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BRIEF COMMUNICATION

P SYSTEM ANTIGENIC DETERMINERS EXPRESSION IN *Ascaris lumbricoides*

Patricia PONCE DE LEÓN & Juana VALVERDE

SUMMARY

The P System antigens have been detected in numerous parasites, bacterias and viruses, nevertheless the clinical significance is still unknown. The aim was to study the presence of P_1 antigenic determiners in *A. lumbricoides* extracts by means of the use of 6 different monoclonal antibodies of well-known concentrations and Ig class. We worked with 14 *A. lumbricoides* extracts. Inhibition Agglutination Test was made in a bromelin enzymatic medium and 4 ºC temperature. Titre, Score and Sensitivity Parameter were determined for each monoclonal antibody against red cells suspension used as revealing system. Ten extracts inhibited the agglutination of all anti P_1 monoclonal antibodies. The 4 remaining extracts only inhibited the agglutination of some of them. It is demonstrated that the extracts have P_1 activity. This activity is independent of titre, Score, Sensitivity Parameter, concentration and Ig class and it depends on the epitope at which the monoclonal antibody is directed.

KEYWORDS: *Ascaris lumbricoides*; P System

The P blood group System was discovered by LANDSTEINER & LEVINE in 1927[4] in the course of a systematic attempt to identify new alloantigens by the immunization of rabbits with human erythrocytes.

The new antigen was named P, but it was later designated P_1 when the antigen now known as P was identified[5,14]. The serology of this system has gradually increased in complexity, and the recognition of antibodies with compound specificities indicated a biochemical relation between P and other blood group systems.

The currently recognized antigens of the P System include two common antigens, P_1 and P, and P_k which has been considered a rare erythrocyte antigen. Approximately 75% of white individuals possess the P_1 antigen, and the P antigen is present on all normal red cells, the erythroblasts and endothelium cells[7].

Many blood group antigens have been found in numerous parasites, nevertheless the clinical significance of these antigenic similitudes between a parasite and its host is not very clear established yet[12].

The antigens of the P System have also been detected in various animal species: *Taenia Echinococcus, Ascaris suum, Fasciola gigantica, Parogonimus westermani, Dicrocelium dentriticum*[13]. Another excellent source for the P_k antigen is the fluid in hydatic cysts[1].

The blood group P substances of tissue cells mediate the adhesion of a series of pathogenic bacterias (*Escherichia coli, Streptococcus suis, Pseudomona aeruginosa* and the Shiga toxin of *Shigella dysenteriae*) and P globoside has been shown to be the cellular receptor for parvovirus B_{19}[9]. At the present time it is accepted that many microorganims and parasites carry blood group antigens which have become evident by Inhibition Agglutination Tests and Immune Fluorescence Techniques. Yet, the clinical significance of this fact is still unknown[13].

The aim was to study the presence of P_1 antigenic determiners in *Ascaris lumbricoides* extracts by means of the use of different monoclonal antibodies of well-known concentrations and Ig class.

We worked with 14 *A. lumbricoides* extracts [AE]. In order to perform our experiments [AE] were prepared. Adult specimens were washed in physiological solution supplemented with 200 mg/ml of streptomycin and 200 mg/ml of penicillin. After that a refrigerated mechanical rupture was performed for 5 days. The supernatants were collected and kept at –20 ºC with a final concentration of timerozal 1:1000[8,9]. Inhibition Agglutination Test was made facing the [AE] against anti P_1 monoclonal antibodies in optimal concentrations. Suspensions of fresh red cells (P_1) were used as a revealing system[6]. The experiments were carried out in a bromelin enzymatic medium (hydrolysis C-terminal peptide bond of Lys, Ala, Tyr and Gly) because the P System antigens are resistant to the action of this enzyme, their reaction capacity being increased. The temperature chosen was 4 ºC because the optimal thermic is room temperature or lower[11]. The anti P_1 monoclonal antibodies were supplied

Laboratorio de Parasitología. Laboratorio de Inmunohematología. Facultad de Cs. Bioq. y Farm. U.N.R Suipacha 531 - 2000 Rosario (Argentina).

by Monoclonal Antibodies against Blood Group Antigens (Monoclonal IV Workshop, Paris, July, 2001), and the Ig concentration in the antibodies was studied by enzyme-linked immunoassays[5]. Titre, Score (S), and Sensitivity Parameter (SP) were determined for each anti P_1 monoclonal antibody against revealing system (P_1 Red cells)[2,10,15].

Anti P_1 monoclonal antibodies characteristics are shown on Table 1.

Table 1
Characteristics of the anti P_1 monoclonal antibodies used in the Inhibition Agglutination Test

Anti P_1 antibody	Ig Class	Concentration (µg/ml)	Titre	S	SP
2.88	Ig M	< 0.5	1/ 256	45	1.02
2.89	Ig M	< 0.5	1/ 256	27	0.63
2.90	Ig A	5.51	1/ 64	24	0.23
2.91	Ig M	87.2	1/ 1024	43	2.78
2.92	Ig M	< 0.5	1/ 256	39	0.85
2.93	Ig M	< 0.5	1/ 16	13	0.06

Ten [AE] inhibited the agglutination of all anti P_1 monoclonal antibodies with red cells (P_1) showing the epitopes present in the [AE] reacted against the six monoclonal antibodies studied.

The 4 remaining [AE] evidenced a different behaviour against anti P_1 monoclonal antibodies. Only some of them were inhibited by the extracts. Inhibition Agglutination Test results of these 4 [AE] are shown on Table 2.

Table 2
Results of the Inhibition Agglutination Test for the extracts with no conventional behavior

[AE] /ANTI P_1	2.88	2.89	2.90	2.91	2.92	2.93
[AE] 1	NI	NI	I	I	I	I
[AE] 2	NI	NI	I	I	I	I
[AE] 3	NI	I	I	I	I	I
[AE] 4	NI	NI	I	NI	I	I

I = Inhibition Agglutination; NI = No Inhibition Agglutination.

It is demonstrated that all the [AE] have P_1 activity, nevertheless it can only be made evident by means of a monoclonal antibody battery. The [AE] activity is independent of the Ig class, antibody Ig concentration, titre, Score and Sensitivity Parameter. The activity depends on the epitope at which the monoclonal antibody is directed. The use of these antibodies for routine or investigation works leads to standardized methods and a better definition of the specificity of antigen-antibody.

RESUMEN

Presencia de determinantes antigénicas P_1 en *Ascaris lumbricoides*

Los antígenos del Sistema P han sido detectados en numerosos parásitos, bacterias y virus, aunque todavía se desconoce su significado clínico. El objetivo fue estudiar la presencia de determinantes antigénicas P_1 en extractos de *A. lumbricoides* mediante el uso de 6 anticuerpos monoclonales de concentraciones y clase de Ig conocidas. Se trabajó con 14 extractos de *A. lumbricoides*. Se realizó la prueba de Inhibición de la Aglutinación en medio enzimático de bromelina y temperatura de 4 ºC. Se determinó el título, Score y Parámetro de Sensibilidad de cada anticuerpo monoclonal frente a la suspensión de glóbulos rojos usada como sistema revelador. Diez extractos inhibieron la aglutinación de todos los anticuerpos monoclonales anti P_1 con la suspensión de glóbulos rojos P_1. Los 4 extractos restantes sólo inhibieron la aglutinación de algunos de ellos. Se demuestra que todos los extractos estudiados tienen actividad P_1. Esta actividad es independiente del título, Score, Parámentro de Sensibilidad, concentración y clase de Ig del anticuerpo monoclonal y depende del epitope al cual esta dirigido el anticuerpo.

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