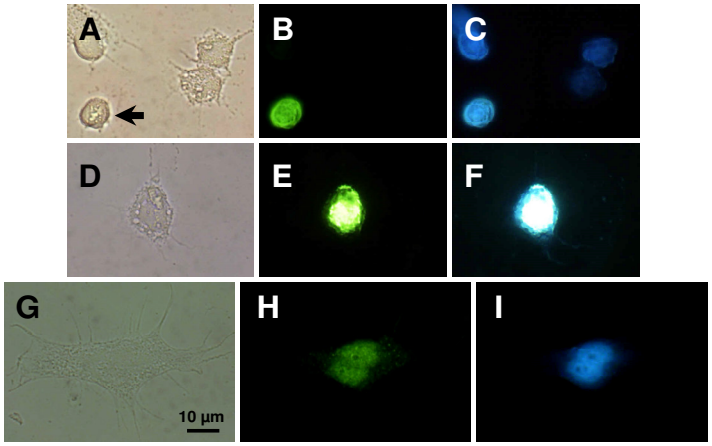

Figure 2. Hemocyte division in circulation. Silkworm larvae on day 2 of 5th instar (V-2) were injected with 0.5 mg/g body weight of BrdU. Circulating hemocytes were fluorescently labeled to probe dividing cells (BrdU-positive cells). In (A), the arrow points to a prohemocyte. Hemocyte in (D) is a granulocyte, and the cell in (G) is a plasmatocyte. Green (B, E, H): cells already incorporated BrdU and was fluorescently labeled following the protocol. Blue (C, F, I): Hoechst 33342 staining of nuclei.

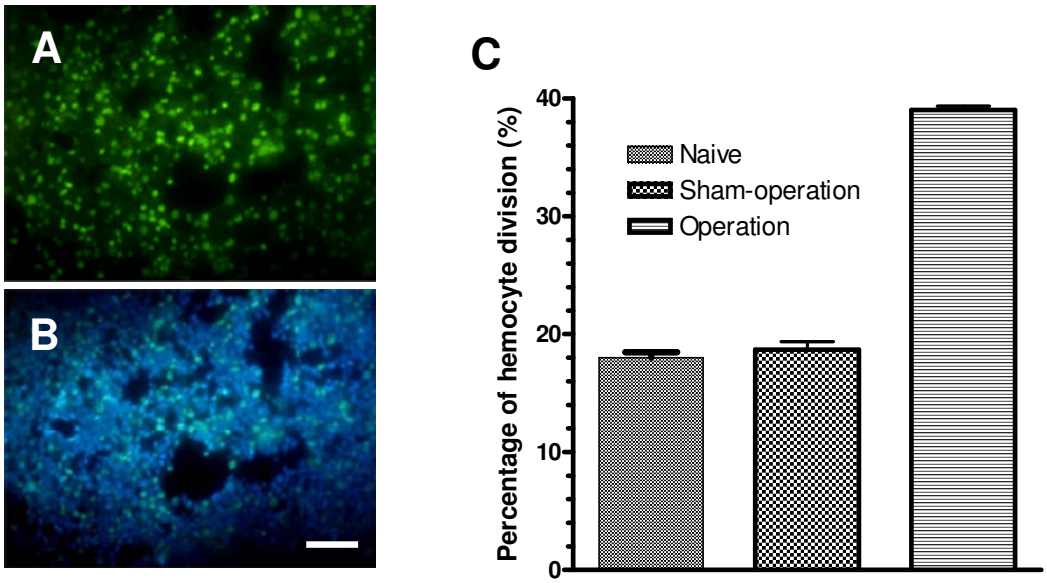

Figure 3. Loss of hematopoietic organs incites circulating hemocytes into intensive division. Larvae (at 12 h after the 5th ecdysis) were operated to remove hematopoietic organs. Silkworm larvae were injected with BrdU to label dividing hemocytes everyday (until pre-pupa stage). (A) Hemocytes from larvae on day 0 of wandering stage (W-0) were pooled by centrifuge for tissue section, and cells were probed for BrdU as shown in Figure 2. (B) Hoechst 33342 was used to label cell nuclei for cell counting. The image was merged using green and blue filters. (C) Silkworm larvae without hematopoietic organs had higher ratio of hemocytes division than the naïve and sham-operated larvae on the wandering stage. For each treatment, 5 - 10 larvae were assayed. This experiment was repeated 3 times with similar results.

hemocytes into intensive division in an unknown mechanism.

Hematopoiesis directly contributed by hematopoietic organs is not able to balance the increasing of hemocytes in circulation

Hemocytes in 2 pairs of anterior and posterior hematopoietic organs of each larva were counted after being mechanically split open to release cells out. Total hemocytes in all hematopoietic organs increase a lot towards V-3 stage and end with a summit on W-0 stage. Then it decreases after W-0. Obviously, hemocytes in all hematopoietic organs of each larva are below 7×10^4 on W-0 (Figure 4A).

During the whole 5th larval stage, total circulating hemocytes can be generally calculated according to the following equation:

Total circulating hemocytes = Body weight × 25% [Ratio of hemolymph to body weight; (Nagata et al., 1980)] ÷ 1.037 [Hemolymph specific gravity; (Sakurai et al., 1955)] × Hemocyte density (on average)

These data were a general calculation on average. In fact, if sessile hemocytes were counted, the total number of circulating hemocytes would have been much higher. According to Figure 4A, the total circulating hemocytes increased from V-0 (day 0 of 5th instar) to W-0 (day 0 on wandering stage) and then decreased significantly after W-0 in naïve and HPO-removed larvae. The values are almost the same among the 2 treatments. The total hemocytes in 2 anterior and 2 posterior hematopoietic organs on average from naïve were put in the same picture. On wandering stage (W-0), the huge difference of hemocytes produced in the hematopoietic organs (6.9×10^4) and hemocytes in circulation (5.1×10^6) of each larva made it unlikely that the increasing of hemocytes in circulation were balanced simply by hemocytes releasing from the organs.

Comparing the net change of circulating hemocytes in amount with that of the total new-born hemocytes from all four hematopoietic organs daily, we found that the difference between them was also significant (Figure 4B). It was clear that, hemocytes produced from all hematopoietic organs in each larva could not balance the net change of circulating hemocytes in amount at any time checked. Although this comparison was not very accurate, there is no better way to show the huge difference of hemocytes in circulation and those produced by hematopoietic organs during the wandering stage when the circulating hemocytes come to the summit.

From W-0 to W-1 stage, circulating hemocytes decrease a lot (Figure 4B). During metamorphosis, the increasing rate of phagocytosis might also accelerate the senescence and death of circulating hemocytes (Wago and Ichikawa, 1979). On the other hand, high concentra-

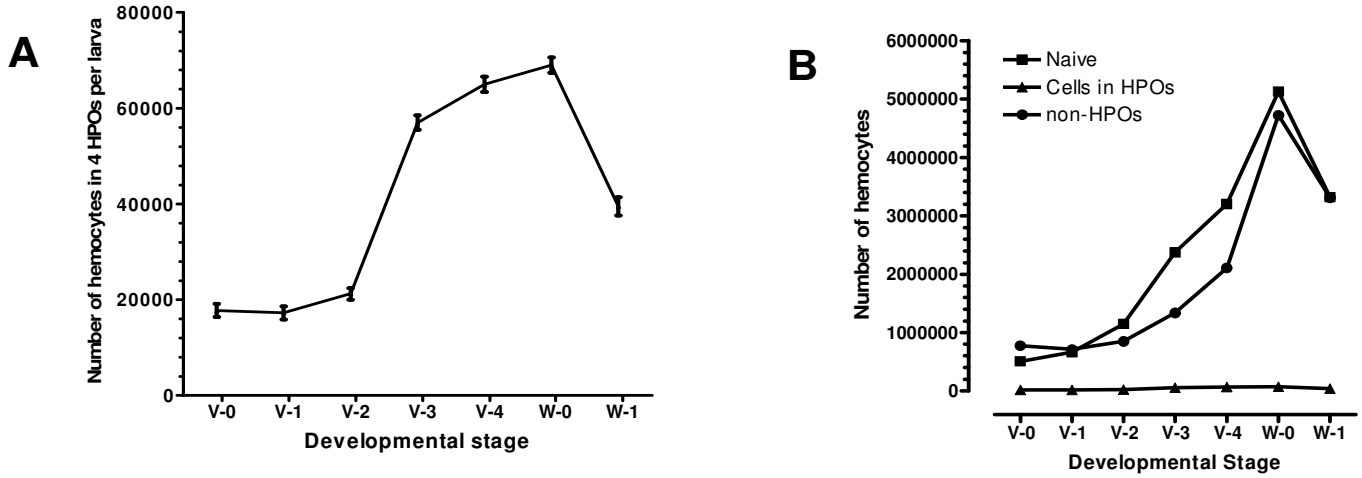

Figure 4. Comparing total hemocytes in 4 hematopoietic organs and in circulation. (A) Stage-dependent changes of hemocytes in hematopoietic organs from V-0 to W-1 (day 1 of wandering stage). Hemocytes were released from 2 pairs of hematopoietic organs of each larva after splitting the organs open to free cells. The number of hemocytes in the organs increased from V-0 to W-0 but decreased after W-0. (B) Total hemocytes in the 2 pairs of hematopoietic organs and in circulation are compared between the naïve and HPO-removed larvae on the whole 5th instar. Total circulating hemocytes of the two treatments were almost the same but were far more than cells in hematopoietic organs of naïve larvae.

tions of ecdysteroids in wandering larvae (Kiguchi et al., 1985) should induce hemocyte apoptosis *in vivo* similar to the case *in vitro* (Ress et al., 1997; Ress et al., 2000), and this may also lead to a decrease in the number of circulating hemocytes.

Hemocytes movement in the hematopoietic organs

The above picture of hematopoiesis *in vivo* obviously shows that the worms need many more hemocytes into circulation than the hematopoietic organs can supply. Therefore, it is necessary to know more about the speed of hemocyte releasing from the hematopoietic organs *in vivo* to understand hematopoiesis better. It is said that hemocytes in hematopoietic organs are released continuously in the silkworm (Akai and Sato, 1971). So far there is no effective method to monitor hemocyte movement in the organs. In order to trace hemocyte release, we labeled dividing hemocytes in the hematopoietic organs with BrdU at 12 h after the 5th ecdysis. Twenty-four hours later, a large number of hemocytes in the organs were observed to have incorporated BrdU, suggesting that cell division was very active during this period (Figure 5A). Within the next 48 h (V-3: 12 h), the BrdU-labeled hemo-cytes showed weak coloration (Figure 5B). One hundred and twenty hours (W-0: 12 h) after labeling, there were still many BrdU-labeled hemocytes but with weak colora-tion (Figure 5C). However, some hemocytes at 120 h post BrdU-labeling still had strong BrdU staining as those observed at 24 h (arrows in C), suggesting that they did not divide as many times as other hemocytes, and interestingly remained in the organs 120 h post-labeling. In the magnified picture of the selected area in Figure 5C, we could see that a lot of BrdU-labeled cells had very weak color, similar to a dark point (Figure 5D). During the wandering stage, hemocytes are finally released from the hematopoietic organs through an opening in the acellular sheath upon the increasing concentration of ecdysteroids in hemolymph (Akai and Sato, 1971; Hinks and Arnold, 1977). The preponderance of BrdU-labeled cells in the hematopoietic organs suggests that hemocytes are not released quickly from the organs even during the wandering stage. If larvae have to depend on hemocyte releasing from the hematopoietic organs to increase circulating hemocytes, the BrdU-positive hemocytes are likely to be very few 5 days after labeling because several generations of cell division and releasing could dilute BrdU significantly. Therefore, we suggest that hemocytes are not released at high speed in the silkworm as previously thought.

DISCUSSION

Before comparing the total hemocytes in hematopoietic organs and in circulation per larva, we once believed that hemocytes released from the hematopoietic organs were enough to account for the increase in the number of circulating hemocytes during the wandering stage. However, the net change of circulating hemocytes in amount before W-0 was in fact much higher than cells produced by all four hematopoietic organs on each day according to Figure 4B. On the other hand, releasing of hemocytes from the hematopoietic organs during the whole 5th instar was not as fast as we expected based on their movement in the hematopoietic organs (Figure 5). In the hemato-

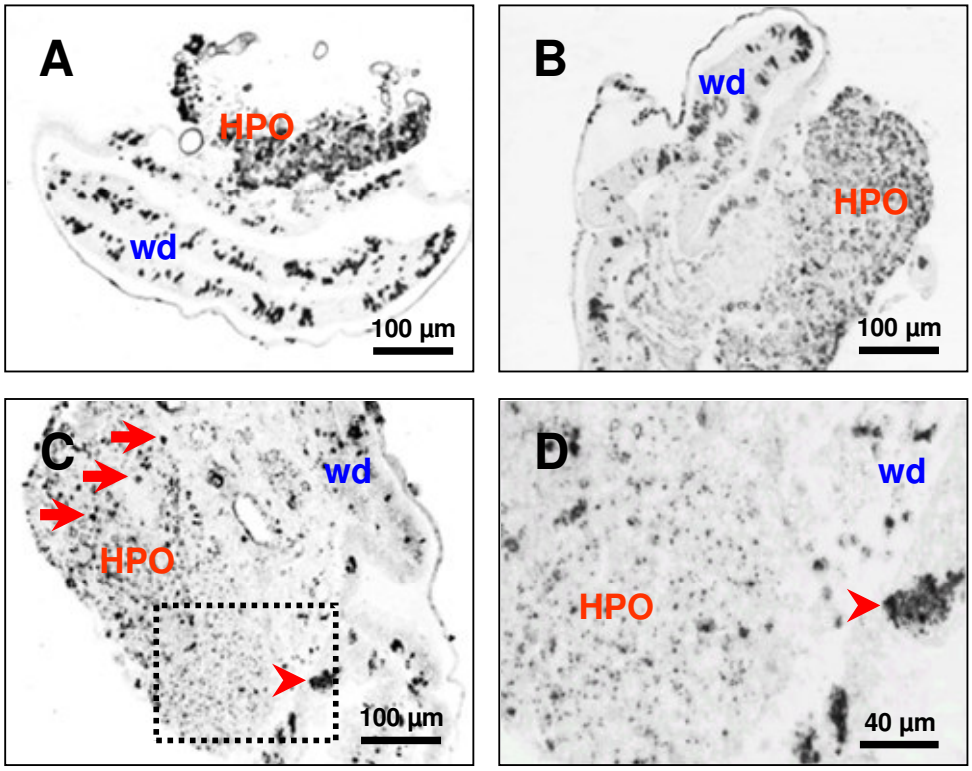

Figure 5. Hemocytes movement in the hematopoietic organs. Naïve larvae at 12 h after the 5th ecdysis were injected with BrdU to label dividing hemocytes in the hematopoietic organs. Hematopoietic organs were sampled 1 (A), 3 (B) and 5 (C) days after BrdU injection. (D) Enlargement of the selected area in (C). Arrows point to some hemocytes with strong color reaction 5 days after labeling. Arrowhead shows the accumulated BrdU-labeled cells in the wing discs. Obviously, some hemocytes (arrow-pointed cells in C) did not divide many times and still stayed in the organ for 5 days until the time we checked. At each pointed time, 5 larvae were checked with similar results. Note: wd: wing disc. HPO: hematopoietic organ.

poietic organs (in BrdU-injected larvae), there were still many BrdU-positive hemocytes with the same color reaction as cells observed on the first day (5 days before) (arrows in Figure 5C). This meant that those arrow-pointed hemocytes did not divide as many times as other hemocytes and even remained in the organs for 5 days post BrdU-labeling. If hemocytes could be released quickly from the hematopoietic organs, the BrdU-labeled cells should have been diluted faster upon several generations of cell division and releasing. At least 5 days later (on W-0) when hemocytes are said to be released violently from the hematopoietic organs due to the increase of ecdysteroids in hemolymph (Akai and Sato, 1971), BrdU-labeled hemocytes in the organs should be fewer than we observed. When the hematopoietic organs were removed from the 4th instar larvae, the number of total hemocytes was not significantly lower than that of the naïve larvae (Ling et al., 2003a). Therefore, hematopoiesis contributed by hematopoietic organs only can not balance the increase of circulating hemocytes during the 5th instar larval development, and the division of circulating hemocytes is also very important even in the naïve larvae. In fact, when circulating hemocytes were labeled with BrdU in naïve larvae on W-0, about 18% circulating hemocytes were capable of cell division. But in the HPO-removed larvae, almost 40% circulating hemocytes could divide (Ling et al., 2003a). So the high ratio of cell division is another important source of hematopoiesis in the HPO-removed as well as in the naïve wandering larvae.

In *Drosophila* larvae, maintenance of circulating hemocytes involves proliferation of cells already in circulation and the production and release of cells from hematopoietic organs (lymph glands) (Lanot et al., 2001; Holz et al., 2003). Proliferation of cells already in circulation appears to be more important for *Drosophila* hematopoiesis during early larval instars, whereas the production and release of cells from hematopoietic organs occurs primarily late in the third instar (Lanot et al., 2001; Holz et al., 2003; Strand, 2008). From our data, we can see that maintenance of circulating hemocytes during the larval stage in silkworm may also depends on both proliferation of cells already in circulation and the release of cells from hematopoietic organs. But unlike *Drosophila*, in which the

lymph glands are a major site of hematopoiesis at late larval stages (Meister, 2004), silkworm seems to be more dependent on hemocyte division for hematopoiesis even at wandering stage (Figuer 1E; Ling et al., 2003a).

In insects, although no other cells are able to differentiate into hemocytes directly, hemocytes were seen to release into culture medium from yolk sac (Locci et al., 1998) and pupae hindgut culture (Judy and Marks, 1971). Jones (Jones, 1962) had concluded that hemocytes of some insects could turn into connective tissues, urate cells, fat body cells and even certain skeletal muscles. But could these changes be reversible? Unfortunately, so far there is no proof of it. However, the HPO-removed silkworm is still a novel model for studying hematopoiesis and hemocyte differentiation further. As we know, human beings and other mammals cannot live without their hematopoietic organs. Although the study of hematopoietic organs and hematopoiesis in *Drosophila* has been leading in the field (Meister, 2004; Crozatier et al., 2007), scientists have no way to physically destroy the *Drosophila*'s hematopoietic organs (lymph glands) because of its small sizes. Therefore, the HPO-removed silkworms offer a good chance to study hematopoiesis further, especially to further determine the mechanism of induced cell division. Since the silkworm genome is getting better understood, it is the opportune time to study the silkworms' novel hematopoiesis using proteomics and genomics methods.

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