Structure and function of the reticular cell in the planarian *Dugesia dorotocephala*

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**Abstract**

Structural and functional characteristics of the reticular cell in the planarian *Dugesia dorotocephala* were studied by light and electron microscopy. Since the reticular cells have numerous glycogen granules, lipid droplets and some lysosomes in their cytoplasm, they are easily distinguishable from other cell types. They migrate into the injured tissue, cover the injured mesenchyme, and also phagocytize debris of degenerating cells. The reticular cells also recognize foreign invaders such as bacteria. The larger aggregates of killed bacteria are encapsulated by reticular cells and eliminated into the intestine, whereas small aggregates are phagocytized by reticular cells. When cell wall extract of bacteria was inserted into the planarian body before insertion of killed bacteria, reticular cells were found to respond more quickly and vigorously to subsequent insertion of killed bacteria, indicating that the reticular cell has an immune response memory. When planarians were treated with 0.3 ppm cadmium sulfate and 0.01 ppm TPA, reticuloma tumors were induced in 76% of exposed planarians, indicating the similarity to blood cell diseases in mammals such as leukemia or lymphoma which are also induced by TPA. When these tumors were transplanted into normal hosts, the tumor cells were attacked by host reticular cells. These observations indicate that planarian reticular cells are primitive blood cells, playing important roles in nutrient transportation, homeostatic control of cells, and in defence and immune surveillance systems.

**Introduction**

In previous papers (Morita & Best, 1974, 1984) we have stated that planarians have a specific type of mesenchymal cell which plays an important role in nutrient transportation and phagocytosis in the body. We have named this type of cell the ‘reticular cell’. This cell type is similar to the ‘fixed parenchymal cell’ described by Pedersen (1961) and Ishii (1965). However, we have demonstrated that the reticular cell is capable of migrating into the injured area, covering the cut-surface of the mesenchymal tissue, and phagocytizing debris of damaged or degenerating cells. These observations indicate that the reticular cell is not a ‘fixed’ type of cell but has the mobility similar to white blood cells such as lymphocytes and macrophages in higher animals. When heat-killed bacteria were inserted into the planarian body, they were phagocytized or encapsulated by the reticular cells (Morita, 1991), indicating that the reticular cell can recognize foreign invaders such as bacteria. Furthermore, we have demonstrated that tumors can be induced in the planarian body by treatment with mammalian carcinogens (Hall et al., 1986a, b). In fact, a type of malignant tumor was induced by treatment with cadmium and 12-O-tetradecanoylphorbol-13-acetate (TPA). Since the tumor consists of a type of cell similar to the reticular cell but transformed, we have named this type of tumor the ‘reticuloma’. Interestingly, the reticuloma seems to be similar to leukemia or lymphoma of mammals which have also been induced by TPA treatment (Berenblum & Lonai, 1970; Armuth & Berenblum, 1974). It becomes clear from the results of these observations that the reticular cell acts like coelomocytes or blood cells of higher animals, although planarians have not yet evolved the vascular system in their body. The present study was designed to provide more evidence for the blood cell theory of the reticular cell.
Materials and methods

Asexual planarians of the species *Dugesia dorotocephala* were used for this study. Healthy planarians, approximately 20–22 mm long, were selected from a laboratory colony that had been maintained in aged tap water and fed twice a week with raw beef liver.

In the first experiments, healthy planarians were decapitated by transverse section just behind the auricles, and the heads were discarded. The decapitated planarians were then maintained in aged tap water without food. The anterior portions of the bodies were dissected 6, 8, 12, 16, 20, 24 and 48 h after decapitation, respectively and prepared immediately for electron microscopy.

In the second experiments, each planarian was immobilized on cold, moist filter paper on a metal plate cooled to below −0 °C, and an incision was made just behind the right eye, and then heat-killed bacteria (*Mycobacterium tuberculosis H37Ra*) were inserted into the incision. Treated planarians were maintained in aged tap water, collected 2, 4, 6, 8, 10, 12, 24 and 48 h after insertion of bacteria, respectively and then prepared for electron microscopy. In the other groups of planarians, a small amount of bacterial cell wall extract (*M. bovis*: RIBI Immuno-Chem. Res. Inc.) was inserted into the incision made just behind the right eye of each planarian. After 5 days of maintenance in normal aged tap water, a small aggregate of heat-killed bacteria was inserted into the incision made in the same site of the previous operation in each planarian. These treated planarians were maintained in aged tap water for 2, 4, 6, 8, 10, 12, 16, 24 and 48 h, respectively and then prepared for electron microscopy.

In the third experiments, planarians were treated with 0.3 ppm cadmium sulfate and 0.01 ppm 12-0-tetradecanoylphorbol-13-acetate (TPA) for 2 weeks and then maintained in normal aged tap water for another 2 weeks. As reported in previous papers (Hall et al., 1986a, b), malignant tumors were induced in about 76% of exposed planarians. Some tumors were transplanted into normal planarian bodies. The host tissues including transplanted tumors were collected a week after transplantation and prepared immediately for electron microscopy.

For electron microscopy, all experimental planarians described above were fixed for 1 h in a mixture of 2.5% glutaraldehyde and 0.5% formaldehyde in 0.1 M phosphate buffer (pH = 7.4). After aldehyde fixation, the target tissues were dissected from the body and postfixed for 1.5 h with 1.0% buffered osmium tetroxide. These specimens were then dehydrated through increasing concentrations of ethanol, replaced with propylene oxide and embedded in Poly/Bed 812 resin. Thick sections (about 0.5 to 1.0 μm in thickness) were cut with glass knives and stained with 1.0% toluidine blue O for light microscopy. Thin sections, cut using a diamond knife, were stained with uranyl acetate and lead citrate (Sato, 1968) and observed using a JEOL electron microscope, 2000EXII.

Results

Glycogen-rich cells, which we named ‘the reticular cell’, are observable everywhere in the mesenchyme of the planarian body. These reticular cells are multipolar, each extending 2 or 3 cytoplasmic processes to form reticular networks among neighboring cells. Their nucleus usually has an irregular shape with frequent convex protrusions which relate morphologically to areas where cytoplasmic processes are extended. The cytoplasms of these reticular cells contains glycogen granules, lipid droplets and lysosomes in addition to the ordinary cellular organelles (Fig. 5). A large number of reticular cells are usually seen in the vicinity of the intestine (Figs 1, 2). Since the intestinal boundary is organized loosely by muscle fibers and primitive connective tissue, either intestinal cells or reticular cells are seen extruding across the tissue and associating closely with each other (Figs 6, 7). In some cases, the debris of degenerating cells, which are encapsulated by reticular cells, were seen near the intestinal boundary (Fig. 7). Light microscopy revealed that large pieces of encapsulated debris appear to be extruded from the mesenchyme tissue and eliminated into the intestine. On the other hand, in the mesenchyme, the cytoplasmic processes of reticular cells are in close association with one another and form gap junctions between them (Fig. 8).

When the planarian body is injured, the reticular cells appear on the damaged tissue 6–8 h after the injury (Fig. 9); some reticular cells appear to extend their cytoplasmic processes and cover the surface of the damaged mesenchymal tissue (Figs 10, 11). More importantly, they phagocytize debris of damaged or degenerating cells. These results indicate that the reticular cell can migrate freely like lymphocytes or macrophages in higher animals (Fig. 3). On the other hand, it is clear that the reticular cell can also recognize foreign invaders such as bacteria. When aggregates of heat-killed bacteria were inserted into the planari-