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Production of intestine-specific monoclonal antibody and interspecific cross-reaction in triclads and polyclads

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ABSTRACT. We have produced a monoclonal antibody (mAb②2B6) specific to intestinal and epidermal cells of *Dugesia japonica*. This mAb②2B6 also reacted with the secretory granules of a kind of sub-epidermal gland cells, epithelial cells and gland cells in the pharynx. The results of the interspecific cross-reaction tests with four other freshwater triclads and four polyclads showed that this mAb②2B6 can be used as a marker, specific to only intestinal cells in both *Planocera multitentaculata* and *Planocera reticulata* of polyclads.

Immuno-electron microscopical research revealed that the cytoplasm of phagocytic cells and the spherical large granules in granular clubs reacted strongly to this mAb in the intestine. Also, mAb positive material seemed to be absorbed by pinocytosis from phagocytic cells into the granular clubs. These observations support the theory that the granular clubs are protein reserve cells, not gland cells for intraluminal digestion.

KEY WORDS: intestine, monoclonal antibody, interspecific cross-reaction, Platyhelminthes.

INTRODUCTION

The intestinal cells of triclads and polyclads consist of two kinds of cells, the phagocytic cell and the granular club. Although some intestine-specific monoclonal antibodies have been produced in freshwater triclads (SHIRAKAWA et al., 1991; ROMERO et al., 1991; SHINOZAWA et al., 1995), the function of the granular club remains obscure. In other orders of Turbellaria, mAb has not been produced. To further clarify the function of intestinal cells in Turbellaria, we tried to produce another intestine-specific monoclonal antibody and examined the localization of this antigen. The interspecific cross-reactions for four freshwater triclads and four polyclads were also examined.

MATERIAL AND METHODS

Animals

Specimens of *Dugesia japonica* Ichikawa & Kawakatsu, 1964 were of the GI strain given by Himeji Institute of Technology. *Dugesia ryukyuensis* Kawakatsu, 1986 was of the OH strain collected from Okinawa Island and strained in Hirosaki University. *Phagocata vivida* (Ijima & Kaburaki, 1916) and *Seidlia auriculata* Ijima & Kaburaki, 1916 were collected from Mt. Iwaki, near Hirosaki. *Bdellocephala brunnea* Ijima et Kaburaki, 1916 was collected from a spring in Hirosaki. *Notoplana humilis* (Stimpson, 1857) and *Pseudostylochus intermedius* Kato, 1939 were collected from the Natsudomari Peninsula, Aomori Pref. *Planocera reticulata* (Stimpson, 1855) and *Planocera multitentaculata* Kato, 1944 were collected from Fukaura, Aomori Pref.

Immunotechnique

The monoclonal antibody was produced by injecting dissociated cells of regenerating *D. japonica* three days after cutting, as antigens, into the mice of the BALB/c strain. The injected dissociated cells were small cells obtained after filtration with a stainless steel mesh of 200 μm, a nylon mesh of 30 μm and a nylon mesh of 20 μm in order of pore size. The procedure for immunization, production of mAb and assay for antibody followed that described in a previous paper (SHIRAKAWA et al., 1991). The ABC method (HSU et al., 1981) was applied to paraffin sections of planarian tissues (fixed in

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6% formalin in a one-quarter strength PBS) to examine the interspecific cross-reaction. After the immunostaining, a counterstaining with Meyer's Haematoxylin was performed. In negative controls, the primary antibody was replaced by mouse myeloma ascites or cloning medium S-Clone SF-B (Sankou Junyaku Co.).

Post-embedding staining using colloidal gold for immuno-electron microscopy followed FAULK & TAYLOR (1971). Specimens were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer for 2 h at 4°C. After being washed in buffer, they were dehydrated in graded ethanols and embedded in LR-White. Thin sections on nickel grids were rinsed with PBS and 1% BSA for 30 min. The sections were incubated for 3-4 h with primary antibody, washed with PBS, then incubated for 2 h with gold-labelled goat anti-mouse antibody IgG+M 15 nm (Zymed) diluted 1:30 in 0.1% BSA-PBS. The sections washed with PBS and distilled water were finally stained with uranyl acetate and lead citrate and observed with a JEM-1210 electron microscope. In negative controls, the primary antibody was replaced by mouse myeloma ascites diluted 1:1000 in PBS.

RESULTS

We have produced a monoclonal antibody, mAb②2B6, specific to intestinal and epidermal cells of *D. japonica*. The isotype was found to be an IgG1 (light chain is *k*) with a mouse-monoclonal isotyping kit (Amersham Co.).

This mAb②2B6 also reacted with a kind of sub-epidermal gland cells, epithelial cells and gland cells in the pharynx of *D. japonica*. The results of the interspecific cross-reaction tests with four other freshwater triclads and four polyclads are shown in Table 1. This antibody did not show an obvious positive reaction in any of the intestines of the other four freshwater triclads, though it reacted with the epidermis of each one. Furthermore this antibody also showed a reaction with the nervous system of *S. auriculata* and *B. brunnea*. The eyes, in particular, showed a strong positive reaction. This antibody also showed positive reaction with the intestinal cells of three kinds of polyclads. Particularly in *P. multitentaculata*, only intestinal cells reacted strongly and negative control showed no reaction (Fig. 1).

Immuno-electron microscopical observation in *D. japonica* showed that the cytoplasm of phagocytic cells and the spherical granules in granular clubs reacted strongly to this mAb in intestine. mAb positive vacuoles scattered in the cytoplasm of phagocytic cells seemed to be absorbed by pinocytosis from phagocytic cells into the granular clubs (Fig. 2). In phagocytic cells, not only these vacuoles but also the matrix of the cytoplasm showed a strong reaction. In granular clubs, a strong reaction was

TABLE 1

Cross reaction tests of mAb②2B6 prepared against *D. japonica* for freshwater triclads and polyclads.

	int.	epi.	s.g.	n.t.	pha.	par.
Tricladida						
Dugesiidae						
Dugesia japonica	++	++	++	-	+	-
Dugesia ryukyuensis	±*	+	+	-	+	-
Planariidae						
Phagocata vivida	-	+	-	-	-	-
Seidlia auriculata	-	+**	+	+	+	-
Dendrocoelidae						
Bdellocephala brunnea	±*	+	+	++	+	-
Polycladida						
Leptoplanidae						
Notoplana humilis	-	-	-	-	-	-
Planoceridae						
Planocera reticulata	+	-	-	-	-	-
Planocera multitentaculata	++	-	-	-	-	-
Diplosoleniidae						
Pseudostylochus intermedius	+	+	+	-	+	+

int., intestine; epi., epidermis; s.g., sub-epidermal gland cell; n.t., nervous tissue; pha., pharynx; par., parenchyma

* Only the surface of intestinal cells showed a weak reaction.

** Only ventral epidermis of head region showed a positive reaction.

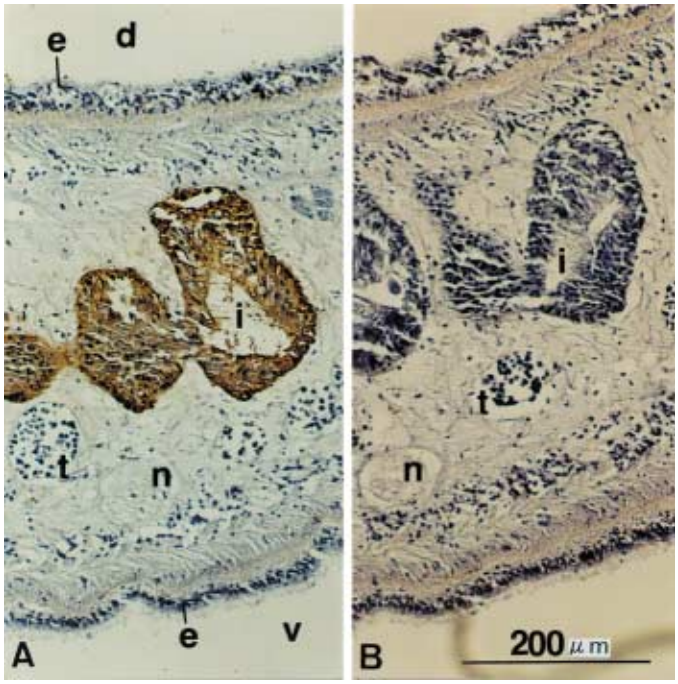

Fig. 1. – Cross-reaction test of mAb②2B6 against freshwater triclad *D. japonica* for polyclad *P. multitentaculata*.
A, Intestine (i) showing intense positive reaction (brown) for this mAb by ABC method. B, Negative control. No reaction is seen. d, dorsal; e, epidermis; n, nerve; t, testis; v, ventral. A and B at the same magnification.

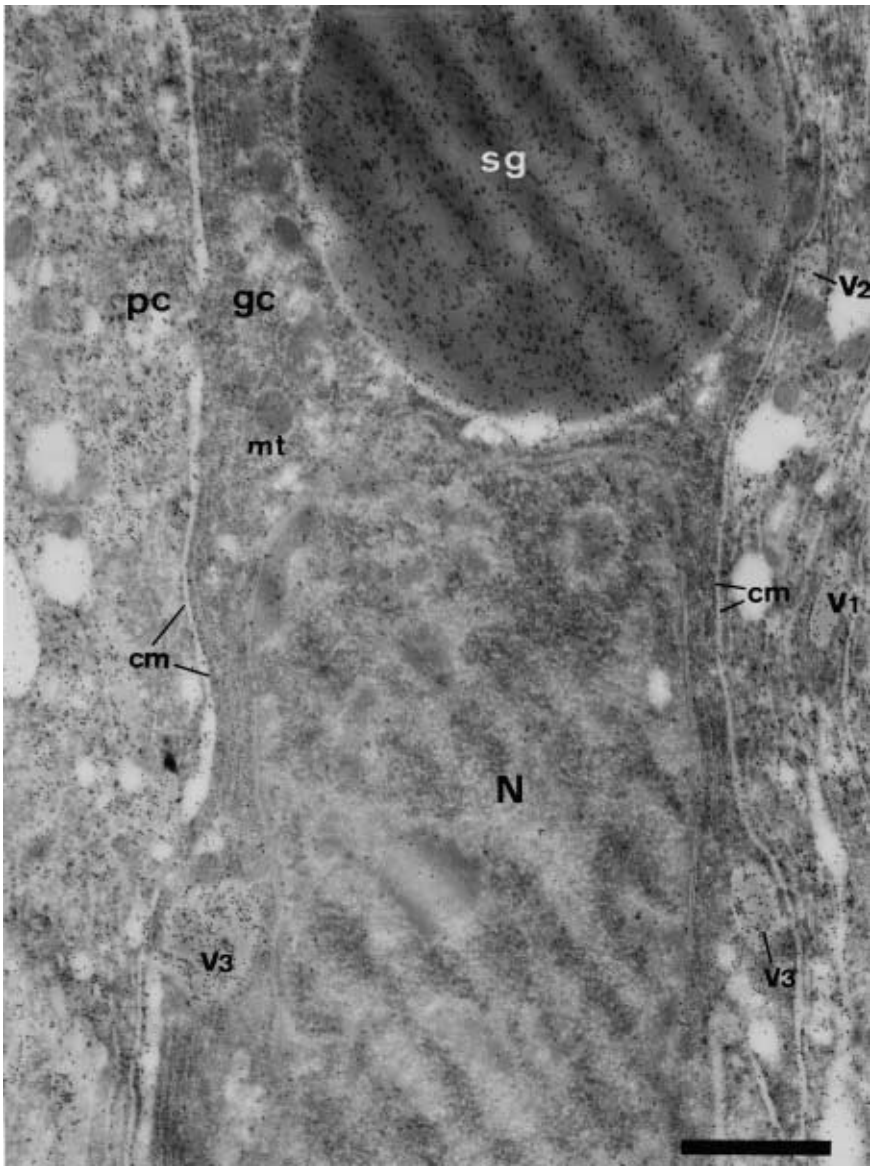

Fig. 2. Immuno-electron micrograph of intestinal cells of *D. japonica* by colloidal gold labeling.
The cytoplasm of phagocytic cell (pc) and spherical large granule (sg) in granular club (gc) react strongly to this mAb. This picture also shows the process of the mAb positive material being absorbed by pinocytosis. Vacuole 1 (V1) is still in phagocytic cell. The limited membrane of vacuole 2 (V2) is fused with the cell membranes (cm) and the contents are being absorbed into the granular club. Vacuoles 3 (V3) have been already absorbed into the granular club (gc). N, nucleus; mt, mitochondria. Bar shows 1 μm.

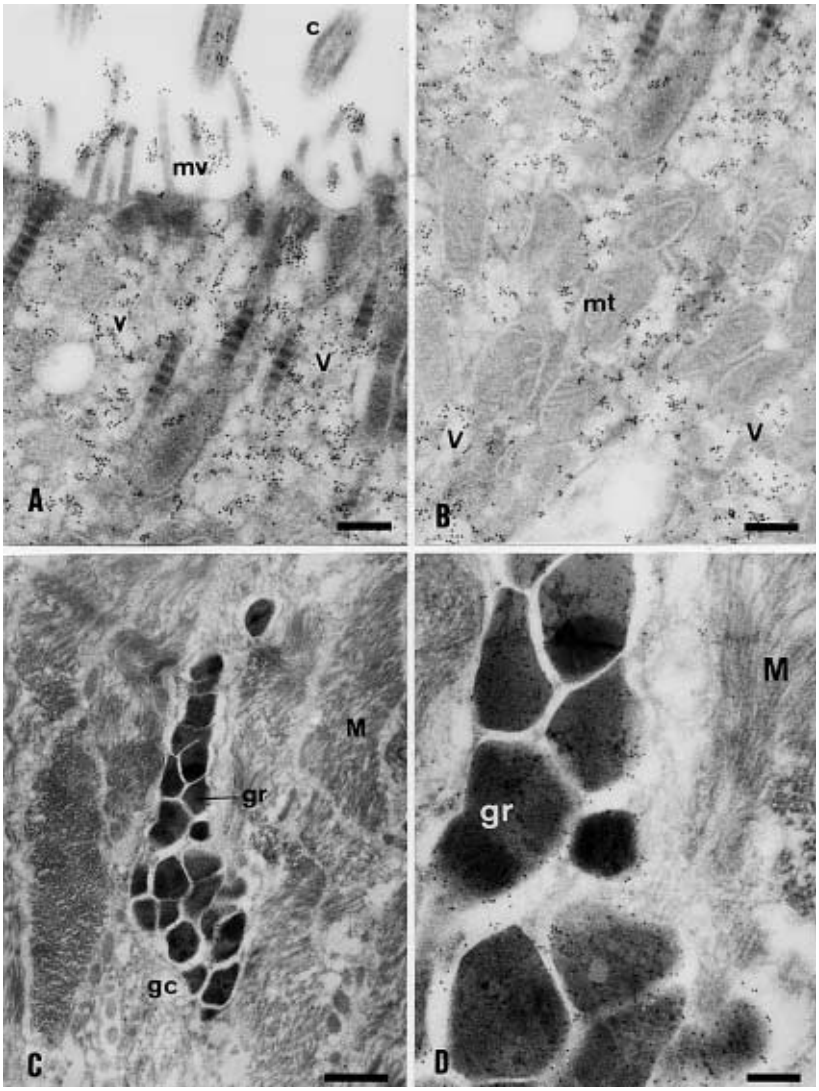

Fig. 3. Immuno-electron micrographs of mAb positive-reaction cells of *D. japonica*. A, upper part of epidermal cell. B, middle part of epidermal cell. C, sub-epidermal gland cell (gc) and muscle cells (M). D, enlarged secretory granules (gr). Gold particles were found on the microvilli (mv), cilia (c) and vacuoles (v) in epidermal cell and on the secretory granules (gr) in the sub-epidermal gland cell. mt, mitochondria. Bars of A, B and D show 250 nm. Bar of C shows 1 μm.

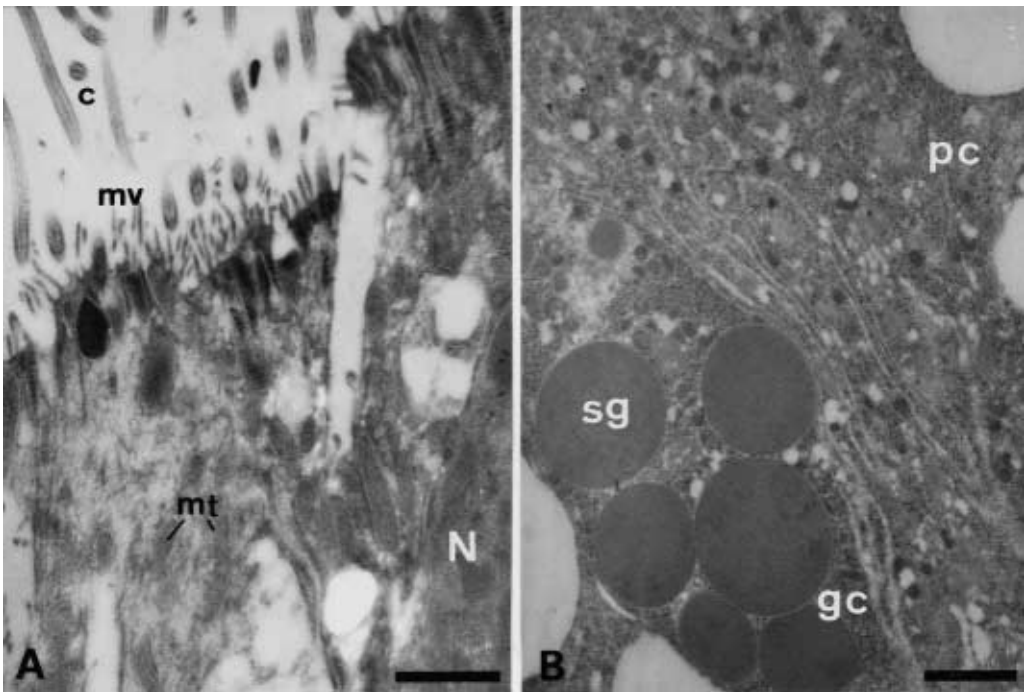

Fig. 4. Immuno-electron micrographs of negative controls. A, epidermis. B, intestine. Few gold particles were found on the both tissues. c, cilium; gc, granular club; mt, mitochondria; mv, microvilli; N, nucleus; pc, phagocytic cell; sg, spherical granule. Bar of A shows 1 μm. Bar of B shows 10 μm.

observed in the vacuoles absorbed by pinocytosis in addition to the spherical large granules (Fig. 2).

In epidermal cells, microvilli, cilia and vacuoles regarded as epitheliosomes (RIEGER et al., 1991) positioned in the upper half of the cells, showed positive reaction to this mAb (Fig. 3, A, B). In sub-epidermal gland cells, secretory granules reacted strongly to this mAb (Fig. 3, C, D).

In negative controls, few gold particles were found on the epidermal cells and two kinds of cells in intestine (Fig. 4).

DISCUSSION

mAb③12B-D reported by SIRAKAWA et al., (1991) reacted strongly and specifically to the gastrodermal and epidermal cells of *Phagocata vivida*. The isotype was found to be an IgG3 and the localization of the antigen was in lipid droplets in phagocytic cells (unpublished data). Thus it was found that mAb②2B6 is different from mAb③12B-D. The localizations of other intestine-specific mbs have not been examined.

The antigens of this mAb②2B6 were distributed in the epidermal cells of the four species examined, though only the ventral epidermis of the head region showed a positive reaction in *S. auriculata*. This results show that a common antigen (epitope) of freshwater triclads is included in the epitheliosomes scattered throughout the epidermis of each species, and that the antigenic determinant is also included in the cells of glands, nervous tissue and the pharynx in some species. The positive reaction of microvilli and cilia on the epidermal cells would be due to the emission of the antigenic determinant from epidermal cells and sub-epidermal gland cells. As the cells injected as the antigen were small cells filtered with nylon mesh of 20μm in pore size, we think that most of the cells were epidermal cells. The results of cross-reaction tests may also support our speculation. It is interesting that the antigenic determinant, including in the epidermal cells of *D. japonica*, also exists in the intestinal cells of polyclads, though *N. humilis* does not have the same antigen. In both *P. multitentaculata* and *P. reticulata*, as this mAb reacts only with intestinal cells, mAb②2B6 can be used as a useful antibody to examine the function of the intestine, and it can also be used as a marker to monitor cellular behavior. In *P. intermedius*, many tissues except nervous tissue reacted to this mAb. We can not use this mAb as a cell marker in this species, because we could not get a single band in western blot analysis of this species (data not shown). In seven other species, this mAb showed no reaction to the parenchyma, so it may be used as a non-mesenchymal cell marker.

The granular clubs in the intestine are filled with spherical granules. The function of this cell remains obscure, and two theories have been presented. One theory is that the cells are protein reserve cells (WILLIER et al., 1925; and others). The other theory is that they are gland cells secreting enzymes for extracellular digestion (JENNINGS, 1974; and others). Our immuno-electron microscopical observation supports the theory that the granular clubs are protein reserve cells. Because the spherical granules and the vacuoles absorbed by pinocytosis from phagocytic cells showed strong positive reaction to the mAb②2B6, it is a possibility that the spherical granules act as a reserve for the protein transported from phagocytic cells.

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