RESEARCH NOTE

SPECIFIC AND CROSS-REACTIVE MONOCLONAL ANTIBODIES TO THE 89-kDa ANTIGEN OF *OPISTHORCHIS VIVERRINI*

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We previously reported the production of monoclonal antibodies (MAbs) to somatic (Billings *et al*, 1990) and excretory-secretory (Amornpunt *et al*, 1991) antigens of *Opisthorchis viverrini*. The characteristics of the antigens to which these MAbs reacted are summarized as shown in Table 1. It was not surprising to note that all MAbs to the worm metabolic products were directed against the 89-kDa component which was previously shown to be the predominant protein in the culture medium in which these adult worms were maintained. It was unexpected, however, to find that these MAbs reacted with different structures of the parasite (Table 1). They also varied considerably with regard to immunological cross-reactivity against a number of closely related flukes of man and animals. Because both Groups 1 and 2 anti-89 kDa MAbs were highly specific for *O. viverrini* (Ov), they were tested on a limited scale for their diagnostic potential (Sirisinha *et al*, 1991). The limited data obtained were highly promising and therefore a larger scale field trial has been undertaken to further evaluate its value in an epidemiological study.

To elucidate the epitopes against which these MAbs reacted, the chemical nature of the 89-kDa antigen was first determined. This was done by subjecting the antigen to SDS-PAGE, blotting onto a nitrocellulose membrane and staining with either Amido black (for protein) or Concanavalin A (for carbohydrate). The results indicated that the 89-kDa antigen is a glycoprotein. This is not surprising as the surface tegument for a number of parasites is known to be highly glycosylated (Sobhon and Upatham, 1990). In order to determine whether or not the anti-89 kDa MAbs were directed against the carbohydrate moiety, a periodate oxidation experiment was performed. The 89-kDa ES antigen was first subjected to SDS-PAGE and blotted on a nitrocellulose membrane as mentioned above and the membrane was subsequently exposed to 50 mM sodium periodate at pH 4.5 for one hour at room temperature (Woodward *et al*, 1985). The membrane was then rinsed and pretreated with 1% glycine prior to being exposed to the MAbs. Positive immunoblotting reaction failed to appear, indicating that the reactive epitope was destroyed by periodate treatment. Thus, these MAbs appeared to be directed toward the carbohydrate moiety of the 89-kDa glycoprotein antigen.

The summary presented in Table 1 pointed out one additional interesting finding. That is, our muscle-reactive MAbs (anti-90 kDa and Group 3 anti-89 kDa) cross-reacted with a large number of closely related flukes. This is not entirely unexpected because one should expect the muscle tissues of these flukes to be highly conserved, particularly among the *O. viverrini, O. felineus* and *Clonorchis sinensis*. However, more recently Chaicumpa and associates (1991) reported the production of highly *O. viverrini*-specific MAbs that also reacted with the muscle system and yet failed to react with even its close relative, *O. felineus* (Table 1). Some of these MAbs gave multiple bands by immunoblotting. It was, however, difficult to be certain of the exact location of these MAbs on the muscular system because the different parasite tissues could not be readily demonstrated from the photographs.

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Table 1

Monoclonal antibodies to *Opisthorchis viverrini.*

Investigators	Immunogens	Immunoreactive component (s)	Major anatomical site (s)*	Cross reactivity with closely relate flukes (ELISA)+
Billings *et al,* 1990	Somatic	90 kDa	M	Of, Cs, Fg, Ph, Ps, C, S
		16 kDa	T	Group 1: Of, Cs, Fg, Ph, Ps, C, S Group 2: Of, Cs, Fg, Ph, Ps
Amornpunt *et al,* 1991	ES	89 kDa	C	Group 1: Ph
		89 kDa	T, TC	Group 2: -
		89 kDa	T, TC, M	Group 3: Of, Cs, Fg, Ph, Ps, C, H
Chaicumpa *et al,* 1991	Somatic	Multiple	M or M and T	-

* M = muscle, T = tegument, TC = tegumental cells, C = Cecum and its content
\+ Of = *O. felineus,* Cs = *Clonorchis sinensis,* Fg = *Fasciola gigantica,*
Ph = *Paragonimus heterotremus,* Ps = *P. siamensis,* C = *Centrocestus* sp, H = *Haplochis* sp.

All of our muscle-associated MAbs (Billings *et al,* 1990) stained exclusively the muscle fibers. No other structures reacted with these MAbs and yet these MAbs strongly reacted with *C. sinensis* and *O. felineus* by ELISA. The discrepancy of the results could be related to the difference in the reactive epitopes to which these antibodies are directed. A direct comparison among these MAbs should clarify this point. A cocktail made from these specific MAbs may provide a more suitable reagent for the detection of antigen to be used in the diagnosis of opisthorchiasis.

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