

## Hemocytes with phagocytic capacity in the ascidian *Ciona intestinalis*

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Phagocytosis is an innate immune response shared by all metazoans allowing elimination and degradation of potentially pathogenic foreign microorganisms. In ascidians a number of studies have shown that hemocytes phagocytose foreign particles, however, our knowledge on the ascidian phagocytes is still fragmentary and sometimes conflicting. Therefore the phagocytic capacity was studied qualitatively and quantitatively in the ascidian *Ciona intestinalis*.

Yeast cells were applied on the monolayer of hemocytes *in vitro*. Using a peptide antibody that reacts with ascidian hemocytes, it was shown immunohistochemically that hemocytes with phagocytic capacity are hyaline amoebocytes, granular amoebocytes, morula cells, and small amoebocytes. The serial tomograms of those hemocytes proved that the yeast cells were really engulfed by those hemocytes. It was firstly shown in this study that the morula cells and small amoebocytes have phagocytic capacity.

The phagocytosis rates of four types of phagocytic hemocytes (Table) evidently show that hyaline amoebocytes are most active phagocytes because nearly 100% of them ingested yeast cells. There is a consensus among the researchers of ascidian hemocytes that hyaline amoebocytes are professional phagocytes. Granular amoebocytes are also active phagocytes because more than 90% of them ingested yeast cells. The other two types of phagocytic hemocytes are considerably less active phagocytes; the phagocytosis rate of morula cells is approximately 33% and that of small amoebocytes is approximately 27%. These results strongly suggest that ascidians have multiple types of phagocytic hemocytes with different roles in innate immunity like in vertebrates.

Table. Phagocytosis rates of the hemocytes of the ascidian *Ciona intestinalis*

Hemocytes	Phagocytic	Non-phagocytic	Phagocytosis rate (%)
Hyaline amoebocyte	1112	13	98.9 ± 0.9
Granular amoebocyte	558	35	93.8 ± 3.9
Morula cell	233	452	33.0 ± 12.8
Small amoebocyte	39	105	27.4 ± 7.9

The number of yeast cells ingested in a hemocyte was counted. Hyaline amoebocytes ingested up to six yeast cells, and the average is 2.62 yeast cells. Granular amoebocytes ingested up to five yeast cells, and the average is 2.38 yeast cells. Morula cells ingested one or two yeast cells, and the average is 1.17 yeast cells. Small amoebocytes were able to ingest only one yeast cell.

Phagocytosis requires combinational receptor-mediated recognition of foreign particles, however,

in ascidians we have scanty knowledge about the ligands and receptors that trigger phagocytosis. In mammals Toll-like receptors, an important family of the receptors, activate phagocytosis when the pathogen-associated molecular patterns of foreign particles attach to them. The high phagocytosis rate (nearly 100%) of hyaline amoebocytes shown in this study possibly assist to investigate these problems about ascidian phagocytes in the future.