HUMAN AUTOANTIBODIES THAT REACT WITH BOTH CELL NUCLEI AND PLASMA MEMBRANES DISPLAY SPECIFICITY FOR THE OCTAMER OF HISTONES H2A, H2B, H3, AND H4 IN HIGH SALT*

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Previous studies have identified two sets of human autoantibodies with apparent dual specificity (1-6). The first set reacts with the Fc fragment of IgG (1, 2) and with an antigenic determinant on core mononucleosomes, the repeating histone-DNA subunit of chromatin (3). The other set, which is the subject of the present report, recognizes a plasma membrane antigen of nucleated cells (4, 5) and an antigen on core mononucleosomes (6), but not IgG. Some of these observations have been confirmed by others (7-9).

The second set, which has been called X-ANA (for cross-reacting anti-nuclear antibodies), is found in the sera of patients with systemic lupus erythematosus (SLE)[1] and related diseases. It is identified and partially purified by incubation of serum with viable human leucocytes at 37°C, and the absorbed antibodies are then eluted; the fraction of the eluate with fluorescence antinuclear antibody (ANA) activity represents cross-reactive-ANA (X-ANA) (4). Because 75–90% of the ANA activity can be absorbed by viable cells (4), it is evident that X-ANA comprise a major fraction of ANA in these sera.

In this report we will present evidence that X-ANA from two patients recognize an epitope on the isolated octamer of the four inner histones in 2 M salt, and that X-ANA do not distinguish between native nucleosomes and nucleosomes reassembled from DNA and inner histone octamers. The findings will be discussed in terms of current concepts of chromatin structure and dual specificity of antibodies.

Materials and Methods

Source and Isolation of X-ANA. The X-ANA containing sera were obtained from two patients, L.H. and J.I., and stored at −70°C because the activity of X-ANA gradually disappears at 4°C. L.H. suffers from SLE; J.I. had drug-induced hepatitis and died of coronary occlusion. X-ANA was partially purified by absorption to and elution from viable human leucocytes, as described (4).

Preparation of Native Nucleosomes. Purification of core mononucleosomes and polynucleosomes

from chicken erythrocyte nuclei (10) on the basis of their different solubility in 0.15 M NaCl (11) have been described (6).

Preparation of Histone Octamer. 1 vol of freshly prepared packed chicken erythrocyte nuclei (10) were lysed in 2 vol of 0.2 mM EDTA, 0.5 mM phenylmethanesulfanyl fluoride (PMSF), 10 mM Tris/HCl·pH 7.2. Solid NaCl was then added to give 2 M and stirred over night at 4°C. The suspension was subsequently centrifuged at 170,000 g for 17 h in a Beckman TI-60 (Beckman Instruments, Inc., Fullerton, Calif.) rotor at 4°C to pellet the DNA. Histone octamer was prepared as described (see Fig. 1 of reference [6]). The preparation contained histones H2A, H2B, H3, and H4; nonhistone proteins or H1/H5 were not detected (Fig. 3 D).

Preparation of High Molecular Weight (Long) DNA. Calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.15 M NaCl, 0.2 mM EDTA, 10 mM Tris/HCl, pH 7.2, to give a concentration of 10 mg/ml, and deproteinized by digestion with 0.2 mg/ml of Proteinase K (Merck AG, Darmstadt, Federal Republic of Germany.) for 1 h at 38°C, followed by two extractions with equal volumes of water-saturated phenol. This DNA was used for reassociation with histone octamer (see below).

Preparation of H1/H5 Depleted Polynucleosomes. 3 ml of freshly prepared polynucleosomes (12 mg DNA/ml) were dialyzed against 0.65 M NaCl in Buffer A, and loaded on a Sephadex G–200 column (2.5 × 80 cm) equilibrated in the same buffer. The material that eluted as a single peak (read at A_{260}) in the void volume was pooled and concentrated by negative pressure dialysis. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) (12) showed that the preparation contained H2A, H2B, H3, H4, but no H1 and H5.

Preparation of H2A and H2B Depleted Mononucleosomes. Native core mononucleosomes were dialyzed against 1.2 M NaCl in Buffer A, and 2 ml were loaded on a Sephadex G–100 column (2.5 × 80 cm) equilibrated in the same buffer. The material eluted as a single peak (A_{260}) in the void volume; 17% SDS-PAGE showed that it contained histones H3 and H4, but no H2A or H2B (Fig. 3 E). The peak fractions were pooled, concentrated, and dialyzed against successively lower NaCl concentrations (1.0 M, 0.3 M, 0.1 M, 0 M) in Buffer A. This preparation was called H2A + H2B-depleted mononucleosomes, and was used in inhibition studies of X-ANA activity without further purification. After centrifugation (24,000 rpm, 21 h) through a 5–20% linear sucrose density gradient, the preparation displayed three peaks (detected by the absorbance at 260 nm of its DNA); the fastest moving peak had a sedimentation coefficient identical to core mononucleosomes. This pattern corresponded to that reported by others (13, 14), and indicates that the material in the fastest moving peak contained DNA in combination with four each of histones H3 and H4.

Preparation of Reconstituted Mononucleosomes and Reversibly Dissociated Nucleosomes. High molecular weight DNA (2 mg/ml) and histone octamer (1 mg/ml) were dialyzed separately against a buffer consisting of 2 M NaCl and 0.1 mM dithiothreitol (DTT) in Buffer A. Equal volumes were then mixed at 4°C and dialyzed against successively lower concentrations of NaCl in Buffer A as described (6, 15). The resulting reconstituted polynucleosomes were subsequently digested with micrococcal nuclease (10 U of enzyme/10 A_{260} U) for 15 min. The mononucleosomes were purified by sedimentation through linear 5–20% sucrose density gradients in Buffer A (see Fig. 1) for 22 h as described (6). Native core mononucleosomes and H1/H5 depleted polynucleosomes were subjected to the same experimental procedures as the reconstituted nucleosomes, and called reversibly dissociated polynucleosomes and core mononucleosomes, respectively. PAGE (6% slabs, 3 h) of mononucleosomes was performed as described (6, 16). The size of nucleosomal DNA was determined, after extraction as described above for long DNA, by PAGE (6% slabs) as described (6, 17).

Detection of Antibody Activity against Nuclear Antigens. ANA activity was detected by indirect immunofluorescence (1). Anti-nucleosome activity was demonstrated by a solid-phase radioimmunoassay (RIA) (6).

Radiolabeling with ^{125}I. Purified IgG anti-nucleosome antibodies (see Results) were radiolabeled by the chloramine-T method (18) to a specific activity of 0.4 μCi/μg.

Preparation of Fab' Fragments of IgG. To produce Fab' from F(ab')$_2$ (19), the solution was made 0.01 M in DTT, incubated under N$_2$ for 60 min; then a twofold molar excess of iodoacetamide was added; incubation was continued for 30 min on ice, followed by dialyses against phosphate-buffered saline (PBS).
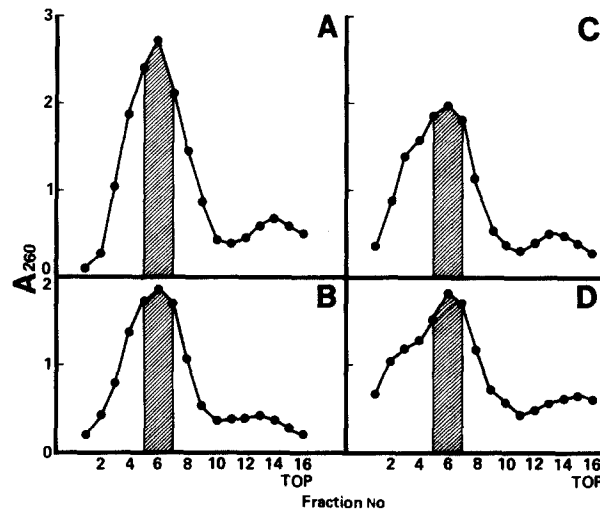
Fig. 1. Preparative ultracentrifugation of different mononucleosome preparations through 5–20% linear sucrose density gradients: (A) Native mononucleosomes, (B) reversibly dissociated mononucleosomes, (C) reconstituted polynucleosomes, (D) reversibly dissociated H1/H5-depleted polynucleosomes. The preparations in C and D were converted to mononucleosomes by micrococcal nuclease digestion prior to ultracentrifugation. See Materials and Methods for details. The hatched areas represent the fractions pooled and saved.

Results

The first aim was to find out whether X-ANA detected a difference between reconstituted and native mononucleosomes.

Compared with previous work (6, 20), we chose a different procedure (21–23) to prepare reconstituted mononucleosomes, starting with high molecular weight (long) DNA instead of short (140–200 base pair) DNA. The resulting reconstituted mononucleosomes could not be distinguished from native core mononucleosomes on the basis of sedimentation in 5–20% sucrose gradients (Fig. 1 C), migration in 6% PAGE (Fig. 2), histone composition (Fig. 3 C), and DNA length (140–200 base pairs) (Fig. 3 H). The same was also found for the two reversibly dissociated mononucleosome preparations.

X-ANA Does Not Distinguish between Native and Reconstituted Mononucleosomes. X-ANA from serum J.I. and L.H., partially purified by absorption to an elution from the plasma membranes of viable leucocytes, were mixed with different amounts of each of the mononucleosome preparation (see legend to Fig. 5), and the remaining antibody activity was determined.

Native and reconstituted nucleosomes were indistinguishable as inhibitors in the fluorescence ANA assay. Thus, both preparations inhibited the activity of X-ANA (L.H.) and X-ANA (J.I.) almost completely at 67–133 μg DNA/ml. The only preparation with weaker inhibitory effect was reversibly dissociated mononucleosomes that required 267 μg DNA/ml to give the same degree of inhibition (Fig. 4).

Native and reconstituted core mononucleosomes also appeared identical in RIA in that both preparations inhibited the binding of X-ANA (L.H.) and (J.I.) 50% at a concentration of 1.6–2.0 μg DNA/ml. One representative experiment with X-ANA
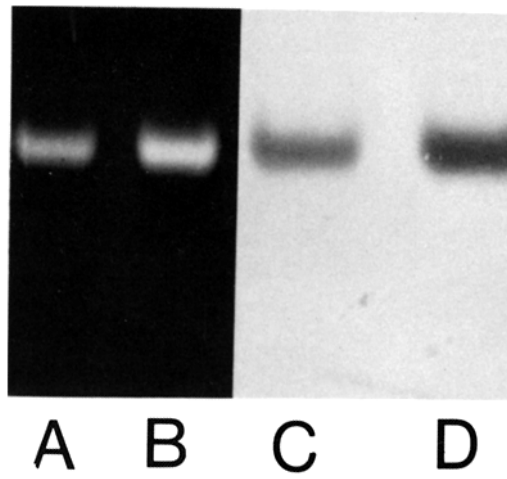
A   B   C   D

Fig. 2. Identical electrophoretic mobility of native and reconstituted mononucleosomes in 6% polyacrylamide gel. A and C: native; B and D: reconstituted mononucleosomes. The gel was stained with ethidium bromide (1 μg/ml) to reveal the migration of DNA in A and B; the gel was then fixed and stained with Coomassie blue to show the migration of histones in C and D.
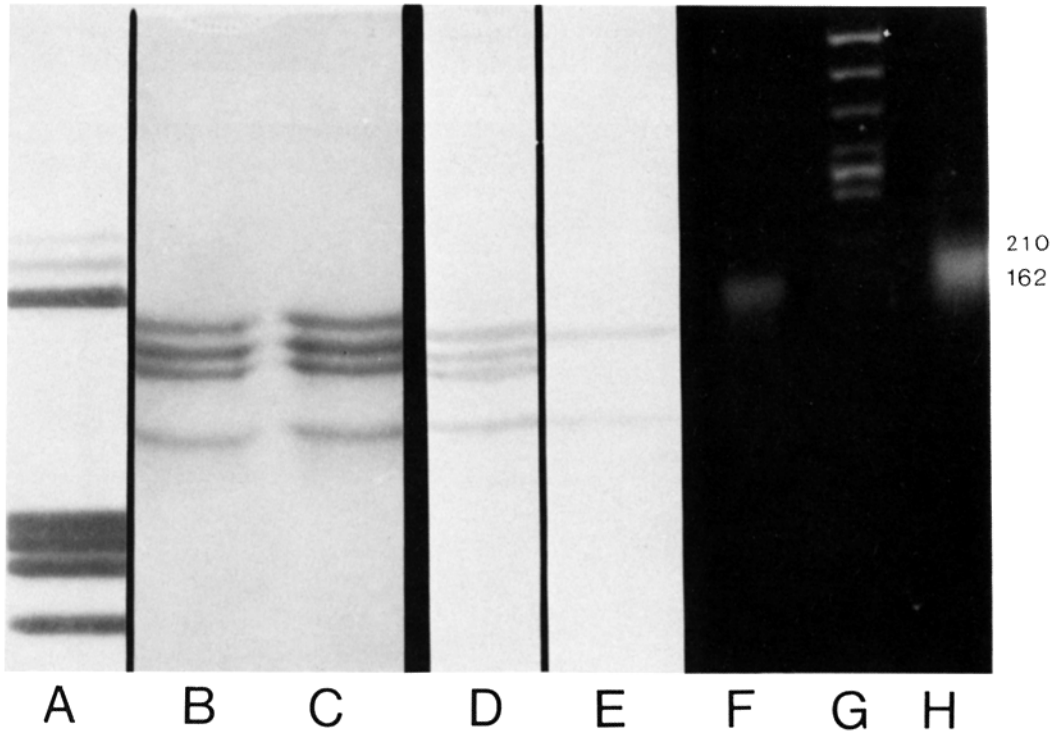
210
162

A   B   C   D   E   F   G   H

Fig. 3. Characterization of purified histone octamer, polynucleosomes, and mononucleosomes by PAGE. A–E shows SDS-PAGE (17%) of histones (12). A: polynucleosomes, B: native mononucleosomes, C: reconstituted mononucleosomes, D: inner histone octamer, E: H2A/H2B-depleted mononucleosomes. F–H show PAGE (6%) of extracted DNA (19). F: DNA from native mononucleosomes, G: Hinc II digest of DNA from phage X174 (New England Biolabs), H: DNA from reconstituted mononucleosomes.
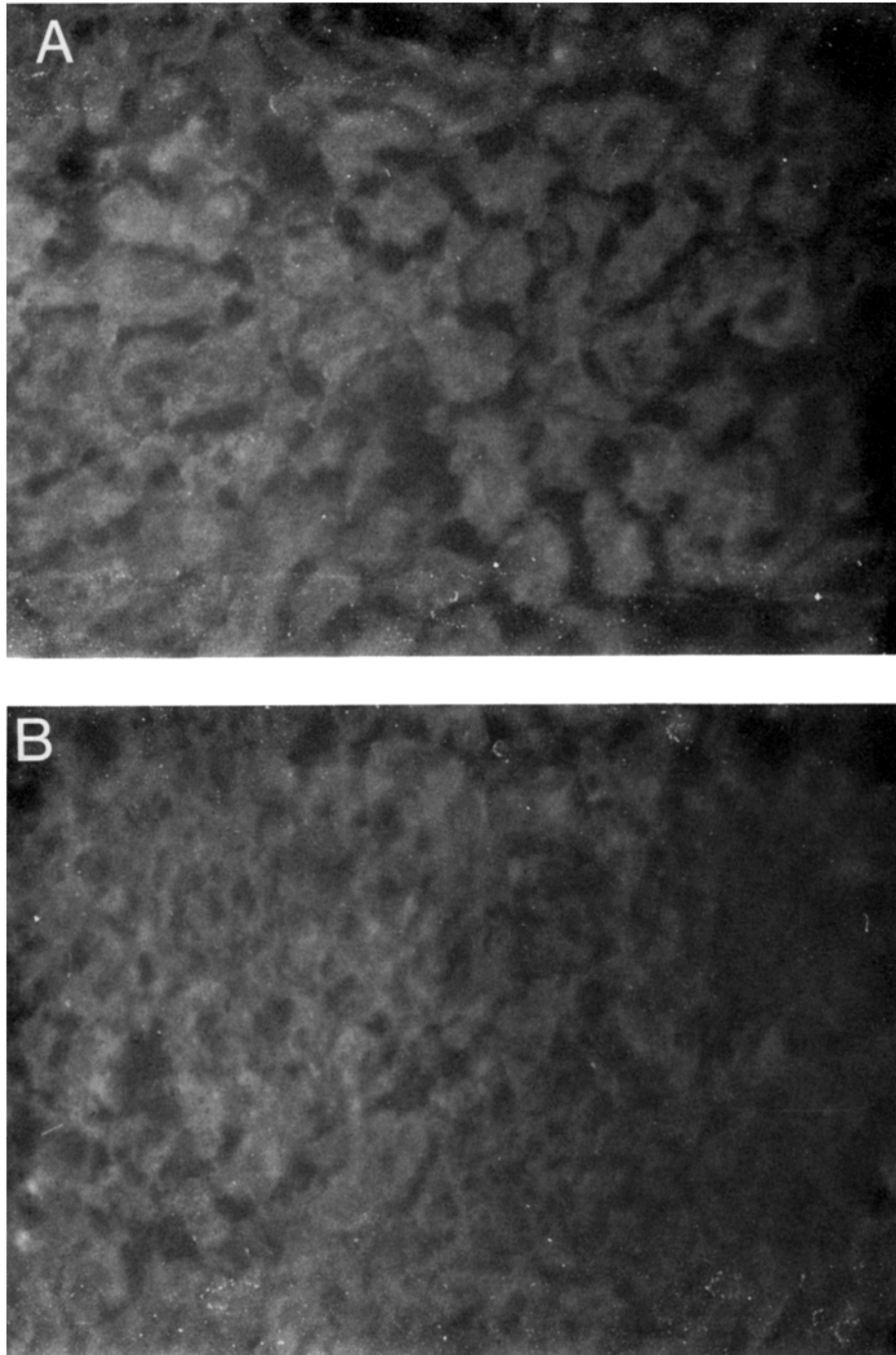
Fig. 4 A and B

Fig. 4. Competitive inhibition of binding of X-ANA (J.I.) to nuclei of tissue sections detected by immunofluorescence. The inhibitors were: A: native mononucleosomes (133 μg/ml), B: reconstituted mononucleosomes (133 μg DNA/ml), C: H2A/H2B depleted mononucleosomes (267 μg DNA/ml). Native and reconstituted, but not H2A/H2B-depleted, mononucleosomes were efficient inhibitors.
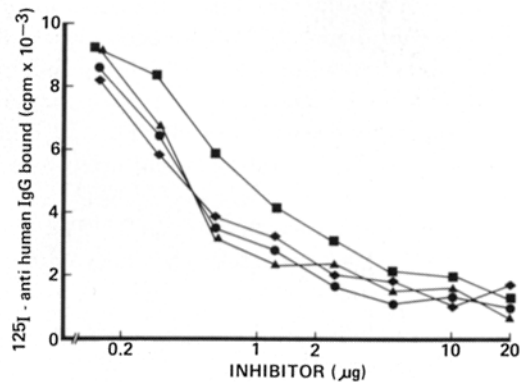
FIG. 4 C

FIG. 5. Competitive inhibition of binding of X-ANA (J.I.) to polynucleosomes detected by solid phase RIA. 200 μl undiluted eluated (X-ANA) was mixed with 50 μl inhibitor, incubated overnight at 4°C, and the binding of X-ANA to polynucleosomes on the walls of plastic tubes was assayed as described (6). The inhibitors were: native mononucleosomes (●), reconstituted mononucleosomes (▲), reversibly dissociated polynucleosomes converted to mononucleosomes (◆), and reversibly dissociated mononucleosomes (■). The latter preparation was a slightly weaker inhibitor than the other three.

(L.H.) is shown in Fig. 5. Again, reversibly dissociated mononucleosomes inhibited X-ANA (L.H.) slightly less effectively than the other three preparations.

The data indicate that the nucleosomal antigen recognized by X-ANA can be completely regenerated by the present procedure for reconstitution of mononucleosomes from long DNA and core histone octamer.

X-ANA Does Not Recognize H2A/H2B-Depleted Mononucleosomes.   H2A/H2B-depleted mononucleosomes did not inhibit X-ANA (L.H. or J.I.) at a concentration of 267 μg DNA/ml in the fluorescence system (Fig. 4), and 160 μg DNA/ml in the RIA (Fig. 6).

Preparation of Highly Pure X-ANA.   The next aim was to determine whether X-ANA recognize the histone octamer. These experiments required very pure Fab' fragments of X-ANA, which were obtained by successive immunospecific binding of X-ANA to its two different ligands, nucleosomes, and plasma membranes. This was achieved as follows.

Polynucleosomes (10 mg in 1 ml of 0.2 mM EDTA, pH 7.2) were mixed with 20 ml serum L.H. The nucleosomes became immediately insoluble at the ionic strength of serum; nevertheless, this did not prevent anti-nucleosome antibodies from binding. After overnight incubation at 4°C, the precipitate was washed three times in PBS, dissolved in 2 ml of 2 M NaCl, 0.5 mM PMSF, 10 mM Tris/HCl, pH 7.2, and loaded on a Sephadex G-200 column in the same buffer at room temperature. The material eluted in two peaks (Fig. 7). The first peak contained mainly free DNA and some IgM (data not shown). The fractions with IgG ANA-activity were pooled as indicated, concentrated, and dialyzed against PBS; immunoelectrophoresis against anti-human serum and SDS-PAGE revealed only IgG (data not shown). Indirect immunofluorescence showed that this preparation contained antibodies that bound to the plasma membrane of viable HeLa cells (not shown). The purified IgG anti-nucleosome antibodies were labeled with ^{125}I. 70% of the labeled antibodies bound to leucocytes; by comparison, >90% of the fluorescence ANA activity was removed when serum L.H. was absorbed by an excess viable leucocytes. The difference is most likely due to some denaturation of the purified antibodies during the experimental manipulations.

The ^{125}I-labeled antibodies were digested (3 h) with pepsin to yield F(ab')₂ fragments (4). The fraction of ^{125}I-F(ab')₂ anti-nucleosome antibodies that recognized plasma membranes was obtained by a second immunoaffinity step that entailed binding to an elution from 2·10⁸ viable human leukocytes (4). The ^{125}I-F(ab')₂ X-ANA were finally converted to monomeric Fab' (Materials and Methods) and used in the following experiment (see below).

X-ANA Recognize the Inner Histone Octamer in 2 M NaCl.   The next experiment was designed to explore whether X-ANA recognize the inner histone octamer in the
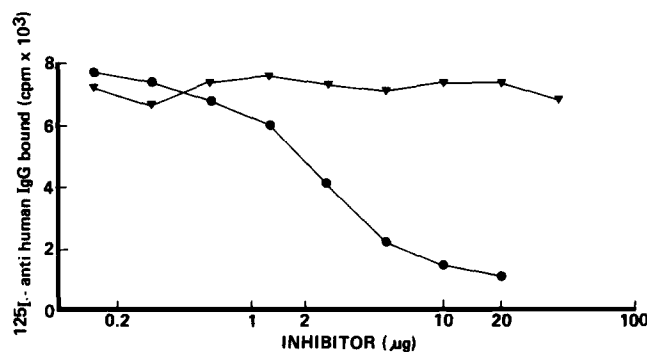

Fig. 6.   H2A/H2B depleted mononucleosomes do not inhibit binding of X-ANA (J.I.) to polynucleosomes. The inhibitors were: native mononucleosomes (●), H2A/H2B depleted mononucleosomes (▼). Assay as in Fig. 5.
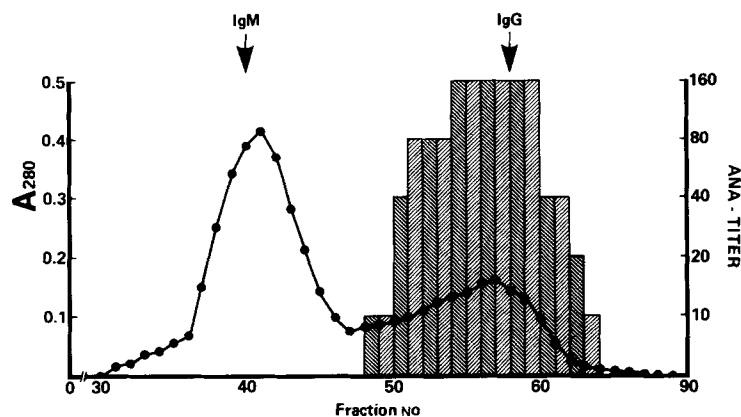
Fig. 7.   Gel chromatography on a column (2.5 × 80 cm) of Sephadex G-200 of the complex between polynucleosomes and proteins from X-ANA containing serum L.H. H1/H5 containing polynucleosomes, 10 mg, were mixed with 20 ml serum; the precipitate was washed and dissolved in 2 ml of the column equilibration buffer (2 M NaCl, 0.5 mM PMSF, 10 mM Tris/HCl, pH 7.2). See Results for details. The column was operated with a flow rate of 20 ml/h, and fractions of 4 ml were collected. The hatched bars represent the IgG-ANA titers of those fractions that were pooled, concentrated to 15 ml, and dialyzed against PBS.

absence of DNA. Evidence for specific binding of ^{125}I-Fab' X-ANA to antigen was obtained by immunosedimentation in linear 5–20% sucrose density gradients. Fab' monomers were used instead of intact IgG because the larger increase in sedimentation rate of liganded Fab' would make it a more sensitive indicator of complex formation than IgG.

A representative experiment, shown in Fig. 8, was done as described in the figure legend. It is evident that in 2 M NaCl and in the presence of mononucleosomes or pure inner histone octamer, the sedimentation rate of the entire ^{125}I-Fab' X-ANA population was accelerated relative to its sedimentation in presence of bovine serum albumin (BSA) (Fig. 8 B). In contrast, in 0.15 M NaCl, ^{125}I-Fab' only complexed with core mononucleosomes; at this ionic strength the presence of the inner histones did not lead to a shift in the sedimentation rate of Fab' (Fig. 8 A). Very similar results were obtained with ^{125}I-Fab' X-ANA (J.I.) (data not shown). The complex formation with inner histones in 2 M salt was highly dependent upon the histone concentration. Thus, 5 mg/ml and 2 mg/ml yielded almost identical shifts in the sedimentation of Fab' (J.I.); by contrast, at 1 mg/ml, the shift was much less pronounced (data not shown).

The observed complex formation between Fab' X-ANA and histone octamer in 2 M NaCl was found to be specific because the sedimentation rate of ^{125}I-labeled Fab' from commercial pooled IgG was not accelerated by either native mononucleosomes or the inner histones (data not shown). We conclude that X-ANA recognize an epitope on the inner histone octamer in 2 M salt. In 0.15 M NaCl, this epitope is not formed unless the histones interact with DNA to generate a nucleosome structure.

Discussion

The data obtained establish that X-ANA recognize an epitope formed when the four inner histones interact in 2 M salt in absence of DNA. The epitope is lost in 0.15
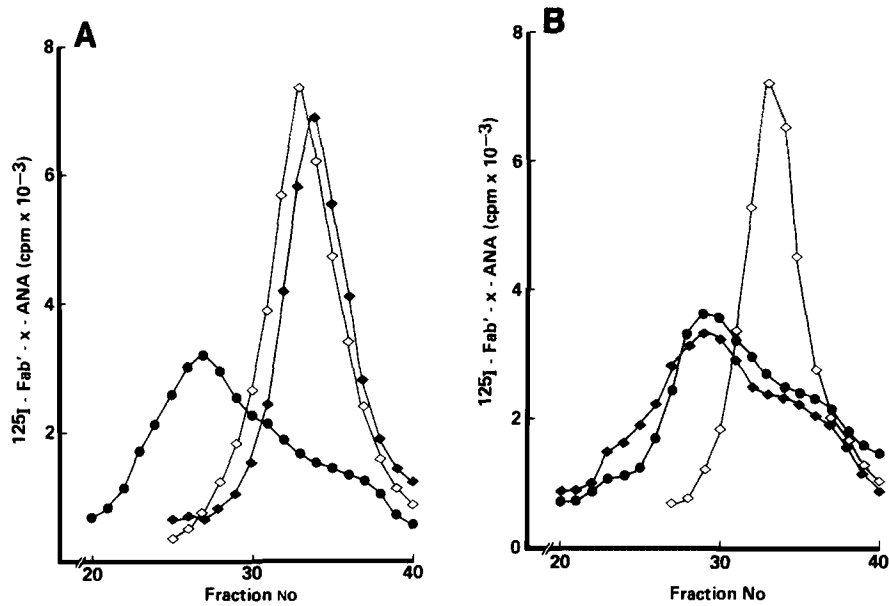
Fig. 8. Complex formation of ^{125}I-labeled Fab′ fragments of X-ANA (J.I.) with mononucleosomes and purified inner histones demonstrated by immunosedimentation in 5–20% linear sucrose density gradients. The sucrose gradient contained 0.15 M NaCl (A), or 2 M NaCl (B), and 10 mM Tris/ HCl, pH 7.2, 0.5 mM PMSF. Fab′, 4·10⁴ cpm in 0.1 ml of the appropriate buffer containing 2% sucrose and 0.02% bromophenol blue, was layered on the sucrose gradients. On top of the Fab′ was carefully layered 2 ml of either BSA (5 mg/ml) (◇), native mononucleosomes (2 mg DNA/ml) (●), or inner histones (2 mg/ml) (◆), all dialyzed against the buffer of the respective sucrose gradients. Ultracentrifugation was performed for 20 h 4°C and 24,000 rpm in an SW27 rotor; fractions of 2 ml were collected from the bottom.

M salt except when the histones interact with DNA to generate a nucleosome structure. This confirms our previous results where the inner histones at physiological ionic strength did not inhibit binding of X-ANA to nuclei of tissue sections or to polynucleosomes (6), and implies that X-ANA recognize a conformation-dependent histone determinant. Because the experiments were conducted with Fab′ fragments of X-ANA, the reactions with histone octamer were most likely mediated by genuine antibody combining sites; evidently, this antigen-antibody interaction is at least partly independent on charge, as otherwise no complexes should have formed in 2 M salt. This implies that nonspecific electrostatic interactions can be ruled out as the basis for complex formation. To our knowledge, the specificity of X-ANA for histone octamer has not been previously described, either in conventionally induced antisera to histones or chromatin (24) or in SLE sera (25, 26).

Because X-ANA stain nuclei in tissue sections (4) and bind to H1 and H5-containing polynucleosomes in 0.15 M salt (6) when chromatin is very compact, one may conclude that the histone epitope is highly accessible to solution components. Current notions place histone amino termini near the surface of nucleosomes on the basis of the ready digestion of these regions in chromatin by trypsin (21, 27). This location of the epitope is also consistent with the fact that trypsin-digested mononucleosomes have lost their ability to bind to X-ANA (6). In addition, the data of the present work demonstrate that H2A, H2B-depleted mononucleosomes, containing

predominantly four each of histones H3 and H4, do not complex with X-ANA, suggesting that the location of the epitope can be further confined to the N-terminal region of either H2A or H2B.

The relation of these data to current concepts of chromatin structure is of interest. The chromatin subunit, called core mononucleosomes, is composed of an octameric histone disc that contains two each of the inner histones H2A, H2B, H3, and H4, surrounded by DNA ~146 base pair long; the nucleosomes are linked by DNA of variable length, resulting in a simple repeating structure (28, 29). Nucleosomes can self-assemble by mixing DNA and inner histones in a solution of high ionic strength (2 M NaCl) that is then gradually lowered by dialysis; the reconstituted particles resemble the native material in most or all structural features (20, 29), but antibodies to nucleosomes have not been included in the analyses. In the absence of DNA, the inner histones can interact to form an octamer, provided the salt and histone concentration is sufficiently high and the pH is neutral (12, 30, 31). Weintraub et al. (21) have reported that the complex of inner histones in 2 M salt, in contrast to 0.2 M salt, is indistinguishable from histones in chromatin by its resistance to trypsin, pattern of reactivity with ^{125}I, and ability to form specific cross-linked products after treatment with formaldehyde. Lowering of the NaCl concentration from 2 M leads to several changes in the inner histones in solution. Thus, in 0.6–0.4 M NaCl, the histone octamer has been shown to be completely dissociated into the H3·H4 tetramer and the H2A·H2B dimer (30, 31); in 0.2 M NaCl, the H2A·H2B homotypic complexes appear to be in different conformations than in 2 M NaCl because the latter, but not the former, ionic strength gives trypsin-resistant histone fragments (21). Electrostatic repulsion between the positively charged histones appears to be the main cause for destabilization of the octamer at physiologic ionic strength.

When we first used X-ANA to compare the structure of native and reconstituted nucleosomes, we found that the latter were 4- to 10-fold weaker inhibitors of X-ANA than the native (6). In the present experiments, we modified the reconstitution method by starting with long rather than short (140–200 base pair) DNA. This preparation of reconstituted nucleosomes inhibited the binding of X-ANA to polynucleosomes with exactly the same efficiency as the native material. In light of our present demonstration that X-ANA recognize a conformation-dependent antigenic site on the inner histones, it can be concluded that this conformation is faithfully regenerated when nucleosomes are reassembled in vitro from DNA and histones in the absence of assembly factors.

The ability of X-ANA to discriminate between inner histones in high and low salt concentration, and the inability to distinguish between native and reconstituted nucleosomes, are in good agreement with conclusions reached from independent nonimmunochemical methods referred to above. In this context, X-ANA may serve as a specific immunochemical probe to test whether the nucleosome assembly factor isolated from the eggs of *Xenopus laevis* (32), or other acidic polypeptides such as polyglutamic acid (33), are able to organize the histones into their native conformations at physiological ionic strength in the absence of DNA. Furthermore, recent evidence indicates that X-ANA occur in close association and may be identical with lupus erythematosus (LE) factor (O. P. Rekuig and K. Hannestad. Manuscript in preparation.), the first antinuclear antibody discovered (34, 35). Because most SLE

patients exhibit a positive LE factor test at some point in the disease, this association implies that X-ANA may also have considerable clinical significance.

The dual specificity of X-ANA is intriguing; three explanations seem possible. (a) Past experience with the structural basis of cross-reactions (36) would lead one to expect that the two antigens share a common or similar epitope. If the epitope is a peptide, the membrane antigen could be a histone or histonelike protein. Alternatively, X-ANA could react with a shared sugar determinant on a glycoprotein; however, the only sugar reported to be linked to histone is poly(ADP-ribose) (37); moreover, the membrane epitope is destroyed by glutaraldehyde (4) and the histone epitope disappears in low salt, all of which argue against the possibility that X-ANA recognize carbohydrate. (b) The two antigenic sites may occupy nonoverlapping spaces in the antibody-combining site and use a different set of contacts. In that case, the epitopes are structurally dissimilar. The recent demonstration by Hannestad and Stollar (3), that certain rheumatoid factors (natural antibodies to the Fc part of IgG) also cross-react with nucleosomes, may be a case in point because no amino acid sequence homology between histones and gamma Ig chains have been reported (38). Other examples of such strange cross-reactions have been found among some myeloma proteins with antibody activity, and among certain polyclonal antihapten antibodies raised in conventionally immunized animals (39, 40). These findings have led to the suggestion that most or all antibodies have the ability to combine well with occasional antigens that do not share common structural features with the eliciting hapten (39, 40). It must be stressed that the evidence for this attractive view is incomplete and has been challenged, particularly with respect to the definition of dissimilarity (41-43). The fact that such a high proportion (75-95%) of polyclonal X-ANA is absorbed by viable cells is difficult to reconcile with multispecificity, assuming that the two specificities are distributed independently among the clones. However, this point cannot be definitively evaluated until the degree of clonal heterogeneity among X-ANA has been ascertained. (c) The dual specificity of X-ANA may be like the cross-reactions between the 2.4-dinitrophenol-group and Menadione (vitamin K3) (41) or levan and inulin (44); although one might not believe that these compounds are similar from their structural formulas, significant similarities are revealed by building molecular models (43, 44). If that is the case, the dual specificity of X-ANA is not different from conventional cross-reactions; that is, a variant of the first explanation.

Summary

Sera of some patients with systemic lupus erythematosus and related diseases contain a polyclonal antibody population (cross-reactive antinuclear antibodies [X-ANA]) that react specifically with both core mononucleosomes and plasma membranes of viable nucleated cells. Native mononucleosomes and nucleosome cores assembled from long DNA and the inner histones were indistinguishable in terms of inhibition of binding of X-ANA to nuclei of tissue sections and to polynucleosomes on the walls of plastic tubes. In contrast, mononucleosomes selectively depleted of histones H2A and H2B did not inhibit these reactions.

A method was developed for isolation of X-ANA from serum that took advantage of the dual specificity of these antibodies.

Immunosedimentation in sucrose density gradients revealed that ^{125}I-labeled Fab' fragments of highly pure X-ANA formed complexes with the inner histones H2A,

H2B, H3, and H4 in 2 M NaCl, but not in 0.15 M salt. These results indicate that X-ANA recognize an epitope of the inner histone octamer in 2 M salt, and that in 0.15 M NaCl this epitope is not formed unless the histones interact with DNA to generate a nucleosome structure. Furthermore, in light of the previous demonstration that the epitope is destroyed by trypsin, it may be localized in the N-terminal region of histone H2A or H2B.

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