Organization of Hematopoietic Tissue in the Intermolt Lobster, *Homarus americanus*

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**ABSTRACT** Hematopoiesis in the American lobster *Homarus americanus*, as in most decapod crustaceans, occurs in a thin tissue covering the dorsal surface of the foregut. This tissue is composed of loosely attached, ovoid lobules containing the hematopoietic precursors and maturing hemocytes. Release of hemocytes into the dorsal hemocoel is accomplished by rupture of a portion of the connective tissue capsule covering the lobule. Cross sections of the lobules contain between 6 and 40 hematopoietic cells, of which approximately 90% constitute stages in granulocyte maturation and 10% are intermediates in hyaline cell maturation. Hematopoietic precursors in these two lines are similar to those recently described in a penaeid shrimp *Stenotis insignis*. The mitotic rate averaged 5.1% (range = 0.7% to 15.8%) in intermolt lobsters, 90% comprised granulocyte precursors. © 1993 Wiley-Liss, Inc.

During the late 1800s and early 1900s, blood cell forming organs, identified by the presence of dividing and maturing hemocytes, were described in several decapods (Allen, 1893; Cuénot, '04; Betances, '21; Bruntz, '07; Kollman, '08). More recent work at the light microscopical level has described the basic architecture of hematopoietic tissue (HPT) in lobsters (Fischer-Piette, '31; Johnson, '80), hermit crabs (Arvy, '52; Charmantier, '72), crayfish (Bohm and Gerach, '83), and shrimp (Demal, '83; Oka, '69; Martin et al., '87). Johnson et al. ('81) have also described the response of this tissue in the lobster to the lethal disease, gaikemia.

There appear to be two basic patterns for crustacean HPT. In penaeid shrimp, the bulk of the HPT occurs as a pair of nodules on the dorsolateral surface of the foregut. Each node is composed of a number of vessels that branch repeatedly from the hematopoietic artery, which in turn branches from the ophthalmic artery a short distance anterior to the heart (Oka, '69; Martin et al., '87). The wall of the hematopoietic artery, as well as the smaller vessels in each node, is thick and contains stem cells and maturing hemocytes embedded in a collagenous extracellular matrix (ECM). In some penaeid shrimp, secondary lobules of HPT have been described at the base of the pericardial lobes (Bell and Lightner, '88).

The HPT of other decapods follows the pattern best described by Johnson ('80) for the blue crab *Callinectes sapidus.* In these crustaceans, the HPT is composed of a series of ovoid lobules that collectively form a thin sheet on the dorsal surface of the foregut. Each lobule is surrounded by connective tissue and contains stem cells, differentiating hemocytes, and mature blood cells. Hemocytes released from lobules pass into the hemolymph spaces between adjacent lobules and enter the general circulation. The purpose of this paper is to describe the HPT of the American lobster *Homarus americanus* at the light and electron microscope levels. This information will assist our understanding of crustacean hematol ogy in three ways. First, new information on lobular HPT (seen in lobsters, crabs, and crayfish) will allow for comparison with nodular HPT (seen in shrimp) and possibly allow for further work on the functional significance of these two plans. Second, we (Hose et al., '90) have developed a classification scheme for circulating hemocytes based on morphological, cytochemical, and functional features. This scheme, which recognizes two lines of hemocyte maturation, is only supported by two studies on hematopoiesis in shrimp (Martin et al., '87; Hose et al., '92). Information on hematopoiesis in the lobster will allow for assessment of the characteristics used to identify two distinct lines of hemocyte matura-
tion in shrimp HPT. Third, information on the morphology of HPT will assist attempts to culture hemocytes by demonstrating the association between stem cells and the ECM that may be significant in liberating the stem cells into culture and providing the ECM that may be necessary to support hemocyte growth.

MATERIALS AND METHODS

Animals

Fifteen lobsters, *Homarus americanus* Milne Edwards, 1837, were obtained from local markets, maintained in marine aquaria at 15°C, and used within 8 hours of purchase. Only intermolt animals were examined in this study.

Tissue preparation

The exoskeleton and epithelial layer of the dorsal cephalothorax were removed to expose the foregut. Fixative (2.5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4, containing 12% glucose) was applied to the dorsal surface of the foregut while this area was excised from the animal and immersed in fixative. The HPT was dissected from the foregut and cut into small pieces and fixed at room temperature for a total of 3 hours. Following a 10-minute rinse in 0.1 M sodium cacodylate containing 24% sucrose, the tissue was post-fixed in 1% OsO₄ in 0.1 M sodium cacodylate for 1 hour and then stained on block for 1 hour in 3% uranyl acetate in 0.1 M sodium acetate buffer. The tissue was dehydrated through a graded series of ethanol solutions, infiltrated, and embedded in Spurr's (69) plastic. Thick sections (0.5 μm) were stained with methylene blue and thin sections (90 nm) were stained with lead citrate and viewed in a Zeiss EM 109 transmission electron microscope (TEM).

Tissue to be examined by scanning electron microscopy (SEM) was fixed as described above except post-fixation and on-block staining were omitted. Following dehydration, the tissue was critical-point dried in a Denton DC-2 apparatus, coated with a gold-palladium mixture in a Technics Hummer II sputter coater, and viewed in a JOEL JSM 35C SEM.

Sample analysis

Light microscopy

Hematopoietic cells were identified and enumerated in 8 to 10 lobules from each of 10 lobsters. Stem cells and hemocytes were categorized into one of the following: hyaline stem cell, hyaline hemocyte, granulocyte stem cell, small-granule stem cell, small-granule hemocyte, or large-granule hemocyte. Capsular cells, infrequently observed, were not enumerated. The basic characteristic used to distinguish between cells of the hyaline and granulocyte lines was the relatively darker cytoplasmic staining of the former (greater electron density for TEM evaluations); this difference was apparent in all cells regardless of the angle of sectioning or its stage in the mitotic cycle. Since stem cells and hemocytes represent a continuum of maturation, cell outlines were used to distinguish these two categories. Stem cells possessed irregular, interdigitated plasma membranes while hemocytes had distinct, frequently rounded cell outlines. The four cell types of the granulocyte line also form a continuum of maturation. However, granulocyte stem cells usually had distinctive nuclear characteristics and contained fewer cytoplasmic granules compared to small-granule stem cells. Characteristics of each cell type are described in the Results section.

Cell counts from 10 replicate lobules were averaged to obtain a profile for each lobster; values from the 10 individuals were averaged to yield overall means. Longest axes of lobules, stem cells, and hemocytes were measured using an ocular micrometer.

All dividing cells were recorded and identified. Hematopoietic cells were included as mitotic from the disappearance of the nuclear membrane during prophase until its reappearance during telophase. Despite the
absence of nuclear characteristics, cells could be assigned into the preceding categories using cytoplasmic features such as the relative density of the cytoplasm and the number and type of cytoplasmic granules. To determine the mitotic index, the number of mitotic hemocytes/precursors was divided by the total number of hematopoietic cells. Mitotic indices for the hyaline and granulocyte lines were calculated by dividing the number of mitotic cells in each line by the total number of cells in that line.

Electron microscopy

TEM was used to describe the ultrastructure of hematopoietic cells and to develop the hemocyte maturation sequence.

RESULTS

General morphology of HPT

The HPT of the lobster is a thin (40–800 μm thick) layer of tissue loosely bound to the dorsal surface of the foregut (Fig. 1). It is covered by an incomplete layer of loose connective tissue and contains muscle fibers, blood vessels, and ovoid lobules containing stem cells and maturing hemocytes. The lobules are most abundant over the dorsal surface of the foregut and become gradually replaced by connective tissue and striated muscle fibers toward the anterior and lateral margins of the foregut.

The hematopoietic lobules are ovoid (Figs. 2, 3) and range in size from 30 μm × 20 μm to 180 μm × 80 μm. Most lobules are clearly separated from adjacent lobules, although a few appear fused. Between nodules are hemal spaces containing hemocytes and in some of the lobsters we examined, Aerococcus uritensis var. homari, the bacterium responsible for the lethal disease gaffkemia, as well (Fig. 3). Blood vessels are occasionally seen passing between nodules but show no special connection with them.

Fig. 5. Homarus americanus. Scanning electron micrograph of a lobule showing several small tears (arrows) in the connective tissue layer. ×825.

Fig. 6. Homarus americanus. Scanning electron micrograph of lobule with connective tissue layer absent from one end, exposing tightly packed maturing hemocytes (H) and cavities (C) from which hemocytes have become detached. ×825.

Fig. 7. Homarus americanus. Photomicrogram of a lobule similar to the one shown in Figure 6, showing absence of the connective tissue layer at one end of the lobule. Hemocytes are apparently being released from the lobule into the adjacent hemal space. ×825.
Each lobule contains a cluster of densely packed stem cells and maturing hemocytes surrounded by a connective tissue layer (Fig. 4). This layer is composed of an outer fibrillar basement membrane (0.2 μm thick) and an inner layer of collagen fibers containing an occasional elongate fibroblast-like cell. This cell has a centrally located nucleus with primarily marginal heterochromatin. The cytoplasm contains mitochondria, dilated rough endoplasmic reticulum (RER), vesicles, free ribosomes, and microtubules.

Examination of the tissue by SEM revealed that some lobules had small irregularly shaped areas where the encapsulating connective tissue layer was leaving back on itself or missing (Fig. 5). In other nodules, judged to be further along in the release of hemocytes, one end of the nodule was devoid of the encapsulating material (Fig. 6). Hemocytes embedded in a loose fibrillar material are clearly seen as well as the cavities from which other hemocytes had detached. At the light microscopic level, many lobules contained areas lacking the capsule from which the hemocytes appeared to be released (Fig. 7). Diapedesis of hemocytes through the encapsulating connective tissue of each nodule was never observed.

**Description of stem cells and maturing hemocytes**

Lobster hematopoietic cells could be categorized into two distinct cell lines, the hyaline cell line and the granulocyte line. Both types of cells were usually present within a single lobule, although lobules composed solely of granulocytes were sometimes observed. The two types of cells were seldom segregated within the lobule; instead, hyaline cells or granulocytes formed small clusters. Frequently, a gradient of maturation was observed with stem cells at one end of the lobule, immature hemocytes intermediate in position, and detached hemocytes grouped at the opposite end where cells were being released into the hemal space.

Hyaline cells were infrequently observed in most intermolt lobsters, comprising 9.5% of the HPT (Table 1, range = 0.9% to 34.9%). Granulocytes formed the bulk of the intermolt HPT, averaging 90.5% of the total cells present. In only one lobster (no. 1) was the bacterium *Aerococcus vitrulans* observed; a few cells within the hemal spaces contained vacuoles filled with bacteria.

Hyaline stem cells (Fig. 8A) were elliptical, averaging 13.2 × 9.3 μm (Table 2). The oval nucleus was composed primarily of heterochromatin, usually present as thick bands. Nucleoli were seldom observed, even using TEM. The nucleus was surrounded by a thin band of dark blue (electron dense at the TEM level) cytoplasm containing a few (<5), small (0.6 μm diameter), ovoid granules. The granular substructure was homogeneous and electron dense. The tiny, round cytoplasmic deposits and striated granules characteristic of shrimp hyaline stem cells were not observed. The cytoplasm also contained a Golgi apparatus and numerous mitochondria.

Hyaline hemocytes (Fig. 8B) were similar in size to hyaline stem cells (10.6 × 6.4 μm in diameter) and ranged as a continuum from the hyaline stem cells to cells indistinguishable from circulating hyaline hemocytes. During maturation, the cell size slightly decreased and the nuclear-to-cytoplasmic ratio

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**Table 1. Differential counts of hematopoietic cells from intermolt lobsters**

<table>
<thead>
<tr>
<th>No. cells/lobe</th>
<th>% Hyaline</th>
<th>% Granulocyte</th>
<th>% Unidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Mean ± SD</td>
<td>Stem cells</td>
<td>Hemocytes</td>
</tr>
<tr>
<td>1</td>
<td>13.9 ± 5.2</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>18.5 ± 2.1</td>
<td>4.2</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>20.2 ± 0.3</td>
<td>28.6</td>
<td>0.9</td>
</tr>
<tr>
<td>4</td>
<td>14.5 ± 0.7</td>
<td>13.8</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>35.2 ± 11.6</td>
<td>4.7</td>
<td>2.0</td>
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<td>6</td>
<td>22.1 ± 5.6</td>
<td>1.2</td>
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<tr>
<td>7</td>
<td>18.6 ± 9.4</td>
<td>25.8</td>
<td>9.1</td>
</tr>
<tr>
<td>8</td>
<td>22.4 ± 11.9</td>
<td>1.8</td>
<td>0.4</td>
</tr>
<tr>
<td>9</td>
<td>38.9 ± 19.2</td>
<td>0.0</td>
<td>1.4</td>
</tr>
<tr>
<td>10</td>
<td>22.9 ± 9.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Grand mean

± SD 22.7 ± 8.2 7.4 ± 9.6 2.1 ± 3.2 29.9 ± 13.6 52.8 ± 18.2 10.0 ± 8.8 6.3 ± 12.2 0.5 ± 1.1

*Ten lobules were scored per lobster and all cells were identified by category. Granulocytes were subdivided into granulocytes (GS), small granulocyte stem cells (SGS), small granulocyte hemocytes (SGH), and large-granule hemocytes (LGH).*
Fig. 8. *Homarus americanus*. A series of transmission electron micrographs showing a hyaline stem cell (A), hyaline hemocyte (B), granulocyte stem cell (C), small-granule stem cell (D), small-granule hemocyte (E), and large-granule hemocyte (F). Insets are light micrographs corresponding to the respective cell types. The darker cytoplasmic staining of the hyaline cells compared to that of the granulocytes is a key differentiating characteristic. Granulocyte stem cells contain fewer granules than do the granular hemocytes, and the latter can also be identified by the fact that their plasma membranes show progressive stages in detachment from the surrounding cells. ×6,000; insets ×1,000.

(N: C) generally increased (Fig. 9). Nuclear characteristic, however, appeared unchanged, with banded heterochromatin also prominent in the hyaline hemocytes which had been released into the adjacent hemal spaces.

**TABLE 2. Cell and lobule dimensions from sectioned lobster hematopoietic tissue**

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Length (µm)</th>
<th>Width (µm)</th>
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</thead>
<tbody>
<tr>
<td>Hyaline cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaline stem cell</td>
<td>13.2 ± 0.4</td>
<td>9.3 ± 0.4</td>
</tr>
<tr>
<td>Hyaline hemocyte</td>
<td>10.6 ± 0.5</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>Granulocyte</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granulocyte stem cell</td>
<td>15.2 ± 0.7</td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td>Small-granule stem cell</td>
<td>11.6 ± 0.5</td>
<td>9.3 ± 0.3</td>
</tr>
<tr>
<td>Small-granule hemocyte</td>
<td>9.7 ± 0.4</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td>Large-granule hemocyte</td>
<td>12.0 ± 0.5</td>
<td>8.0 ± 0.4</td>
</tr>
<tr>
<td>Lobule diameter</td>
<td>52.8 ± 3.9</td>
<td></td>
</tr>
</tbody>
</table>

As the hyaline hemocytes matured, the number of cytoplasmic granules increased from 0–3 per section in stem cells, to roughly 10 in hemocytes detaching from the lobules, to approximately 15 in cells released into the adjacent hemal spaces. The latter figure is similar to the mean of 14 granules per section reported in circulating hyaline hemocytes (Hose et al., '90). No changes in granular substructure could be observed.

As in penaeid shrimp (Hose et al., '92), maturing granulocytes could be grouped into four different cell types, although intermediates between progressive types were observed. The earliest is the granulocyte stem cell followed by the small-granule stem cell, the small-granule hemocyte, and finally the large-granule hemocyte. Some nuclear characteristics of the two types of stem cell differ between the lobster and penaeid shrimp. However, the cells retain similar cytoplasmic features, notably the absence of cytoplasmic...
granules in the granulocyte stem cell, the presence of increasing numbers of granules as the small-granule stem cell matures, and the progressively decreasing N:C during maturation. These trends continued through maturation into the large-granule hemocyte (Fig. 10).

Granulocyte stem cells (Fig. 8C) could be recognized by their large size (15.2 × 10.0 µm). These cells had an elliptical nucleus predominately consisting of euchromatin, with one to three prominent (2.5 µm diameter) nucleoli. At the light microscopical level, the plasma membrane was indistinct and the cytoplasm stained light blue. Using TEM, up to two small, ovoid cytoplasmic granules could be observed. The granule contents were usually electron-lucent and had a fibrillar substructure. Numerous mitochondria and abundant RER were also present. Granulocyte stem cells were common, averaging 20.9% of the hematopoietic cells (Table 1). In most individuals, these cells comprised between 14% and 52% of the total; in one lobster, only 0.9% were granulocyte stem cells.

Small-granule stem cells (Fig. 8D) were relatively smaller (11.6 × 9.3 µm, Table 2). Like the granulocyte stem cell, they had light blue-staining cytoplasm; however, small-granule stem cells were distinguished by the presence of many (3–20 per cross section), small (<1.0 µm diameter), ovoid-cylindrical cytoplasmic granules. In addition to granules with a fibrillar substructure, numerous granules were composed of a homogeneous, electron-dense material. Cylindrical granules were always electron dense. Usually a single (1.8 µm diameter) nucleolus was observed within the nucleus although some cells contained two. A thick band of marginal chromatin was present. The remainder of the nucleus was composed of euchromatin and the nuclear shape varied from round to indented. Small-granule stem cells were usually the most frequent category observed, with a mean of 52.8% (range = 32% to 80%, Table 1).

Small-granule hemocytes (Fig. 8E) were detached from the adjacent cells and resembled their circulating counterparts both in nuclear characteristics and the number of granules per section (Hose et al., ’90). Compared to small-granule stem cells, the N:C ratio was lower and more cytoplasmic granules were usually present (>20 per cross section). These round or oval cells averaged 9.7 × 7.4 µm (Table 2). Using light microscopy, the circular-elliptical, centrally located nucleus was banded by heterochromatin. The interior of the nucleus appeared to be composed solely of euchromatin although sometimes up to two small nucleoli were apparent at the TEM level. Small-granule hemocytes comprised an average of 10% of the hematopoietic cells (Table 1), ranging from 0.8% to 24.5%.

Large-granule hemocytes (Fig. 8F) were distinguished by the presence of larger (1.3–2.0 µm diameter), cylindrical, electron-dense cytoplasmic granules in addition to numerous small granules. Compared to small-granule hemocytes, large-granule hemocytes had a lower N:C ratio, larger size (12.0 × 8.0 µm), and a more eccentrically placed nucleus. Large-granule hemocytes were less frequently observed than the less mature granulocytes, forming an average of 6.3% of the HPT. However, half of the lobsters did not contain any (<1%) large-granule hemocytes and in one individual, these comprised 40.1% of the total.

Mitotic index

The average mitotic rate of intermolt lobsters was 5.1%, with a range from 0.7% to 15.8% (Table 3). The cell types capable of division were hyaline stem cells, granulocyte and small-granule stem cells (Figs. 9, 11) and, rarely, small-granule hemocytes. Only 10.3% of the dividing cells were hyaline stem cells, whereas the granulocyte line contributed 89.7% of the total. Most of the dividing cells were small-granule stem cells (78.6%) with the remainder (11.1%) being granulocyte stem cells.

In half of the lobsters, no division of hyaline stem cells was observed. Hyaline cells were generally uncommon in the HPT; however, in one individual (no. 3), 29% of the hematopoietic cells were of hyaline origin, yet no mitosis was observed. Another individual (no. 7) had 35% hyaline cells and only 4.8% were dividing. Yet in three lobsters (nos. 5, 6,
Fig. 10. *Honors americanus.* Montage of transmission electron micrographs of a lobule containing only maturing granulocytes. Some granulocyte stem cells (GS; GS* is same cell shown in Fig. 5C) lack cytoplasmic granules, while others contain a few. Note the prominent, occasionally multiple, nucleoli. More mature cells, the small-granule stem cells (SG), show an increase in size and number of granules, most of which are ovoid to cylindrical. Prominent nucleoli are less common in these cells. In more mature granulocytes (MC), the granule size and number increase further, the marginal chromatin becomes thicker, and the nucleocytoplasmic ratio generally decreases. ×3400.

and 8), from 1.2% to 6.5% of the total cells were of hyaline origin but mitotic indices for the hyaline cells ranged from 17.4% to 60%.

In contrast, all of the lobsters had some mitosis of granulocytes, usually in small-granule stem cells. Division was observed only in small-granule stem cells in 4 of the 10 individuals with this cell type contributing between 33.3% and 80% of the total in the remaining lobsters. Mitotic rates for small-
granule stem cells averaged 5.4% and varied between 0.9% and 10.3%. Division of granulocyte stem cells was observed in four animals where these cells constituted between 20% and 50% of the total; overall, they comprised 11.1% of the dividing cells. A mean percentage of 3.9% of the granulocyte stem cells were mitotic; individual values ranged from 0% to 11.4%.

**DISCUSSION**

The HPT in the American lobster is composed of a series of ovoid lobules, each containing maturing hemocytes and surrounded by a thin layer of connective tissue. We have previously proposed characteristics that are useful in identifying two main categories of circulating hemocytes (Hose et al., '90). Identification is important because hyaline cells and granulocytes perform distinct physiological functions. Hyaline hemocytes have a high nuclei-cytoplasmic ratio, contain relatively few cytoplasmic granules, and initiate coagulation of the hemolymph (Omori et al., '89). Granulocytes may be further subdivided into small-granule and large-granule hemocytes. Both contain lysosomal enzymes and primarily function in defense against foreign particles. Although there is some overlap of function, small-granule hemocytes are responsible for phagocytosing bacteria while large-granule hemocytes encapsulate larger foreign materials such as fungal hyphae (Hose and Martin, '89). As in intermolt penaeid shrimp (Hose et al., '90), granulocytes and their precursors predominated in the HPT of intermolt lobsters. About one third of the hematopoietic cells of shrimp were of hyaline origin compared to only 10% in the lobster. The lower value in the lobster corresponds to its relatively lower percentage of circulating hyaline cells (22% vs. 50% for the shrimp). Differences in the ultrastructure of hyaline precursors were identical to those previously described for the circulating hyaline hemocytes in shrimp and lobsters (Hose et al., '90). These variations in granule number and substructure (striated in shrimp vs. homogeneous in lobster) and the appearance of the tiny deposits which confer the dark staining to the cytoplasm (aggregated in shrimp compared to dispersed in lobster) probably reflect differing mechanisms of coagulation. In penaeid shrimp, which are examples of Tait’s ('11) coagulation type C, the entire hyaline hemocyte explodes and releases the clotting material into the hemolymph. The clot is formed primarily by coagulated proteins. Hyaline cells of the American lobster (Tait’s type A) release clotting proteins through localized breaks in the plasma membrane. In type B decapods, the prominent feature of the clot are aggregated hemocytes bound together by coagulated hemolymph. Species with type B coagulation have lower percentages of circulating hyaline hemocytes than species possessing type C coagulation.

In the present study, we have identified both hyaline cells and granulocytes dividing within the same lobules. We have been unable to identify a mitotic cell with features common to the hyaline cell and the granulocyte. While the earliest hyaline and granulocyte stem cells both lack granules, the cytoplasm of the former is always more electron dense and the chromatin patterns are different. Our inability to identify a common pre-

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**TABLE 3. Mitotic index of intermolt lobsters**

<table>
<thead>
<tr>
<th>No. cells dividing</th>
<th>Total no. cells</th>
<th>% Dividing cells</th>
<th>% Hyaline</th>
<th>% Granulocyte</th>
<th>% Hyaline dividing</th>
<th>% Granulocyte dividing</th>
<th>Mitotic index by category</th>
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</thead>
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<tr>
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<tr>
<td>1</td>
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<td>139</td>
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<td>145</td>
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</table>

Grand mean ± SD: 5.1 ± 4.5 10.3 ± 14.8 11.1 ± 16.7 78.8 ± 22.4 12.0 ± 20.0 3.9 ± 5.1 5.4 ± 3.7
Fig. 11. H. americanus. Transmission electron micrographs of dividing granulocyte stem cell (A) and of small-granule stem cell (B). The cytoplasmic staining of the granulocytes is lighter than that of hyaline stem cells (Fig. 9). This comparison is also shown in (C), a light micrograph of a dividing hyaline stem cell (arrow) and a granulocyte stem cell (double arrow). Two dividing small-granule hemocytes (arrows) are shown in (D). A, ×6,000; B, ×5,000; C, D, ×1,350.

cursor lends support to the idea that there are two distinct lines of hemocyte maturation in the decapods.

Production of hemocytes occurs almost exclusively within the HPT since mitotic hemocytes are rarely observed in the peripheral circulation. Cells released from the lobules (hyaline hemocytes and small- and large-granule hemocytes) appear identical to circulating cells. However, large-granule hemocytes are not common in the HPT, suggesting that they can also develop from circulating small-granule hemocytes.

Cell division leads to enlargement of the lobules and may explain the observed variation in the size of the lobule seen by SEM. In contrast, the hematopoietic tubules of penaeid shrimp remain uniform in size due to the extensive collagenous matrix which forms the wall of the tubule. Cell proliferation is observed only as an increase in the cellularity of the wall. Possible regulation of hematopoesis by the connective tissue matrix or capsule has not yet been explored.

Control of hemocyte production and release is probably correlated with the molt
hematopoietic tissue in lobsters

cycle as seen in shrimp (Hose et al., '92) and blue crab (Johnson, '80). In all decapods studied, granulocyte production is maximal during the intermolt phase and hyaline cell production is greatest during the edcsiall interval. This synchrony reflects the integral role played by hyaline hemocytes during molting: these cells produce proteins which harden the new exoskeleton (Vacca and Fingerman, '83).

The mechanism by which maturing hemocytes are released into circulation is not clear. In the shrimp, hemocytes migrate into the lumen of the hematopoietic tubule, which is continuous with the ophthalmonic artery (Martin et al., '87), but diapedesis of lobster hemocytes through the connective tissue layer surrounding each lobule into the hemal spaces has not been observed. Instead, SEM images suggest that the connective tissue layer is degraded beginning with one or a small number of localized spots and expanding until one end of the investing layer is removed and the hemocytes gain free access to the hemolymph. It seems likely that the stem cells may play an important role in the dissolution of this layer. We have not observed "healing" of a ruptured nodule, but the mechanisms involved in rupture and repair of the nodules deserve further attention.

Finally, the results presented here may be useful to efforts to culture crustacean tissue, particularly the HPT for which a continuous culture has not been established (see Chang and Brody, '89). Primary cultures of HPT have been made from shrimp (Chen et al., '87, '89), lobster, and crayfish (Brody and Chang, '89) of cells that migrated from minced tissue explants, presumably the hemocytes from the hemal spaces adjacent to the hematopoietic lobules. Decapod hemocytes survive in culture but do not divide. It seems unlikely that previous efforts successfully ruptured individual lobules by mincing; a prior collagenase dissociation such as that described by Brody and Chang ('89) for testicular tissue may be necessary for liberating the stem cells from the lobules. This situation appears similar to early attempts to culture mammalian HPT (Friedenstein et al., '74) and future efforts should focus on the possibility that continuous culture of decapod hematopoietic cells requires attachment to an ECM.

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