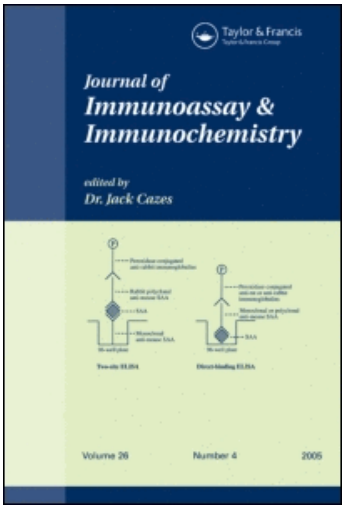

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Detection of Immunoprecipitated Systemic Lupus Erythematosus Antigens by Immunoblot Analysis

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DETECTION OF IMMUNOPRECIPITATED SYSTEMIC LUPUS ERYTHEMATOSUS ANTIGENS BY IMMUNOBLOT ANALYSIS

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ABSTRACT

When antigens are isolated from staphylococcal protein A immunoprecipitation pellets for analysis by SDS polyacrylamide gel electrophoresis and immunoblotting, severe background problems, due to the presence of antisera and bacterial proteins, can result. We describe a procedure for the analysis of immunoprecipitated systemic lupus erythematosus antigens (e.g., La, Ro, and Sm) which significantly reduces this background while retaining sensitivity with respect to antigen detection. We have adapted a method previously described (MacSween, J. M. and Eastwood, S. L. Methods Enzymol. 1981; 73:459-471) in which lithium diiodosalicylate is used to separate the immunoprecipitated antigen from a covalent antibody-staphylococcal protein A complex. In addition, a modified series of immunoblot incubations was employed, in which antigenic proteins were identified by incubating blots with the antiserum used for the original immunoprecipitation (e.g., La) followed by protein A-biotin and avidin-alkaline phosphatase. Overall, the procedure is straightforward and may be applicable to other immunoblot systems.

(KEY WORDS: immunoblot; immunoprecipitation; antigen detection; avidin-biotin systems; ribonucleoprotein characterization)

INTRODUCTION

Certain cellular and viral RNAs associate with cellular proteins to form ribonucleoproteins (RNPs). Recent evidence indicates that some of these RNPs may be involved in the post-

transcriptional regulation of gene expression, either at the level of mRNA processing or protein translation (1,2). Based upon selective recovery by immunoprecipitation with certain autoimmune antisera, such as produced by patients having systemic lupus erythematosus (SLE), several of these RNPs have been grouped into four classes: Sm, (U1)RNP, La, and Ro. (U1)RNP and Ro are subclasses of Sm and La, respectively. Thus, Sm antisera immunoprecipitate RNPs which contain U1, U2, U4, U5, U6, and the less abundant U7-U12 RNAs associated with at least nine distinct proteins, whereas (U1)RNP antisera immunoprecipitates U1 RNA associated with five of the proteins found in the Sm RNPs and three proteins unique to (U1)RNP (3-5). Only a subset of the proteins associated with each of these RNP classes are actually antigenic. Antigenic proteins include the 70, 34(A), and 22(C) kDa proteins recognized by (U1)RNP antisera, and the 29(B'), 28(B), 16(D), and sometimes the 12(E) kDa proteins recognized by Sm antisera. La RNPs consist of a 45-50 kDa antigenic protein which binds to a polyuridylate stretch at the 3' end of nascent cellular and viral RNA polymerase III transcripts, while Ro RNPs contain in addition to the La protein, the 57-60 kDa Ro antigen as well as a recently described 52-54 kD antigen (6-13).

We have found (14) that a specific subset of human cytomegalovirus (HCMV) RNAs are immunoprecipitated from infected fibroblast cells by La, Ro, and Sm, but not by (U1)RNP, antisera. As part of our characterization of these viral ribonucleoprotein complexes, we wanted to determine the identity of any antigenic

proteins (cellular or viral) that are associated with, and perhaps responsible for, the immunoprecipitation of these HCMV RNAs. Immunoprecipitation of these viral RNAs employs the same protocol as is used for immunoprecipitation of cellular La, Ro, and Sm ribonucleoprotein RNAs. The cell extract from either uninfected or HCMV infected cells is incubated with a monospecific autoimmune antiserum (e.g., La), and the RNP-antibody complexes are precipitated by the addition of Pansorbin. Pansorbin is a preparation of killed Staphylococcus aureus which has protein A attached to the peptidoglycan part of the cell wall; protein A binds to the Fc region of primarily IgG, as well as certain classes of IgA and IgM (15). However, when proteins were isolated from these immunoprecipitates for analysis by denaturing gel electrophoresis and immunoblotting, severe background problems resulted due to the autoimmune antisera and staphylococcal proteins still present in the samples. When ^{35}S-labeled proteins are immunoprecipitated and analyzed by electrophoresis there is, of course, no background from antisera or Pansorbin proteins. However, whereas immunoprecipitation of ^{35}S-labeled proteins identifies the total profile of proteins present in the RNP particles, (and this profile can be misrepresented by differential labeling of individual proteins), we were interested in determining which of these proteins are actually antigenic. Certain of the (U1)RNP and Sm ribonucleoproteins have been isolated biochemically, an approach which avoids the presence of antisera and Pansorbin proteins (3,4). However, to identify those

antigens responsible for immunoprecipitation of HCMV RNAs, we wanted to use a protocol analogous to that used for RNA immunoprecipitation. In this report we describe an immunoblot procedure by which we were able to dramatically reduce the background due to immunoprecipitated immunoglobulin and staphylococcal proteins while retaining the sensitivity of antigen detection. The procedure is relatively simple and may be applicable in other cases where antigen identification and/or purification is most easily accomplished via immunoprecipitation and immunoblotting.

MATERIALS AND METHODS

Antisera and Antigens

The La, Ro, and Sm (human) antisera used for these studies had been tested (Alpha Antigens, Inc.) against Center for Disease Control reference antisera and determined to be monospecific. To confirm that the La, Ro, and Sm antisera used were monospecific, it was determined (14) that the electrophoretic profiles of the cellular La, Ro, and Sm RNAs immunoprecipitated with each antisera were identical to characteristic RNA profiles previously described (5,6,10). In addition, when total cell lysates were analyzed by electrophoresis and subsequent immunoblotting using La or Sm antisera, the only proteins detected corresponded to known La and Sm antigens, respectively (3-13). Partially purified La and Sm antigen preparations were obtained from Immunovision, Inc. (Springdale, AZ).

Ribonucleoprotein Immunoprecipitations

Ribonucleoproteins were immunoprecipitated essentially as previously described (4,14). Briefly, human fibroblast cells (strain MRC-5; ATCC No. CCL 171) were harvested, resuspended (1.5×10^8 cells/ml) in TBS (TBS: 150 mM NaCl, 50 mM Tris-HCl, pH 7.4), and after gentle sonication, adjusted to 1 unit/µl RNasin (Promega-Biotec), 1 mM dithiothreitol (DTT), 400 µg/ml carrier yeast tRNA, and 1 mM phenylmethylsulfonyl fluoride (PMSF). After centrifugation at 12,000 x g for 10 min to remove cell debris, the supernatant was precleared by incubation at 2°C for 15 min with an equal volume of 10% staphylococcal protein A (Pansorbin; Calbiochem-Behring) in NET-2 buffer (NET-2: 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 0.05% NP-40). Preclearing was done to remove cellular components which bind nonspecifically to the staphylococcal cells. The staphylococcal protein A was removed by centrifugation (5 min at 12,000 x g) and the supernatant incubated with a monospecific antiserum (e.g., Sm, La, or Ro; 20-60 µl antiserum added per 1.5×10^7 cells) at 2°C for 15 min with gentle mixing. Each antiserum was titered to determine the amount necessary for quantitative immunoprecipitation of the viral RNPs. Staphylococcal protein A (5-10 µl/ul antiserum) was then added and after a 15 min incubation at 2°C, the antibody-antigen complexes were pelleted and washed five times with NET-2 at 2°C. For initial attempts at analysis of both total and antigenic protein profiles, the pelleted immunoprecipitates were boiled in disruption buffer and electrophoresed on SDS polyacrylamide gradient gels.

Covalent Conjugation of Antibody to Staphylococcal Protein A and Elution of Antigen

Immunoprecipitated antigens were eluted from covalent staphylococcal protein A-antibody complexes essentially as previously described (16-18). Briefly, antisera (e.g., Sm, La, or Ro; 10 mg/ml IgG) were incubated with at least one equivalent of 10% staphylococcal protein A (1 ml of 10% staphylococcal protein A binds 2.1 mg IgG) for 30 min at 25°C. The antibodies were then covalently cross-linked to the protein A moiety by incubation in 0.5% paraformaldehyde for 45 min at 37°C. After washing four times in NET-2, the staphylococcal protein A-antibody complexes were incubated with the precleared cell supernatant (prepared as described under Ribonucleoprotein Immunoprecipitations) for 20 min at 2°C. The immunoprecipitates were then pelleted, washed five times in NET-2, and the antigens eluted from the antigen-antibody-protein A complex by incubation in 0.1 M lithium diiodosalicylate (LIS) or 3 M $MgCl_2$ for 20 min at 25°. After centrifugation to remove the antibody-staphylococcal protein A complex, the supernatant (containing eluted proteins) was dialysed to remove the LIS or $MgCl_2$. The eluted proteins were then concentrated using a speed vac (Savant), and electrophoresed on SDS polyacrylamide gels as described below.

Protein Electrophoresis and Immunoblotting

Total immunoprecipitation pellets or LIS/$MgCl_2$ eluted proteins were boiled in disruption buffer (0.02% bromophenol blue, 20% glycerol, 4% SDS, and 300 mM β-mercaptoethanol) and electro-

phoresed through SDS polyacrylamide gradient (4-30%) gels (19). The proteins were then electroblotted to 0.22 μm nitrocellulose (Schleicher and Schuell) using 25 mM Tris-HCl, pH 8.3, 0.19 M glycine, and 20% methanol as the transfer buffer (20). After removal of molecular weight markers (detected by amido black staining), filters were incubated overnight in TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20) containing 1% BSA to block nonspecific protein binding sites. Antigenic proteins were then identified as described below.

Identification of Antigenic Proteins

Two distinct series of incubations were used. In the first series, filters (blocked overnight in TBST-1% BSA as described above) were incubated for 2 hr (25°C) in TBST-1% BSA containing the primary antiserum (i.e., either La, Ro, or Sm antiserum) diluted 1:50 or 1:100. After three 10 min washes in TBST, the filters were then incubated for 1/2-1 hr in TBST-1% BSA containing anti-human IgG conjugated to alkaline phosphatase (Promega-Biotec) diluted 1:15,000. The filters were again washed for 3 x 10 min in TBST, and the antigenic proteins then visualized by incubating filters in AP buffer (AP buffer: 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) containing the alkaline phosphatase substrate reagents nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as described in the ProtoBlot system (Promega-Biotech).

In the second series of incubations, filters which had been incubated with the primary antiserum (La, Ro, or Sm) and washed in TBST as described above, were then incubated for 1 hr in TBST-1%

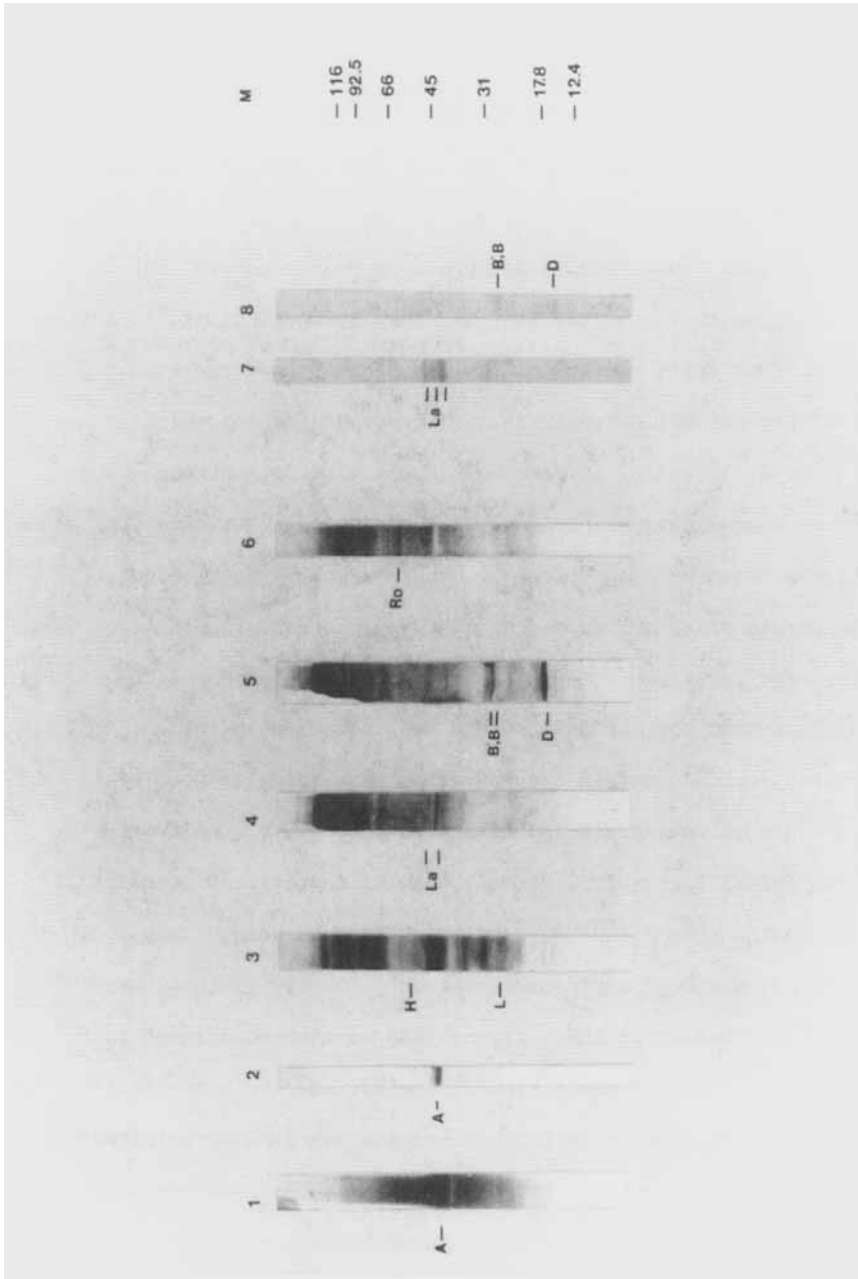
BSA containing protein A conjugated to biotin (Polysciences, Inc.) diluted 1:10,000. The filters were subsequently washed 3 x 10 min in TBST, and then incubated for 30-45 min in TBST-1% BSA containing avidin conjugated to alkaline phosphatase (Polysciences, Inc.) diluted 1:25,000. The best working dilutions for a specific application should, however, be determined empirically. After washing in TBST, antigenic bands were visualized as described above.

RESULTS AND DISCUSSION

Immunoblot Analysis of Proteins Contained in Immunoprecipitation Pellets

To determine the profile of antigenic proteins associated with La, Ro, and Sm immunoprecipitated HCMV RNAs, proteins were initially immunoprecipitated using a method identical to that used for the immunoprecipitation of the RNAs (see Methods; Ribonucleoprotein Immunoprecipitation). Briefly, cell sonicates were preincubated with staphylococcal protein A (Pansorbin) to remove those cellular components which bind nonspecifically to the staphylococcal cells. After removal of the staphylococcal protein A by centrifugation, the supernatants were incubated with a monospecific (i.e., La, Ro, or Sm) human autoimmune antiserum, and antigen-antibody complexes pelleted upon the addition of an additional aliquot of staphylococcal protein A. The pelleted immunoprecipitates were boiled in disruption buffer, electrophoresed on SDS polyacrylamide gels, and electroblotted to nitrocellulose. Blots were then incubated with the same antiserum

as was used for the original immunoprecipitation (e.g., La, Ro, or Sm), followed by incubation with anti-human IgG conjugated to alkaline phosphatase. The results of such an experiment in which cellular La, Ro, and Sm ribonucleoproteins were immunoprecipitated from uninfected cells are shown in Figure 1. For the purposes of this report, immunoprecipitations from uninfected vs. infected cells gave analogous results, and thus immunoprecipitations from uninfected cells are shown. It can be seen that there is significant background, not only in La, Sm, or Ro immunoprecipitations (lanes 4, 5, and 6, respectively), but also for corresponding amounts of either staphylococcal protein A, or antisera (La) alone (lanes 1 and 3, respectively). There was less background when sepharose-protein A rather than staphylococcal protein A was used (compare lanes 2 vs. 1, respectively). However, we had found that with respect to both antigen and RNA immunoprecipitation, that the staphylococcal protein A preparation was easier to work with, and gave a more consistant and efficient recovery. Despite the substantial background, we were able to tentatively identify La, Ro, and Sm (B', B, and D) antigenic bands in sample lanes. The identification of antigens was based primarily on the known molecular weight of the antigens (see co-electrophoresed La and Sm standards, lanes 7 and 8, respectively), and the specificity of the identified bands to their corresponding immunoprecipitation. As described by others (11) the 48-50 kD La protein degrades to a 43 kD product, as well as other less abundant species (Fig. 1, lanes 4 and 7). The Sm 13 kD E protein was not detected by these



sera. When total proteins immunoprecipitated were examined by including [^{35}S]methionine in the media (data not shown), the profile of immunoprecipitated proteins was as expected, with the 70 kDa, A, B', B, C, D, E, F, and G proteins detected in Sm immunoprecipitates, a 48 kD protein in La and Ro immunoprecipitates, and an approximately 60 kDa protein antigen in Ro immunoprecipitates with, as expected, no background from the antisera or staphylococcal protein A.

Upon analysis of the incubation conditions (data not shown), it was found that a) dilution of the primary and/or secondary antisera did not improve the relative intensity of antigen bands with respect to background, b) incubation of blots with an E. coli extract (Promega-Biotec) reduced, but did not eliminate,

FIGURE 1. Immunoblot analysis of immunoprecipitation pellets. Immunoprecipitation pellets, or individual components contained within immunoprecipitation pellets, were electrophoresed through SDS polyacrylamide gels, blotted to nitrocellulose, and then probed, first with monospecific antiserum (either La, Ro, or Sm), and then with anti-human IgG conjugated to alkaline phosphatase. Lanes 1, 2, and 3 are 40 µl of 10% staphylococcal protein A (which binds 2.1 mg IgG/ml), 8 µl sepharose-protein A (which binds 10 mg IgG/ml), and 4 µl La antiserum, respectively, each probed with La antiserum. Bands labeled A, correspond to protein A, while those labeled H and L correspond to heavy and light immunoglobulin chains, respectively. Lane 4 is one plate equivalent of a La immunoprecipitation pellet probed with La antiserum. One plate equivalent corresponds to the immunoprecipitation of proteins from 3 x 10^6 cells with 4 µl La antibody and 40 µl 10% staphylococcal protein A. Lanes 5 and 6 are Sm and Ro immunoprecipitation pellets probed with Sm and Ro antisera, respectively. La antigen bands, Sm B', B, and D antigen bands, and Ro antigen are indicated. Lanes 7 and 8 are partially purified La and Sm antigen standards (Immunovision, Inc.), probed with La and Sm antisera, respectively. Lane M corresponds to co-electrophoresed molecular weight markers (sizes given in kDa).

background due to staphylococcal proteins, c) no signal resulted upon incubation of blots with only primary antisera, and d) the staphylococcal protein A background was not seen upon incubation of blots with secondary antibody alone, but antisera bands were still detected. La and Sm antigen preparations, which by silver-stain analysis (not shown) contain many protein bands, only revealed characteristic La and Sm antigen bands by immunoblot analysis (Fig. 1, lanes 7 and 8, respectively). In addition, when total cell lysates were electrophoresed, blotted, and probed with either La or Sm antisera, the only bands visualized corresponded to La and Sm antigens, respectively. It appeared then, that the staphylococcal and antisera proteins, although denatured upon electrophoresis, were present in such amounts as to produce background bands which could prevent the detection of ribonucleo-protein antigenic bands.

Recovery of Antigen from Immunoprecipitation Pellets

In that the background using total immunoprecipitates was problematic, it appeared that a method to separate the antisera and staphylococcal proteins (if using staphylococcal protein A) from the immunoprecipitated antigens might be required. We therefore adapted a procedure originally described by MacSween and Eastwood (16,18) to our immunoblot procedure. Briefly, antibodies were covalently cross-linked to the protein A moiety of the staphylococcal cells by incubation in 0.5% paraformaldehyde at 37°C. The staphylococcal protein A-antibody complex was then

incubated with antigen (the precleared cell sonicate containing cellular La, Ro, or Sm RNPs) and after 20 min at 2°C, antigens bound to the protein A-antibody complex pelleted by centrifugation. To selectively elute antigens from the protein A-antibody complex, the pellets were incubated with either 0.1 M lithium diodosalicylate (LIS) or 3 M $MgCl_2$. After dialysis of the eluted proteins to remove the LIS or $MgCl_2$, samples were analyzed by electrophoresis and immunoblotting as described above.

Figure 2 shows the results of such an experiment. Silver stain analysis (Fig. 2A) of a total La immunoprecipitation pellet (lane 1) as compared to an eluted fraction (lane 2) indicated that there was a significant reduction in sample complexity; some of the more prominent bands in the eluted fraction are indicated. Figure 2B shows an immunoblot of La immunoprecipitation pellets eluted with either LIS or $MgCl_2$. Immunoreactive proteins eluted with either LIS (lanes 1 and 2) or $MgCl_2$ (lanes 3 and 4) correspond to the 48-50 and 43 kD La antigens and to some extent, some of the more predominant antisera proteins; the predominant band which runs above the upper La antigen band corresponds to the heavy immunoglobulin chain. Most of the antigen is eluted in the first elution step (lanes 1 and 3), with comparatively small amounts recovered during a second elution (lanes 2 and 4). In addition, it can be seen that while $MgCl_2$ seems to elute more of the La antigen, it also elutes more of the antisera proteins than does LIS; compare LIS eluant (lane 1) and pellet after elution

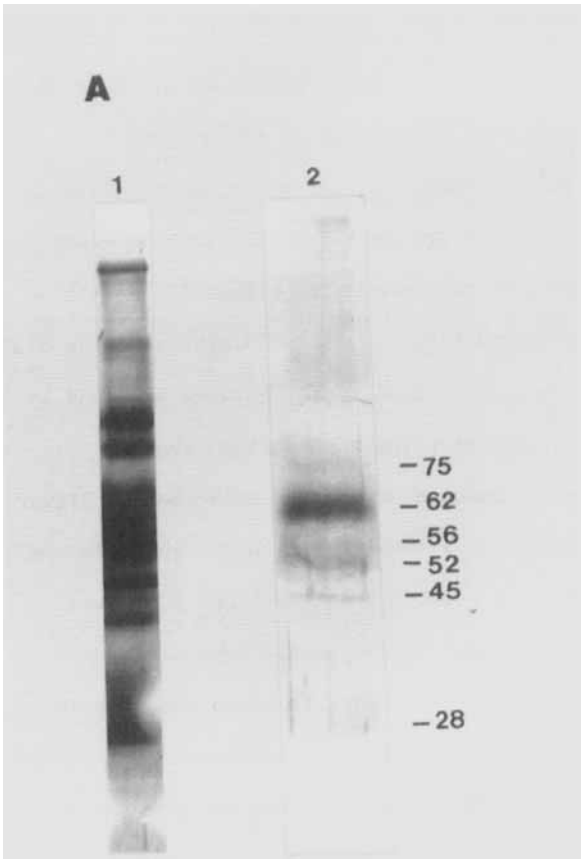


FIGURE 2. Elution of antigen from antibody-Pansorbin complex. A) Proteins eluted from La antibody-protein A conjugates with 3 M $MgCl_2$ are compared by silver stain analysis to proteins found in total La immunoprecipitation pellets. Lane 1, total immunoprecipitation pellet, one plate equivalent; lane 2, $MgCl_2$ eluted proteins, two plate equivalents. Molecular weights were determined by comparison to co-electrophoresed standards and are given in kD. B) Proteins eluted from La antibody-protein A pellets (two plate equivalents) are compared to proteins detected in the total La immunoprecipitation pellets by immunoblot analysis. Bands corresponding to La antigen are indicated. Lanes 1-7, correspond to samples in which La antibody was covalently cross-linked to staphylococcal protein A prior to incubation with antigen. Lanes 1 and 2, LIS eluted proteins, first and second elutions of the immunoprecipitation pellet, respectively; lanes 3 and 4, $MgCl_2$ eluted proteins, first and second elutions, respectively; lane 5; total immunoprecipitation pellet, before elution with either LIS or $MgCl_2$; lanes 6 and 7; immunoprecipitation pellet after elution with $MgCl_2$ or LIS, respectively. Lanes 8-14 correspond to samples treated the same as those in 1-7 except the cross-linking of La antibody to staphylococcal protein A was eliminated. Lanes 8 and 9, LIS eluted proteins, first and second elutions, respectively; lanes 10 and 11, $MgCl_2$ eluted proteins, first and second elutions, respectively; lane 12, total immunoprecipitation pellet, before elution with either LIS or $MgCl_2$; lanes 13 and 14, immunoprecipitation pellet after elution with $MgCl_2$ or LIS, respectively. Lane 15, co-electrophoresed La antigen standard.

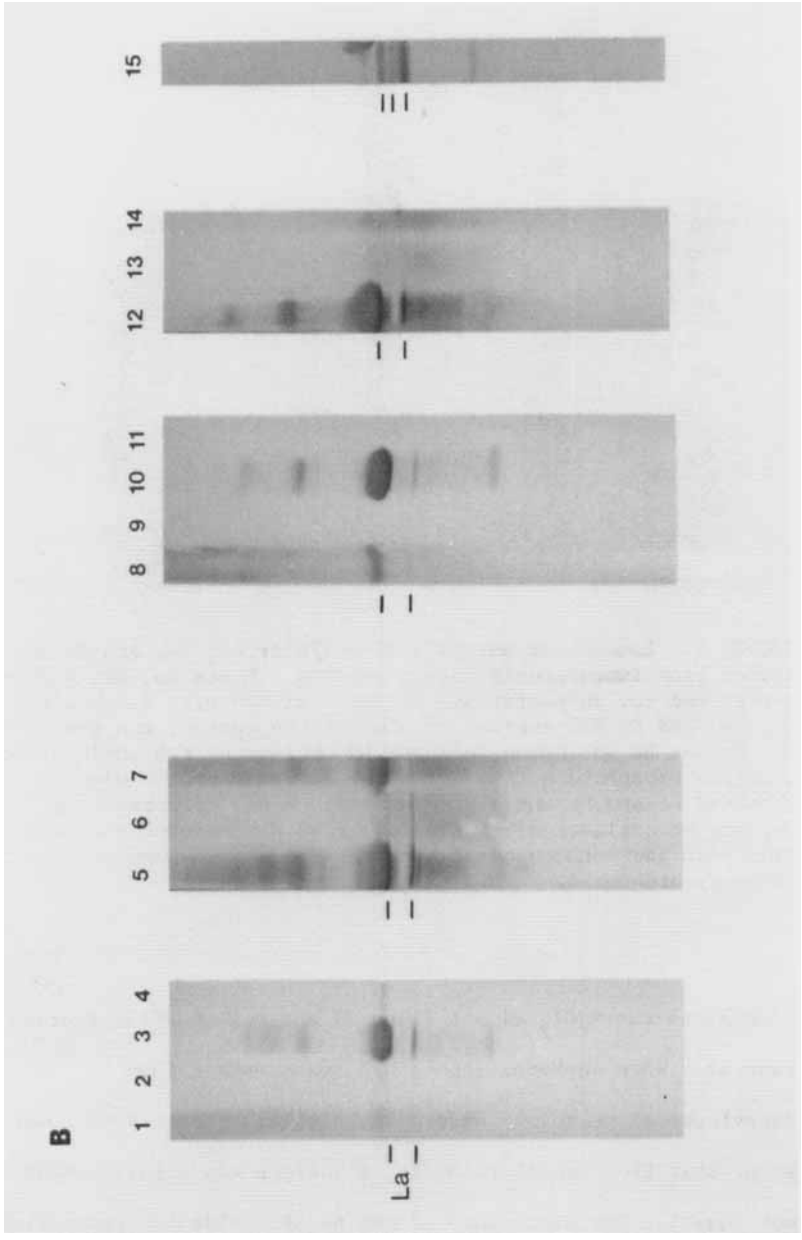


FIGURE 2, continued

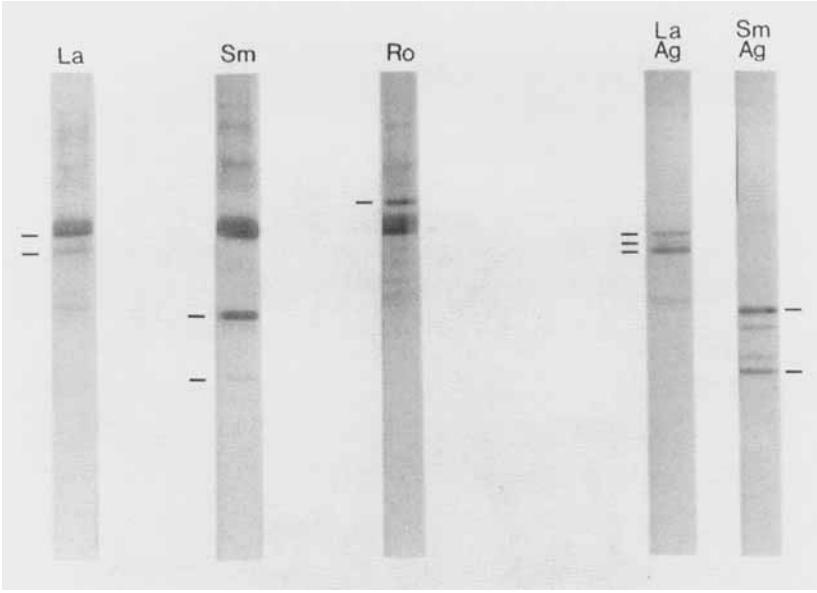


FIGURE 3. Immunoblot analysis of cellular La, Sm, and Ro antigens eluted from immunoprecipitation pellets. Lanes La, Sm, and Ro correspond to, respectively, proteins eluted with 0.1 M LIS from La, Sm, and Ro RNP-antibody-protein A conjugates, and probed with La, Sm, or Ro antiserum followed by anti-human IgG conjugated to alkaline phosphatase. La Ag and Sm Ag lanes are co-electrophoresed La and Sm antigen standards. Bands corresponding to La, Sm, and Ro antigens are indicated. The predominant band which runs just above the upper La antigen band corresponds to the heavy immunoglobin chain.

(lane 7) to the $MgCl_2$ eluant (lane 3) and pellet after elution (lane 6). When sepharose-protein A beads rather than staphylococcal protein A were used, similar results were found, except that the overall recovery of antigen was significantly less (not shown). The efficiency of the paraformaldehyde cross-linking step was examined, and it was found that the protein A-antibody complex was significantly more stable to LIS elution than to $MgCl_2$

elution; compare proteins remaining with the staphylococcal protein A pellet after elution in cross-linked (lanes 6 and 7, $MgCl_2$ and LIS, respectively) vs. uncross-linked (lanes 13 and 14, $MgCl_2$ and LIS, respectively) samples. In agreement with the earlier work of MacSween and Eastwood (16,18), we found the paraformaldehyde cross-linking step to have a minimal effect on antibody reactivity. Thus, when the La antigen present in total immunoprecipitates was compared (by densitometry) to that eluted by LIS from paraformaldehyde cross-linked immunoprecipitates, approximately equal amounts (70-95%) of the antigen were detected in LIS eluted immunoprecipitates (see for example Fig. 4B, lanes 1 vs. 2). In general, this was found to reflect the somewhat variable elution of antigen from the antigen-antibody complexes, rather than a difference in the total amount immunoprecipitated (data not shown).

As the immunoglobulin heavy chain runs close to the 48-50 kD La antigen band, LIS elution was used for the identification of immunoprecipitated cellular La, Ro, and Sm antigens shown in Figure 3. It can be seen that there was a significant reduction of background banding as compared to that seen in Figure 1 (lanes 4, 5, and 6). There was, however, still some background primarily due to the interaction of the secondary antibody (anti-human IgG) with the immunoglobulin proteins remaining in the eluants.

Modification of Immunoblot Incubations

In an attempt to further reduce the background, a modified series of immunoblot incubations were employed (see Methods).

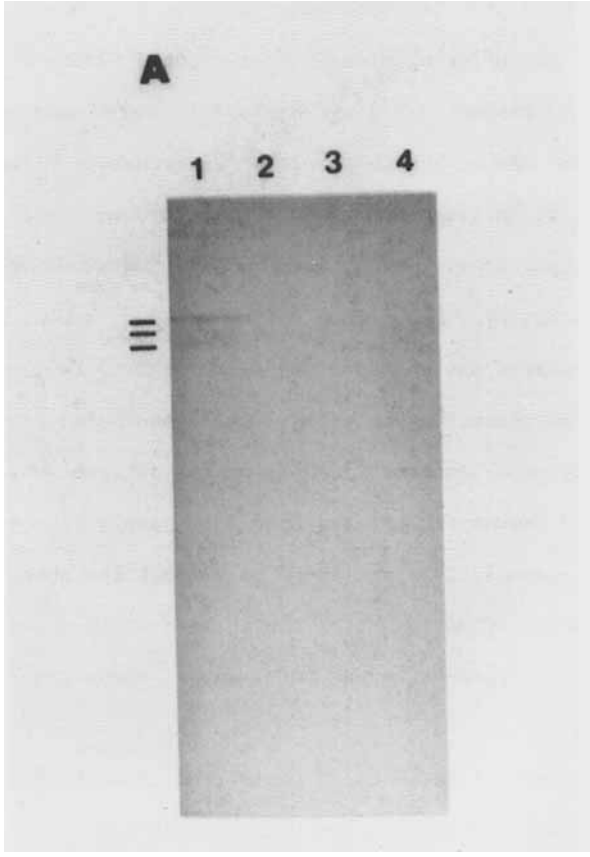


FIGURE 4. Probing of immunoprecipitates with a modified series of immunoblot incubations. After incubation with a monospecific SLE antiserum (i.e., La, Ro, or Sm) blots were incubated with protein A conjugated to biotin followed by avidin conjugated to alkaline phosphatase. A) The primary antiserum was La. Lane 1, La antigen standard; lane 2, La antibody; lane 3; sepharose-protein A; lane 4, staphylococcal protein A; lanes contain same amount of sample as was used in Figure 1. B) Total La, Ro, and Sm immunoprecipitates (lane 1) or proteins eluted with 0.1 M LIS from La, Ro, and Sm immunoprecipitates (lane 2). The primary antiserum was the same as that used for the original immunoprecipitation (i.e., La, Ro, and Sm, respectively). La, Ro, Sm antigenic bands are indicated.

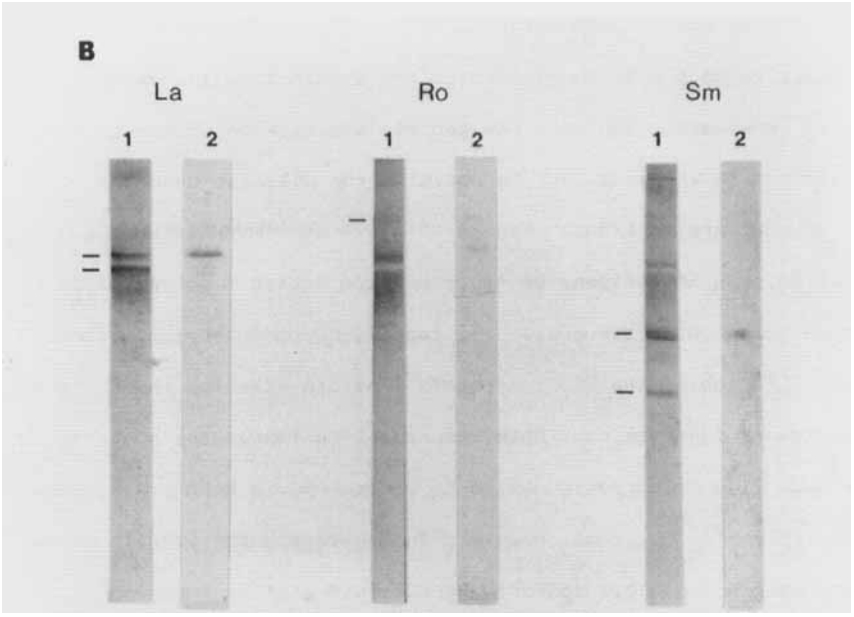


FIGURE 3, continued

After incubation with primary antiserum (e.g., La, Ro, or Sm), blots were exposed to protein A conjugated to biotin, and then avidin conjugated to alkaline phosphatase. It was reasoned that protein A should interact primarily with an intact Fc region, and thus should preferentially associate with the primary antiserum (La, Ro, or Sm) bound during immunoblot incubations, rather than the denatured immunoglobulin proteins present on the blot. A reduction in background was seen (Fig. 4A), particularly with respect to antisera background banding (Fig. 4A, lane 2 vs. Fig. 1, lane 3) as well as staphylococcal protein A background (Fig. 4A, lane 4 vs. Fig. 1, lane 1). It was found that the

background due to both antisera and staphylococcal proteins was sensitive to the concentration of the avidin-alkaline phosphatase used (presumably due to a nonspecific association of the avidin), and thus it was necessary to optimize the dilution used for different applications. Figure 4B shows an immunoblot of cellular La, Ro, and Sm antigens using either the entire immunoprecipitation pellet or after elution of the immunoprecipitation pellet with LIS, using the biotin-protein A/avidin-alkaline phosphatase series of incubations. Upon comparison to immunoblot shown in Figure 1, a dramatic reduction in background in both experiments is apparent. We found, however, that although the profile using the entire immunoprecipitation pellet was greatly improved (compare Fig. 4B, lanes 1 to Fig. 1, lanes 4, 5, and 6), blots were consistantly cleaner, and therefore easier to interpret, when LIS eluted antigens were used (Fig. 4B, lanes 2). It was of interest that regardless of the procedure used, we consistantly detected an approximately 48-50 kD protein in Ro immunoprecipitates. Such lower molecular weight (50-54 kD) Ro antigens have been previously described; this particular reactivity, however, appears to correlate with the 50 kD band described by Elkon and Jankowski (11), rather than the 52-54 kD activity described by others (12,13). In addition to the probings shown in Figure 4, a series of self and cross-probings, using LIS eluted immunoprecipitates and the biotin-protein A/avidin-alkaline phosphatase incubations, were done (data not shown). In all cases, the antigenic profiles were as expected. For example, when Sm [or

(U1)RNP] immunoprecipitates were probed with (U1)RNP antisera, the (U1)RNP 70 kDa, A, and C antigens were detected, whereas when (U1)RNP immunoprecipitates were probed with Sm antisera, the B', B, and D Sm antigens were detected. In addition, none of the SLE immunoprecipitates (i.e., La, Ro, Sm, or [(U1)RNP] showed any evidence of antigenic bands when probed with normal or unrelated (e.g., anti-centromere) antisera.

In summary, we describe a procedure which enables the analysis of immunoprecipitated La, Ro, and Sm RNP antigens by immunoblotting. It is reasonably straightforward to analyze either total or biochemically purified cell proteins for the presence of antigenic species, or to analyze ^{35}S-labeled proteins for the profile of total proteins in immunoprecipitates. However, the analysis of antigenic proteins in immunoprecipitation pellets can be complicated by the presence of immunoglobulin and, if staphylococcal protein A is used, staphylococcal proteins. We found that with the preferential elution of the antigen from antibody-protein A complexes using the chaotropic agent LIS, and the use of a modified series of immunoblot incubations which take advantage of the selectivity of protein A for intact Fc regions, we were able to significantly reduce the background due to antisera and staphylococcal proteins present in immunoprecipitated samples. For our purposes, the use of both modifications significantly improved antigen detection. Elution of the antigen by LIS reduced but did not eliminate the staphylococcal protein A and antisera background, whereas probing blots with biotin-protein

A followed by avidin-alkaline phosphatase consistantly resulted in much cleaner blots with LIS eluted antigens than with total immunoprecipitation pellets. There are, of course, other approaches which can be used to reduce immunoblot background from antibody and/or staphylococcal proteins. For example, the precipitating antibody can be directly cross-linked to a solid phase, and after immunoprecipitation, antigens eluted from this complex. Alternatively, the primary antibody used during immunoblot incubations (e.g., La, Ro, or Sm) can be cross-linked to a chromogenic agent (e.g., horseradish peroxidase), thus eliminating the need for a secondary antibody and much of the resultant background. These methods, however, typically require significant expertise with immunochemical manipulations, often entailing chromatographic purification of the antibody, coupling (to either the matrix or chromogenic agent), and subsequent purification and isolation of the modified antibody. We attempted coupling La antisera to horseradish peroxidase, but found the yields of modified antiserum to be prohibitively low (≤10%). The approach we have described is presented as a simpler alternative, requiring minimal time and materials to establish a working system. In addition, it is particularly well suited to those situations in which a) antibody supply is limited, b) many different antibodies need to be characterized rapidly by immunoprecipitation (e.g., a clinical setting), or c) minor cross-reactivities in an antibody preparation need to be retained. Thus, the procedure described herein may be applicable with respect to immunoblot analysis in other systems where samples have

been immunoprecipitated, and thus contain staphylococcal protein A and/or antisera proteins, as well as the antigenic proteins of interest.

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