

TECHNICAL NOTE

Absorption of IgG does not enhance toxoplasma IgM and IgA immunoblotting

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Total IgG, IgM and IgA levels and toxoplasma IgG, IgM and IgA immunoblotting patterns were assayed in 10 sera before and after IgG absorption with Protein G – Sepharose 4. Removal of IgG (mean reduction 96%) was accompanied by a significant reduction in the level of IgM (mean reduction 56%) and IgA (mean reduction 53%) in nine of the 10 sera. The absorbed supernates showed fewer and weaker IgM bands in five sera, but IgA immunoblotting patterns were unaffected by absorption. There was no benefit in removing IgG in toxoplasma IgM and IgA immunoblotting.

Introduction

Measurement of specific IgM antibody poses technical problems in immunoassays [1]. One problem is false negativity caused by specific IgG competing for antigens and blocking binding sites. This is largely overcome in enzyme-linked immunosorbent assays by the use of capture techniques to specifically bind IgM from serum to the solid phase and eliminate IgG interference from the assay. In systems in which IgM capture is not an option, such as immunoblotting, a frequently used approach is to remove the competing IgG before performing the assay [2–5]. Several commercial products are available to remove IgG and they have been used in various ways in different studies. It is assumed that IgG removal will result in improved detection of specific IgM or IgA and effective pre-treatment is assessed by titrating IgG in the test system and repeating the treatment as necessary to eliminate IgG activity [6]. Few studies have indicated whether the absorption method affects IgM or IgA antibody levels or immunoblotting patterns.

Pre-treatment of sera to remove IgG is time-consuming and expensive [7]. In this study, the levels of IgG, IgM and IgA were measured before and after IgG absorption and the toxoplasma IgG, IgM and IgA immunoblotting patterns in sera were compared before and after treatment.

Materials and methods

Sera

Ten sera were tested. Four sera were toxoplasma-specific IgM positive by in-house ELISA [8] and commercial ELISA (Toxonostika IgM, Organon Teknika, The Netherlands) with total toxoplasma antibody between 500 IU and 4000 IU in the Sabin-Feldman dye test (DT) [9]. Three further samples were created by diluting one of these sera (DT 500 IU) in toxoplasma antibody negative serum to give IgM positive samples with DT titres of 125, 65 and 30 IU, respectively. Three sera with DT titres of 125 IU, 65 IU and 30 IU and negative for IgM were also tested; to conserve toxoplasma sera they were absorbed only with Protein G-Sepharose 4. Six other sera were absorbed with both Protein G-Sepharose 4, and Sepharose 4 not coated with Protein G.

Absorption

A reagent-serum centrifugation method of absorption was used that was adapted from methods described by others [3, 5]. Protein G-Sepharose 4 Fast Flow or Sepharose 4 Fast Flow not coated with Protein G (Pharmacia Diagnostics) was washed three times in sterile phosphate-buffered saline (PBS) pH 7.3, and a slurry of 75% settled gel to 25% PBS was prepared; 0.3 ml of the slurry was transferred to microtubes and centrifuged to pellet the gel. The supernate was decanted and 0.3 ml of serum was added and incubated at room temperature for 16 h with continuous shaking. The absorbed serum supernate was removed and stored at 4°C.

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Measurement of serum IgG, IgM and IgA

The levels of total IgG, IgM and IgA in sera before and after absorption were measured by rate nephelometry with a Beckman Array Analyser (Beckman, High Wycombe).

SDS-PAGE and immunoblotting

Toxoplasma SDS-PAGE and immunoblotting were performed as described previously [10]. Toxoplasma antigen was prepared from tachyzoites harvested from the peritoneal cavities of cotton rats infected by intraperitoneal inoculation 66 h previously with the RH strain of toxoplasma. The exudate was washed three times in PBS, the pellet was resuspended in distilled water, frozen and thawed three times, and disrupted for 3 min in a sonic bath. Electrophoresis was performed at a constant current of 6 mA for 16 h with a stacking 3.5% gel and a separating 12% slab gel in a discontinuous SDS buffer system. Separated proteins were transferred to nitrocellulose sheets in an LKB 2005 Transphor Electroblothing unit at 4°C for 2 h at 0.8 A. The sheet was blocked in PBS containing non-fat milk 5% for 1 h, rinsed in PBS containing Tween 20 0.05%, dried and stored in the dark at room temperature. The 10 toxoplasma sera were tested before and after absorption for IgG, IgM and IgA antibody by a standard immunodetection method [10] with strips cut from the sheet. Taking account of the reduction in IgM and IgA associated with absorption, sera were tested at a 1 in 100 dilution for IgG and IgA and 1 in 20 and 1 in 100 dilutions for IgM with goat anti-human IgG, IgM and IgA peroxidase conjugates (Sigma) as appropriate.

Statistical analysis

Where appropriate, data were subjected to statistical analysis by Student's paired *t* test.

Results

The IgG, IgM and IgA levels in the 10 toxoplasma sera before and after treatment with Protein G-Sepharose 4 were significantly different ($p < 0.001$). In eight of the 10 sera the IgG level was reduced by 93–>97% (mean 96%), the IgM level by 45–60% (mean 54%) and the IgA level by 42–58% (mean 51%). In one serum (Tx3), the IgG was reduced by only 70% and the levels of IgM and IgA were reduced by only 13% and 20%. In another serum (Tx8), a reduction in the level of IgG of >97% was accompanied by reductions of 72% and 70% in the levels of IgM and IgA, respectively. The IgG, IgM and IgA levels were significantly reduced ($p < 0.001$) in six sera after absorption with Sepharose 4 not coated with Protein G. The reduction in IgM and IgA was similar to that observed after Protein G-Sepharose 4 absorption. Absorption with Protein G-Sepharose 4 reduced the IgM and IgA levels by between 30–39% (mean 35%) and the IgG levels by 96–97% (mean 96%). Absorption with Sepharose 4 not coated with Protein G reduced the levels of IgM, IgA and IgG by 28.5–43.4% (mean 34%).

The IgG immunoblotting reactivity was eliminated in the nine toxoplasma sera in which the IgG level had been reduced by >93%, but was almost unaffected in serum Tx3 where the level had been reduced by 70% (Table 1). Absorption with Protein G did not enhance IgM immunoblotting and the absorbed supernates showed fewer and weaker IgM bands than the equivalent untreated serum with five of the 10 sera (Table 1). In all five sera, IgG had been totally removed by absorption. When the sera and supernates were tested at 1 in 100 and 1 in 20 dilutions, band intensity increased with the 1 in 20 dilution, but there was no difference in the band patterns of absorbed and unabsorbed sera (Fig. 1). Toxoplasma IgA immunoblotting produced very few weak bands with four of the 10 sera and reactivity was unaffected by absorption of IgG (Table 1).

Table 1. Number of IgG, IgM and IgA immunoblotting bands detected before and after absorption with Protein G-Sepharose 4 Fast Flow

Serum no.	Serology		Number of immunoblotting bands detected					
			IgG		IgM		IgA	
	Dye test (IU)	ELISA IgM	Serum	Absorbed	Serum	Absorbed	Serum	Absorbed
Tx1	1000	Positive	6	0	7	3	1*	1*
Tx2	1000	Positive	9	0	1	1	1*	1*
Tx3	4000	Positive	12	10	4	4	1*	1*
Tx4	500	Positive	7	0	4	4	1*	1*
Tx5	125	Positive	4	0	2	2*	0	0
Tx6	65	Positive	4	0	2	2*	0	0
Tx7	30	Positive	4*	0	2	2*	0	0
Tx8	125	Negative	12	0	0	0	0	0
Tx9	65	Negative	1	0	0	0	0	0
Tx10	30	Negative	4*	0	2*	1*	0	0

*Weak bands.

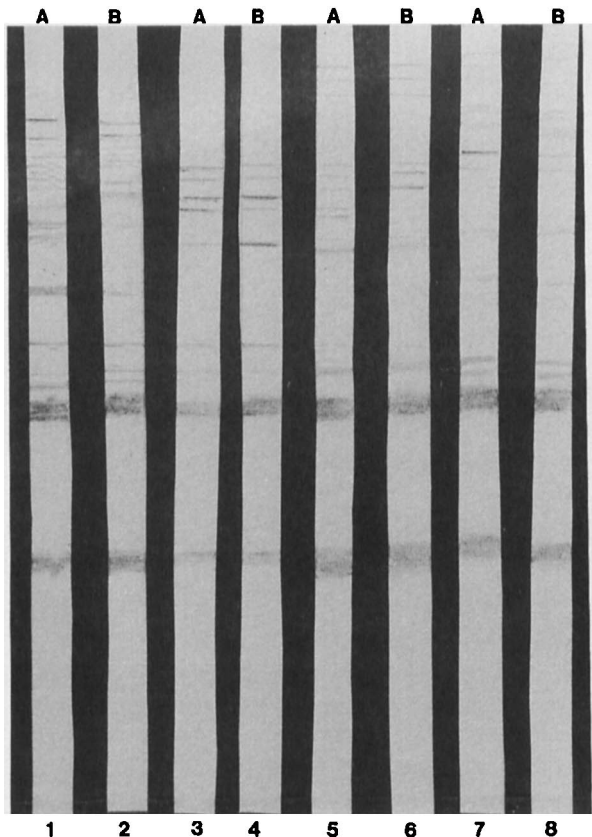


Fig. 1. Anti-toxoplasma bands detected by IgM immunoblotting: (A) serum 1 in 20 dilution; (B) absorbed supernate 1 in 20 dilution. Lanes 1 and 2, Tx1; 3 and 4, Tx2; 5 and 6, Tx3; 7 and 8, Tx4.

Discussion

Pre-treatment of sera to remove IgG is now a standard procedure in most IgM and IgA immunoblotting studies [2–5]. However, the effect on serum IgA and IgM levels is often not stated and only treated serum is tested for IgA and IgM by immunoblotting. In the sera in the present study, a reduction of >90% in the IgG level was commonly accompanied by a reduction of 30–60% in the levels of IgM and IgA. There was no evidence when comparing absorption with Sepharose 4 alone with Protein G-Sepharose 4, that Protein G binds IgM or IgA. The reduction in IgM and IgA is probably due to dilution which, from the reagent volumes used, could be predicted to be *c.* 50%. Nevertheless, the significant reduction in the levels of IgM and IgA could offset any benefits of pre-treating sera before IgM and IgA testing.

Total IgG levels in serum had to be reduced by >90% before toxoplasma IgG blots became negative. Even when all the IgG blotting activity had been removed, IgM and IgA blotting was not enhanced. Indeed, pre-treatment of sera reduced rather than increased IgM

and IgA immunoblotting sensitivity. This is contrary to the findings of other studies in which removal of IgG has been shown to improve rubella virus IgM and IgA immunoblot sensitivity significantly [5]. It is possible that in immunoblotting with a complex organism like toxoplasma, rather than a virus, IgG, IgM and IgA do not necessarily compete and antibody is produced sequentially to different epitopes as the immune response matures.

If a positive effect of IgG removal was simply being masked by a reduction in serum IgM and IgA levels then it would have been expected that testing at a higher serum concentration would give better results. In fact, IgM blotting patterns were identical when absorbed sera were tested as dilutions of 1 in 100 and 1 in 20. As the reduction in IgM and IgA observed in the samples was very variable, accurate comparison of serial specimens from the same patient would require time-consuming adjustment of the IgM or IgA concentrations. These results show that there is no benefit in removing IgG prior to toxoplasma IgM and IgA immunoblotting.

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