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Immunobiological Studies on Schistosomiasis Japonica with Hybridoma Technology (II) Immunohistochemical Reactivity with the Nervous Systems and the Stomach Glandular Cells of a Heterophil Monoclonal Antibody Obtained from Spleen Cells of *Schistosoma japonicum* Infected Mice

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Abstract

We previously reported that a monoclonal antibody (MoAb) obtained from the spleen cells of *Schistosoma japonicum* infected mice was a novel heterophil antibody. This monoclonal antibody (84B3) immunohistochemically reacted with nervous tissue and glandular cells of the stomach from sheep, cows, mice, and man. The distribution of 84B3 reactive antigens in the nervous system differed from species to species. The molecular weights of the major antigens, as determined by Western blotting, were 50 kDa in the human stomach, 67 kDa in the bovine 4th stomach, and greater than 94 kDa in the gray matter of bovine spinal cord.

Key words: monoclonal antibody, *Schistosoma japonicum*, heterophil antigen

Introduction

In the previous paper, we described two monoclonal antibodies (MoAb) obtained from spleen cells of BALB/c mice infected with *Schistosoma japonicum* (Sj) (Yamashita *et al.*, 1989). One of them (84B3) reacted with sheep red blood cells (SRBC) and goat red blood cells (GRBC), but neither reacted with mouse red blood cells, Sj adult worm antigen nor Sj egg antigen, suggesting that 84B3 is a heterophil MoAb. Further study demonstrated that 84B3 may detect a novel heterophil antigen different from known ones such as Paul-Bunnel, Forssman and Hanganutziu-Deicher antigens. In the course of our study of the specificity of MoAb 84B3, we found by chance that 84B3 immunohistochemically reacts with peripheral nerves in sheep spleen. This unexpected result led us to examine the specificity of the reactivity of 84B3 more precisely using the immunohistochemical method. This study demonstrates that 84B3 reacts with the nervous system and glandular cells in the stomach of the four species examined.

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Materials and Methods

1) Production of MoAb

Details of the method for production of MoAb 84B3, are described in the preceding paper (Yamashita *et al.*, 1989). Briefly, spleen cells of BALB/c mice infected with cercariae of Sj were hybridized with P3-X63-Ag8-653 myeloma cells. The supernatants reactive with SRBC were selected using a membrane radioimmunoassay, and antibody positive clones were obtained by limiting dilution.

2) Immunohistochemistry

Tissues were fixed with 10% formalin, and embedded in paraffin. Specimens were cut to 3 μm. Some specimens were stained with hematoxylin-eosin, PAS, or Alcian blue. For immunohistochemical study we used indirect enzyme im-

munostaining as described by Nakane and Kawaoi (1974). Briefly, after deparaffinization and dehydration, the specimens were treated with methanol containing 3% H_2O_2 to suppress endogenous peroxidases. Nonspecific reaction was inhibited by blocking with 3% bovine serum albumin for 1 hr at room temperature. After washing with PBS, the specimens were treated with MoAb 84B3 (1/300 dilution of culture supernatants, which gave the immunologically specific reaction as determined in the preliminary experiments.) for 1 hr at room temperature. The specimens were then washed with PBS, and treated with 1/300 diluted horse-radish peroxidase conjugated rabbit anti-mouse Ig (Dakopatts; Glostrup, Denmark) for 1 hr at room temperature. The final step was the exposure to DAB solution containing 0.01% H_2O_2 and counterstaining with hematoxylin. The specimens which were positively stained only when both of the first and second antibodies were added, were estimated as reaction positive. As a control, irrelevant MoAbs were used.

3) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

Mucosal tissue from the human stomach or the bovine 4th stomach, and the gray matter of bovine spinal cord, were lyophilized and then delipidized with diethylether. The supernatants obtained after centrifugation at 1700 g for 5 min were further centrifuged at 15000 g for 30 min. To separate the proteins, each supernatant was dissolved in buffer as described by Laemmli (1979) (3 mg protein/ml) and heated for 3 min to 100°C. The details of SDS-PAGE and Western blotting were described in the previous paper (Yamashita *et al.*, 1989).

4) Absorption of MoAb 84B3 with the tissues.

MoAb 84B3 at 1/10 dilution was mixed with an equal volume of crude antigen of the human gastric mucosa (protein concentration: 18mg/ml) prepared for SDS-PAGE analysis or of packed SRBC. The reaction mixtures were incubated for 2 hrs at 37°C, and then at 4°C overnight. The mixtures of human gastric antigen and MoAb 84B3, and that of SRBC and the MoAb were centrifuged at 15000 g for 1hr and 1000 g for 10 min respectively. The supernatants were used as absorbed MoAb 84B3.

Results

1) Organ and tissue distribution of MoAb 84B3 reactive antigens in several different species.

Various organs of the sheep, cow, man, and mouse were examined for immunohistochemical reactivity with MoAb 84B3. In the sheep, the cerebellum, the peripheral nerves, and glandular cells of the fourth stomach (Fig. 1b) gave a positive reaction, however the parenchymal cells of the other organs examined were negative. In the nervous system, the strongest reaction was observed in ganglional cells (Fig. 1c) and the intermuscular nerve plexus (Fig. 1d) of the stomach, the small intestine, and the colon, and the peripheral nerve fibers of all the organs examined. A moderate reaction was observed with motor neurons and nerve fibers of spinal gray matter (Fig. 1a). Furthermore, a weak reaction was observed in the molecular layer and in the granuler layer of the cerebellum. In the stomach, diffuse positive staining was observed in the cytoplasm of glandular cells of the fundic gland (Table 1). PAS and alcian blue staining revealed that reaction positive glandular cells were not mucin producing cells.

In the cow, the nervous system (Figs. 2a, 2c) and glandular cells of the fourth stomach gave a positive reaction, similar to results in the sheep. The distribution of antigens in the spinal cord, and the fourth stomach was the same as in the sheep. A difference in staining between the cow and the sheep was observed as far as distribution of the antigens of the peripheral nerves. In the cow, the reaction was positive in the ganglional cells and intermuscular nerve plexus (Fig. 2d) of the fourth stomach, the small intestine and colon, and the peripheral nerve fibers of the heart and the ovarium, whereas the peripheral nerve fibers of the other organs examined were antigen negative (Table 1). Antigens of the fourth stomach were found in the cytoplasm of the large glandular cells, but were absent in the mucin

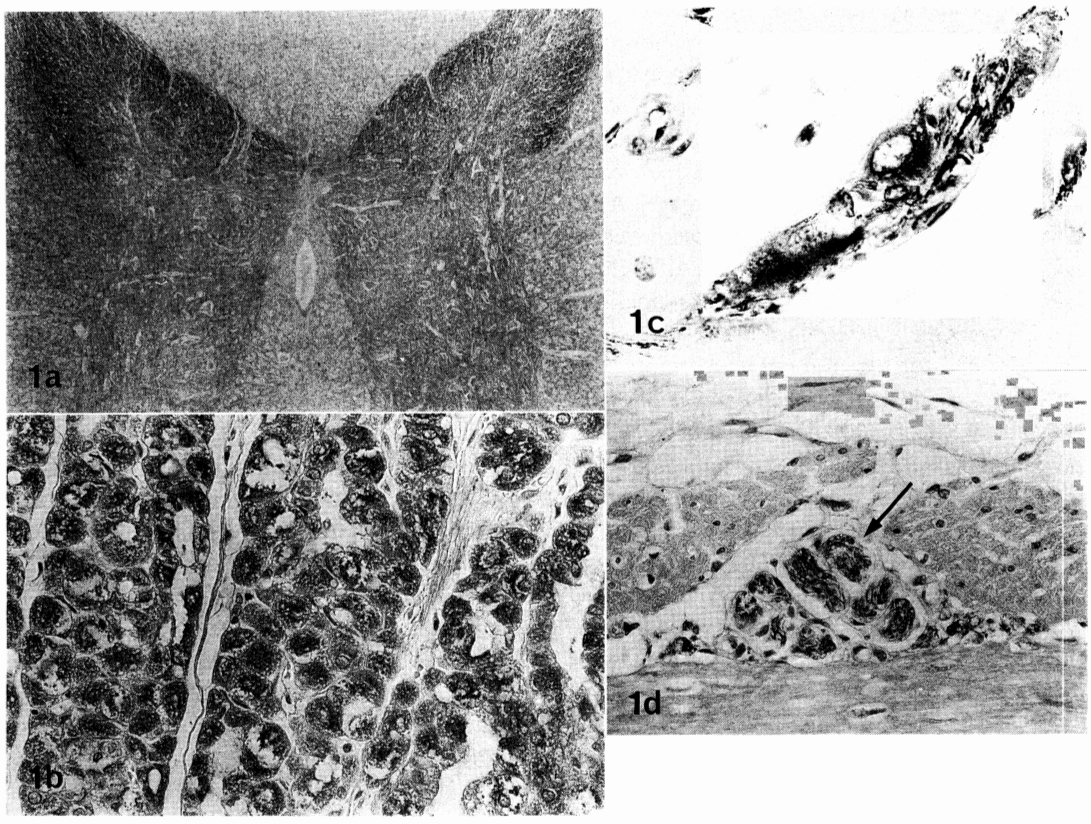

Fig. 1. Immunohistochemical staining of various sheep tissues with MoAb (84B3). In spinal gray matter the motor neuron and the nerve fibers was positive (a), ×40. In the 4th stomach virtually all of the glandular cells reacted positively (b), ×400. The submucosal ganglional cells (c), as well as the intermuscular nerve plexus (d, arrow) reacted very strongly, ×100.

Table 1 Immunohistochemical reactivity of the monoclonal antibody (84B3) to various organs

Organ	Species			
	sheep	cow	mouse	man
Cerebrum	(−)	(+)	(−)	(−)
Cerebellum	(+)	(+)	(+)	(−)
Spinal cord	(+)	(+ + +)	(−)	(−)
Peripheral nerve	(+ +)	(+ +)	(−)	(+ +)
Lung	(−)	(−)	(−)	(−)
Liver	(−)	(−)	(−)	(−)
Stomach	(+ +)	(+ +)	(+ +)	(+ +)
Intestine	(−)	(−)	(−)	(−)
Kidney	(−)	(−)	(−)	N.D.
Heart	(−)	(−)	(−)	N.D.
Spleen	(−)	(−)	(−)	N.D.
Ovary	N.D.	(−)	N.D.	(−)

(−): negative, (+): slightly positive, (+ +): moderately positive, (+ + +): intensity positive, N.D.: not done.

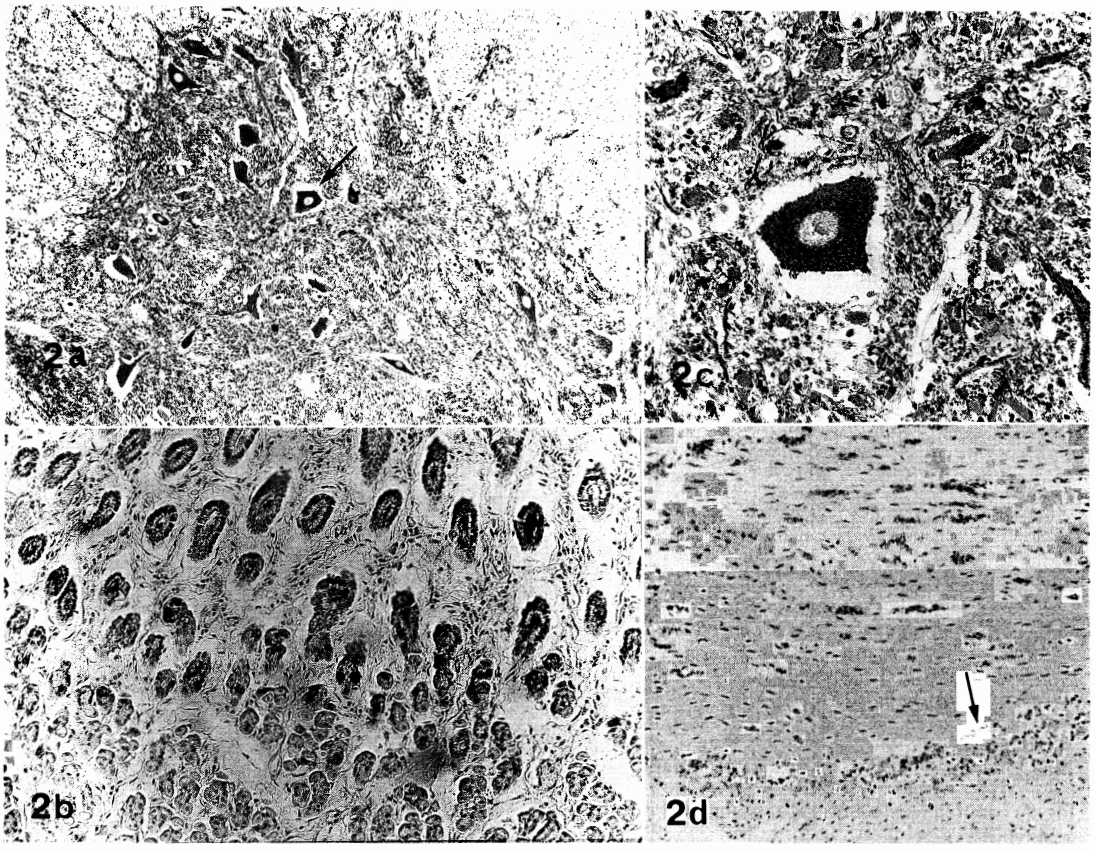

Fig. 2. Immunohistochemical staining of various bovine tissues with MoAb (84B3).
(a) In the spinal cord, the motor neuron (arrow) and nerve fibers in the gray matter reacted positively, ×20. (c), ×100. (b) In the 4th stomach some of the glandular cells, ×400, and (d) the intermuscular nerve plexus (arrow) reacted positively, ×200.

producing cells (Fig. 2b). In the mouse, the antigens were found in the celebellum (Fig. 3a), and the glandular cells of the stomach (Fig. 3b), but not in any peripheral nerves (Table 1).

In man, the antigens were detected only in the submucosal ganglional cells (Fig. 4b) and chief cells (Figs. 4a, 4c) of the stomach. In man, in contrast to the sheep and the cow, the antigens were negative in the spinal cord, the intermuscular nerve plexus of the stomach and the colon, and the peripheral nerves of the other organs (Table 1).

To confirm that the antigens on SRBC are identical to those detected in the tissue immunohistochemically, MoAb 84B3 preabsorbed with homogenized human gastric mucosal tissue and SRBC was used for immunohistochemical staining. The immunohistochemical reactivity of MoAb 84B3 with chief cells and submucosal ganglional cells of the human stomach was completely abolished by the absorption with either SRBC or gastric tissue.

2) Western blotting analysis of the antigens reactive with MoAb 84B3.

The molecular weights of the antigens in tissues reactive with MoAb 84B3 were determined by Western blotting. As shown in Fig. 5, the main band was at 50 kDa in human gastric mucosa, mainly 67 kDa and to a lesser degree 55 kDa and

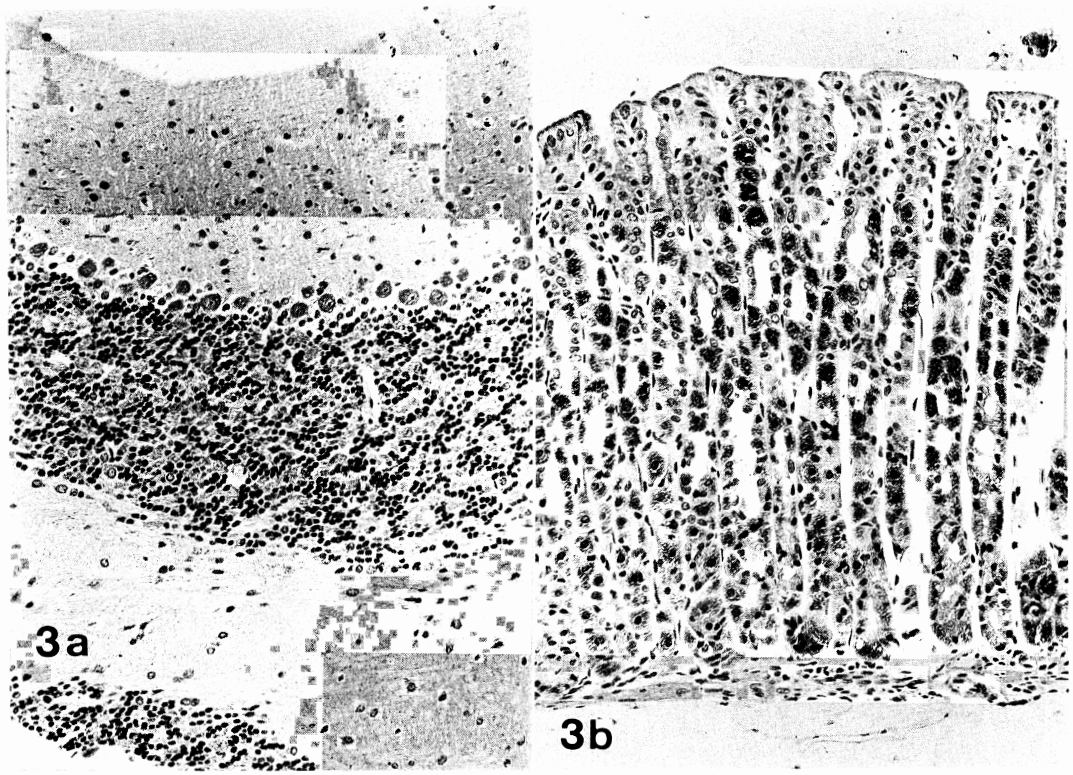

Fig. 3. Immunohistochemical staining of the cerebellum (a) and the glandular stomach (b) of the BALB/c mouse with MoAb (84B3).
(a) Purkinje cells and some components in the molecular layer and in the granuler layer were positive. ×400.
(b) Some of the glandular cells reacted positively, ×400.

45kDa in the bovine fourth stomach, and more than 94 kDa in bovine spinal cord (Fig. 5).

Discussion

In this paper, we report a heterophil MoAb obtained from spleen cells of Sj infected mice that reacts not only with xenogeneic red blood cells but with the nervous system and glandular cells of the stomach. These experiments extend the results of our previous reports on the specificity of heterophil antibodies produced in Sj infected mice (Asahi *et al.*, 1985) and that of heterophil MoAb obtained from these mice (Yamashita *et al.*, 1989). It is unlikely that MoAb, 84B3, is a minor clone in antibody producing cells in Sj infected mice, and that it reacts by chance with the nervous system and glandular cells in the stomach for the following reasons: 1) The specific reactivity of 84B3 with red blood cells from various species is almost identical with that of sera from Sj infected mice, in that both react mainly with SRBC and GRBC (Asahi *et al.*, 1984; Yamashita *et al.*, 1989). 2) Sera from Sj infected mice immunohistochemically stained nervous tissue and glandular cells of the stomach of certain species (Atsumi, unpublished result). 3) In contrast to the previous findings that heterophil antibodies detected in hosts infected with various parasites are mainly of the IgM class, the heterophil antibody in Sj infected mice is resistant to 2-ME treatment (Asahi *et al.*, 1984). The immunoglobulin class of 84B3 is also not IgM, (IgG_1 as reported in the preceding paper (Yamashita *et al.*, 1989). These results clearly show that 84B3 detected a novel heterophil antigen which has not

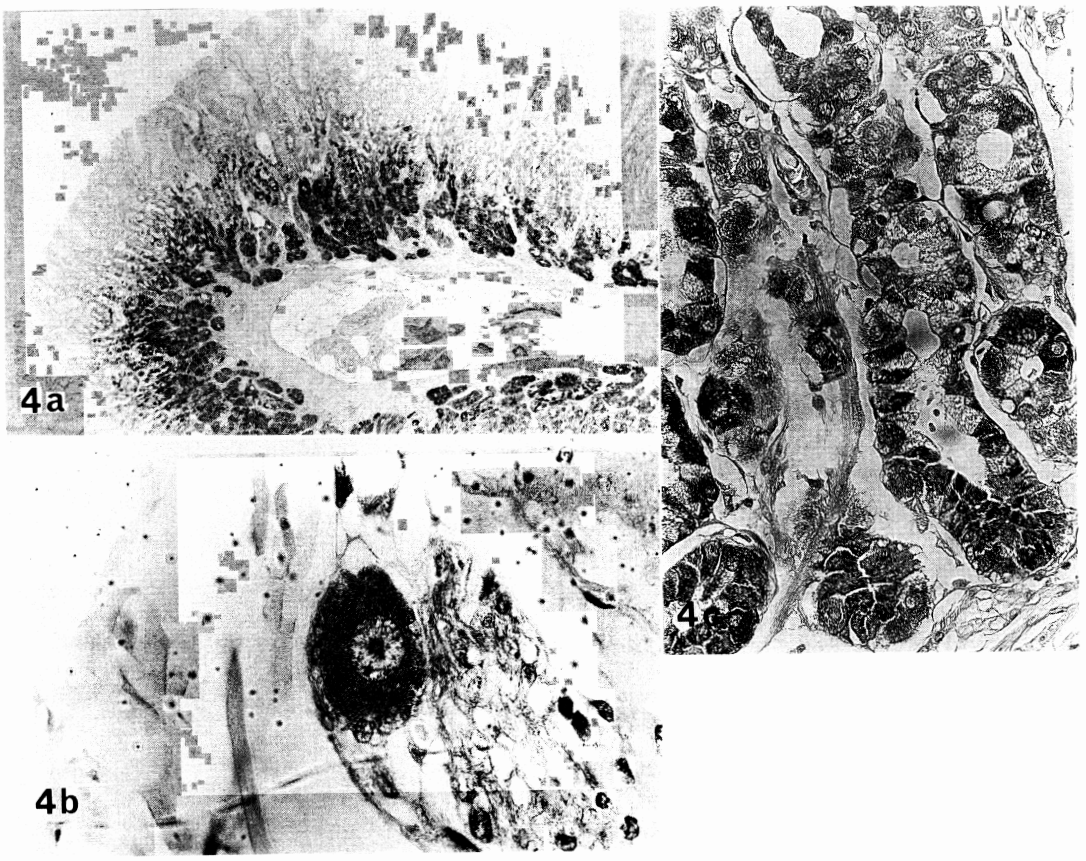

Fig. 4. Immunohistochemical staining of the human stomach with MoAb (84B3).
(a) The cytoplasm of the chief cells reacted positively, ×40. (c), ×400. (b) The submucosal ganglional cells reacted slightly positively, ×400.

been identified (Damian, 1964; Goldring *et al.*, 1976). MoAb 84B3 reacted with tissues of the mouse. This result indicates that MoAb 84B3 is not a heterophil antibody in the strict sense of the word (Zilton *et al.*, 1971; Kawabata *et al.*, 1981). To our knowledge, we do not know of a heterophil antibody with reactivity to autologous tissues. In this sense, MoAb 84B3 may be a novel kind of heterophil antibody (Kano *et al.*, 1977; Hakomori *et al.*, 1977; Mori *et al.*, 1979; Nishimaki *et al.*, 1979). Furthermore, absorption of MoAb, 84B3 with various tissues such as guinea pig kidney usually used for determination of the specificity of classical heterophil antibodies clearly indicates that the specificity of MoAb 84B3 is different from Paul-Bunnel, Hanganutzui-Deicher and Forssman antibodies (Sendo, unpublished results). Results of Western blotting in the present experiments showing that the molecular weight of antigens from the human and bovine stomach, and bovine gray matter are different from each other indicates that a common epitope on different molecules reacts with 84B3. Although the molecular nature of 84B3 reactive substances is quite unknown, the large difference in molecular weight of each molecule reactive with 84B3 suggests that these substances may be other than neuro – intestinal hormones. Further biochemical studies on these substances should provide answers concerning their molecular natures. The distribution of MoAb 84B3 reactive antigens in neural tissue differs

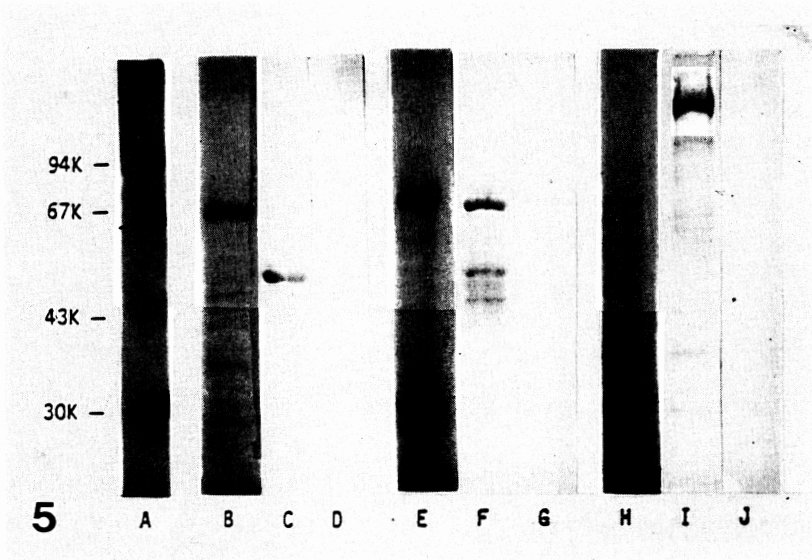

Fig. 5. Western blotting analysis of human stomach and bovine 4th stomach and gray matter of the bovine spinal cord using monoclonal antibody 84B3.
Lane A: molecular weight standards, lane B, C, D: human stomach, lane E, F, G: bovine 4th stomach and lane H, I, J: bovine spinal gray matter. Lanes B, E, F: stained with Coomassie brillant blue, lane C, F, I: reacted with 84B3. Lane D, G, J: treated with secondary antibody (negative control).

from species to species. We do not have any information to explain these differences so far. The antigen may be expressed on different parts of neural tissue from a phylogenetic point of view. The role of this novel heterophil antibody in the pathogenesis of schistosomiasis japonica is also unclear, a situation similar with other heterophil antibodies detected in various infections (Mauss, 1941; Dammin and Weller, 1945; Henderson-Begg, 1946; Soulsby, 1958; Hauba *et al.*, 1969; Hauba *et al.*, 1974; Nishimaki *et al.*, 1979). However, considering that MoAb 84B3 reacted with the cerebellum of syngeneic mice, autoantibodies of which the reactivity is similar to MoAb 84B3 may play a role in the pathogenesis of encephalopathia in human schistosomiasis japonica, the cause of which is still unknown (Ariizumi, 1963; Hayashi, 1979; Chen and Mott, 1988).

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