Effectivity of crude versus purified mycobacterial secretory proteins as immunogen for optimum antibody production

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Monospecific antibodies have been successfully utilized in antigen detection, which is better indicator of active infection. Mycobacterium tuberculosis excretory secretory (M tb ES) antigens such as ES 31, ES 41 and ES 43 (31 kDa, 41 kDa and 43 kDa protein, respectively) have been shown to be present in Mycobacterium tuberculosis H37Ra culture filtrate and are of diagnostic interest. To study the immunogenic potential of crude versus purified antigen, goat was immunized with M tb detergent soluble sonicate (DSS) antigen as well as purified antigen fraction (ESAS 7) containing ES 31 antigen. Both anti-DSS IgG antibody and anti ESAS 7 IgG antibody were found to be reactive with ES 31 antigen upto 0.06 ng concentration of antibody by ELISA. Crude DSS antigen was found to be quite effective in producing high titre antibodies and showed further high reactivity with other ES antigens (ES 41 and ES 43) of diagnostic interest.

Keywords: Antigen ELISA, Immunogen, Mycobacterial ES antigens, Mycobacterium tuberculosis.

Tuberculosis, caused by Mycobacterium tuberculosis (M tb) is a chronic re-emergent disease of global importance and ranks among top ten causes of death in the world. Serological diagnosis based on antibody or antigen detection is of considerable interest. Mycobacterial antigens M tb ES 31, ES 41 and ES 43 have been shown to be present in circulating blood of tuberculosis patient and isolated from M tb H37Ra culture filtrate. Further, ES 31 and ES 41 have been demonstrated in patients’ sera. Affinity purified antibody has been shown to be useful in M tb ES 31 antigen detection. In the present study, an attempt was made to study the effectivity of crude M tb DSS antigen versus purified M tb ES 7 antigen containing ES 31 kDa protein to produce high titre antibody.

Goat was immunized with M tb detergent soluble sonicate (DSS) antigen as well as M tb ESAS 7 antigen. M tb DSS antigen was prepared from M tb H37Ra bacilli taken from 2 weeks spent thyroxine-supplemented LI medium. Bacilli were inactivated with phenol (5%), homogenized and sonicated with 30 sec burst at 1 min intervals for 30 min. The sonicate was then incubated with sodium dodecyl sulphate (SDS)-extraction buffer [containing SDS (5%), 2 mercaptoethanol (5%) and 8 M of urea in 0.01M of phosphate buffer solution (PBS; pH 7.2)] in boiling water bath for 5 min. The antigen was extracted for 24 hr at 4 °C with the same buffer. The supernatant was collected by centrifugation at 16000 g for 20 min at 4°C, followed by dialysis and protein concentration was estimated by Lowry’s method. M tb ESAS 7 antigen was isolated by 50% of ammonium sulfate precipitation of M tb excretory secretory (ES) antigen, followed by SDS PAGE fractionation as described earlier. Antibodies to DSS antigen were raised in goat by immunizing (im) with DSS antigen (500 μg/ml) and Freund’s incomplete adjuvant (1 ml) on day 0, 20, 33 and 45. Immune sera were collected on day 32, 44, 57, 60 and thereafter fortnightly. Anti DSS IgG was isolated from immune sera by 33% saturation with ammonium sulfate followed by DEAE ion exchange column chromatography. Similarly, anti ESAS 7 IgG was isolated from goat immune serum. M tb ES 31, ES 41 and ES 43 antigens were isolated from M tb culture filtrate as described earlier.

Indirect peroxidase ELISA was carried out to study the reactivity of anti DSS IgG and anti ESAS 7 IgG to specific M tb ES antigens viz., ES 31, ES 41 and ES 43. Briefly, microtitre wells were sensitized with optimally diluted (100 ng/100 μl) antigens (ES 31, ES 41 and ES 43) with 0.06 M carbonate buffer, pH 9.6. The unbound sites were saturated with bovine serum albumin (2%) in the same buffer. The wells were incubated with optimally diluted (1μg/100μl/well and serial 10-fold dilutions) antibodies (anti DSS IgG, anti ESAS 7 IgG and normal goat IgG) in 0.01 M phosphate buffer saline with Tween 20 (PBS/T, pH 7.2). Following washing, rabbit anti goat IgG peroxidase (1:1000) was used as conjugate. The immune reaction was observed by incubating the
wells with o-phenylene diamine (OPD) substrate (100 µl) in citrate phosphate buffer (pH 5) and H₂O₂ (3%) in dark at room temperature. By adding 3N HCl (100 µl) the color development was arrested and the wells were then read in ELISA reader at 492 nm.

Reactivity of anti DSS IgG was observed upto 1:1000 dilution (1 ng/100 µl) of protein with ES 31, ES 41 and ES 43 antigens, while reactivity of anti ESAS 7 IgG was observed upto 1:1000 dilution (1 ng/100µl) of protein with ES 31 antigen and upto 1:10 dilution (100 ng/100µl) of protein with ES 41 and ES 43 antigens. The results are presented in Table 1.

Several other studies have reported purified antigens such as A-60, 38, 65, 30/31, 23, 19 and 14 kDa antigens, which improve the ELISA test characteristics, but turned out with varied results due to heterogeneous humoral response to different antigens of *M. tuberculosis*. However, for developing an efficient antibody assay, independent of host-immune response in tuberculosis patient, isolation of mono-specific antibodies to specific antigens will be of interest. In earlier studies, carried out in our laboratory *Mtb* ES 31, ES 41 and ES 43 antigens have been isolated and found to be diagnostically useful. Further, presence of *Mtb* ES 31 and ES 41 antigens has been demonstrated in serum of tuberculosis patients. In this study, production of an efficient antibody was explored by immunizing goat, with crude DSS antigen as well as purified ESAS 7 protein fraction. The study showed that both anti DSS IgG and anti ESAS 7 IgG were equally efficient in producing anti ES 31 antibody, while anti DSS IgG was found to be reactive with all three namely ES 31, ES 41 and ES 43 antigens. This was possibly due to absence of ES 41 and ES 43 antigens in the purified ESAS 7 protein fraction. Thus, *Mtb* DSS antigen was quite effective in producing antibodies in bulk quantities in goat and could be a better candidate-immunogen for isolating monospecific antibodies against certain mycobacterial excretory secretory proteins for developing multi-antigen assay for diagnosis of tuberculosis.

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References


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<th>Table 1 — Reactivity titre of crude and purified antibodies with specific ES antigens</th>
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<td>IgG antibodies used*</td>
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<td>Anti DSS IgG</td>
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<td>1:1000</td>
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<td>Anti ESAS 7 IgG</td>
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*Initial protein concentration of IgG was 10 µg/ml.

**Final antibody dilution showing positive reaction.