

## TUNICATES AND THEIR IMMUNE MECHANISM

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(Received September 11, revised September 16, 1992)

**Abstract** Tunicates (Urochordata) belong to Chordata, the phylum which also includes vertebrates, as the most primitive group in phylogeny of the group. In spite of earlier reports about allogeneic recognition in tunicates, molecular basis of it remains unknown. Functions of immuno-potent cells and components of their cytoplasmic granules are not defined, either. Memory in immuno-defense reactions have not been proved. Thus, many immunological problems still remain unsolved in tunicate. However, recent studies about cytokines and hemopoiesis add new aspects to tunicate immunology.

*Key Words* : Tunicate, Immune system, Hemocytes, Hemopoiesis

### Phylogeny, common and unique features of tunicates

Tunicate is a member of the group called Urochordata (Tunicata), a subphylum consisting phylum Chordata with other subphyla Cephalochordata and Vertebrata. As a mem-

ber of chordates, tunicates have many characteristics common to vertebrates<sup>1)</sup>; bilaterally symmetrical form, and pharyngeal gill slits, dorsal notochord and nerve cord present at some stage in development (Fig. 1). Tunicates also have "heart" (not really like a heart of vertebrates since tunicate lacks

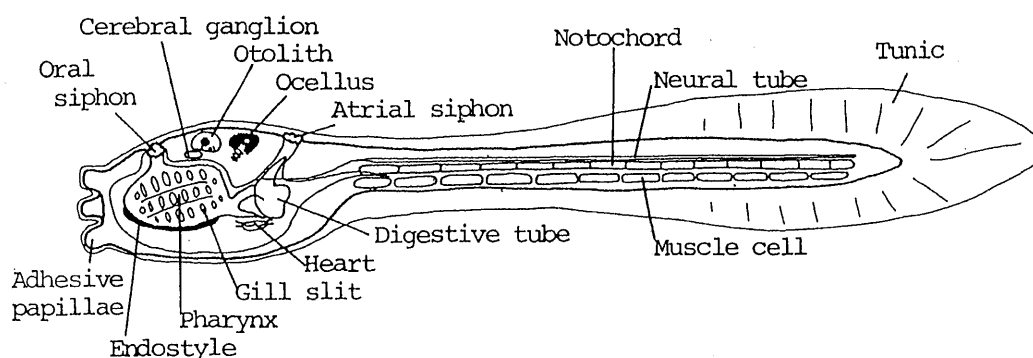


Fig. 1 Schematic image of tadpole larva of tunicate, at swimming stage, with some prototypic characters of vertebrates. It shows tactic behavior, positive for light at first and negative for gravity later. The sensory organs for that are probably ocellus for light and otolith for gravity. Muscle cells have sarcomeres as striated muscle. It will adhere on some substratum and undergo metamorphosis very quickly to lose notochord, neural tube, and muscle cells of tail part.

blood vessels which are sealed by endothelium), and endostyle which corresponds to thyroid gland of vertebrates. Tadpole larva has primitive sensory organs for light and gravity, ocellus and otolith respectively, in relation with tactic behaviors against the light and gravity. Thus tunicates are interesting and important in relation with the evolution of many physiological systems of vertebrates. Tunicates may still retain physiological and morphological features of ancestral animals of vertebrates.

On the other hand, tunicates exhibit extremely unique features in asexual reproduction and colony formation in many species, hermaphroditic sexual reproduction, and metamorphosis during development. These uniqueness of tunicates also provides us with a unique experimental model to solve many problems common to many animals including vertebrates.

In immunological aspect, colonial tunicates undergo asexual reproduction to form colonies of clone animals, providing unique experimental model for allogeneic recognition<sup>2,3</sup>. In addition, tunicate is one of rare materials in which developmental fate of all blastomeres and embryonic cells was defined, providing an advantage to investigate the development of immune system. Recent findings of invertebrate cytokines led a development of new field in tunicate immunology<sup>4</sup>.

#### Immune system of tunicates

Any immune organs such as thymus, lymph nodes or spleen are absent in tunicates, although there is a possibility that primitive immune organ as a site of immune reactions could be discovered in future. So far, immuno-defense reactions seem to occur in hemolymph and connective tissue of all part of the body. In addition, tunic outside the epidermis also provides the field for immuno-defense reactions.

Tunicates have no blood vessels sealed by endothelial cells, and the "blood" consists of hemolymph and free cells wandering through tissue cavities. Naturally, hemocytes are free to migrate out of those cavities into surrounding loose network of fibrous structures. Cir-

ulation of hemolymph is not apparent. Instead, hemocytes stream reciprocally back and forth inside tubular spaces within connective tissue, being pushed by contraction of "heart", muscular contraction inside gill, and contraction of the body.

#### *Allogeneic recognition*

Attacking reaction between allogeneic hemocytes, "contact reaction" in *H. roretzi*<sup>5</sup>, and proliferation of particular hemocytes in *S. clava*<sup>6</sup> upon allogeneic stimuli were reported. Genetic control of these allorecognition may resemble to mammalian MHC gene system<sup>7</sup>. However, the studies about "contact reaction" in combinations of allogeneic animals' revealed that tunicate histocompatibility as a phenotype differs from the mammalian's. In tunicates, allogeneic individuals sharing at least one allele of histocompatibility genes seem to be acceptable<sup>8</sup>. An animal supposed to have "BC" as histocompatibility genes would be acceptable for both animals having genes "AB" and "CD", while "AB" animal is not compatible with "CD" animal. Similar phenomenon was reported about the compatibility at colony fusion of colonial species<sup>9</sup>. Molecular basis for such allogeneic recognition have not been revealed yet. It is not proved yet that tunicate has a memory of immune response (adaptive immune reaction). Recently, a report suggested the presence of memory of allogeneic stimuli<sup>6</sup>.

#### *Humoral factors*

There are not sufficient data to construct whole image about the role of humoral factors in tunicate immuno-defense. Lectins, which are assumed as immuno-defensive molecules in many invertebrates, are also present in tunicates<sup>10</sup>. However, their physiological roles have not been established in most cases (lectins might act as regulation molecules to orchestrate immuno-defense reactions like cytokines in vertebrates).

Immunoglobulin has not been detected in protochordates, and it has been of special interest if ancestral molecule of immunoglobulin or immunoglobulin gene superfamily (IgGSF) would exist in tunicates<sup>11,12</sup>. IgGSF molecules are distributed widely as cell adhe-

sion molecules and cell surface receptors, having roles as recognizing molecules on cell surface<sup>13,14</sup>), including fasciculin of insect<sup>15</sup>). Naturally, there are a prediction that tunicates also have some molecules of IgG SF in immunological recognition.

Antibiotic and anti-tumor substances were also detected in hemolymph. Didemnin from Caribbean tunicate is a well known example which is now on the test for clinical use<sup>16</sup>). However, almost none of those substances has been studied with respect to their physiological roles. Enzymes in hemolymph possibly play various roles in immuno-defense system as those in vertebrate serum. Complement-like activity has not been detected.

Cytokines are another important humoral components which regulate various cellular activities in immuno-defense system. Interleukin-1 (IL-1) like activity has been detected already, and IL-1 activity was blocked by anti-human IL-1 antibody<sup>4</sup>). Tunicate IL-1<sup>17</sup>) and recombinant human IL-2<sup>18</sup>) stimulated the proliferation of tunicate hemocytes. These data suggest the presence of cytokines in tunicates.

#### *Cellular components*

Cells involved in immune responses would include epithelial cells, epidermis, and some stromal cells in connective tissues. But, only hemocytes were proved as cellular components of tunicate immuno-defense system so far. Since tunicates do not have closed circular system, any of free-wandering cells in tissue cavities could be assumed as hemocytes. Furthermore, tunic overlaying outside epidermis also contains numerous cells of similar appearances with hemocytes inside the body<sup>19</sup>), and those cells are often counted as hemocytes.

Hemocytes (including free cells in tunic) contribute to rejecting reactions against allogeneic colony-fusion in colonial species<sup>2</sup>) and "contact reaction<sup>5</sup>)". Many of hemocytes exhibit active phagocytosis against foreign particles, and also many of them contain granular components that will be discharged after contact with foreign cells or bacterial endotoxin.

In spite of many studies about hemocytes, confusions and controversies still remain

even in their classifications. There are so much variety of hemocytes in different species that each species may need different classification or identification criteria of hemocytes, even if we could divide them into three categories; hemoblasts, phagocytes and granule-containing cells<sup>20</sup>). Hemoblasts are the candidates for hemopoietic stem cells, and granule-containing cells were believed to discharge various materials upon immune responses. Lymphocytes might exist as the fourth group, whereas the lymphocytes in former classifications were often considered also as hemopoietic stem cells. The cells which play the same functions as vertebrate lymphocytes have not been proved yet.

#### **Hemocytes and hemopoiesis in solitary tunicates *Halocynthia roretzi* and *Styela clava***

Hemocytes of *Halocynthia roretzi* has ten<sup>21,22</sup>) and *Styela clava* has four types<sup>23</sup>) of hemocytes. All four types of hemocytes in *S. clava* seemed to have corresponding groups in *H. roretzi*, and the rest 6 types of *H. roretzi* did not have corresponding types in *S. clava*.

#### *Hemocyte functions*

Phagocytosis is probably a primarily important function in their immune system. In *H. roretzi*, phagocytosis against sheep red blood cells was specifically performed by two types of hemocytes, p1- and p2-cells<sup>21</sup>). In contrast in *S. clava*, three out of four types of hemocytes exhibited active phagocytosis against yeast particles. Since phagocytosis seems to be mediated by specific receptors against target particles in vertebrates<sup>24</sup>), it may be possible that phagocytic activity of hemocytes varies with biochemical characters of target particles. Opsonin activity in hemolymph was detected recently in *S. clava* using yeast-particles as targets<sup>25</sup>). However, specificity of opsonin and the cells producing opsonin molecules are still unknown.

Granulated or vacuolated cells are most abundant in both species and they occasionally discharge their granules or vacuoles. For example, vacuolated cells in *H. roretzi* discharge cytoplasmic vacuoles and lose most of cell volume upon allogeneic and xenogeneic stimulation. However, substances contained

in those vacuoles and molecular mechanism of the vacuole discharge is not known. Although many humoral factors such as antibiotic substances<sup>26)</sup>, an enzyme<sup>27)</sup> and lectins were found in hemolymph of *H. roretzi* so far, there is no detail investigation about particular cells to secrete them. Thus, the content of cytoplasmic granules or vacuoles of hemocytes is still important subjects to be investigated.

### Hemopoiesis

Hemopoiesis would be one of the most important subjects in current tunicate immunology. Hemopoiesis have not been studied well and less understanding of hemocyte differentiation becomes main problem to disturb the investigations about cellular immuno-defense. There is only a few observation about hemopoietic tissues which described hemoblasts as candidates for hemopoietic stem cells in *S. clava*<sup>28)</sup>. Hemoblasts are small round cells having a little cytoplasm, a large nucleus and a characteristic large nucleolus which is visible under light microscopy. Inside hemopoietic nodules which are aggregations consisted of ten to

hundred hemocytes, a few hemoblasts were surrounded by many mature hemocytes such as granulocytes (Fig. 2). Autoradiographs of *in vivo*<sup>29)</sup> and *in vitro* culture suggested that the hemoblasts proliferated actively.

In *Styela clava*, in which hemopoietic nodules were found in connective tissues of most part of the body, we can select any convenient portion for the investigation. Even it may be possible to harvest hemopoietic cells by syringe. Although there is no methods established for hemocyte culture, an important attempt to culture hemopoietic tissue was accomplished successfully using pharyngeal explants<sup>30)</sup>. Before the culture of isolated hemocytes will be established, this tissue culture is the most probable candidate for experimental model in various analyses including the bio-assay of hemopoietic factors.

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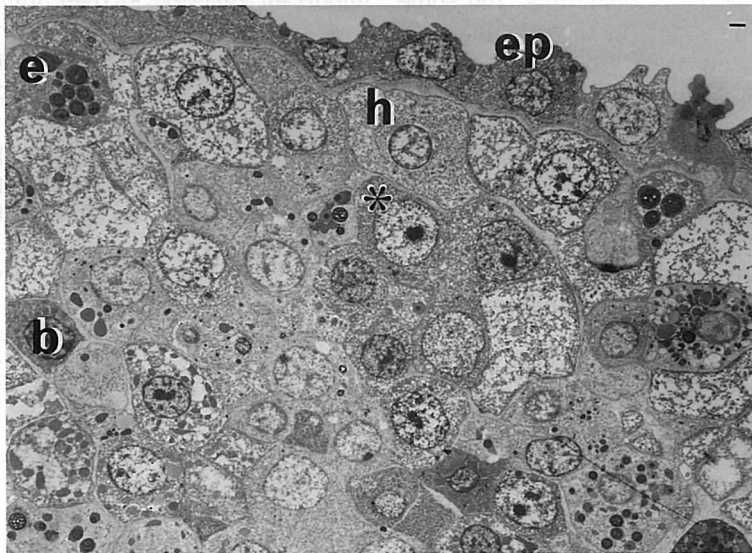


Fig. 2 Transmission electron micrograph of hemocyte nodule at pharynx of *Styela clava*. Hemoblasts were surrounded by numerous number of differentiated hemocytes. ep; epithelial cells, e; eosinophilic granulocyte, b; basophilic granulocyte, h; hyaline cells, \*; hemoblast, Bar=1  $\mu$ m.

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