Characterization of a 31-kda specific antigen from *Parastrongylus cantonensis* (Nematoda: Metastrongylidae)

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Abstract

A 31 kDa specific antigen of Parastrongylus cantonensis was previously shown in immunoblotting to be potentially useful for diagnosis of human parastrongyliasis. The present showed that this 31-kDa component appeared to be a glycoprotein. Immunoblotting using serum from a patient with parasitologically confirmed parastrongyliasis revealed no inhibition of its antigenicity following treatment with periodate. As the 31-kDa specific band could still be observed after carbohydrate destruction, the sugar residue of this glycoprotein antigen is therefore not responsible for the specificity in immunodiagnosis of human *Parastrongylus cantonensis* infection.

Key words: Parastrongylus (=*Angiostrongylus*) cantonensis; specific antigen; immunoblotting

Introduction

Human parastrongyliasis (angiostrongyliasis) is caused by a neurotropic metastrongyloid nematode *Parastrongylus* (=*Angiostrongylus*) cantonensis. The definitive diagnosis is to recover the worms in the cerebrospinal fluid (CSF) or eye of the patients (Cross, 1987; Durette Desset *et al.*, 1993), but such occasions are rare. Attempts have been made to diagnose the infection by using various immunological methods to detect antibodies. However, the antigenic reagents currently available for diagnosis lack the necessary specificity. Indeed, the crude antigens of *P. cantonensis* are known to cross-react with the heterologous antibodies produced against a few other parasites especially *Gnathostoma spinigerum*, a clinically related parasite (Tharavanij, 1979; Dharmkrong-at *et al.*,1989;Morakote *et al.*,1989). Our recent work in analysing somatic antigens of *P. cantonensis* by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting using sera from patients suffering from parastrongyliasis has led to the indentification of a 31-kDa protein as one of the immunodominant antigens (Eamsobhana, 1994). This antigens reacted with all parasitologically proven parastrongyliasis sera but not with those of other parasitic infections and normal healthy individuals (Eamsobhana *et al.*, 1997). It appears to be an ideal protein for use as a diagnostic antigen. However, the nature of the 31-kDa specific component has not been studied. As such, it will certainly be interesting to determine its biochemical and immunochemical properties.

Materials and Methods

Antigens

Adult worms of *Parastrongylus cantonensis* were obtained from the pulmonary arteries and hearts of infected albino rats as previously described (Eamsobhana *et al.*, 1997). Male and female worms were washed and homogenized separately in normal saline with a glass tissue grinder. The suspension was then sonicated and left overnight at 4°C to allow elution of antigens. Soluble antigens were obtained as the supernatant after centrifugation at 4,000 rpm at 4°C for 15 min. Protein content of the extracts was determined using a protein assay kit II (Bio-Rad Laboratories, U.S.A.).

Serum

The parasitologically confirmed parastrongyliasis serum used in the study was that of a patient from whom a fourth-stage larva of *P. cantonensis* was recovered from the CSF. The serum was kept at -70°C in small aliquots until use.

SDS-PAGE and immunoblot analysis

The adult worm antigens of *Parastrongylus cantonensis* were separated by SDS-polyacrylamide gel, using a 12% gel as described by Laemmli (1970). Approximately 10 µg protein per well was loaded onto the gel. The protein bands were visualized by staining with Coomassie brilliant blue R 250. For transblotting, the separated antigens from the gel

were electroblotted onto a 0.45 µm nitrocellulose membrane (Sigma Chemical Company, U.S.A.), by the method of Towbin *et al.* (1979). The non-specific binding sites on the membrane were blocked by soaking it in a solution of 5% skimmed milk in phosphate buffered saline (PBS), pH 7.4 for 1 hr at room temperature. The strip was then allowed to react with the test serum at a dilution of 1:200 in 1% bovine serum albumin (BSA) in PBS, pH 7.4 overnight at 4°C. After thorough washing, the strip was incubated with peroxidase-conjugated rabbit anti-human immunoglobulins (Dakopatts, Denmark) at a dilution of 1:1,000 in PBS, pH 7.4 for 1 hr at room temperature followed by further washing. The blot was developed with the chromogenic substrate solution containing 60 mg of 4-chloro-1-naphthol (Bio-Rad Laboratories, U.S.A.) in 20 ml of cold absolute methanol mixed with 60 µl of 30% H_2O_2 in 100 ml of PBS, pH 7.4. The reaction was terminated by rinsing the membrane strip with distilled water.

Lectin-binding analysis

To detect glycoconjugate of the antigen, specific carbohydrate-binding molecules namely concanavalin A and wheat germ agglutinin were used.

The electroblotted membrane strips were incubated in a blocking solution as mentioned above for 1 hr with agitation, followed by washing. The strips were then treated with either 45 µg protein/ml of peroxidase-labeled concanavalin A or wheat germ agglutinin (Sigma Chemical Company, U.S.A.) in PBS, pH 7.4 containing 0.2% BSA and 0.2% gelatin for 2 hr at room temperature with gentle rocking. After washing with washing buffer, the membrane strips were finally incubated with the substrate, as described above.

Oxidation of sugar residues

Carbohydrate structures were treated with periodate according to Vieths *et al.* (1995). Briefly, the electroblotted membrane strip was incubated with 0.1 mol/l $NaIO_4$ in 0.01 mol/l sodium acetate, pH 5, for 2 hr in the dark. The membrane was then washed, and the blotting procedure followed as described above. The other membrane obtained from the same gel was incubated only in the acetate buffer, to serve as control.

Results

SDS-PAGE analysis and Coomassie brilliant blue staining of the somatic extracts from adult male and female worms of *P. cantonensis* revealed many proteinous components of high and low molecular weight. The specific component of 31 kDa appeared as a poorly stained band (Fig.1 lane E). Immunoblotting with a serum of patient with confirmed parastrongyliasis reacted strongly with the 31-kDa band of the adult worm antigens (Fig.1 lane A).

Lectin-binding analysis demonstrated that the specific antigen of 31 kDa contained carbohydrate moiety which precipitated strongly with wheat germ agglutinin (Fig.1 lane C), but not with concanavalin A (Fig.1 lane B). Oxidation of sugar residues after periodate treatment of the transferred proteins did not result in a loss of recognition of this particular 31-kDa band (Fig. 1 lane D). The carbohydrate moiety is not responsible for the antigenicity of this 31-kDa antigen.

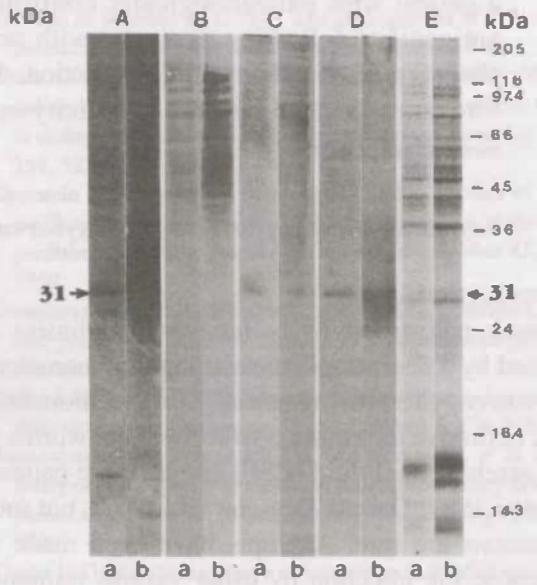

Fig. 1. Positive (lanes A, C and D) and negative (lane B) patterns of immunoblotting against *Parastrongylus cantonensis* adult male (a) and female (b) worm antigens. Note the presence of a 31-kDa specific band in the positive patterns. A: parastrongyliasis patient's serum; B: peroxidase-labeled concanavalin A; C: peroxidase-labeled wheat germ agglutinin; D:serum of patient with confirmed parastrongyliasis after treatment with periodate. Protein profiles of the adult worm antigens stained with Coomassie blue are shown in lane E. Molecular weight markers are indicated on the right-hand side.

Discussion

Parastrongylus cantonensis is well known as a major causative agent of eosinophilic meningitis and eosinophilic meningoencephalitis in humans in Southeast Asia and the Pacific islands (Cross, 1987). A definite diagnosis of the infection can only be made following a recovery of the worm from a patient, but this is very rare. Therefore, the most practical, useful and dependable diagnostic

assays for human parastrongyliasis are the immunological tests. However, the tests are hampered by the cross-reactive nature of the antigens used (Tharavanij, 1979; Ko, 1989).

In our previous work, an analysis of the *P. cantonensis* specific antigens by comparing the serum reactivities between patients with parastrongyliasis and those with other parasitic infections in immunoblot, identified a diagnostic 31-kDa antigen from the third-stage larvae and adult worms of *P. cantonensis* (Eamsobhana, 1994; Eamsobhana *et al.*, 1997). In the present study, Coomassie brilliant blue staining of the SDS-PAGE gel showed this specific antigen to be a protein. However, as it stained poorly for protein, it may be a heavily glycosylated antigenic component. Further study using lectin-binding assays revealed this specific antigen of *P. cantonensis* to be a glycoprotein. It formed a precipitated complex with wheat germ agglutinin but not with concanavalin A. This indicates that the sugar residue of the 31-kDa antigen is probably a *N*-acetylglucosamine. Wheat germ agglutinin has been reported to possess two binding sites for *N*-acetylglucosamine per sub-unit and the binding is highly specific (Nagata & Burger, 1974).

Like many other parasite antigens, *P. cantonensis* antigens are complex protein mixtures. Among them, glycoprotein antigens have been reported to cause much of the cross-reactions due to sugar residues. These cross-reactive epitopes are known as cross-reactive carbohydrate determinants (Weil *et al.*, 1990). To find out whether the carbohydrate moiety of 31-kDa glycoprotein was responsible for the antigenicity, periodate treatment as described by Vieths *et al.* (1995) was used. Our study clearly showed that the serum from the parastrongyliasis patient contained a specific antibody not directed to carbohydrate determinant of 31-kDa antigen. A 31-kDa band was still present after periodate oxidation of transferred proteins. If the antibody was directed against the carbohydrate determinant, this specific reaction of 31-kDa band would be eliminated, due to carbohydrate destruction after treatment with periodate. Since carbohydrate determinants cause much of the cross-reactivity among nematodes (Weil *et al.*, 1990), perhaps the use of periodate-treated antigens could enhance the specificity of this 31-kDa component. As the specificity and sensitivity of the immunodiagnostic tests to detect specific antibodies depend on purity and specificity of the antigens used, this study provides the information required for further purification of this 31-kDa antigen for use in immunodiagnosis of human parastrongyliasis. Experiments involving the purification of this specific, diagnostic glycoprotein antigen of *P. cantonensis* through biochemical processes are being conducted.

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