USE OF MONOCLONAL ANTIBODIES FOR THE CHARACTERIZATION OF NOVEL DNA-BINDING PROTEINS RECOGNIZED BY HUMAN AUTOIMMUNE SERA

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The presence of serum antibodies reacting with nucleic acids, nuclear proteins, or nucleoprotein complexes is one of the hallmarks of systemic lupus erythematosus (SLE)[1] and related disorders (for a recent review, see reference 1). For reasons that are not well understood, antinuclear antibodies in these disorders often appear to react preferentially with nucleoprotein complexes (1–9). Serum autoantibodies to RNA-protein complexes, including the Sm and RNP (2–5), Ro (6, 7), and La (8, 9) antigens have provided important information regarding the molecular biology of ribonucleoproteins and the immune responses to these antigens in autoimmune diseases. Fewer instances of autoantibodies to DNA-protein complexes have been described (10–15). The best-characterized example is that of antihistone antibodies (14–15).

Previous studies from our laboratory (16 and Reeves, Conner, and Kunkel, unpublished observations) have identified a number of high molecular weight nuclear protein antigens, recognized by small subsets of human autoimmune sera, that are distinct from the Sm, RNP, Ro, and La antigens. It would be of interest to know whether or not these poorly characterized autoantigens are also ribonucleoprotein or deoxyribonucleoprotein complexes. The usefulness of autoimmune sera in characterizing these new antigens is limited by the small number of available sera, and by the presence of more than one autoantibody specificity in most autoimmune sera. Monoclonal antibodies (mAb) reacting with the antigens would eliminate many of the problems encountered in using autoimmune sera. Although nuclear proteins are characteristically poor immunogens, mAb against Sm (17), DNA (18), and RNA (19) have previously been obtained from autoimmune mice. In the present studies, antinuclear mAb against a DNA-binding protein complex have been obtained by immunization of BALB/c mice. The deoxyribonucleoprotein complex recognized by the mAb is also recognized

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[1] *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; mAb, monoclonal antibody; MCTD, mixed connective tissue disease; NP-40, Nonidet P-40; PAS, protein A Sepharose; RAM, rabbit anti-mouse immunoglobulin antibodies; SDS, sodium dodecyl sulfate; SDS-PAGE, SDS–polyacrylamide gel electrophoresis; SLE, systemic lupus erythematosus; TF IIIA, RNA polymerase transcription factor IIIA; TKM, 10 mM Tris, 5 mM KCl, 5 mM $MgCl_2$, pH 7.5 buffer.

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by sera from certain patients with SLE, mixed connective tissue disease (MCTD), and scleroderma. The availability of mAb has facilitated partial characterization of the antigen as well as the detection of autoantibodies to the proteins in autoimmune sera.

Materials and Methods

Immunization and Cell Fusion. Human B cell nuclei were isolated from the plasmacytoid cell line 2p68 (a hypoxanthine-guanine phosphoribosyl transferase–deficient variant of ARH-77; 20) by resuspending washed cells at 5×10^7 cells/ml in 10 mM Tris, 5 mM KCl, 5 mM $MgCl_2$ (TKM) containing 0.3% Nonidet P-40 (NP-40) on ice for 5 min. Nuclei were pelleted at 1,800 rpm for 10 min and washed three times in detergent-free TKM before resuspending in the same buffer at 3×10^7 nuclei/0.1 ml. 6-wk-old BALB/c mice (The Jackson Laboratory, Bar Harbor, ME) were immunized intraperitoneally with 3×10^7 nuclei emulsified in complete Freund's adjuvant on day 0 and day 14, and with 3×10^7 nuclei without adjuvant on day 28. Spleen cells were isolated 3 d after the last immunization, fused with the mouse myeloma cell line SP2/0, and grown in selective medium using standard techniques (21, 22).

Screening and Cloning of Hybridomas. A three-step screening protocol was used to isolate hybridomas that secreted antinuclear antibodies. The first step was an enzyme-linked immunosorbent assay (ELISA), using a sonicated nuclear extract as antigen. 2p68 nuclei were isolated and washed as described above. The final nuclear pellet was resuspended in 150 mM NaCl, 10 mM sodium phosphate, pH 7.2, and sonicated three times on ice for 20 s. After extracting for 20 min on ice, the cell extract was spun at 8,740 *g* for 5 min. The cleared extract was used to coat 96-well microtiter plates (Nunc; Vanguard International, Neptune, NJ) overnight at 4°C. Hybridoma supernatant was added to the antigen-coated and blocked wells, followed by a peroxidase-conjugated goat anti-mouse IgG + IgM second antibody (Tago Inc., Burlingame, CA) and phenol-aminoantipyrine substrate (23). Hybridoma supernatants that were positive by ELISA were then screened by immunofluorescence (IF) using, as substrate, either cytocentrifuged 2p68 cells fixed in methanol at −20°C (24), or HEp-2 cells grown on coverslips and fixed in the same manner. The fixed cells were incubated with 50 μl hybridoma supernatant for 30 min, washed, and incubated with 20 μl of 1:20 rhodamine-conjugated goat anti-mouse Ig (Tago Inc.) for 30 min at 22°C. Hybridoma supernatants that were positive by IF were characterized further by immunoprecipitation of radiolabeled cell extracts (see below).

Hybridomas that secreted antinuclear antibodies were cloned in soft agar and ascites was produced by injecting 10^6 cloned hybridoma cells intraperitoneally into pristane-primed BALB/c mice (22). IgG from the resulting ascites was obtained by ammonium sulfate precipitation followed by ion exchange chromatography on DE-52 (Whatman Laboratory Products Inc., Clifton, NJ) as described (22). Ig subclasses of the mAb were determined by radial immunodiffusion using subclass-specific anti-mouse Ig antibodies (Miles Laboratories Inc., Elkhart, IN).

Immunoprecipitation Using Murine mAb. Protein A–Sepharose (PAS) (50 μl of a 50% suspension in water; Pharmacia Inc., Piscataway, NJ) was coated with rabbit anti-mouse Ig antibodies (RAM) (30 μg affinity-purified RAM per 50 μl 50% PAS) for 1 h at 22°C. The RAM-coated beads were then incubated with 10 μl ascites or 1–3 ml hybridoma culture supernatant for 3 h at 22°C. After incubation, the mAb-coated PAS-RAM beads were washed three times with 10 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, and 1 mg/ml ovalbumin, and added to a [^{35}S]methionine-labeled cell extract. The cell extract was obtained by labeling 1.5×10^6 2p68 cells/ml in methionine-free RPMI 1640 containing 10% dialyzed fetal bovine serum and 0.5 mg/l cold methionine, plus 50 μCi/ml [^{35}S]methionine (New England Nuclear, Boston, MA; translation grade; sp act, ~1,100 Ci/mmol) for 12 h. Cells were collected by centrifugation, washed three times, and resuspended in lysis buffer (150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM iodoacetamide, 2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, and 0.3% NP-40), then sonicated on ice three times, 20 s each time, and extracted for 30 min. The extract was centrifuged

5 min at 8,740 *g* in a Beckman microfuge (Beckman Instruments Inc., Palo Alto, CA), and precleared by incubating with 10 μl 50% PAS per 10^6 cell-equivalents for 2 h at 4°C. The PAS was removed by centrifugation before adding 0.5–1.0 × 10^6 cell-equivalents of precleared cell extract per immunoprecipitate to the mAb-coated beads at 4°C for 1–3 h. The beads were then washed extensively as described (4) and boiled for 5 min in loading buffer containing 0.1 M dithiothreitol and 2.5% sodium dodecylsulfate (SDS) (4). The eluted radioactive proteins were separated on 10–20% SDS-polyacrylamide gradient gels (4), stained with Coomassie Blue, destained, and fluorographed (25).

Immunoprecipitation Using Human Autoimmune Sera. The proteins immunoprecipitated by human autoimmune sera were analyzed by a modification of the procedure used for murine mAb. Human serum (10 μl) was centrifuged at 8,740 *g* for 5 min and added directly to 0.5–1.0 × 10^6 cell-equivalents of [^{35}S]methionine-labeled cell extract for 1 h at 22°C, before adding 50 μl 50% PAS for 1–3 h, at 4°C. The beads were washed and eluted, and the proteins were separated on SDS-polyacrylamide gels and fluorographed as above.

Limited Proteolysis. [^{35}S]methionine-labeled proteins were cut out of 10–20% gradient gels using a razor blade, rehydrated, and subjected to limited proteolysis using the method of Cleveland et al. (26). Proteins were digested in gels with chymotrypsin (Worthington Biochemical Corp., Freehold, NJ; 10 μg per lane) or staphylococcal V8 protease (Miles Laboratories Inc.; 2 μg per lane); the resulting fragments were resolved on 20% SDS-polyacrylamide gels containing 1 mM EDTA (26).

ELISA for Autoantibodies in Human Sera. I used an ELISA to screen human autoimmune sera for antibodies against the proteins recognized by murine mAb. mAb were purified from ascites, as described above, and dialyzed into 50 mM Tris, pH 7.95 (23). 96-well microtiter plates were coated with the purified mAb at 10 μg/ml for 12 h at 4°C, washed, and blocked with 10% fetal bovine serum. Antigen (100 μl) was then allowed to adhere to the antibody-coated wells for 3 h at 22°C. The antigen consisted of either a sonicated whole cell extract in lysis buffer (see above), or else cytoplasmic or nuclear fractions of cells (adjusted to 150 mM NaCl), which were obtained as outlined in the immunization protocol above, both at 5 × 10^7 cell equivalents/ml extract. After washing the wells, 100 μl of diluted human serum was added to the wells for 2.5 h at 22°C. The wells were washed again, and 100 μl of affinity-purified peroxidase-conjugated goat anti-human kappa and lambda chain antibodies (Tago Inc.), diluted 1:1000, was added for 1 h at 22°C. After washing, aminoantipyrine substrate (23) was added for 1 h at 22°C, and optical density was read at 490 nm in a Titertek Multiskan (Flow Laboratories Inc., Rockville, MD). The specificity of the ELISA was confirmed by the radioimmunoprecipitation technique described above, using both the mAb and the positive autoimmune sera to precipitate specific antigens.

The antigenic sites recognized by human autoantibodies were examined using a modification of the above ELISA technique. Preliminary experiments indicated that two mAb, 162-11 and 111-12, recognized different antigenic sites on the same proteins (see Results). In view of this finding, the microtiter plates were coated with either 162-11 or 111-12 mAb, and antigen was allowed to adhere to the coated wells. Serial dilutions of human sera were then added to the antigen-coated wells for 1 h at 22°C, after which 100 μl of the other mAb (i.e., 111-12 or 162-11, respectively), coupled to alkaline phosphatase (27), was added (at a 1:80 dilution) for 2.5 h, at 22°C. The wells were washed and *p*-nitrophenyl phosphate substrate (Sigma Chemical Co., St. Louis, MO) in diethanolamine buffer, pH 9.8 was added for 1 h at 22°C. The reaction was stopped by adding NaOH to 1.0 N, and optical density was read at 405 nm. The percent inhibition of binding of the alkaline phosphatase–labeled mAb was calculated, and the dilution at which the serum inhibited binding of the mAb by 50% was determined.

ELISA for Measuring Antigen. Since mAb 162-11 and 111-12 recognized different portions of the same molecule (see Results), the ELISA technique could be modified for the specific detection of antigen. Microtiter plates were coated with one mAb as above, the antigen to be tested was added to the coated wells, and antigen binding to the first

antibody was quantitated by measuring antigen binding to the second mAb which had been labeled with alkaline phosphatase.

Sucrose Gradient Analysis. Human B cells (2p68) were metabolically labeled with [^{35}S]methionine, and a cell extract was obtained by sonicating cells in detergent-free lysis buffer, as described above. The extract was cleared by centrifugation at 8,740 *g* for 10 min, and 0.2 ml (~1.5 × 10^7 cell-equivalents) was layered onto each 11.5-ml 5–20% sucrose gradient. The gradients were centrifuged for 16 h in a SW-40 rotor at 40,000 rpm at 5°C; 1.3-ml fractions were collected and immunoprecipitated with 162-11 mAb, as described above, followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and fluorography.

In other experiments, 5 × 10^7 cell-equivalents of an unlabeled 2p68 cell extract were separated on each 5–20% sucrose gradient, and the antigens recognized by mAb were quantitated in aliquots of the fractions, using an ELISA, as described above. The remainder of each fraction was analyzed for the nucleic acids immunoprecipitated by the mAb (see below).

Analysis of Nucleic Acids. mAb-coated RAM-PAS beads were prepared as described above and added to nonradioactive, sonicated cell extracts (5 × 10^7 cell equivalents/ml lysis buffer), or to fractions from sucrose gradients, using the method of Lerner and Steitz (2) with minor modifications. The immunoprecipitates were washed extensively, and the washed beads were resuspended in 300 μl 150 mM NaCl, 50 mM Tris, pH 7.5, 2 mM EDTA. The beads were sequentially extracted with 2 vol phenol, phenol/chloroform (1:1, vol/vol), and 2 vol chloroform, followed by ethanol precipitation of the nucleic acid from the aqueous layer. The nucleic acid pellet obtained by 15 min of centrifugation at 8,740 *g* was resuspended in loading buffer and separated on 7% polyacrylamide gels containing 7 M urea, as described (2). Gels were stained for 30 min in ethidium bromide (10 μg/ml in electrophoresis buffer), and nucleic acid was visualized by UV transillumination.

In Situ Enzyme Sensitivity of Nuclear Antigens. HEp-2 cells were grown on coverslips and treated with methanol for 5 min at −20°C, followed by solubilization of membranes for 1 min in 150 mM NaCl, 5 mM $MgCl_2$, 10 mM sodium phosphate, pH 7.2 and 0.5% Triton X-100 (24). The cells were then incubated with either pancreatic ribonuclease A (RNase) (Sigma Chemical Co.), or deoxyribonuclease I (DNase) (Worthington Biochemical Corp.), both at a concentration of 50 μg/ml in 150 mM NaCl, 5 mM $MgCl_2$, and 10 mM sodium phosphate, pH 7.2; or with buffer alone for 30 min at 22°C. IF staining was then performed using the mAb and rhodamine-conjugated goat anti-mouse Ig antibodies, as described above.

Results

Preparation and Antigen Specificity of Antinuclear mAb. BALB/c mice immunized with isolated human B cell nuclei developed high titers of antinuclear antibodies, as shown by IF. When spleen cells from two of these animals were fused with SP2/0, 130 hybridoma supernatants were obtained that had greater than background reactivity with the 2p68 nuclear extract, as determined by ELISA. Six of these supernatants demonstrated strong IF nuclear reactivity, five had both nuclear and nucleolar reactivity, and five had only nucleolar reactivity. Hybridoma supernatants that demonstrated strong nuclear reactivity were selected for further study using the radioimmunoprecipitation technique.

In immunoprecipitation experiments using [^{35}S]methionine-labeled 2p68 cell extracts, three of the cloned antinuclear mAb (designated 162-11, 111-12, and 1177-31) specifically immunoprecipitated proteins with relative mobilities of approximately 70,000 and 80,000 daltons (Fig. 1). The proteins immunoprecipitated by these three mAb produced a pattern on SDS-polyacrylamide gels similar to that previously observed using certain human autoimmune sera (unpublished

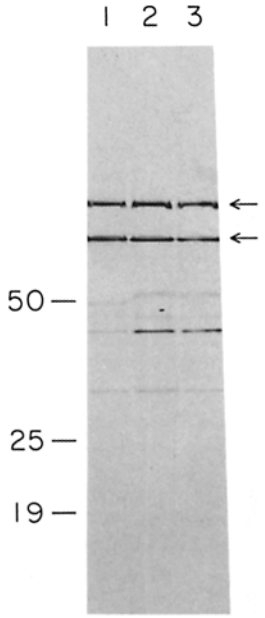

FIGURE 1. Antigens recognized by monoclonal antinuclear antibodies. [^{35}S]methionine-labeled 2p68 cell extract was immunoprecipitated using RAM-PAS coated with cloned mAb 162-11 (lane *1*), 111-12 (lane *2*), or 1177-31 (lane *3*) followed by analysis of the precipitates by SDS-PAGE. All three mAb specifically immunoprecipitated proteins with relative mobilities of approximately 70,000 and 80,000 daltons (arrows). Molecular weight markers are indicated on the left.

results). In addition, all three of the mAb stained 2p68 and HEp-2 cells in an intense, speckled, nuclear pattern and a diffuse nucleolar pattern (Fig. 2), similar to that seen with the autoimmune sera. Staining of the cytoplasm by the mAb and sera was variable, but less intense than staining in the nucleus (Fig. 2). Because of the similarities observed between mAb and autoimmune sera, the antigens recognized by the mAb were compared directly, by SDS-PAGE, with the antigens recognized by the human autoimmune sera. Initially, two human sera, one from a patient with SLE and one from a patient with MCTD, were identified that immunoprecipitated proteins with mobilities identical to those precipitated by the mAb (Fig. 3). Additional radioactive proteins were also immunoprecipitated by the human sera, due to the presence of anti-Sm and anti-RNP antibodies, respectively, in these two sera (Fig. 3; compare lanes *2* and *3* with the pattern obtained using an anti-Sm reference serum in lane *4*). The mAb, however, precipitated only the 70,000 and 80,000 dalton proteins and not the Sm- and RNP-specific proteins (Fig. 3, lane *1*).

To confirm that the 70,000 and 80,000 dalton proteins (p70 and p80) immunoprecipitated by the mAb and human autoimmune sera were identical, the individual radioactive bands (arrows, Fig. 3) were cut out of a 10–20% gradient gel and subjected to limited proteolysis (26). The chymotryptic peptides generated from p80, which had been immunoprecipitated by autoimmune serum, were identical to the peptides generated from p80, which had been immunopre-

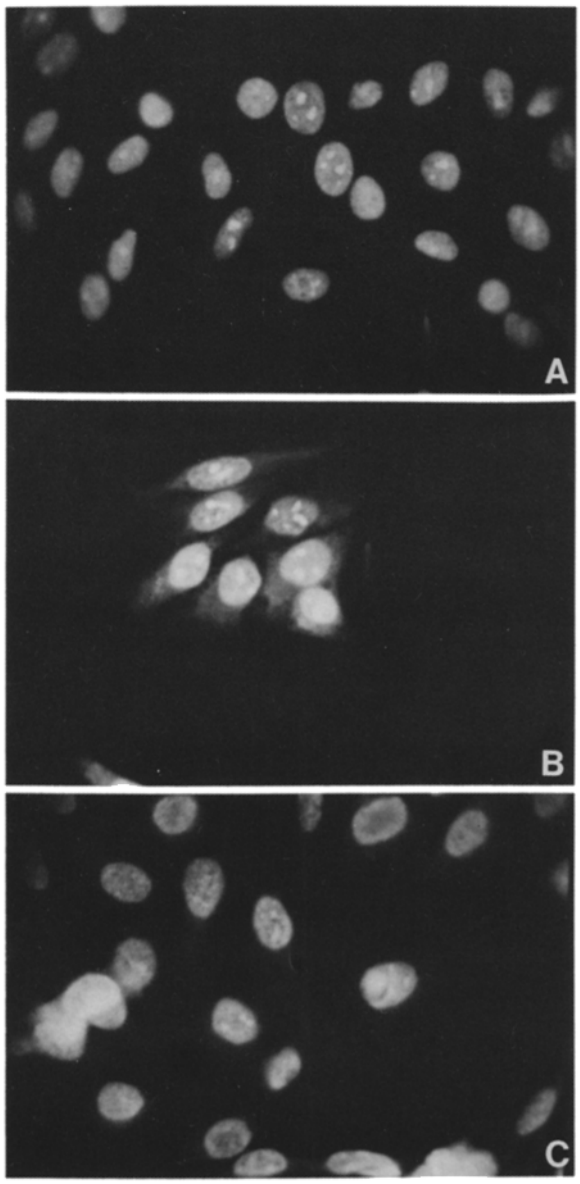

FIGURE 2. Immunofluorescence staining of HEp-2 cells by mAb. HEp-2 cells were grown on coverslips, fixed in methanol (24), and incubated for 30 min with 50 μl of hybridoma supernatant from mAb 162-11 (*A*), 111-12 (*B*), or 1177-31 (*C*). Slides were washed and incubated with 20 μl of a 1:20 dilution of rhodamine-conjugated goat anti–mouse Ig antibodies for 30 min, then examined and photographed using an Ortholux epifluorescence microscope at a magnification of 630. All three mAb demonstrated intense speckled nucleoplasmic, as well as diffuse nucleolar staining using HEp-2 cells as substrate. Cytoplasmic staining was variable.

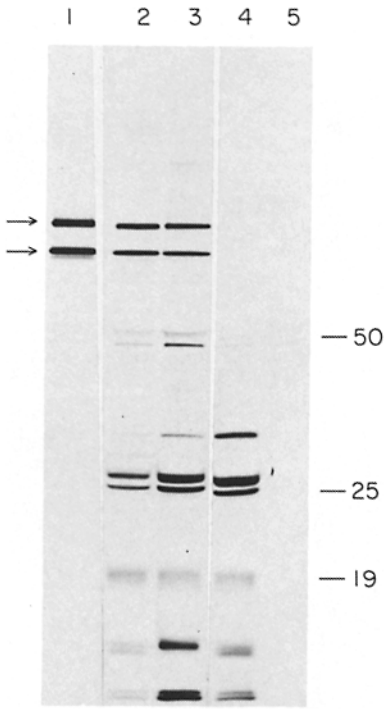

FIGURE 3. Comparison of proteins immunoprecipitated by mAb 162-11 and autoimmune sera. [^{35}S]methionine-labeled 2p68 cell extract was immunoprecipitated using mAb 162-11 (lane *1*) or autoimmune sera from patients with SLE (lane *2*) or MCTD (lane *3*). Immunoprecipitates were analyzed by SDS-PAGE on a 10–20% gradient gel, and fluorographed. The mAb, as well as the sera in lanes *2* and *3*, immunoprecipitated two proteins with relative mobilities of approximately 70,000 and 80,000 (arrows). The sera used in lanes *2* and *3* also contained Sm and RNP antibodies, respectively, which immunoprecipitated the characteristic Sn and RNP proteins (4, 5), ranging from 9,000 to 33,000 daltons (cf., lane *4*, which illustrates the pattern using an anti-Sm reference serum). Lane *5* shows the pattern obtained using normal human serum. Molecular weight markers are indicated on the right.

cipitated by the mAb 162-11 (Fig. 4, lanes *1* and *2*, respectively). The chymotryptic peptides generated from p70, which had been immunoprecipitated by autoimmune serum, were identical to the peptides generated from p70, which had been immunoprecipitated by 162-11 antibodies (Fig. 4, lanes *3* and *4*, respectively). Similar results were obtained when the fragments generated by limited proteolysis using staphylococcal V8 protease were analyzed (not shown). These experiments verified that the proteins recognized by the mAb and autoimmune sera were identical. In addition, the prominent differences observed between the fragments obtained by limited proteolysis of p70 and those obtained by limited proteolysis of p80, suggested that p70 is probably not a proteolytic degradation product of p80 (Fig. 4, compare lanes *1* and *2* with lanes *3* and *4*).

Characterization of the mAb. The mAb specific for P70 and P80 were further characterized by radial immunodiffusion using subclass-specific anti-mouse Ig antibodies. Two of the mAb (111-12 and 1177-31) were of the IgGl subclass, and the third, 162-11, was IgG2a.

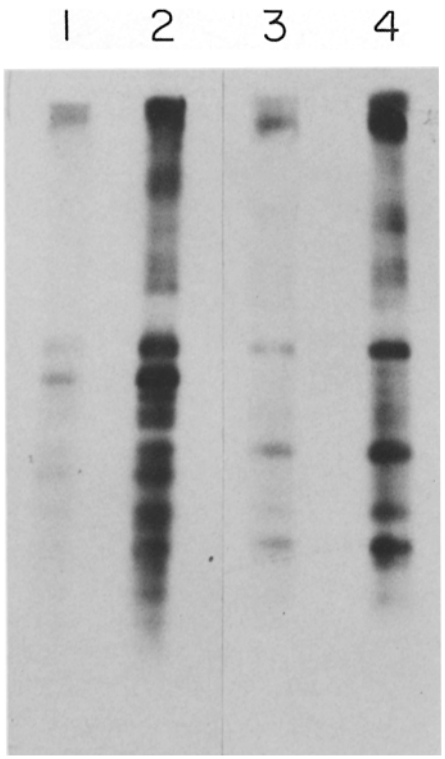

FIGURE 4. Limited proteolysis of p70 and p80 using chymotrypsin. P70 and p80 bands immunoprecipitated by mAb 162-11 or by autoimmune serum were cut out of a lightly fixed 10–20% gradient gel and rehydrated. Gel slices were then placed in the slots of a 20% gel containing 1 mM EDTA along with 10 μg chymotrypsin per lane (26). The proteins were electrophoretically eluted from the gel slices and digested in the gel for 30 min with the current turned off. The fragments were then separated by electrophoresis and fluorographed. (Lane *1*) Chymotryptic peptides from p80 previously immunoprecipitated by autoimmune serum JMi (see Table I); (lane *2*) chymotryptic peptides from p80 previously immunoprecipitated by 162-11; (lane *3*) chymotryptic peptides from p70 previously immunoprecipitated by autoimmune serum JMi; (lane *4*) chymotryptic peptides from p70 previously immunoprecipitated by 162-11.

The epitopes recognized by the mAb were compared by measuring the ability of an unlabeled mAb to compete in ELISA with either 162-11 or 111-12 antibodies that had been conjugated to alkaline phosphatase (Fig. 5). Ascites containing either 111-12 or 1177-31 antibodies, at a dilution of 1:1000, completely inhibited the binding of alkaline phosphatase–labeled 111-12; ascites containing 162-11 antibodies failed to inhibit binding of the 111-12 conjugate, even at a 1:10 dilution (Fig. 5*A*). Ascites containing unlabeled 162-11 antibodies strongly inhibited binding of alkaline phosphatase–conjugated 162-11; ascites containing 1177-31 antibodies inhibited weakly at 1:10 and 1:100, and ascites containing 111-12 antibodies inhibited weakly only at 1:10 dilution (Fig. 5*B*). These experiments indicate that 162-11 and 111-12 antibodies bind to different epitopes on the p70 and p80 molecules, and that 1177-31 recognizes an epitope that is either the same or physically near the epitope recognized by 111-12.

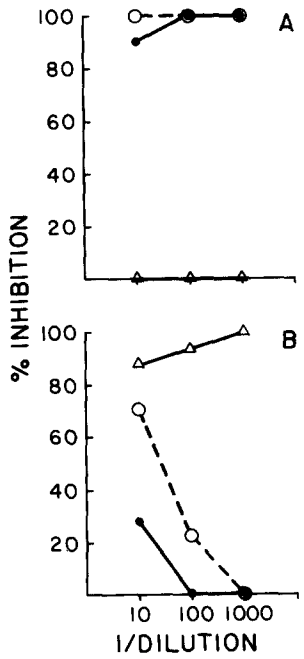

FIGURE 5. Antigenic sites recognized by mAb. Wells of microtiter plates were coated with 162-11 (*A*) or 111-12 (*B*) antibodies, blocked, and incubated with 100 μl of 2p68 cytoplasmic extract (5×10^7 cell-equivalents/ml) for 3 h at 22°C. 100 μl of serially diluted 111-12 (●) 1177-31 (○), or 162-11 (△) ascites was then added to the washed wells for 1 h before adding 100 μl of 1:80 alkaline phosphatase–conjugated 111-12 (*A*) or 100 μl of 1:80 alkaline phosphatase–conjugated 162-11 (*B*) for an additional 2 h. Results are expressed as percent inhibition of binding of alkaline phosphatase–conjugated 111-12 (*A*) or 162-11 (*B*).

Characterization of p70/p80. A number of autoantigens recognized by sera from patients with autoimmune disorders consist of proteins bound to nucleic acid (1–16). The possibility that p70 and p80 might also be nucleoproteins was investigated by determining the sensitivity of the nuclear IF staining to pretreatment with RNase or DNase, as described (10). All three mAb demonstrated both speckled nucleoplasmic and diffuse nucleolar staining of untreated HEp-2 cells (Fig. 6*A*). Nucleoplasmic staining by 111-12 and 1177-31 was almost completely removed by DNase pretreatment (Fig. 6*B*), while staining by 162-11 was partially sensitive (not shown). The nucleolar staining was consistently unaffected by DNase pretreatment (Fig. 6*B*), but was completely sensitive to pretreatment with RNase (Fig. 6*C*). The speckled nucleoplasmic staining was completely resistant to RNase, however (Fig. 6*C*). Staining by the autoimmune sera displayed similar patterns of enzyme sensitivity, except that the nucleoplasmic staining was only partially sensitive to DNase pretreatment, consistent with the presence of additional autoantibody specificities (such as anti-Sm and anti-RNP) in these sera (not shown). These experiments suggest that the proteins might be associated with DNA in the nucleoplasm and with RNA in the nucleolus.

To further investigate this possibility, p70 and p80 were immunoprecipitated using 162-11–coated RAM-PAS beads (see Materials and Methods). The immunoprecipitated p70/p80 was then subjected to treatment with DNase, RNase, or

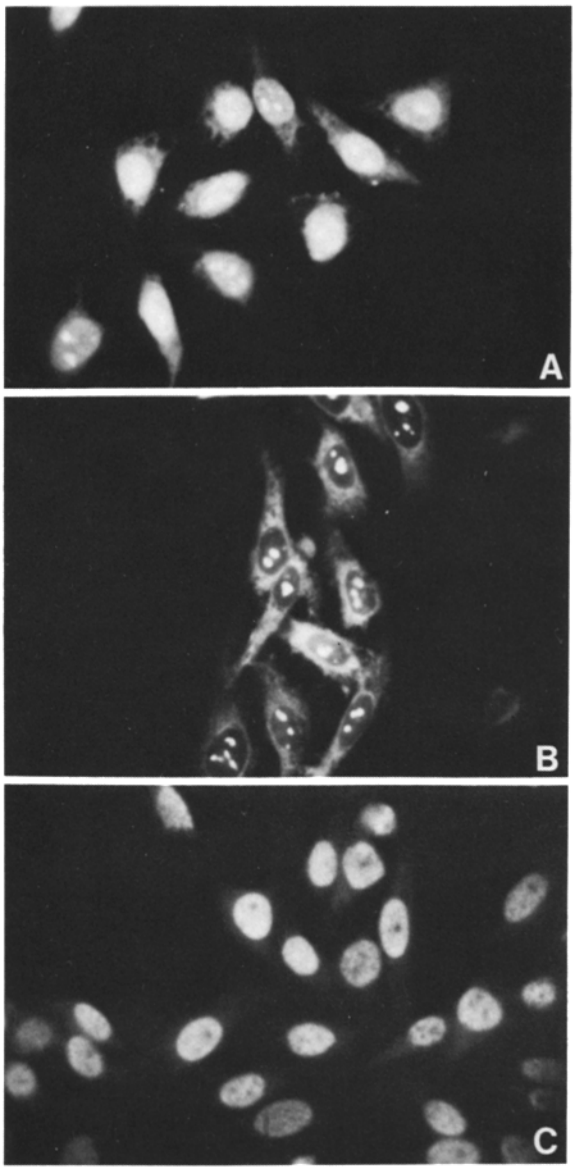

FIGURE 6. In situ enzyme sensitivity of antigens recognized by mAb. HEp-2 cells were grown on coverslips, and treated for 5 min with methanol, at −20°C, followed by 0.5% Triton X-100 in saline for 1 min. The slides were then treated with DNase or RNase (50 μg/ml in 150 mM NaCl, 10 mM sodium phosphate, pH 7.2, 5 mM $MgCl_2$), or with buffer alone for 30 min at 22°C, followed by washing and blocking. The cells were then incubated with 50 μl of 111-12 hybridoma supernatant for 30 min, washed, and incubated with 20 μl of a 1:20 dilution of rhodamine-conjugated goat anti-mouse immunoglobulin antibodies. (*A*) Buffer control incubated with 111-12; (*B*) DNase pretreatment before incubation with 111-12; (*C*) RNase pretreatment before incubation with 111-12.

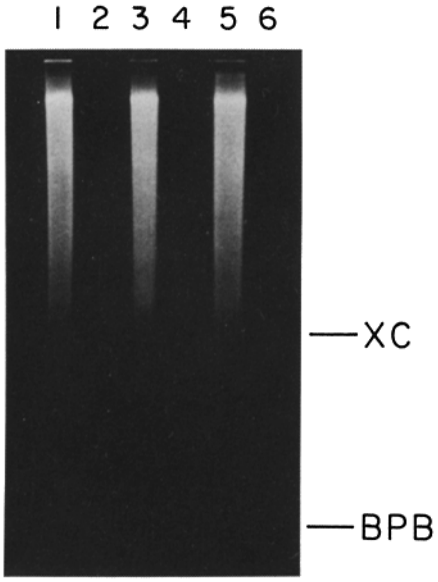

FIGURE 7. Gel analysis of nucleic acids in p70/p80 immunoprecipitates. 2p68 cell extract (5 × 10^7 cell-equivalents per lane) was immunoprecipitated using 50 μl of packed 162-11-coated RAM-PAS beads. Extract was added to the beads for 15 min at 4°C and the beads were washed extensively. The beads were then incubated for 15 min at 22°C with 100 μl of 150 mM NaCl, 10 mM sodium phosphate, pH 7.2, 5 mM $MgCl_2$, or with 100 μl of DNase (500 μ/ml in the same buffer) or RNase (500 μg/ml in the same buffer), followed by washing, phenol/chloroform extraction, and ethanol precipitation of nucleic acids from the aqueous phase. Nucleic acids were collected by centrifugation and analyzed on a 7% polyacrylamide, 7 M urea gel run until marker dye front was 2 cm from the bottom of the gel. 162-11 immunoprecipitate (Lane *1*) Untreated; (lane *2*) treated with DNase; lane *3*, treated with RNase. Other immunoprecipitates were washed on the beads and phenol/chloroform extracted. Nucleic acids isolated by repeated ethanol precipitations were then incubated with 50 μl of DNase or RNase (at the same concentration as above) for 10 min at 22°C, followed by phenol/chloroform extraction and gel analysis of the ethanol-precipitated nucleic acids. (Lane *4*) protein-free nucleic acid from p70/p80 immunoprecipitates treated with DNase; (lane *5*) protein-free nucleic acid from p70/p80 immunoprecipitates treated with RNase. (Lane *6*) Nucleic acid immunoprecipitated by normal human serum–coated PAS. RAM-PAS alone and RAM-PAS coated with irrelevant mAb produced the same pattern as obtained using normal human serum (not shown). Positions of xylene cyanol (*XC*) and bromphenol blue (*BPB*) marker dyes are indicated.

buffer alone, followed by phenol-chloroform extraction and ethanol precipitation of the nucleic acids present in the treated immunoprecipitates. When the nucleic acid isolated from buffer-treated immunoprecipitates was analyzed on 7% polyacrylamide, 7 M urea gels, a smear of high molecular weight nucleic acid was seen after ethidium bromide staining (Fig. 7, lane *1*). The nucleic acid in 162-11 precipitates was completely sensitive to DNase treatment, but was unaffected by RNase treatment (Fig. 7, lanes *2* and *3*, respectively). Identical results were obtained when the material immunoprecipitated by the mAb was first extracted with phenol-chloroform, and the protein-free nucleic acid (obtained by repeated ethanol precipitations) was treated with DNase or RNase (Fig. 7, lanes *4* and *5*, respectively). Hybridoma supernatants without p70/p80 specificity, and normal human sera, did not immunoprecipitate the smear of high molecular weight DNA (Fig. 7, lane *6*).

To eliminate the possibility that the mAb recognized DNA, the three mAb were tested for anti-DNA activity using an ELISA (28). None of the mAb reacted with double- or single-stranded DNA in this assay (data not shown). In other experiments, 162-11–coated RAM-PAS beads were added to the nucleic acids isolated from p70/p80 immunoprecipitates. The protein-free nucleic acid obtained by phenol-chloroform extraction and ethanol precipitation could no longer be immunoprecipitated by mAb, indicating that the antigenic determinants reside in the protein moieties of the complex.

Tissue Distribution of p70/p80. The tissue distribution of p70/p80 was examined by IF staining of human cell lines and normal human tissues. All human cell lines tested stained positively for p70/p80 using the mAb, including B cell lines (2p68, CESS, JOSH-7, EB-3, RPMI 8226, 0467, HS Sultan, 8866, and Raji), and T cell lines (KE37, CEM-T), as well as HEp-2 (laryngeal carcinoma), and K562 (erythroleukemia). Normal human T and B cells as well as macrophages and fibroblasts were also positive by IF. A rat T cell line (CTLL) and mouse B and T cell lines (SP2/0 and HT-2) were negative for nuclear staining in IF assays, but had a high cytoplasmic background. Preliminary experiments suggest that murine cells contain small amounts of p70/p80, which can be immunoprecipitated by the mAb from [^{35}S]methionine-labeled whole cell extracts, and that most of this p70/p80 may be cytoplasmic (data not shown).

Sucrose Gradient Analysis. Since limited proteolysis experiments suggested that p70 was not a proteolytic degradation product of p80, we investigated how three mAb were capable of immunoprecipitating two distinct protein molecules. Two possibilities were considered: that the mAb reacted with two molecules containing similar or identical epitopes, or that p70 and p80 were physically associated, and thus coprecipitated. To distinguish between these possibilities, [^{35}S]methionine-labeled cell extracts were ultracentrifuged through 5–20% sucrose gradients, and each fraction was immunoprecipitated using 162-11 mAb (Fig. 8). In these experiments, the p70 and p80 molecules were always found in the same fractions, and the relative autoradiographic intensities of p70 and p80 in a given fraction were always roughly the same (Fig. 8). The bulk of the radioactively labeled p70 and p80 migrated at ~10 S (IgG, which has a molecular weight roughly the same as the sum of p70 and p80, migrates at ~7 S). No activity was seen at 4 S, the mobility of bovine serum albumin (~68,000 daltons), suggesting that p70 and p80 are physically associated, and that little or none of the free p70 and p80 subunits is present. Identical results were obtained by immunoprecipitating with the autoimmune sera (not shown). In other experiments, the mobilities of p70 and p80 were unaffected by reduction, indicating that the proteins are not disulfide-linked (not shown).

Although most of the p70/p80 activity sedimented at ~10 S, some activity was also detectable in the 10–20 S fractions after 12 h labeling with [^{35}S]methionine (Fig. 8, lanes *6–8*). This rapidly migrating activity was further analyzed by monitoring the sucrose gradiant fractions for p70/p80 using a sensitive, highly specific ELISA for the antigens. Nonradioactive whole cell extracts were centrifuged through 5–20% sucrose gradients. An aliquot of each fraction was tested for p70/p80 activity by ELISA (Fig. 9, *top*), and the remainder was analyzed for nucleic acid bound to p70/p80 by immunoprecipitation onto 162-11–coated

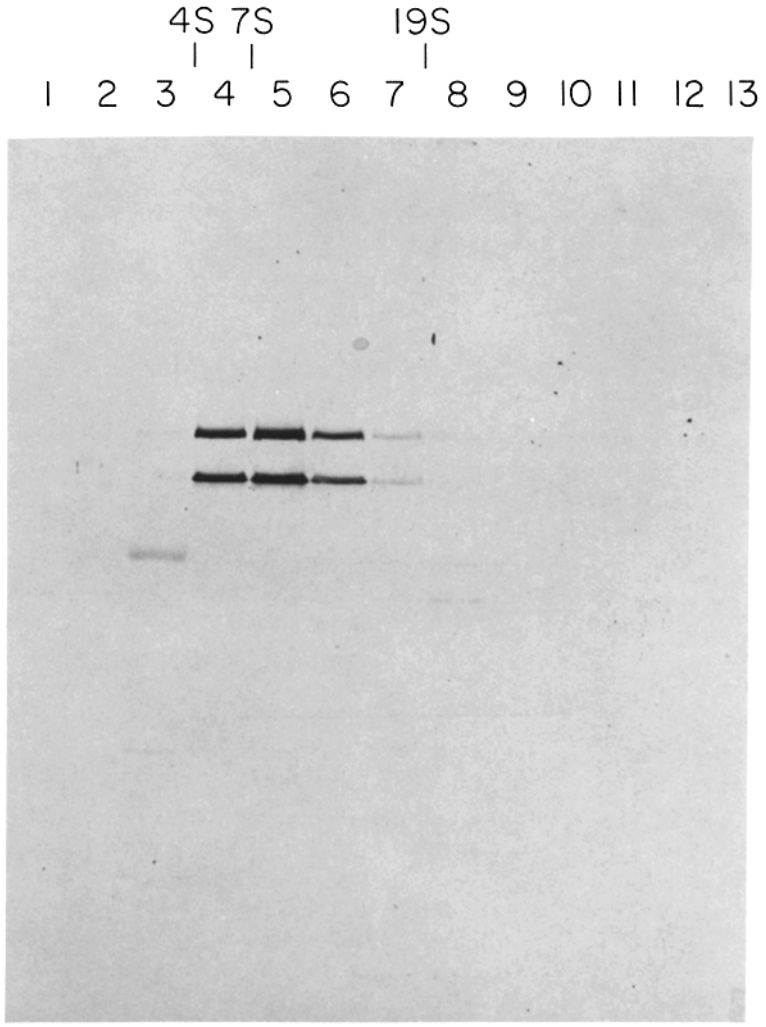

FIGURE 8. Sucrose gradient analysis of [^{35}S]methionine-labeled p70/p80. 2p68 whole cell extract (1.5×10^7 cell-equivalents) was centrifuged through a 5–20% sucrose gradient at 40,000 rpm for 16 h in an SW-40 rotor; 1.3-ml fractions were collected and analyzed for p70/p80 by immunoprecipitation using 162-11-coated RAM-PAS beads, followed by SDS-PAGE and fluorography. Positions of bovine serum albumin (4 S), human IgG (7 S), and human IgM (19 S) in the gradients are indicated. The peak of p70/p80 activity appears at ~10 S (fraction *5*) while smaller amounts of the proteins are detectable from 10–20 S (fractions *6–8*).

RAM-PAS beads. Nucleic acid was extracted and separated on 7% polyacrylamide, 7 M urea gels, as described (Fig. 9, *bottom*). In these experiments, two peaks of p70/p80 activity were detected by ELISA, one sharp peak at ~10 S, and a second, broader peak at ~20 S (Fig. 9, *top*). The bulk of the DNA immunoprecipitable by 162-11 was found in the 10–20 S peak (Fig. 9, *bottom*), although smaller amounts of DNA were present in the 10 S peak. Thus, the 10–20 S peak appeared to be distinguishable from the 10 S peak by virtue of the fact that the

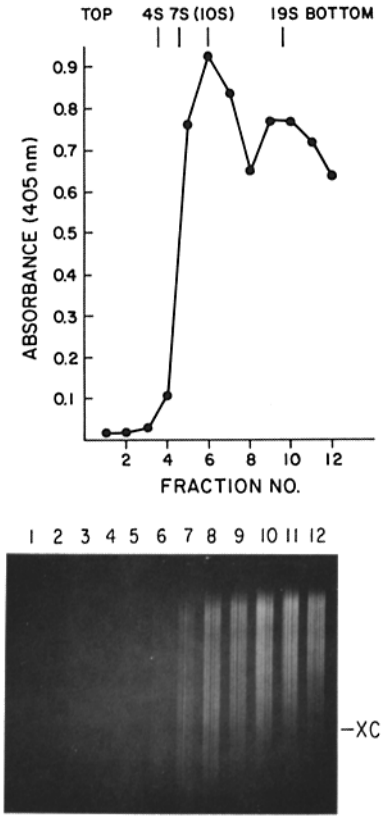

FIGURE 9. (*Top*) Sucrose gradient analysis of p70/p80. ELISA for p70/p80 proteins in the fractions. 2p68 whole cell extract (6×10^7 equivalents) was centrifuged at 40,000 rpm through a 5–20% sucrose gradient for 16 h in a SW-40 rotor; 1.3-ml fractions were collected. Aliquots of 175 μl from each fraction were analyzed for p70/p80 protein by ELISA. The microtiter plate was coated with 111-12, and p70/p80 bound to the mAb was detected using a 1:80 dilution of 162-11 conjugated to alkaline phosphatase. Optical density was read at 405 nm, 1 h after adding substrate. Positions of BSA (4 S), human IgG (7 S), and human IgM (19 S) in the gradient are indicated; the calculated mobility of 10 S is also shown. (*Bottom*) Analysis of nucleic acids bound to p70/p80 in the fractions. 0.7 ml from each of the fractions analyzed above was incubated with 162-11-coated RAM-PAS for 20 min at 4°C. Nucleic acids bound to p70/p80 in the immunoprecipitates were extracted with phenol/chloroform, ethanol precipitated from the aqueous phase, and separated on a 7% polyacrylamide, 7 M urea gel. Fraction numbers correspond to those above; mobility of xylene cyanol (*XC*) is indicated.

10–20 S peak contained a larger amount of DNA (Fig. 9), and was labeled much more weakly by [^{35}S]methionine in 12 h metabolic labeling experiments (Fig. 8).

Antibodies to p70/p80 in Autoimmune Disease. The availability of several mAb recognizing different epitopes on the p70/p80 complex (Fig. 5) permitted analysis of the binding specificities of human autoantibodies to p70/p80 using solid phase immunoassays. Sera were obtained from patients meeting the American Rheumatism Association revised clinical criteria for SLE (29), and from patients seen at The Rockefeller University Hospital, with MCTD and scleroderma. All sera were screened for the presence of antibodies to p70/p80 by ELISA. Since p70/p80 is a DNA-binding protein complex, the possibility of anti-DNA antibodies binding to the antigen on microtiter plates was of concern. To avoid this problem, 2p68

TABLE I
Autoantibodies to p70/p80 in Human Diseases

Patient	Dx*	Reciprocal titer p70/p80 antibodies	Inhibition of binding‡		ANA§	dsDNA/ssDNA‖
			111-12	162-11		
JMi	MCTD	>3,906,250	2,800	1,600	sp/n	23.7/40.7
CKi	SLE	781,250	170	16,000	d/sp/n	17.2/55.4
DJo	SLE	156,250	130	120	sp/n	66.5/77.3
JKr	PSS	156,250	<50	<50	d/n	2.6/3.8
ACr	SLE	31,250	<50	Neg¶	d	2.6/4.6
CTu	SLE	6,250	<50	100	d/sp/n	13.4/60.2
MHe	SLE	6,250	<50	>1,250	sp	12.8/89.5
DJa	MCTD	6,250	<50	75	d/sp	4.2/24.1
LGl	SLE	250	<50	Neg	d/sp/n	100/97.6
ADe	PSS	250	<50	<50	d/n	3.8/79.4
ATo	SLE	250	<50	70	sp/n	64.8/58.5
DWo	SLE	Neg	<50	Neg	d/sp/n	100/100
WRe	Normal	Neg	Neg	Neg	Neg	2.1/5.4

* Diagnosis: SLE, systemic lupus erythematosus; MCTD, mixed connective tissue disease; PSS, progressive systemic sclerosis (scleroderma).
‡ Reciprocal dilution of serum required to inhibit binding by mAb 111-12 or 162-11 by 50%.
§ Pattern of immunofluorescent, antinuclear antibody staining on HEp-2 cells; d, diffuse, sp, speckled; n, nucleolar.
‖ dsDNA, antibodies to double-stranded DNA (percent binding by RIA); ssDNA, antibodies to single-stranded DNA (percent binding by RIA).
¶ Not detectable.

cytoplasm was separated from nuclei, and the cytoplasmic extract was used, instead of a whole cell extract, as a source of p70/p80. Control sera with high levels of anti-DNA antibodies in the Farr assay (30), but no antibody to p70/p80 by radioimmunoprecipitation, failed to bind to wells that had been coated with the cytoplasmic extract (Table I, patient DWo). The 11 autoimmune sera that reacted strongly in the ELISA were tested by radioimmunoprecipitation for the ability to precipitate p70/p80; each serum was found to contain p70/p80 autoantibodies by this criterion.

20 of 51 SLE sera (39%), 6 of 11 MCTD sera (55%), and 6 of 15 scleroderma sera (40%) contained at least low levels of antibodies to p70/p80 as determined by ELISA. 11 sera (7 SLE, 2 MCTD, and 2 scleroderma sera; Table I) contained p70/p80 antibodies at titers between 1:250 and 1:4,000,000. Most of these sera also demonstrated bright speckled or diffuse nuclear staining and diffuse nucleolar staining in HEp-2 cells, and most contained antibodies to both native and single-stranded DNA (Table I). The remainder of the sera that were positive by ELISA had a <1:250 titer of antibodies to p70/p80, and the presence of these antibodies was not consistently confirmed by radioimmunoprecipitation.

Since the 111-12 and 162-11 mAb recognize different epitopes of p70/p80, the presence, in human sera, of autoantibodies to these two epitopes (or nearby epitopes) could be determined by the ability of human serum to inhibit binding of the alkaline phosphatase–labeled mAb (Table I and Fig. 10). In general, autoimmune sera that contained high levels of p70/p80 antibodies inhibited the binding of both 162-11 (Fig. 10*A*) and 111-12 (Fig. 10*B*) to the antigen. Sera with smaller amounts of p70/p80 antibodies also appeared to react with both

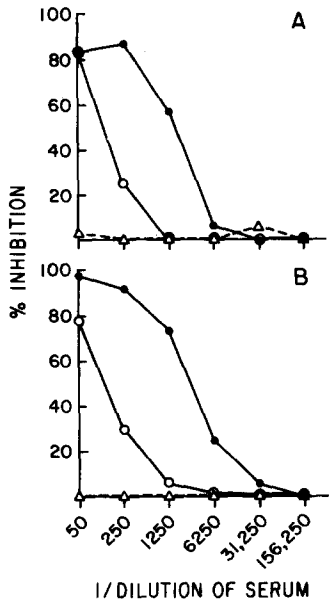

FIGURE 10. Inhibition of binding of mAb to p70/p80 by human sera. Microtiter plates were coated with 111-12 antibodies (*A*) or 162-11 antibodies (*B*) and p70/p80 was allowed to bind to the coated wells. Serially diluted human serum (100 μl) was then added to the wells for 1 h before adding 100 μl of 1:80 162-11–alkaline phosphatase conjugate (*A*) or 100 μl of 1:80 111-12–alkaline phosphatase conjugate (*B*) for 2.5 h at 22°C. Substrate was added, and absorbance was read at 405 nm 1 h later. Results are expressed as percent inhibition of binding of the alkaline phosphatase–conjugated antibody without added serum. Sera are from Table I: JMi (●), DJo (○), normal human serum (Δ). (*A*) Inhibition of 162-11 binding; (*B*) inhibition of 111-12 binding.

epitopes, but inhibition was at the lower limits of the sensitivity of the assay. These results suggest that human autoantibodies to p70/p80 are likely to be directed against several epitopes on the antigen—the humoral immune response is polyclonal. In addition, these results suggest that murine mAb obtained by immunizing non-autoimmune mice recognize either the same determinants or determinants physically near those recognized by the human autoantibodies.

Discussion

Antinuclear mAb were produced in BALB/c mice and used to identify and partially characterize two DNA-binding proteins with relative mobilities of approximately 70,000 and 80,000 daltons (p70 and p80) which are recognized by certain human autoimmune sera. The fact that the human autoantibodies and the murine mAb recognize the same antigen was established by three observations: (*a*) the proteins immunoprecipitated by the mAb and sera had exactly the same mobilities (Fig. 3); (*b*) peptide maps of the proteins immunoprecipitated by the mAb and sera were identical (Fig. 4); and (*c*) the autoimmune sera, but not control sera, inhibited binding of the mAb to p70/p80 in direct competition experiments (Fig. 10). In addition, in preliminary sequential immunoprecipitation experiments, incubation of cell extracts with autoimmune sera containing anti-p70/p80 antibodies could remove the p70 and p80 proteins recognized by the mAb (unpublished results).

The studies presented here strongly suggest that, as is the case for anti-Sm, -RNP, -Ro, and -La antibodies (1–9), the antigenic sites recognized by anti-p70/p80 autoantibodies and mAb are located on the protein moieties of the p70/p80–DNA complex, rather than on nucleic acid. This interpretation is supported by several findings. First, the mAb and sera recognize very similar or identical determinants, and although most of the autoimmune sera contained antibodies to both single- and double-stranded DNA, the mAb failed to react with either in ELISA. Conversely, a number of sera that contained extremely large amounts of anti-DNA antibodies failed to react with p70/p80 (Table I). Second, the mAb did not immunoprecipitate the nucleic acid attached to p70/p80 after the proteins were extracted, and in preliminary experiments, radiolabel was immunoprecipitated by both the autoimmune sera and the mAb after DNase treatment of cell extracts (unpublished results).

The antigen recognized by the three mAb and autoimmune sera described here represents one of the few non-histone, DNA-binding proteins known to be recognized by autoantibodies in human disease. Other examples include anticentromere antibodies (10, 11), the so-called Ku antigen (13), and Scl-70 (12). It seems likely that, as in the case of RNA-binding proteins (1–9), several DNA-binding protein antigens will be found that are recognized by autoimmune sera from patients with SLE and related disorders. The identification and characterization of nucleic acid–binding proteins, such as p70/p80, which are recognized by autoantibodies, may lead to a better understanding of the reasons that this class of proteins is particularly susceptible to autoimmune attack in SLE and other disorders. By using autoimmune sera and mAb to study the structure and function of nucleic acid–binding proteins, certain features common to a number of such autoantigens may be identified. For example, mAb could be used to probe the DNA-binding regions of cellular and viral proteins to look for structural similarities and possible immunological cross-reactivities. In addition, detection of autoantibodies that react with the DNA-binding domain of p70/p80 or other DNA-binding proteins might be of particular interest. If such antibodies are capable of inducing antiidiotypic antibodies, a portion of these antiidiotypic antibodies might have specificity for DNA. An example of this type of mechanism is the production of acetylcholine receptor antibodies by an auto-antiidiotypic response to antibodies against a synthetic acetylcholine agonist (31). The polyclonality of anti-p70/p80 antibodies in the autoimmune sera and the presence of large amounts of anti-DNA antibodies in many of these sera provides some circumstantial evidence in favor of this idea.

The relationship of p70/p80 antibodies to the clinical manifestations of autoimmune disease is unclear at present. Antibodies to p70/p80 are found in high titers in sera from a small number of patients with SLE, MCTD, and scleroderma (Table I), and lower levels of p70/p80 antibodies are detectable by ELISA in ~⅓–½ of patients with these disorders. It is difficult, at present, to be certain whether the small amount of p70/p80 antibodies in this latter group of sera is significant, since some of the sera are capable of immunoprecipitating radiolabeled p70/p80 while others do not consistently precipitate the proteins. In view of the relatively small number of identified sera that contain high levels of p70/p80 antibodies, further studies will be necessary to determine whether the

presence of these antibodies in the sera might define a clinical subset of autoimmune disease.

A limited analysis of the specificities of p70/p80 autoantibodies did not reveal significant differences in the epitopes recognized by SLE, MCTD, or scleroderma sera (Talbe I). This finding contrasts with the observed differences in the recognition of different epitopes on U1-RNP particles by SLE- and MCTD-derived autoantibodies (5, 16, 32). Additional studies are in progress to determine whether or not more subtle differences might exist in the antigenic sites recognized by sera from patient with different clinical diseases. The competition experiments also suggest that the humoral immune response to p70/p80 is polyclonal. It will be of interest to determine whether autoantibodies against these different epitopes are truly heterogeneous, or whether, as has been postulated in other systems, they might contain cross-reactive idiotypic determinants, suggesting derivation from a common precursor clone (33–35).

The antigen recognized by the mAb and autoimmune sera described here appears to be different from the other autoantigen systems, including Sm, RNP, Ro, and La, based on biochemical and immunologic studies. Scl 70 has a mobility similar to p70 and may bind to DNA (12), but is not physically associated with an 80,000 dalton protein, and is found only in sera from scleroderma patients (1, 12). Preliminary studies on the "Ku" antigen system indicate that this antigen consists of two proteins that are remarkably similar to p70/p80 in molecular weight and DNA-binding capacity (13). Anti-Ku antibodies are found mainly in scleroderma-polymyositis overlap syndrome, however (13, 36), and reportedly do not have nucleolar reactivity in IF. Exchange of sera will be necessary to further clarify differences or similarities between p70/p80 and the Ku and Scl-70 antigens.

By analogy with the RNA-protein autoantigens, Sm, RNP, Ro, and La (1–9), p70/p80 and other DNA-protein autoantigens may play important roles in the regulation of certain aspects of cellular metabolism. Autoimmune sera and mAb to these proteins may therefore provide insight into cellular processes such as cell growth and gene expression. With this in mind, we have begun to characterize p70/p80 using both murine mAb and human sera.

Sucrose gradient analysis suggests that two forms of p70/p80 exist. The significance of these two forms is, at present, unclear. The 10 S form is rapidly labeled by [^{35}S]methionine and is associated with only small amounts of DNA. This form may represent free p70/p80 protein complexes that are not associated with DNA, in which case, the small amount of DNA immunoprecipitated by the mAb in the 10 S fractions (Fig. 9) might result from overlap of the 10 S peak with the broad 10–20 S peak. Alternatively, the p70/p80 in the 10 S peak might be associated with small DNA fragments that contribute relatively little to the mobility of the proteins in sucrose gradients. The existence of a free form of p70/p80 would be consistent with the recent observation that much of the total p70/p80 immunologic activity is recovered in the cytoplasmic fractions of human B cells, while the remainder is nuclear and nucleolar (unpublished results). The rapidly sedimenting form of p70/p80 is only weakly radioactive after 12 h metabolic labeling with [^{35}S]methionine, but contains a significant portion of the total p70/p80 immunologic activity measured by ELISA. This form migrates as

a broad peak at 10–20 S in sucrose gradients and contains relatively large amounts of DNA. The most likely explanation for these findings is that the 10–20 S peak consists of p70/p80 bound to DNA that has been sheared into fragments of different lengths by sonication of the cell extracts. This conclusion is supported by the progressive increase in average molecular weight of the DNA precipitated by the mAb from 10 to 20 S on sucrose gradients (Fig. 9, *bottom*). The inefficient [^{35}S]methionine-labeling of this peak of activity may be due to slow turnover of p70/p80 bound to DNA. This interpretation would be consistent with the postulated existence of an extremely stable association of certain gene-regulating proteins with DNA through many cell divisions (for recent review, see 37). Experiments are in progress to investigate the functional significance, if any, of the two different forms of p70/p80, and to determine whether the 10 S form of the antigen contains DNA.

I have been unable to verify whether, as suggested by enzyme digestion studies, the nucleolar form of p70/p80 is attached to RNA. This may be due, in part, to the large amount of fragmented DNA in p70/p80 immunoprecipitates. These DNA fragments may obscure an RNA band on nucleic acid gels and prevent its visualization by ethidium bromide staining (see Fig. 7). Alternatively, contamination by DNase might be responsible for the sensitivity of nucleolar IF staining to RNase treatment. I feel that this is unlikely, however, since: (a) the speckled nucleoplasmic staining was completely resistant to RNase treatment despite its sensitivity to DNase, and (b) the nucleolar staining was completely resistant to DNase despite its RNase sensitivity (see Fig. 6). Experiments are in progress to resolve this issue. If, as appears to be the most likely possibility, p70/p80 is capable of binding to both DNA and RNA, this might play an important regulatory role. RNA polymerase III transcription factor IIIA (TF IIIA) is another protein that has the capacity to bind both DNA and RNA; TF IIIA binds to both 5 S RNA and to the noncoding strand of the 5 S gene (38, 39). Binding to 5 S RNA competes with binding to the 5 S gene, and is thought to regulate transcriptional activity of the gene (38, 39).

In sum, these studies indicate that DNA-binding proteins may be more frequent targets of autoimmunity in SLE and related disorders than was previously recognized. Along with previous studies on RNA-binding proteins (1–9), the present findings suggest that nucleic acid–binding proteins, as a class, may be especially prone to autoimmune attack in these disorders. The reasons for this are not clear at present, but might be related to common structural and/or functional features of nucleic acid binding proteins or to similarities in the autoantibodies recognizing these proteins (i.e., cross-reactive idiotypes). mAb to these proteins should be useful tools for investigating the structure and function of nucleic acid binding proteins, and may eventually lead to a better understanding of autoimmunity as well.

Summary

Autoantibodies to a DNA-bining heterodimer consisting of 70,000 and 80,000 dalton subunits were identified in 30–50% of human autoimmune sera from patients with systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), and scleroderma. Three murine monoclonal antibodies (mAb) against

the heterodimer were produced in BALB/c mice by immunizing with isolated human B cell nuclei. By immunofluorescence, the mAb and autoimmune sera demonstrated both speckled nucleoplasmic staining and diffuse nucleolar staining in all human cell types examined. The nucleoplasmic staining was sensitive to DNase but not RNase pretreatment, while the nucleolar staining was sensitive to RNase but not DNase pretreatment. Biochemical characterization of the 70,000 and 80,000 dalton proteins using the mAb indicated that two forms of the antigen, with different mobilities on sucrose gradients, are present in human B cells. A 10 S form consists of the physically associated 70,000 and 80,000 dalton proteins, while a larger, 10–20 S form probably represents the same two proteins bound to DNA. Binding of the proteins to nucleolar RNA could not be confirmed in biochemical studies. These studies indicate that non-histone, DNA-binding proteins may be more frequently recognized by autoantibodies in SLE, MCTD, and scleroderma than has been previously recognized. Along with previous studies on RNA-binding proteins such as Sm, RNP, Ro, and La, the present findings suggest that nucleic acid–binding proteins, as a class, may be particularly frequent targets of autoimmunity in SLE and related disorders.

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