Chulalongkorn Medical Journal

Volume 30
Issue 8 *August 1986*

Article 5

8-1-1986

Gnathostoma antigen: An analysis of pprotien profiles

W. Saksirisampant

K. Bunmanavong

P. Kraivichian

นิพนธ์ต้นฉบับ

Gnathostoma antigen : An analysis of protein profiles.

Wilai Saksirisampant*
Kanda Bunmanavong* * Phisai Kraivichian*

Saksirisampant W, Bunmanavong K, Kraivichian P. Gnathostoma antigen : An analysis of protein profiles. Chula Med J 1986 Aug ; 30 (8) : 739-742

Somatic antigens of third stage Gnathostoma larva were prepared and analyzed for their antigenic mosaic by SDS-polyacrylamide gel electrophoresis under reducing condition. These soluble extracts, not entirely unexpected; showed highly complicated protein components. As many as 36 peptides could be demonstrated when the gel was stained by Coomassie Brillient blue R. Their molecular weights (MW) varied between 113.0 and 14.9 kilodaltons (K), the most intense bands occurring at 41.0 and 39.5 K. However, these various protein profiles should be identified further for their reactive, rather than non-reactive or (and) cross reactive component(s), and specifically isolated for use in the serodiagnosis of gnathostomiasis.

วิไล ศักดิ์ศิริสัมพันธ์, กานดา บุญมานะวงศ์, พิสัย กรัยวิเชียร. Gnathostoma antigen : An analysis of protein profiles. จุฬาลงกรณ์เวชสาร 2529 สิงหาคม ; 30 (8) : 739-742

จากการวิเคราะห์หาความซับซ้อนของโปรตีนองค์ประกอบในแอนติเจน โรคพยาธิตัวจี๊ดที่เตรียมขึ้นจากตัวอ่อนระยะที่ 3 นั้น พบว่าภายใต้ภาวะริดิวซ์ของวิธี SDS-polyacrylamide gel electrophoresis แอนติเจนนี้มีโปรตีนองค์ประกอบซับซ้อนมากถึง 36 ชนิด โดยมีน้ำหนักโมเลกุลในแต่ละแถบโปรตีนเรียงกันตั้งแต่ 113.0 ถึง 14.9 กิโลดาลตัน แถบที่มีน้ำหนักโมเลกุลตำแหน่ง 41.0 และ 39.5 กิโลดาลตัน มีความเข้มมากที่สุดจากสีย้อมชนิด Coomassie Brillient blue R. อย่างไรก็ดีแถบของโปรตีนต่าง ๆ ที่พบควรที่จะทดลองต่อไปเพื่อชี้ว่าองค์ประกอบใดมีความจำเพาะเจาะจงในโรคพยาธิตัวจี๊ดเพื่อแยกเอาส่วนนี้มาใช้ในงานวินิจฉัยทางภูมิคุ้มกันต่อไป

* Department of Parasitology, Faculty of Medicine, Chulalongkorn University.
* * Fourth year Medical-technology student, Faculty of Medicine, Chulalongkorn University

Gnathostomiasis is one of the important parasitic diseases in Thailand and many other countries in Southeast Asia including Phillippines, Indonesia, Vietnam, Cambodia, Burma and Malaysia. Highest incidence of the disease is found in Thailand.[1] The causative agent is Gnathostoma spinigerum[2] whose third stage larva is generally the infective stage which do not normally develop into the adult stage in human.[3]

At present there is no satisfactory diagnostic tool for this disease, although many attempts have been made with various highly sensitive immunological methods employing the somatic antigen of third stage larvae. These assays of high sensitivity lead to decreased or loss of specificity which is principally due to the complex antigenic mosaic of the parasite antigens.[4] When compared with bacterial or viral antigens, helminthic antigens are more complicated in their antigenic components and exhibit high contents of non-reactive and/or cross reactive moieties.[4]

Suntharasamai et al previously demonstrated that an enzyme-linked immunosorbent assay for gnathostoma serum antibodies showed cross reaction in patients with opisthorchiasis, filariasis, paragonimiasis including malaria, hook worm, strongyloid and enterobius infections.[5] In addition, not only in a radio-immunoassay system with serum from animals experimentally infected with Opisthorchis sp. and Toxocara cati[6], but also in intradermal tests in patients with other parasitic infections crossed reaction could be demonstrated.[7,8,9]

In developing a specific diagnostic antigen or potential antigen for G.spinigerum infection, it would be more advantageous if one could use a well characterized antigenic component (s). Therefore, the analysis of protein profiles of the third stage larval somatic antigen is necessary in order to gain understanding in the serodiagnosis of Gnathostoma infection.

Meterials and Methods

Third stage larvae (L_3) of G. spinigerum were originally obtained from the Department of Helminth, Faculty of Tropical Medicine of Mahidol University and have been maintained by cycling through cats, cyclops and mice in our department. L_3 were havested from mice after six weeks of experimental infection with oral force-feeding of second stage larvae in infected cyclops as described by Daengsvang et al.[10]

Preparation of somatic L_3 antigen : About one thousand L_3 were cleaned by several washes in steriled normal saline under the dissecting microscope. The larvae were homogenized in a ground glass tissue grinder with a small volume of steriled normal saline. The homogenate was then sonicated under an Ultrasonic Disintegrator (Soniprep 150, MSE Scientific Instrument, England) at an amplitude of 10 microns for 1-min and repeated until most intact cells were broken as judged from a microscopic examination. The sonicated suspension was centrifuged at 11,500 rpm, 4°C for 25 min. The supernatant of L_3 extract was determined for its protein concentration by a micro-modification of the Folin-Ciocalteau method[11] using bovine serum albumin as a standard.

Polyacrylamide gel electrophoresis (PAGE) : The complexity of the protein components extracted from somatic L_3 was subjected to PAGE for analysis. The procedure was the discontinuous buffer system which was carried out in the presence

of 1% sodium dodecyl sulfate (SDS) essentially as described by Laemmli.[12]

In the experiment, a slab gel containing 10% acrylamide was set in the descending direction and left to polymerize at room temperature. Both unknown and standard proteins were treated with a sample buffer containing 0.0025 M. Tris, 1% SDS, 10% glycerol and 5% mercaptoethanol, and heated in a boiling water-bath for 2 min. A constant current of 25 mA was passed through and the gel, after electrophoresed was stained with 0.25% Coomassie Brillient Blue R.

Molecular weight calibration : A protein standard (Sigma Chemical Company, St. Louise, U.S.A.) which includes bovine serum albumin (67.0 kilodaltons), egg albumin (45.0 kilodaltons), trypsinogen (24.0 kilodaltons), β-lactoglobulin (18.4 kilodaltons) and lysozyme (14.3 kilodaltons) were used to construct a standard curve. Unknown protein bands were determined for their molecular weights with the help of protein markers.[13]

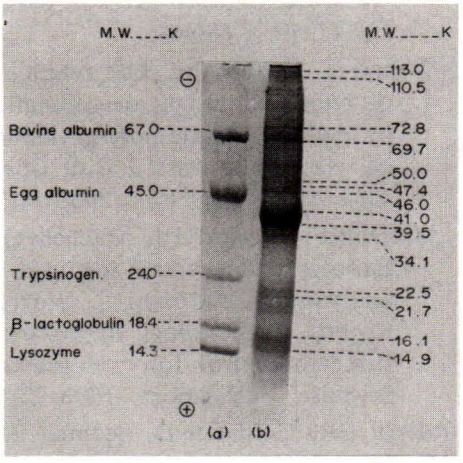

Figure SDS-PAGE patterns of third stage larval extract (b). Molecular weights of some major components shown on the right were estimated by calibrating with protein markers (a).

Results

The somatic extract of L_3, after being prepared, was subjected to the SDS-PAGE to analyze its complexity of protein profiles. After having been electrophoresed and stained, the electrophoretic pattern of these antigens showed highly complicated protein components, containing at least 30-36 peptide bands. These proteins or peptide profiles had molecular weights ranging from 113.0 to 14.9 kilodalton (K).

Molecular weights of some major components are shown in the figure. The bands with molecular weights of 41.0 K and 39.5 K are the major components of L_3 somatic antigen, judging from the intensity of staining. Other components with molecular weights from slightly less than 110.5 K to as high as 72.8 K, less than 69.7 K to as high as 50.0 K, less than 34.1 K to as high as 22.5 K and less than 14.9 K were presented in trace quantities.

Discussion

Result of the above characterization of L_3 somatic antigens by SDS-PAGE under reducing condition is the representative example of several repeated runs using the same set of standard proteins. The antigenic make up of these antigens, not entirely unexpected, is highly complex.

Many previous similar demonstrations of the complicated antigenic mosaic have been documented for several other helminthic antigens.[14] In those studies, using developmental immunobiochemical technology, a few protein compenents appeared to have been related to specific antibodies induced whereas others were responsible for the non-reactive and cross reactive components.[14] In our studies, there were at least 30-36 peptide components stained with Coomassie Brilliant Blue R. Indeed, more than 36 peptides could be demonstrated

by using an increased sensitive staining of silver mitrate[15] (data not shown).

The specific serological component (s) of the gnathostoma antigen should be further identified and then isolated to obtain the potential diagnostic agent.

Acknowledgements

The authors are grateful to Paisarl Yingyourd for his suggestions concerning the preparation of the third stage larva.

References.

1. Daengsvang S. Gnathostomiasis in Southeast Asian. Southeast Asian J Trop Med Public Health 1981 Sep; 12 (3) : 319-332
2. Daengsvang S. Gnathostoma spinigerum owen : 1836. In : SEAMIC Southeast Asian Medical Information Center. A monograph on the genus Gnathostoma and Gnathostomiasis in Thailand. Tokyo: Japan, 1980; 4-47
3. Prommas C, Daengsvang S. Further report of a study on the life cycle of Gnathostoma spinigerum. J Parasitol 1936 ; 22 : 180-6
4. Bridget MO, Savigny D DE. Immune response to nematodes. In : Cohen S. Warren KS, eds. Immunology of Parasitic Infections. London : Blackwell Scientific Publications, 1982. 715-757
5. Suntharasamai P, Desakorn V, Migasena S, Bunnag D. ELISA for immunodiagnosis of human gnathostomiasis. Southeast Asian J Trop Med Public Health 1985 June ; 16 (2) : 274-279
6. Kasemsuth R, Panut-Ampon P, Sanghirun C. Study on the diagnosis of Gnathostoma infection in cats by radioimmunoassay. Southeast Asian J Trop Med Public Health 1981 Sep ; 12 (3) : 410-412
7. Yamaguchi T. Immunological studies on human gnathostomiasis. (Japan with English abstract) J Kurume Med Assoc 1952 ; 15 : 26-34
8. Ando T. A study of Gnathostoma spinigerum. (Japan with English abstract) Acta Medical 1957 ; 27 : 2342-2359
9. Morisita T, Kobayashi M, Nagase K, Nishida Y, Iwanaga H, Sumi M. Non-specificity of intradermal test with gnathostoma antigen. (Japan with English summary) J Parasitol 1969 ; 18 : 120-129
10. Daengsvang S. Penetration of the skin by Gnathostoma spinigerum larvae. Ann Trop Med Parasitol 1970 Sep ; 64 (3) : 399-404
11. Kabat EA, Mayer MM. Experimental Immunology 2 ed. Springfield : Charles C Thomas, 1967. 557-558
12. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T 4. Nature (London) 1970 Aug 15 ; 227 (5259) : 680-685
13. Weber K, Osborn M. The reliability of molecular weight determination by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem 1969 Aug ; 244 (16) : 4406-4412
14. Anders RF, Howard FJ, Mitchell GF. Parasite antigens and methods of analysis. In : Cohen S, Warren KS eds. Immunology of Parasitic Infections. London : Blackwell Scientific Publications, 1982; 28-73
15. Merril CR, Goldman D, Sedman SA, Ebert MH. Ultrasensitive stain for protein in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science 1981 Mar 27 ; 211 (4489) : 1437-1438

จุฬาลงกรณ์เวชสารได้รับต้นฉบับเมื่อวันที่ 4 เดือน กุมภาพันธ์ พ.ศ. 2529