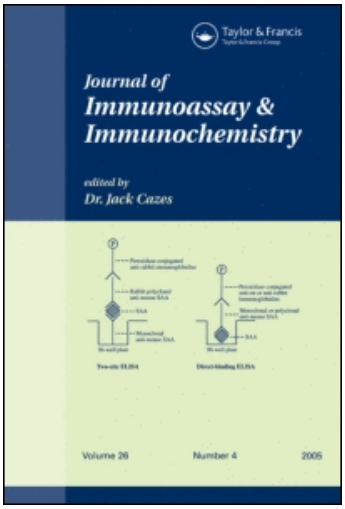

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ENHANCED ANTIBODY ACTIVITY IN SERUM DEPLETED OF ANTIGEN

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ABSTRACT

Presence of antigen and antibodies in a sample may interfere with the antibody, as well as with antigen detection assays. In such a situation, avidity of the probing antigen or antibody plays the key role in the assay. In the present study, using monoclonal antibodies against a mycobacterial antigen, lipoarabinomannan, patient serum is depleted of mycobacterial antigen by capture immunoradiometric assay and this antigen-depleted serum is tested for anti-lipoarabinomannan antibodies by inhibition immunoradio-metric assay. It is observed that serum, after depletion of antigen, revealed enhanced antibody activity compared to initial levels. It, therefore, appears that the avidity of the probing monoclonal antibody may detach the antigen from the loosely attached complexes and renders the complexed antibody free, thus increasing the reactive antibody molecules in the serum.

INTRODUCTION

Detection of antigen and antibodies in body fluids has been the basis of immunodiagnosis of various diseases. Detection of pathogen antigen is possible, usually, when the antigen is in excess, compared to the anti-pathogen antibodies. Such a phenomenon is possible when the antigen is produced abundantly. However, quite often, detection of antigen is not possible for the reason that it is complexed with antigen. Antigen antibody complexes (immune complexes) are known to be involved in the pathogenesis of variety of diseases.(1,2) Detection of disease-specific immune complexes and its implication in diagnosis of tuberculous meningitis has been recently evaluated.(3) In mycobacterial infections, like tuberculosis and leprosy, detection of antigen in serum may not be always possible for the reason that the antigen is meagre in the serum(4) and even the minute amount is presumably bound with the antibody available in the serum, thus forming immune complex. The present study reports the impact of probable presence of antigen on antibody levels in a set of serum samples from leprosy patients.

EXPERIMENTAL

Monoclonal antibody ML34,(5) which binds to mycobacterial lipo-arabinomannan (LAM), is used to capture LAM antigen from

Table 1.

	Pre Absorption of Antigen		Post Absorption of Antigen	
Name of Patient	LAM Antigen (CPM) 1:1	Anti-LAM Antibody (%binding) 1:5, 1:25	LAM Antigen (CPM) 1:1	Anti-LAM Antibody (%binding) 1:5, 1:25
1. BPS	200	7, 65	150	1, 29
2. F	680	8, 90	350	1, 21
3. GP	220	8, 60	120	1, 21
4. RB	180	11, 100	100	2, 29
5. MS	400	1, 30	300	0, 2.15
6. KPS	380	69, 87	270	24, 90

CPM: Counts/minute; sample taken as LAM antigen positive if CPM is > 200 (Patil et al., 1995); %binding: (100 − %inhibition); sample taken as anti-LAM antibody positive if %binding is $< 50\%$ (Patil et al., 1995).

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serum samples on a microtiter plate, and a second antibody ML34, which is labelled with ^{125}I is used as a probing counterpart in the antigen capture immunoradiometric sandwich assay.(6) For detection of antibody, *M. leprae* soluble antigen is coated to a microtiter plate and serum sample is competed with monoclonal antibody ML34 labelled with ^{125}I.(6) Depletion of antigen in the serum is done by coating microtiter plate with monoclonal antibody ML34 and incubating the test serum for 2 hours in the microtiter plate wells. After incubation, the serum is aspirated, which will be fairly depleted of LAM antigen. This antigen depleted serum sample is retested for antibody levels.

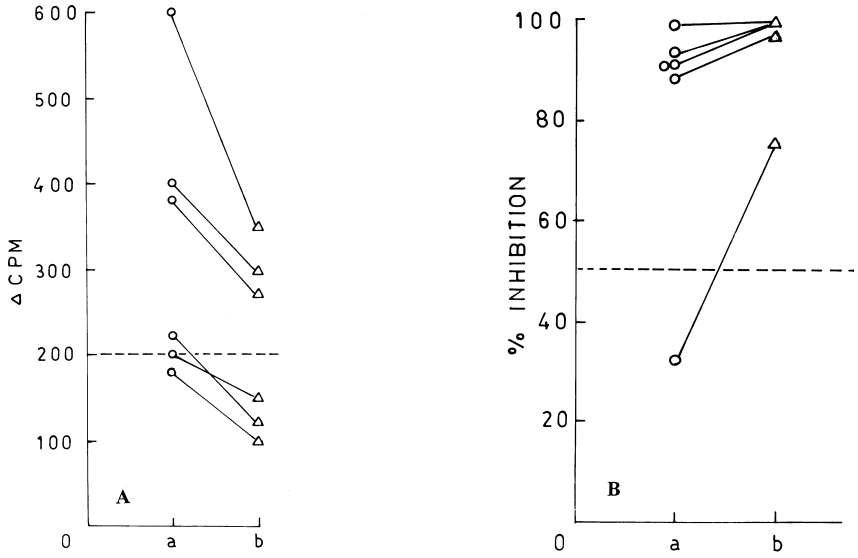

Figure 1. (A) Immunoradiometric sandwich antigen capture assay using ML-34 monoclonal antibody. CPM-counts per minute, a: Antigen detection in serum before antigen absorption; b: Antigen detection in serum after absorption. CPM ≥ 200 in a sample indicates antigen positivity. The drop in CPM is observed after absorption. (B) Immunoradiometric antibody detection by competition inhibition. a: Antibody detection in pre-absorbed serum; b: Antibody detection in post-absorbed serum. Inhibition of binding of labelled monoclonal antibody to more than 50% is taken as antibody positive. The increase in inhibition of binding of radiolabelled monoclonal antibody is observed post absorption of antigen from the serum.

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RESULTS AND DISCUSSION

The findings reveal that LAM antigen and anti-LAM antibody could be possible to detect in the same sample (Table 1). Probably, the avidity of serum antigen towards the monoclonal antibody ML34, which is coated to the plate, is more than the available antibody in the serum itself. Hence, even the complexed antigen is attracted towards the monoclonal antibody, leaving its serum antibody partner. This type of immunological infidelity might occur in loosely attached complexes (lesser avidity) like polysaccharide and anti-polysaccharide antibody complexes, thus making it possible to detect antigen. It is also observed that antibody titer increases in serum samples which are depleted of antigen by antigen capture (Figure 1A & 1B). The rise in antibody titer might probably be because of the freed binding sites of antibody molecules which were earlier taken up by antigen. In such a situation, it may also be possible that a sample with antigen excess, which was antibody negative earlier, may become antibody positive (as seen in pt. No.6) after depletion of antigen.

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