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# Procainamide elicits a selective autoantibody immune response

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## SUMMARY

The specificity of the in vivo humoral immune response elicited by procainamide was examined by solid-phase assays, immunofluorescence, immunoprecipitation and a cytotoxicity assay. Serial samples obtained from patients during their procainamide therapy showed a progressive increase in antibodies to histones and denatured DNA, and both activities decreased after discontinuation of therapy. In contrast antibodies to tetanus, human IgG (rheumatoid factor) and heterologous lymphocytes were unaffected by procainamide treatment, indicating that they were not drug-induced. Of 29 sera examined by protein-A-facilitated immunoprecipitation, four sera had antibody to ribosomal RNA and three sera immunoprecipitated a 40kD protein. Antinuclear antibodies were invariably present but absorption studies showed that these activities were due to anti-histone antibodies. These results indicate that procainamide-induced autoimmunity is characterized predominantly by an anti-histone and anti-denatured DNA immune response.

**Keywords** procainamide-induced autoantibodies

## INTRODUCTION

A disease resembling systemic lupus erythematosus is induced in 10–20% of patients undergoing anti-arrhythmic therapy with procainamide (Blomgren, 1973; Lee & Chase, 1975). This clinical condition as well as the accompanying laboratory abnormalities in both symptomatic and asymptomatic patients are clearly drug-induced because withdrawal of therapy results in gradual cessation of symptoms and return to a normal serology.

Most patients develop anti-nuclear antibodies (ANA) after prolonged exposure to procainamide (Woosley *et al.*, 1978). Antibodies to nucleoprotein occur in many patients (Molina *et al.*, 1969; Klajman *et al.*, 1970; Blomgren, Condemi & Vaughan, 1972). Histones reconstitute the ANA activity of sera from patients with procainamide-induced lupus (Fritzler & Tan, 1978; Grossman & Barland, 1981) and in approximately 1/3 of asymptomatic procainamide-treated patients (Klajman *et al.*, 1983). Whether ANA, anti-nucleoprotein and anti-histone are separate antibody populations or are activities of essentially the same specificity has not been formally examined.

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Reports of other autoantibodies in drug-induced lupus have also appeared including anti-ribonucleoprotein (Winfield, Koffler & Kunkel, 1975) antibodies to denatured DNA (dDNA) and other polynucleotides (Koffler *et al.*, 1969; Koffler *et al.*, 1971; Blomgren *et al.*, 1972; Winfield & Davis, 1974; Klajman *et al.*, 1975; Fritzler & Tan, 1978), rheumatoid factor (Blomgren *et al.*, 1972), anti-native DNA (Molina *et al.*, 1969; Klajman *et al.*, 1975), anti-Sm (Klajman, Farkas & Ben-Efraim, 1972) and lymphocytotoxic antibodies (Bluestein *et al.*, 1979). However, a diverse array of antibody activities has not been observed in some reports (Fritzler & Tan, 1978; Grossman & Barland, 1981; Portanova *et al.*, 1982; Klajman *et al.*, 1983), suggesting that factors related to methodology or patient selection may account for these apparent discrepancies. In retrospective studies involving single serum samples, the pre-existance of an abnormal serology due to age, sex or underlying disease cannot be excluded. Definition of the humoral immune response induced by procainamide may help to focus attention toward more viable mechanisms underlying this form of autoimmunity.

In the present study autoantibodies in sera from patients treated with procainamide were enumerated by a variety of techniques designed to probe for antibody diversity. Distinction between pre-existing and drug-induced antibodies could be made by following patients serially throughout procainamide therapy and after therapy was discontinued. We found only a limited spectrum of drug-induced antibodies and show that ANA induced by procainamide is due to anti-histone antibodies.

## MATERIALS AND METHODS

*Patient population.* Patients in this study were treated with procainamide (Procan-SR, Morris Plains, NJ, USA) to suppress ventricular arrhythmia. Patients with a history of underlying rheumatic disease prior to therapy were excluded from the study. Sera and clinical information from patients in the prospective group were obtained at the start of therapy and at subsequent intervals. Another group of patients had clinical drug-induced lupus and presented to the Scripps Clinic Division of Cardiovascular Diseases with constitutional symptoms, arthralgias, myalgias, pleuritis, pericarditis and/or arthralgias as previously described (Rubin *et al.*, 1985). Serum was obtained at this symptomatic stage and at intervals following withdrawal of procainamide treatment. The third group consisted of patients who had been treated with procainamide for at least 2 years without developing symptoms and from whom only a single serum sample was available.

*Enzyme-linked immunosorbent assays (ELISAs).* All ELISAs were carried out in a similar manner based on the methodology previously described (Rubin, Joslin & Tan, 1983). Briefly, sera were diluted 1:200 in phosphate-buffered saline containing 0·05% tween–20 (PBS-tween) containing bovine serum albumin and gelatin at 1 mg/ml and either 0·75 mg/ml bovine gammaglobulin (BGG) for the anti-histone and anti-chromatin assays or 5 mg/ml BGG for the anti-DNA, anti-tetanus and rheumatoid factor assays. After incubation of 0·2 ml in the antigen-coated plate for 2 h, bound antibody was detected with peroxidase-conjugated anti-human immunoglobulin. Antihuman IgM was used for the rheumatoid factor assay and a polyvalent antibody reactive with the three major Ig classes and light chains was used for the other assays. Both reagents were obtained from Tago Inc. (Burlingame, CA, USA).

Total histones were obtained from Calbiochem-Behring (La Jolla, CA, USA) and further purified by DNase I treatment and acid extraction. The quality of the preparation was evaluated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970) and found to consist of the five major histone classes. DNA (Calbiochem-Behring, La Jolla, CA, USA) was treated with proteinase K and phenol extracted and had no detectable protein. DNA was heat denatured just prior to use by boiling for 10 min and quickly cooling. Tetanus toxoid fluid (Lederle, Pearl River, NY, USA) was used at a dilution of 1:300 for coating wells. Purified human IgG (Miles, Elkart, IN, USA) was used at 2·5 $\mu$g/ml to coat wells for the rheumatoid factor assay. Chromatin was isolated from frozen calf thymus (Pel-Freeze, Rogers, AR, USA) as previously described (Rubin & Moudrianakis, 1975) and the histone composition of a typical preparation is

shown in Fig. 2. For coating plates, chromatin was diluted to 3 μg/ml in 0·2 mM EDTA, pH 7·5, 0·5 μg was added to each well and the solution adjusted to PBS using × 10 PBS.

*Absorption of sera with chromatin.* To 10 μl serum was added 13 μl of concentrated (× 6) serum diluent (containing 30 mg/ml BGG) and 57 μl 0·2 mM EDTA containing increasing concentrations of chromatin. After agitation for 2 h at room temperature 240 μl of serum diluent was added (containing 0·75 mg BGG/ml) and the chromatin was pelleted by centrifugation. A portion of the supernate was diluted seven times with serum diluent and used for ELISA. Another portion was diluted up to 12 times in PBS for determination of ANA activity.

*ANA determination.* Hep-2 cells (Bion, Ridge, IL, USA) were used as the substrate, and serum dilutions were chosen to produce a sub-maximum ANA intensity (2–3$^+$). Twenty-five micro-litres of chromatin-treated serum was incubated with the substrate for 30 min. After appropriate washes and incubation with fluorescein-conjugated anti-human Ig (polyvalent, Tago, Inc. Burlingame, CA, USA), ANA intensity was scored using a Leitz Ortholux II fluorescence microscope at × 625 magnification.

*Immunoprecipitation.* HeLa cells were labelled with $^{35}$S-methionine or $^{32}$P inorganic phosphate (Francoeur & Mathews, 1982). Cells were lysed by freeze/thawing in 10 mM Tris-HCl, pH 7·4, 150 mMNaCl, 1·5 mM $MgCl_2$, 0·5% Nonidet P-40, and the extract clarified at 15,000 × **g** for 15 min. Twenty microlitres of each test serum was incubated for 1 h with 200 μl of a 10% (v/v) suspension of protein A-Sepharose (Pharmacia, Piscataway, NJ, USA) in immunoprecipitation (IP) buffer (50 mM Tris-HCl, pH 7·4, 150 mM NaCl, 5 mM EDTA, 0·5% Nonidet P-40, 0·5% sodium deoxycholate, 0·1% sodium dodecyl sulfate (Francoeur & Mathews, 1982) and 23 units/ml Kallikrein inactivator (Calbiochem-Behring, La Jolla, CA, USA). After washing Sepharose beads (× 5) in IP buffer, a 30 μl aliquot of radiolabelled HeLa cell extract containing 30 μg protein (pre-absorbed with virgin protein-A sepharose beads) was added. After incubation for 90 min with constant mixing, the beads were washed (× 5) in IP buffer. Half the beads which had been incubated with $^{32}$P-labelled extract were treated with proteinase K at 1 mg/ml for 30 min at 37°C and the other half was left untreated to preserve both RNA and phosphoprotein. Samples were analyzed by 15% SDS-PAGE (Laemmli, 1970) followed by autoradiography.

*Lymphocytotoxicity assay.* Anti-lymphocyte antibodies were determined by the two step microcytotoxicity method (Terasaki, Mottironi & Barnett, 1970) as previously described (Searles, *et al.*, 1983). Targets were fresh peripheral blood mononuclear cells from three normal donors. Undiluted test serum was incubated with each target for 1 h at 4°C. After addition of rabbit complement and incubation for 2 h at 22°C viability was assessed by exclusion of 5% eosin. Normal human sera gave ⩽ 5% dead cells; 20% killing was considered significant.

## RESULTS

*Selective induction of antibodies to histones and dDNA*

Five patients were followed serially during their therapy with procainamide, the duration of which varied from 9–21 months (mean = 13 months). None of these patient developed lupus symptoms during this period but four patients showed a progressive increase in anti-dDNA and all developed antibodies to histones. In contrast anti-tetanus antibodies did not significantly increase in any patient. Rheumatoid factor (RF) (IgM anti-human IgG) was detected in low levels in approximately half the samples, but this activity did not increase significantly during therapy, indicating RF was not drug-induced in these patients. These serological changes are expressed in Fig. 1a as percentage increase in each antibody during procainamide therapy, demonstrating the selective nature of antihistone and anti-dDNA antibody induction.

*Effect of discontinuation of procainamide therapy on antibody levels*

Patients treated with procainamide who presented with inflammatory symptoms associated with drug-induced lupus were followed during their convalescence after discontinuation of procainamide. This group consisted of seven patients, one of whom had been followed throughout the evolution of his disease and was included in Fig. 1a during his asymptomatic period.

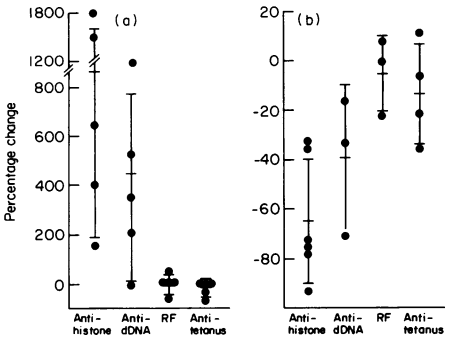


**Fig. 1.** Change in four antibody activities in patients undergoing therapy with procainamide (a) and after discontinuation of therapy in another group of patients (b). Patients in group a were treated for an average of 13 months, and antibody activity at this time was compared to the initial activity, which was always in the normal range. Group b patients were taken off procainamide because of lupus symptoms, and antibody levels at this time were compared to levels in sera obtained 2–15 months later (mean interval = 7 months). The mean percentage change ± 1 s.d. is represented by the horizontal lines.

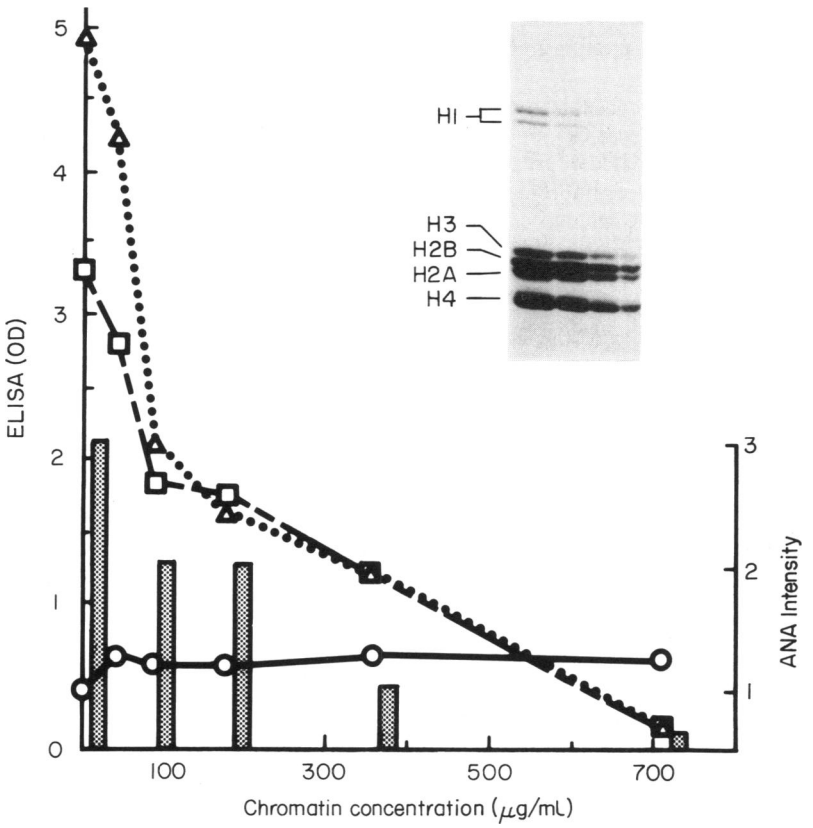


**Fig. 2.** Effect of absorption with chromatin on various antibody activities. Increasing amounts of calf thymus chromatin were incubated in the serum and bound antibody was removed by centrifugation. Remaining antibody activity for chromatin (△), histone (□) and tetanus toxoid (○) were assayed by ELISA. ANA was evaluated on Hep-2 cells (intensity, (■)). The inset shows SDS-PAGE profiles of proteins extracted from chromatin used in the absorption studies. Total protein 5, 10, 20 and 30 μg was applied to separate lanes. Bands identified as histones are labelled.

All these patients had IgG antibodies to the histone complexes H2A-H2B as previously described (Rubin *et al.*, 1985), and this antibody activity was monitored after withdrawal of procainamide. As shown in Fig. 1b the level of anti-(H2A-H2B) decreased with time, concomitant with subsidence of symptoms. Anti-dDNA was detected in low levels in approximately half the sera and this activity also tended to decrease in parallel with the anti-histone levels. In contrast antibodies to tetanus toxoid showed only a modest decrease and IgM RF was also largely constant throughout the period of observation.

*Anti-nuclear antibodies in procainamide treated patients are predominantly anti-histone*

Previous studies suggested that histone reactive antibodies account for the bulk of the ANA in drug-induced lupus (Portanova *et al.*, 1982). Direct support for this notion was sought by absorption studies. Chromatin was used as the anti-histone antibody immunoabsorbant because specific antibody is removed due to chromatin insolubility in physiological environments and the bulk of chromatin proteins consist of the five major histone classes (Fig. 2). Although native DNA accounts for approximately one-half the mass of chromatin, anti-native DNA was not detected in any sera from procainamide-treated patients (data not shown).

The effect of absorption of sera with chromatin on various antibody activities was examined, and typical results are shown in Fig. 2. Absorption of anti-chromatin antibodies coincided with removal of anti-histone antibodies, suggesting that histones and nucleohistones (chromatin) have a similar antigenic composition. Anti-tetanus activity did not decrease, indicating that chromatin did not cause nonspecific removal of immunoglobulin. ANA activity decreased in parallel with a loss of anti-histone antibody, suggesting that histone reactive antibodies account for the ANA in this serum.

Ten additional sera were subjected to absorption with the highest concentration of chromatin, and the remaining ANA was examined on Hep-2 cells and mouse kidney sections. As shown in Table 1, chromatin eliminated or greatly reduced the ANA of all sera on both substrates. Inability to remove the entire nuclear fluorescence is probably not due to specific antibody as normal sera often produce weak ANA reactivity, especially on Hep-2 substrates. These data support the view that ANA in serum from procainamide-treated patients is due to anti-histone antibody.

**Table 1.** Chromatin absorption of drug-induced antinuclear antibodies

| | ANA Intensity* | | | | | |
|---|---|---|---|---|---|---|
| | Hep-2 cells | | | Mouse kidney section | | |
| Patient | Dilution | Control absorbed | Chromatin absorbed | Dilution | Control absorbed | Chromatin absorbed |
| S.A. | 1:64 | 2+ | tr | 1:32 | 3+ | tr |
| W.A. | 1:512 | 3+ | 1+ | 1:256 | 3+ | 1+ |
| L.Ca. | 1:128 | 2+ | tr | 1:64 | 3+ | 1+ |
| L.C. | 1:64 | 2+ | 1+ | 1:32 | 3+ | tr |
| F.D. | 1:512 | 3+ | tr | 1:256 | 3+ | tr |
| A.M. | 1:64 | 3+ | 1+ | 1:32 | 1+ | tr |
| R.M. | 1:64 | 3+ | 1+ | 1:32 | 3+ | 1+ |
| R.P. | 1:64 | 2+ | 1+ | 1:32 | 1+ | − |
| E.S. | 1:128 | 4+ | 1+ | 1:64 | 3+ | tr |
| B.S. | 1:128 | 3+ | 1+ | 1:64 | 3+ | 1+ |

* Sera were diluted 1:8 and treated with chromatin at 713 μg/ml or control solvent in 5 mg/ml BGG for 2 h. Samples were diluted four-fold, centrifuged and the supernatants diluted again to the varying final extents shown. (tr) Trace immunofluorescence.

**Table 2.** Cytotoxic anti-lymphocyte antibodies in procainamide (Pr)-treated patients

| Patient | Duration of Pr therapy (months) | Clinical status* | Cytotoxicity (mean % killing ±s.d.) |
|---|---|---|---|
| F.D. | 6 | Ar,Pe,Pl,At,M,F | 20±17 |
| V.B. | 25 | M,Pl,Pe | 5±5 |
| E.W. | 21 | Pl,Pe | 80±0 |
| R.H. | 120 | asymptomatic | 60±0 |
| A.H. | 2 | asymptomatic | 0±0 |
| W.J. | 17 | asymptomatic | 0±0 |
| J.F. | 0·5 | asymptomatic | 27±6 |
| | 2·5 | asymptomatic | 33±15 |
| | 10 | asymptomatic | 33±12 |
| R.P. | 0·5 | asymptomatic | 7±6 |
| | 2 | asymptomatic | 10±17 |
| | 8 | asymptomatic | 10±17 |
| J.S. | 0 | asymptomatic | 23±6 |
| | 2 | asymptomatic | 3±3 |

* Symptoms: (Ar) arthritis; (At) arthalgias; (F) fever; (M) myalgias; (Pe) pericarditis; (Pl) pleuritis.

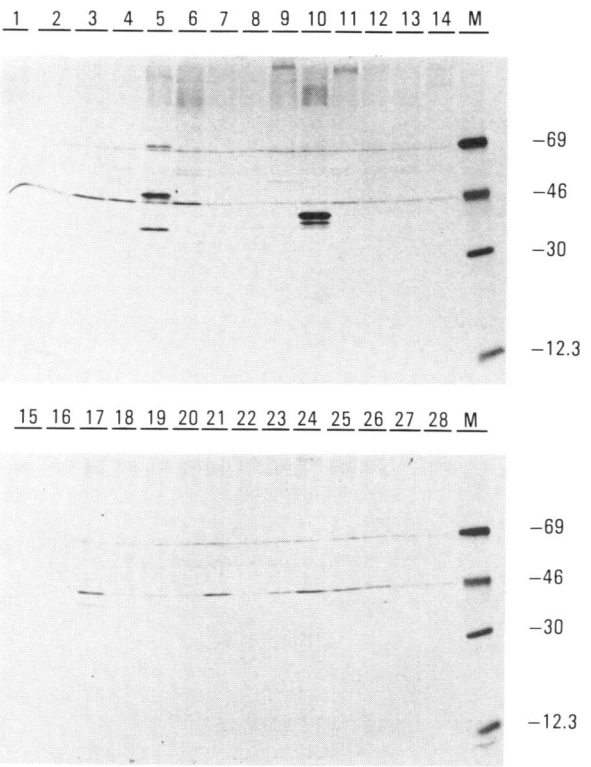


**Fig. 3.** Immunoprecipitation of $^{35}$S-methionine labelled extracts of HeLa cells by sera from a variety of procainamide-treated patients. Labelled proteins bound to antibody were separated by SDS-PAGE and subjected to autoradiography.

*Anti-lymphocyte Antibodies*

Cold reactive lymphocytotoxic antibodies (LCTA) were assayed on lymphocyte targets from three donors. Properly stored samples of serial bleeds from three patients, all of whom remained asymptomatic, and single samples from six other patients were available. As shown in Table 2 moderate to high LCTA activity was detected in 1/3 symptomatic and 2/6 asymptomatic patients. Low LCTA was found in another patient from each group. However, two of the five antibody positive patients had LCTA activity at the start of procainamide therapy, indicating that these antibodies were probably not drug-induced. Pre-therapy samples from the other three positive patients were unavailable so it could not be determined if LCTA in these patients was drug-induced.

*Autoantibodies detected by immunoprecipitation*

The capacity of sera from drug treated patients to bind radiolabelled proteins and nucleic acids from HeLa cells was determined by protein-A-facilitated immunoprecipitation. Positive control sera included samples with anti-Sm and anti-SS-B/La, and autoradiograms of antigens precipitated by these sera showed the expected array of proteins and U-RNAs for Sm and the 45kD phosphoprotein, pre-5-SRNA and pre-tRNA for SS-B/La (Lerner *et al.*, 1981) (data not shown).

$^{35}S$ labelled proteins precipitated by a variety of sera from drug treated patients are shown in Fig. 3. Two bands (at 46kD and 67kD) were common to all sera (and to normal sera) and are presumably due to nonspecific binding of actin and an unidentified protein. Four of 29 sera showed unique protein bands (one negative serum not shown). A 40kD protein was precipitated by three of these sera, one of these sera (serum no. 10) also precipitated a 38kD protein, and the fourth serum (serum no. 5) precipitated three proteins of 37kD, 48kD, and 70kD.

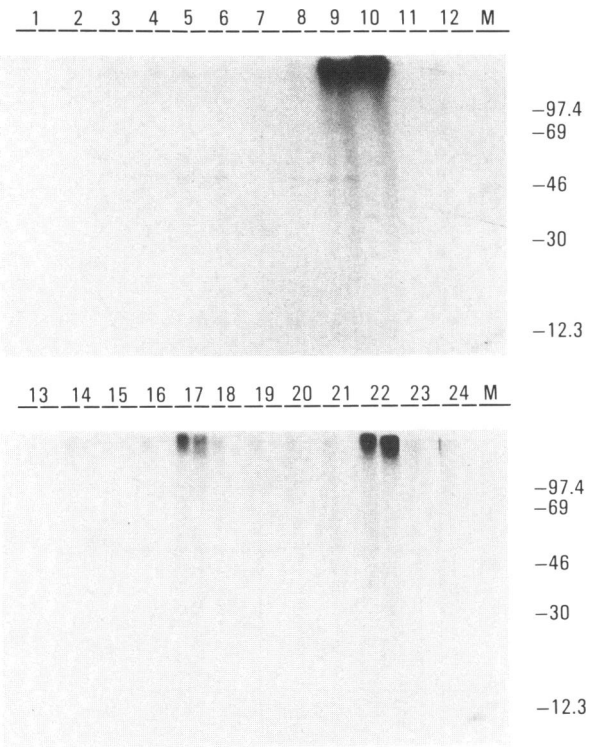


**Fig. 4.** Immunoprecipitation of $^{32}P$-labelled macromolecules from HeLa cells by sera from a variety of procainamide-treated patients. Half of each immunoprecipitate was subjected to digestion with proteinase K prior to SDS-PAGE. The total $^{32}P$ (phosphoprotein + RNA) is in the left lane and the RNA component in the right lane under the number corresponding to each patient.

Material from $^{32}$P-labelled HeLa cell extracts precipitated by 24 of 29 sera tested is shown in Fig. 4. To distinguish between $^{32}$P labelled phosphoproteins and nucleic acids, half the precipitated material was subjected to protease digestion prior to electrophoretic separation. Both the total $^{32}$P-labelled antigens and the nucleic acid only material are shown in Fig. 4. Four sera precipitated substantial $^{32}$P macromolecules which were largely insensitive to protease digestion. This nucleic acid was approximately the same size and heterogeneity for all sera, and analysis on agarose gels showed that it consisted of 18S and 28S RNA (data not shown). Two of these sera (nos 10 & 17) also precipitated the 40kD protein shown in Fig. 3. Therefore 6/29 sera had antibody to a discrete RNA and/or protein antigen in HeLa cells, but the remainder of the sera (79%) had no autoantibodies detectable by immunoprecipitation.

## DISCUSSION

Antibodies elicited by procainamide were unambiguously identified in patients from the prospective study group because serum samples were available throughout the induction period. Anti-histone and anti-dDNA antibodies appeared in most patients after 2 months of therapy, and their levels progressively increased so that after 1 year there was an average of a nine-fold increase in anti-histone and a four-fold increase in anti-dDNA. In contrast, antibodies to tetanus toxoid or rheumatoid factor did not significantly change during this period, suggesting that procainamide did not induce a nonspecific polyclonal activation or otherwise cause a global antibody response. The specific induction of anti-histone and anti-dDNA antibodies is also reflected in the decrease in these antibodies following withdrawal of procainamide.

Other autoantibodies have been reported in symptomatic and asymptomatic procainamide-treated patients (Blomgren *et al.*, 1972; Klajman *et al.*, 1972; Winfield *et al.*, 1975; Klajman *et al.*, 1975; Bluestein *et al.*, 1979). We searched for additional antibody activities by immunofluorescence using mouse kidney sections and Hep-2 cells and by immunoprecipitation of HeLa cell extracts. In addition to patients in a prospective study group and a group with clinical lupus, we included patients who had been treated for years with procainamide without developing lupus symptoms in order to increase the number and variety of samples. Little additional antibody activities were detected by these techniques in any of the patient groups. Anti-nuclear antibody were invariably present, but absorption studies indicated that histone-reactive antibodies accounted for the ANA activities. Although it is generally assumed that DNA is necessary to confer antigenicity to nucleohistone, the capacity of sera to react with naked histones and the correlation and quantitative similarity between anti-histone and anti-chromatin activities (data not shown) suggest that histones alone retained full antigenicity.

Extracts of radiolabelled cells have become a widely used source of nuclear and cytoplasmic antigens reactive with autoantibodies in human sera (McNeilage, Whittingham & MacKay, 1984). However, we detected antibody activity in only six of 29 sera from drug-treated patients by immunoprecipitation of HeLa cell extracts. Although most of the sera had anti-histone antibodies, histones were not detected because of their low methionine content and/or insolubility in the cell lysate. Four sera precipitated ribosomal RNA and two of these sera also precipitated a 40kD protein. Three of these patients had been treated with procainamide for an average of 7 years without developing drug-induced lupus and the fourth patient had drug-induced lupus at the time the sample was obtained. Winfield *et al.* (1975), reported an anti-ribonucleoprotein activity predominantly in patients recently placed on procainamide after an acute myocardial infarction. However, none of the four patients in the present study had recent myocardial infarctions when sampled, and RNA was not immunoprecipitated by other sera obtained from patients soon after initiation of therapy. Matter, *et al.* (1982) reported an antibody (Ga) from SLE patients which precipitated high molecular weight RNA and a 34kD protein, similar to the activity we observed in a few patients. Further studies are directed to determining whether the observed activity is related to anti-dDNA antibodies which were present in high levels in all these sera.

Anti-lymphocyte antibodies (Bluestein *et al.*, 1979) and rheumatoid factor (Blomgren *et al.*, 1972) have been reported in procainamide-induced lupus. We also detected these antibodies in

prevalences similar to those previously reported. However, where serial samples were available, patients with elevated RF or LCTA invariably had those activities at the start of procainamide therapy, and their levels did not increase during treatment. It appears, therefore, that LCTA and RF were not drug-induced in these patients. The common presence of these antibodies in the procainamide-treated population may be related to the high incidence of RF and LCTA in the elderly (Hallgren, *et al.*, 1970; Goodwin, Searles & Tung, 1982).

The basis for the selective elicitation of antibodies to histones and dDNA by procainamide is not clear. Direct immunization by drug-modified antigen cannot readily account for the diverse array of anti-histone antibody specificities within the patient population (Rubin *et al.*, 1985). Furthermore, the obvious lack of primary structure similarity between histones and dDNA, the absence of unique antigenicity and immunogenicity of procainamide-DNA complexes compared to DNA alone (Blomgren *et al.*, 1972) and the inability of procainamide to block anti-dDNA (Klajman *et al.*, 1970), ANA (Wittingham *et al.*, 1972) or anti-histone (unpublished observations) suggest that loss of tolerance by immunization with drug-altered antigens or by procainamide as the hapten is unlikely to explain drug induction of autoantibodies.

Long-term exposure to procainamide may have an enhancing effect on humoral immunity as suggested by the presence of increased numbers of immunoglobulin secreting cells (Ochi *et al*, 1983) and the elevated secretory capacity of lymphocytes isolated from procainamide-treated patients (Miller & Salem, 1982). Since anti-histone and anti-dDNA are the predominant autoantibodies induced by procainamide, it might be expected that chromatin, the naturally occurring histone-DNA complex, may be rendered immunogenic in a predisposed host by virtue of its unique multivalent structural features or possibly due to an unusual in-vivo localization or degradative pathway after release from cells. Investigations from several directions including the nature of the putative immunogen, the identity of the cells which regulate autoantibody expression as well as the mechanism underlying procainamide-induced hyper-B cell activity may be needed to unravel the basis of this form of autoimmunity.

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