BRIEF REPORT

USE OF MAGNETIC PARTICLES IN THE PURIFICATION OF IgM ANTIBODIES AGAINST *Taenia solium*

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ABSTRACT

The use of L protein coupled magnetic particles for the concentration and purification of immunoglobulin M (mIgM) monoclonal antibodies against Taenia solium was evaluated. Three concentration methods and different elution times were evaluated and the ratio of particles to the ratio of mIgM was optimized. It is demonstrated that: 1) with the use of magnetic particles, a previous concentration of mIgM is not required, which reduces the manipulation of the antibodies and improves the recovery, 2) the use of a binding buffer can be omitted, since the pH of most cell culture supernatants are neutral, and 3) longer elution times (~ 45 minutes) are needed to increase recovery to a level greater than 80%. The study demonstrates that the use of L protein-coupled magnetic particles is a simple and efficient tool for mIgM concentration and purification.

Keywords: Purification; Concentration; *Taenia solium*; Cysticercosis; Monoclonal Antibodies; Peru (source: MeSH NLM).

INTRODUCTION

Neurocysticercosis (NCC) is a nervous system infection caused by the invasion of the larval stage of *Taenia solium* [(1)], and is considered one of the major infectious causes of epilepsy [(2)]. The detection of circulating antigens is important in the definitive diagnosis of NCC because it allows the detection of an active infection [(2-4)]. The use of monoclonal antibodies (Mabs) improves the specificity of antigen detection techniques, but few Mab actually exist to improve diagnosis [(5)].

https://doi.org/10.17843/rpmesp.2020.371.4628

The Cysticercosis Working Group in Peru produced and characterized 21 monoclonal antibodies (Mabs) that recognize *T. solium* antigens in serum and urine of infected patients [5-6]. Eight of the Mabs generated are M-isotype (mIgM) antibodies. The mIgM generate a more intensive signal (optical density >3.0) in antigen-capture ELISA tests compared to immunoglobulin G (IgG) type antibodies. It is proposed that this difference is due to the presence of tandem repeated aminoacid sequences in the parasite antigens [6], which are more efficiently recognized by immunoglobulin G (IgG) antibodies [7].

Immunoglobulin purification and concentration procedures are mainly developed for IgG [8-10]. However, these methods may not be as effective for IgM purification. Due to its large size, IgM (~950 kDa) is more susceptible than IgG (~150 kDa) to denaturation and precipitation by changes in temperature, pH and conductivity, even at low concentrations [11].

Protein A and protein G have been used for a long time in the purification of antibodies, mainly IgG type that have the constant fraction (Fc) exposed since it's the one they use for interaction [12]. However, neither of these two proteins can be used for IgM purification, because in this antibody, the Fc is hidden [12-13]. Unlike proteins A/G, the L protein (LP) binds to the variable domain of the kappa light chain which is exposed in all antibody isotypes without interfering with the antigen binding site. LP offers the additional advantage of not reacting with bovine and caprine IgG which are generally present in serum-enriched cell culture supernatants of these animals [14-15].

The use of magnetic particles (MP) can replace the pre-concentration steps, decreasing antibody handling. Likewise, antibody binding occurs in solution, and not on a static surface as in chromatographic columns, providing a 3D interaction between IgM and LP. Moreover, it allows the separation of purified IgM using a magnetic force or a magnet without the need for centrifugation or precipitation which makes this method applicable in both small-scale and large-scale purification, in laboratories that do not have specialized infrastructure or using automated platforms.

In this study, the use of LP-coupled MP (MP-LP) was evaluated in the concentration and purification of anti-*Taenia solium* mIgM.

KEY MESSAGES

Motivation for the study: The use of monoclonal antibodies improves the specificity of antigen detection techniques in neurocysticercosis, but few monoclonal antibodies actually exist to improve diagnosis.

Main findings: The use of magnetic particles coupled to L protein allows the purification of IgM-type monoclonal antibodies by applying magnetic force, this way eliminating the need for centrifugation or precipitation, thus decreasing the quantity of handling steps, which prevents early denaturation and precipitation.

Implications: The optimization of a simple platform based on magnetic particles and L protein allowed the concentration and purification of IgM antibodies from hybridoma supernatant. This platform can be adapted for laboratories that do not have specialized infrastructure, since it does not require the use of columns or ultracentrifuges.

THE STUDY

Two mIgM clones (TsW5 and TsW8) specific to T. solium cyst proteins were used. The production and maintenance of hybridomas were performed according to the protocols described above [5]. Briefly, cell cultures of hybridomas were maintained in advanced DMEM medium (Dulbecco's modified Eagle's medium, Thermo Fisher, USA) supplemented with 10% fetal bovine *HyClone* serum (Thermo Fisher, USA), at 37 ºC in 5% CO_2. Hybridoma supernatants (HS) were collected after one to two weeks of incubation, filtered with 0.22 µm nitrocellulose filters and stored at 4 ºC for up to four days.

Evaluation of HS concentration methods

Three methods of HS concentration were evaluated: pressure filtration (Amicon® Stirred Cells), passive ultrafiltration (Minicon® Concentrator) and ultrafiltration by centrifugation (Amicon® Ultra Centrifugal Filters), all filters with a minimum cut-off of 10 kDa (all from Merk Millipore, USA). The protein concentration of the supernatants was determined using the Bradford method (Bradford Protein Assays, Thermo Scientific, USA), before and after being concentrated; the concentration factor was calculated by dividing the protein concentration of the concentrated supernatant with the protein concentration of the initial supernatant. An equal volume of initial supernatant was evaluated in all three concentration methods. Since the protein concentration will mainly reflect the presence of albumin and other proteins, the recovery percentage (RP) in terms of mIgM activity was also calculated and measured by ELISA test using a crude extract of *T. solium* cysts as antigen, and a secondary peroxidase-labelled antibody to mouse IgM produced in goat (Peroxidase-Labeled Antibody To Mouse IgM, Produced in

Goat, KPL Laboratories, USA) [5]. Optical density (OD) was measured at 590 nm using the Spectra-Max-340 (Molecular Devices, Sunnyvale, California, USA). The RP of mIgM was calculated by dividing the optical density of the concentrated supernatant by the optical density of the initial supernatant multiplied by 100.

Purification of mIgM

Superparamagnetic particles (0.5 µm of mean radius) coupled with recombinant LP (PierceTM Protein L Magnetic Beads, Thermo Scientific, USA) were used. To determine which concentration of LP-MP recovers more than 90% mIgM in HS, five different HS volumes were incubated (100 µl, 250 µl, 500 µl, 750 µl and 1500 µl) with 400 µg of LP-MP in a total volume of 1500 µl of Tris-buffered saline (TBS, Tris 25 mM, NaCl 150 mM, pH 7.4). A volume of 100 µl of HS was used as a reference, since the ELISA test showed that no mIgM was left in the HS after incubation with LP-MP, i.e., all mIgM present in the medium was captured. Incubation of HS and LP-MP was performed for 45 minutes, at room temperature (RT) and under constant agitation (500 rpm, LabRoller). After incubation, the mIgM + PM-PL compounds were separated from the medium using a magnetic holder (DynaMagTM-2 Magnet, Thermo Scientific, USA) for one minute. For the separation of mIgM from PM-PL, 2M glycine, pH 2.0 at ambient-temperature, was used with constant agitation for 15, 45 and 60 minutes (Figure 1). The mIgM solution was neutralized with 1M Tris, pH 8.5, then the buffer exchange to phosphate-buffered saline was performed. The purified mIgM was concentrated by ultrafiltration by centrifugation (10 kDa cut).

Purity evaluation of eluates

To evaluate purity of mIgM, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed under reducing conditions using 10% polyacrylamide gel and Coomassie blue stain.

RESULTS

In regard to protein concentration, the highest concentration factor was obtained when supernatants were concentrated using pressure filtration (4.4 times), followed by passive ultrafiltration (3.9 times) and ultrafiltration by centrifugation (2.5 times). In terms of mIgM activity measured by ELISA test, all three methodologies produced a RP above 80% (pressure filtration: 92.5%-95%, passive ultrafiltration: 84.2%-93.9% and ultrafiltration with centrifugation: 82.1%-

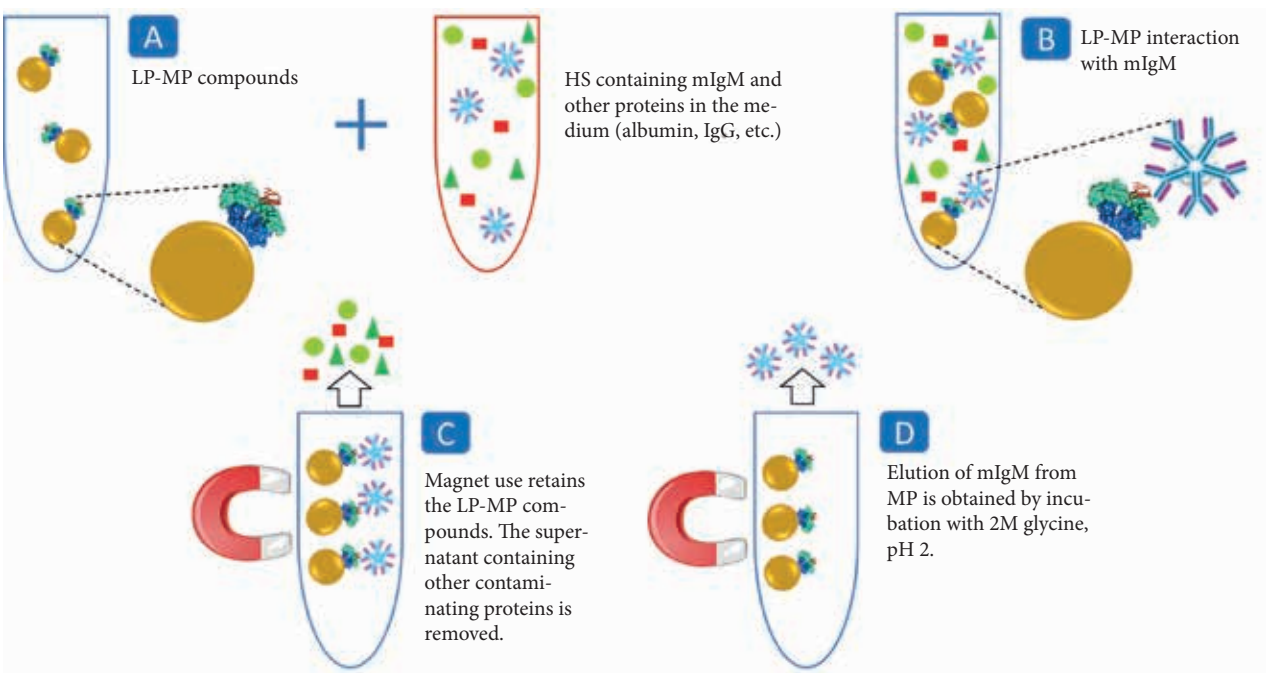

Figure 1. Schematic representation of the mIgM monoclonal antibody purification using magnetic particles coupled to L-protein (figure developed by the authors). A) and B) Monoclonal IgM antibodies (mIgM) present in the hybridoma supernatant (HS) will bind in solution to the L-protein (LP) coupled to the magnetic particles (LP-MP). C) The magnetic particles (MP) are separated from the HS with the help of a magnetic holder. D) The purified mIgMs are eluted from the LP-MP using an acidic glycine solution.

https://doi.org/10.17843/rpmesp.2020.371.4628

84.3%). However, protein precipitation was observed twice using pressure filtration, for this reason, to minimize mIgM precipitation and due to the lower cost and shorter time needed for concentration (15 minutes), compared to the other methods (~12 hours for passive ultrafiltration or ~5 hours for pressure filtration), it was decided to use ultrafiltration by centrifugation for future analyses.

The optimal ratio of µg LP-MP to µl HS was evaluated using the ratio of 400 µg LP-MP to 100 µl HS as a reference. The percentage of functionally active mIgM that were not captured or eluted by PM-PL was calculated by subtracting the OD value of HS after incubation with LP-MP minus the OD value of HS at the reference ratio, multiplied by 100. It was found that the lowest percentage loss was obtained using 400 µg LP-MP with 250 µl HS (0.6%) and the highest RP was obtained using 500 µl HS (99.3%) (Table). It is concluded that the best way to recover mIgM is when the ratio of µg of LP-MP to µl of HS is 0.8.

To determine if a pre-concentration step is needed before incubation of HS with LP-MP, we compared the RP obtained with 50 µl of concentrated HS (initial volume 2500 µl, concentration factor 50x) and 2500 µl of unconcentrated HS, obtaining a RP of 95.46% (coefficient of variation, CV 0.77%) and 96.93% (CV 0.43%) when a concentration and non-concentration step was carried out, respectively, which indicates that a pre-concentration stage is not required.

In this study, a glycine buffer, pH 2.0, was used for the separation of mIgM from LP-MP. According to the manufacturer's recommendations, the elution or separation time with acidic solution should not exceed 15 minutes to prevent degradation of the antibody. Considering the activity of the antibody, we observe that the elution time of 15 minutes has a RP of 78.5%. However, when the time increases to 45 minutes the RP increases to 94.1%. Conversely, if the time is increased to 60 minutes, the RP decreases to 83%, probably due to some degree of denaturation caused by the pH.

In the electrophoresis results, different proteins, mainly albumin (67 kDa), were observed before purification (Figure 2). After the LP-MP purification process, only two bands of 73 kDa and 25 kDa, corresponding to the heavy and light chains, respectively, of IgM, were observed, confirming the purity of the eluates.

The number of times that LP-MP can be re-used for antibody purification using the same mIgM clone was also evaluated. A RP of 81.9% was observed after reusing the same batch of LP-MP 21 times.

DISCUSSION

It is shown that magnetic particles can be used to concentrate IgM from a solution in a single step [16], reducing antibody manipulation that can lead to degradation and denaturation. When evaluating pre-concentration methods, protein precipitation was observed twice using pressure filtration, possibly due to the mechanical forces and temperature rise that led to IgM desolubilization. This is an advantage of MP over the use of chromatographic columns, since with the use of columns, preconcentration is necessary, probably because the attraction and frequency of interaction opportunities are lower compared to when using particles [17-18], and also to avoid passing several times the culture through the column, especially when it is higher than 1 ml volume, which can be tedious. The use of magnetic particles favors the interaction of mIgM and LP in solution in a single step, and also prevents the loss of mIgM immunogenic activity, since this can occur during the binding of IgM to other resins [16]. Based on the results of electrophoresis under reducing conditions, it was shown that the use of L-protein produces a highly

Table 1. Optimization of the monoclonal IgM antibody purification protocol from hybridoma culture supernatants using magnetic particles coupled to L-protein.

Ratio of µg LP-MP to µl HS	HS Initial Vol. (µl)	OD of HS after Incubation with LP-MP (A)	Activity from mIgM that has not been captured or eluted from LP-MP (%) (A-Reference) *100)	mIgM initial activity in total volume (B)	mIgM activity in the eluate (C)	Recuperation (%) (C/B*100)
4	100	0.088 ± 0.005	Reference	7.12	6.63	93.12
1.6	250	0.094 ± 0.004	0.60	16.58	15.48	93.40
0.8	500	0.115 ± 0.004	2.70	33.00	32.80	99.30
0.53	750	0.146 ± 0.003	5.80	49.70	45.33	91.20
0.4	1000	0.182 ± 0.010	9.40	66.30	57.41	86.60
0.26	1500	0.328 ± 0.006	24.00	99.50	55.72	56.00

400 µg of LP-MP were added to the HS in a final volume of 1500 µl of Tris-buffered saline solution as a binding buffer
Antibody activity was measured by ELISA test to determine the ability of the antibody to recognize sonic cysts of *T. solium* LP-MP: L-protein coupled magnetic particles; HS: hybridomas supernatant; OD: optical density measured at 590 nm

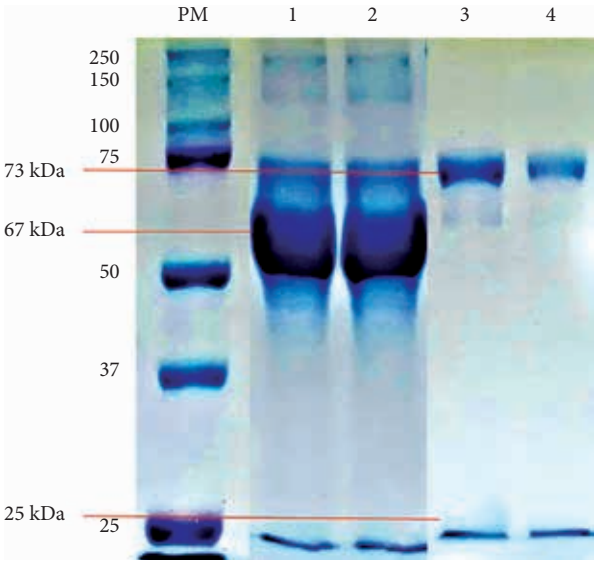

Figure 2. Coomassie blue staining after polyacrylamide gel electrophoresis with 10% sodium dodecyl sulfate of monoclonal IgM antibodies (TsW5 and TsW8) before and after purification with L protein coupled magnetic particles.

MW: Molecular weight marker in kDa (Precision Plus Protein, Bio-Rad). Channel 1 and 2: Hybridoma supernatant (TsW8 and TsW5, respectively) before purification with L-protein coupled magnetic particles, albumin (67 kDa) is observed. Channel 3 and channel 4: Purified IgM monoclonal antibody (TsW8 and TsW5, respectively) after incubation with L-protein coupled magnetic particles, light and heavy chains of the antibody 25 kDa and 73 kDa are observed, respectively, without the presence of albumin.

purified IgM eluate without IgG contamination present in bovine serum that is used as a supplement in cell culture [14].

A longer mIgM elution time than recommended by the manufacturer was needed (45 minutes vs. 15 minutes), probably due to the high affinity between IgM and LP (Ka = 1010 M- 1) [19]. Other studies have reported that IgM is able to withstand exposure to acidic pH [20]. In our study, the short handling time (two hours total) compared to classical affinity chromatography methods (ten hours to two days) may have contributed to the lower risk of denaturation of mIgM and increased the likelihood of resistance to acidic elution [12]. Our RP (> 90%) is higher than previously reported using MP coupled with anti-IgM antibodies (70%), probably due to the higher affinity of LP to IgM [17].

We present the results of concentration optimization and mIgM purification from hybridoma supernatant using a simple platform based on the use of magnetic particles and LP. This methodology can be adapted to non-specialized protein concentration laboratories since it does not require the use of columns or ultracentrifuges. It was demonstrated that HS does not need to be concentrated. Therefore, if the use of LP-MP is to be implemented on a medium or large scale, it is necessary to have magnetic supports for 50 ml tubes. The average time for the separation of LP-MP from the medium should be between three to five minutes, in order to ensure the total recovery of MP. It is demonstrated that incubation of HS and LP-MP can be carried out at ambient-temperature or 4 °C. However, the incubation should be at 4 °C to avoid degradation of the analyte in the sample.

Acknowledgements: We thank Dr. Cristina Guerra and Dr. Miguel Orrego for facilitating access to hybridoma supernatants. To Adriana Paredes, for her guidance in the initial steps of the study. To Helena Jahuira, and Giuliana Oyola Lozada for coordinating the purification procedures. To Heydi Toro for the administrative and financial coordination of the project. To Doctors Patricia Sheen, Manuela Verastegui, and José Espinoza for providing us with access to their laboratory equipment. To Dr. Theodore Nash Sukwan Handali for corrections and suggestions in writing the article.

Authorship contributions: LAP and YCS participated in the conception, design and writing of the article, in the analysis and interpretation of data, and in the final approval of the version for publication. HHG, JAB, PPW, YC participated in the design and critical review of the manuscript, and in the final approval of the version for publication. CE, LMT, YS, KSM participated in collection of data, in the critical review of the manuscript, and in the final approval of the version for publication. All authors are responsible for all aspects of the manuscript, to ensure that issues regarding the accuracy or completeness of any part of the manuscript will be properly investigated and resolved.

Funding sources: This study was funded by the National Council for Science, Technology, and Technological Innovation (FONDECYT - CIENCIA ACTIVA) [115-2015-FONDECYT-DE] and the NIH/ Fogarty Training Grant [TW001140].

Conflicts of interest: All authors have none to declare.

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https://doi.org/10.17843/rpmesp.2020.371.4628

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