

Antibodies to the five histones and poly(adenosine diphosphate-ribose) in drug induced lupus: implications for pathogenesis

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SUMMARY Certain drugs are a frequent source of antinuclear antibody (ANA) induction, and ANA is invariably present in the few patients who progress to the drug induced lupus syndrome. This report concerns the fine specificity of the ANA response to hydralazine, penicillamine, and sulphasalazine therapy. Using highly purified individual histones in fluorimetric assays, antihistone antibodies are always detectable, often in large amounts, but the pattern of response to individual histones is variable and not drug specific. In addition to the response to the three histones H1, H2B, and H3 reminiscent of idiopathic systemic lupus erythematosus, antibody to histone H2A predominates in some drug induced cases. Contrary to previous thought, histones are not the sole target of the antinuclear response: we also demonstrate a significant correlation between ANA titre and antibody to poly(adenosine diphosphate-ribose). Like the histones, this is a macromolecule that can bind to deoxyribonucleic acid (DNA). It is proposed that drug induced damage to chromatin leads to ANA production, while drug induced impairment of complement activity may then enable these autoantibodies to mediate the lupus syndrome.

Key words: antinuclear antibody, adverse drug reaction, hydralazine, D-penicillamine, sulphasalazine, prizidilol.

Antinuclear antibodies (ANA) often develop during long term therapy with a number of drugs, though the drug induced lupus syndrome is less common.¹⁻⁴ Such ANA are associated with the LE cell phenomenon and generally give the homogeneous pattern of nuclear immunofluorescence⁵⁻⁹ characteristic of chromatin antigens such as DNA and histones. With few exceptions (captopril and penicillamine), very little antibody is induced to native DNA, and recently attention has turned to the histones. By an immunofluorescence technique on acid extracted tissue reconstituted with histones, antihistone antibodies were said to be the sole ANA specificity in patients with the lupus syndrome induced by procainamide or isoniazid,⁵ yet in the

case of hydralazine such antibodies were not detected,^{6,7} or were restricted to patients with active drug induced lupus syndrome.⁸ By radio-immunoassay,⁷ antihistone antibodies were found to be induced by hydralazine as well as procainamide, but only the larger amounts induced by procainamide were detectable on tissues reconstituted with histones. It was suggested that these drugs also differ in the type of histone to which antibodies are induced, but it has remained an open question whether other nuclear antigens are involved.

The five histones are small, highly conserved, DNA binding proteins, rich in basic amino acids but lacking sequence homology.¹⁰ In idiopathic systemic lupus erythematosus (SLE), antihistone antibodies are common, albeit often in modest amounts, and they react mainly with histones H1, H2B, and H3.¹¹⁻¹³ In rheumatoid arthritis with vasculitis the response is chiefly to histones H2A, H2B, and

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Table 1 Serological results and clinical details of the patients under study

Patient code	Drug	Disease under treatment	Lupus syndrome	Rheumatoid factor	Antibody to soluble cellular antigens	DNA binding (%)*	Poly(ADP-ribose) binding (%)*	ANA titre	ANA on histone reconstituted tissue	Histone giving peak binding in fluorimetric assay
A	Hydralazine	Hypertension	+	-	-	20	47	2560	ND	H3
B	Hydralazine	Hypertension	+	-	-	20→ND†‡	10→5	640→40	-	H2B
C	Hydralazine	Hypertension	+	-	-	13	59	≥2560	-	H1
D	Hydralazine	Hypertension	+	-	-	18	38	640	ND	H1
E	Hydralazine	Hypertension	+	-	-	18	26	160	-	H3
F	Hydralazine	Hypertension	+	-	-	10	24	640	-	H2A
G	Hydralazine	Hypertension	+	-	-	12	25	640	-	H2A
H	Hydralazine	Hypertension	+	-	-	10	ND	≥2560	-	H1
I	Hydralazine	Hypertension	+	-	-	ND	59	≥2560	-	H3
J	Hydralazine	Hypertension	-	-	-	ND	26	160	-	H2B
K	Hydralazine	Hypertension	-	-	-	ND	6	40	ND	H1
L	Penicillamine	Rheumatoid arthritis	+	+	-	67→14	32→21	640→40	ND	H2B
M	Penicillamine	Rheumatoid arthritis	-	+	-	62→14	53→48	≥2560→160	ND	H1
N	Sulphasalazine	Ulcerative colitis	+	-	-	31→7	ND	≥2560→160	ND	H2B
O	Sulphasalazine	Ulcerative colitis and MCTD†	-	-	+(RNP)†	19	ND	≥2560	ND	H2B

*Normal DNA binding<30%; normal poly(ADP-ribose) binding<20%.

†MCTD=mixed connective tissue disease; RNP=ribonucleoprotein; ND=not done.

‡Horizontal arrows indicate change in titre after drug withdrawal.

H4.^{13 14} For the present study we have used the same highly purified histones and the same solid phase fluorimetric assay as in our previous studies¹³⁻¹⁵ to describe the fine specificity and variety of antihistone antibodies induced by three drugs in common use—hydralazine, sulphasalazine, and D-penicillamine.^{1 16 17} We go on to show that the drug induced antinuclear response involves not only histones but at least one other chromatin associated antigen, poly(adenosine diphosphate-ribose).

Patients and methods

As shown together with the serological and diagnostic data in Table 1, serum was obtained from 10 patients with drug induced lupus and four patients with a high or rising titre of ANA during drug therapy. In addition, a patient with mixed connective tissue disease and ulcerative colitis was studied while under treatment with sulphasalazine. Eight healthy controls were included in the histone binding assays. A further 25 sera were included in the Farr assay for antibody to poly(adenosine diphosphate-ribose); these were serial samples from nine patients who developed ANA during treatment with the antihypertensive drug prazidilol.¹⁸

The drug induced lupus syndrome was diagnosed on clinical grounds^{1 3} and confirmed by its resolution after withdrawal of the drug. The most frequent features were arthralgias or arthritis, myalgias, malaise, weight loss, anaemia, and raised erythrocyte sedimentation rate.³ In addition patient A had cutaneous vasculitis,¹⁹ patient L developed increased DNA binding by the Farr assay,²⁰ and patient N had a pleural effusion. All these clinical features resolved within a few weeks of discontinuing therapy with the offending drug.

FLUORIMETRIC ASSAY

The fluorimetric assay was performed as described in detail elsewhere,¹⁴ using histones purified and generously provided by Dr E W Johns.²¹ Polystyrene EIA cuvettes (Gilford) coated with tyrosine-glutamic acid (1:1) copolymer of mol.wt 66 000 (Miles-Yeda) followed by histone were incubated with 5% serum in 0.1% Tween borate-saline buffer pH 8.2 and then fluorescein isothiocyanate (FITC) labelled sheep antihuman immunoglobulin, IgG or IgM (Wellcome). For the IgG subclass assays, FITC labelled sheep antiserum was replaced by unlabelled sheep antihuman IgG subclass specific antiserum (Miles) (1 vol antiserum: 5 vol normal rabbit serum: 24 vol borate-saline-Tween 20 buffer) followed by an additional overnight incubation with FITC labelled rabbit antiserum IgG (Miles) (1 vol conjugate: 5 vol normal rabbit serum: 114 vol Tween

buffer). The conjugate was released by 1 ml 0.1 M NaOH containing 0.1% sodium dodecyl sulphate. Fluorescence was measured on a fluorimeter (Locarte, London) and expressed in arbitrary units.

ANA DETERMINATION

Sera were tested in fourfold dilutions, 1/10–1/2560, on rat liver frozen sections using FITC labelled sheep antihuman whole immunoglobulin (Wellcome, Dartford).³

HISTONE RECONSTITUTION ASSAY

Using the method of Tan *et al.*,^{5 22} serum dilutions (1/10–1/2560) in phosphate buffered saline (PBS) were tested by indirect immunofluorescence on acetone fixed, mouse kidney frozen sections which were (a) extracted with HCl (0.1 M) for 30 min at room temperature, washed, and incubated with PBS (30 min); or (b) extracted with 0.1 M HCl, washed, and reconstituted by incubation with total histones or histone fractions (Millipore) at 25 µg/ml in PBS (30 min); or (c) incubated in PBS throughout.

FITC labelled sheep antihuman immunoglobulin (Wellcome) was employed, and a positive control antihistone serum was kindly provided by Dr E M Tan.

POLY(ADENOSINE DIPHOSPHATE-RIBOSE) BINDING ASSAY

Antibody to ³H labelled poly(adenosine diphosphate-ribose), synthesised *in vitro* from [³H]nicotinamide adenine dinucleotide and kindly provided by Dr M Tavassoli and Professor S Shall, was measured by the Farr assay as described in detail previously.²³ Sera from 20 healthy controls and 205 patients with various idiopathic autoimmune diseases were studied at the same time.²³

OTHER AUTOANTIBODIES

Antibody to ³H labelled native DNA (Amersham, UK) was measured by the Farr assay²⁴; antibodies to soluble cellular antigens were sought using counterimmunoelectrophoresis²⁵; rheumatoid factor was detected by the slide latex test (Ortho Diagnostics, UK).

Results

ANTI-HISTONE ANTIBODIES BY FLUORIMETRIC ASSAY

Controls

Using the five purified histones, normal ranges were established for the whole immunoglobulin (Ig), IgG, and IgM specific assays (Table 2). In the IgG subclass specific assays three control sera gave binding values of 0.02–0.05 with IgG₁, IgG₂, and IgG₄, and of 0.03–0.07 with IgG₃.

Table 2 Histone binding values (fluorimeter units) using sera from healthy controls in assays specific for total immunoglobulin (Ig) and the IgG and IgM classes

	H1	H2A	H2B	H3	H4
Ig (n=8)					
mean	0.098	0.086	0.068	0.096	0.092
mean+2SD	0.12	0.11	0.90	0.12	0.10
IgG (n=5)					
mean	0.030	0.026	0.025	0.034	0.035
mean+2SD	0.043	0.034	0.029	0.039	0.043
IgM (n=5)					
mean	0.047	0.040	0.040	0.067	0.051
mean+2SD	0.058	0.049	0.054	0.117	0.062

SD=standard deviation; mean+2SD was set as the upper limit of normal.

Hydralazine

Histone binding was raised in all 11 cases of hydralazine induced ANA (Figs 1 and 2), whether the patient had the lupus syndrome (A-H) or not (I-K). Antibodies to all five histones were above normal in each case, but often reactivity with one or two histones was predominant. Peak binding involved H1, H2A, H2B, and H3 in different cases;

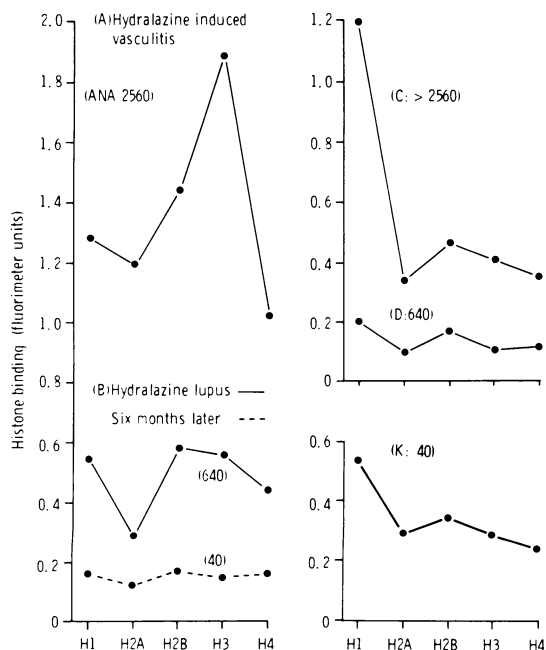


Fig. 1 Patterns of immunoglobulin binding to the five individual histones obtained with sera from four patients with the hydralazine induced lupus syndrome (A-D) and one with hydralazine induced ANA (K). The ANA titres are shown in parentheses.

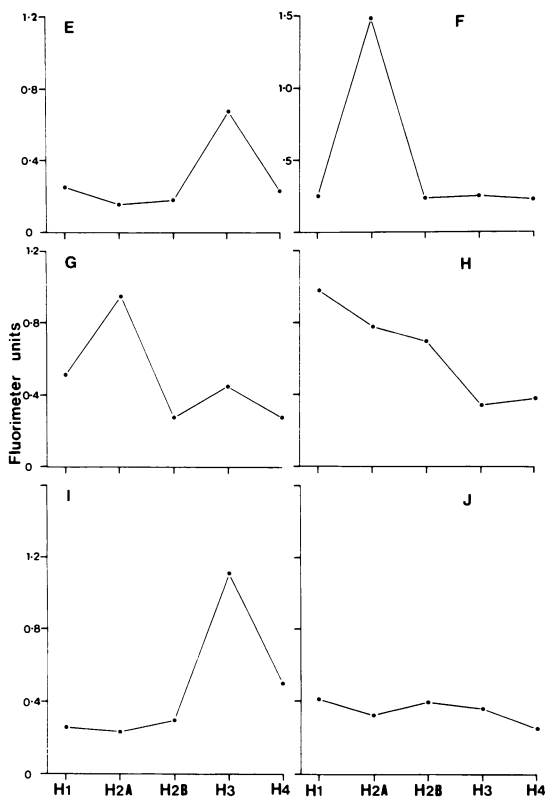


Fig. 2 Patterns of immunoglobulin binding to the five histones obtained with serum from four patients who had hydralazine-lupus (E-H) and two patients with just hydralazine induced ANA (I, J).

there was no consistent pattern. The highest binding values were obtained with serum from patient A who had drug induced cutaneous vasculitis.¹⁹ Patient B was followed up longitudinally and showed parallel falls in ANA titre and histone binding over the six month period after discontinuing hydralazine therapy. Six sera were tested for IgG and IgM class specific histone binding (Fig. 3); the response was chiefly IgM antibody in three cases (E, I, J), IgG in one case (F), and mixed in two cases (G, H). The IgG subclass reactivity (Fig. 4) mirrored the pattern of whole IgG binding to a varying extent. Histone binding was generally higher than in idiopathic SLE,¹³ but some sera (D, J, K) gave modest binding despite high ANA titres.²⁶

D-Penicillamine

Two patients (L, M) under treatment with D-penicillamine for rheumatoid arthritis were selected

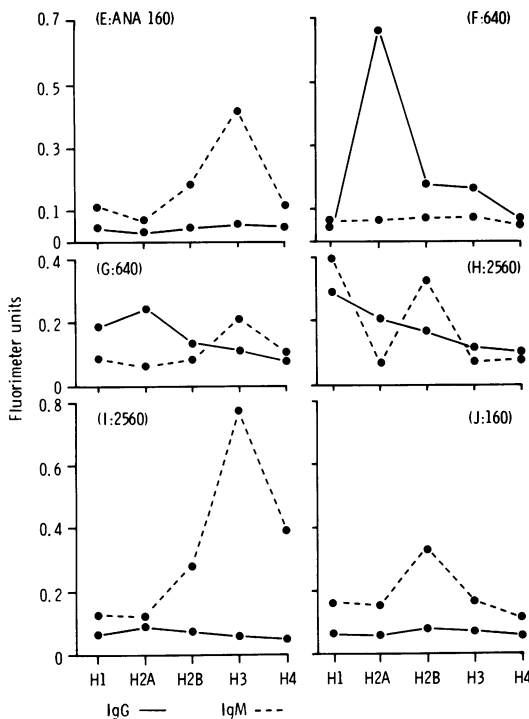


Fig. 3 Patterns of IgG and IgM binding to the histones using the same sera as in Fig. 2. ANA titres are shown in parentheses.

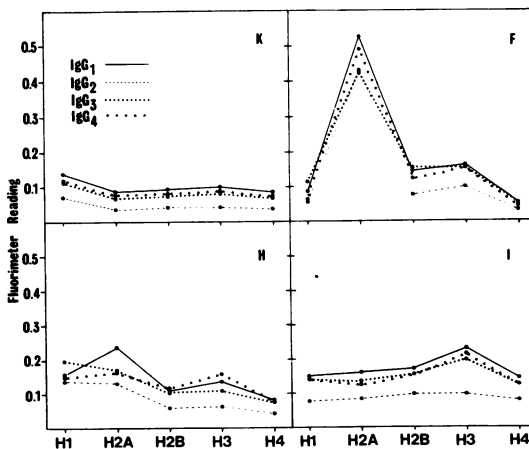


Fig. 4 Patterns of IgG subclass specific histone binding using sera from two patients with hydralazine-lupus (F, H) and two with ANA without lupus (I, K).

because high DNA binding activity had appeared during therapy; in one case (L) there was a clear cut clinical deterioration with malaise, fever, and worsening arthritis that resolved on drug withdrawal. Both sera gave raised histone binding (Fig. 5), but the patterns differed, showing similar rises to all histones in one case (L) and a peak of anti-H1 activity in the other (M). In both cases, ANA titre, histone binding, and DNA binding fell in the months after drug withdrawal.

Sulphasalazine

Serum from two ANA positive patients receiving sulphasalazine therapy for ulcerative colitis showed raised histone binding. In both cases this was predominantly to H2B (Fig. 5). One patient (N) had drug induced lupus, whereas in the other (O) the rheumatic disease (mixed connective tissue disease) was idiopathic and preceded sulphasalazine therapy.

ANTIHISTONE ANTIBODY ON TISSUE

SECTIONS RECONSTITUTED WITH HISTONES

We compared our fluorimetric assay with the older immunofluorescence ANA technique for antihistone antibodies.²² Serum samples from six patients with hydralazine induced lupus and from two patients with ANA but without lupus were tested for antihistone antibodies by immunofluorescence on tissue sections that had been extracted with acid and reconstituted with total histones or the H2A/H2B fraction. In no case was antihistone antibody detected in this way (Table 1), though the positive control serum reacted strongly. We conclude that the antigens recognised by hydralazine induced ANA are not generally reconstituted by this technique. Various explanations will be discussed but these data raise the possibility that some non-histone antigen is involved.

ANTIBODY TO POLY(ADENOSINE

DIPHOSPHATE-RIBOSE) INDUCED BY DRUGS

We reported previously that antibodies to the cellular macromolecule poly(adenosine diphosphate-ribose) can be induced by three drugs that induce ANA.²³ The relation between ANA titre and the Farr assay result for poly(adenosine diphosphate-ribose) binding is shown in Fig. 6. The data include those from Table 1 (concerning hydralazine and penicillamine) and additional serial measurements made in nine patients who developed ANA during treatment with prizidolol, an antihypertensive agent structurally related to hydralazine.¹⁸ Six (75%) of eight patients with drug induced lupus and nine (64%) of 14 further patients with drug induced ANA at a titre of at least 1/160 gave poly(ADP-ribose) binding above 20%. By way of

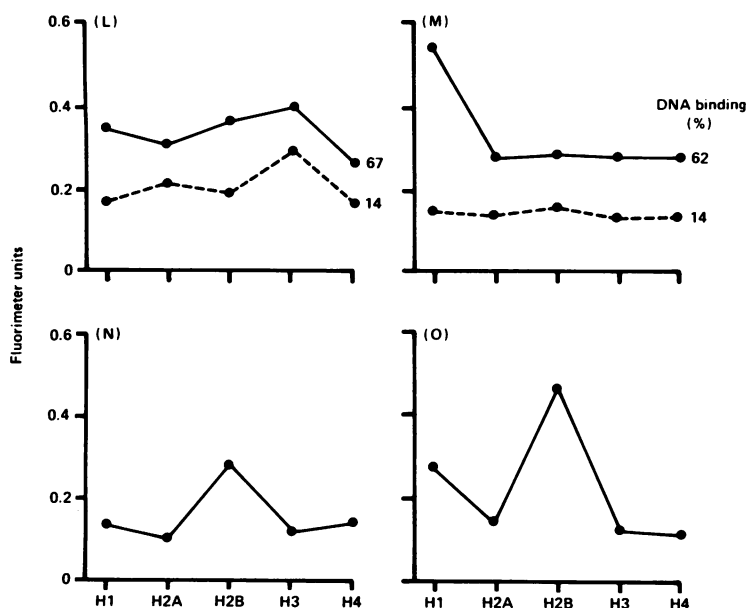


Fig. 5 Upper panels: histone binding and DNA binding induced by D-penicillamine (L, M) just before (—) and about six months after (---) cessation of therapy with this drug. Lower panels: histone binding patterns in a patient with sulphasalazine induced lupus (N) and a patient with pre-existing mixed connective tissue disease and ulcerative colitis who was treated with sulphasalazine (O). (Reproduced by permission of the University Department of Medical Illustration, Royal Infirmary, Manchester M13 9WL, UK.)

comparison only 54% of 61 SLE sera and 3% of 144 other rheumatic and autoimmune disease controls gave binding values of 20% or more; the highest binding value shown in SLE was 64%.²³ The correlation evident in Fig. 6 between ANA titre and the Farr assay result for poly(adenosine diphosphate-ribose) binding is confirmed by regression analysis ($p < 0.01$).

Discussion

Our results show that high levels of antihistone antibody can be induced by therapy with hydralazine, penicillamine, and sulphasalazine, but that the pattern of response to individual histones is variable. Antihistone antibody is not the sole antinuclear specificity involved, since antibody to another chromatin associated antigen, poly(adenosine diphosphate-ribose), is present in amounts that correlate with the ANA titre.

Antihistone antibodies occur in a variety of conditions^{13 14 22} and are not therefore specific for drug induced lupus. The antihistone response in drug induced lupus is generally greater than in idiopathic SLE, and the pattern is more variable, with histones H1, H2A, H2B, and H3 predominant in different cases. As in SLE, there was relatively little antibody to histone H4, but in contrast with SLE (where the response to H2A is usually small and never predominant¹³), H2A gave peak binding in two of the eight cases of hydralazine-lupus. The various patterns of antihistone response exclude a systematic bias in the sensitivity of the assays for antibody to each histone. This is emphasised by disease related differences in the pattern of response¹³: in SLE the response to H1 greatly exceeds that to H4, whereas the reverse is true in rheumatoid vasculitis (Fig. 7). The various patterns of drug induced histone binding showed no correlation with the titre of ANA, the presence or

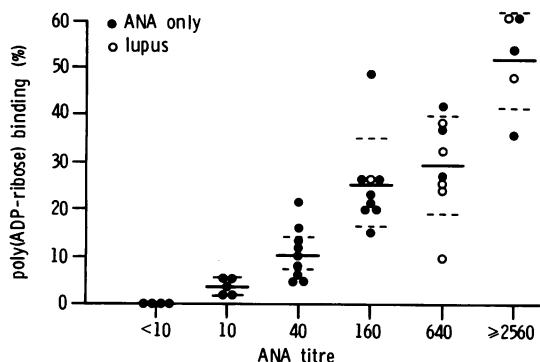


Fig. 6 Relation between poly(adenosine diphosphate-ribose) binding measured by the Farr assay and ANA titre measured on rat liver substrate (○=drug induced lupus; ●=drug induced ANA without lupus). The data include 15 measurements on 12 patients shown in Table 1 and a further 25 serial measurements in nine hypertensive patients who developed ANA while being treated with prizidilol.^{18 23}

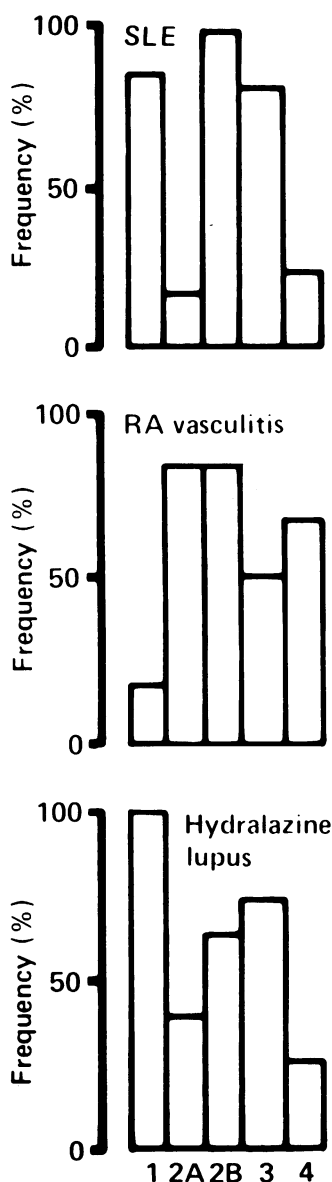


Fig. 7 Disease related patterns of antihistone response. The histograms compare idiopathic SLE, rheumatoid arthritis with vasculitis, and the lupus syndrome induced by hydralazine (data from this study and Ref. 13), showing the frequency with which each histone gave binding values ranked in the top three as opposed to the bottom two results for each serum. When SLE and rheumatoid vasculitis are compared the differences for H1, H2A, and H4 are all significant ($p < 0.001$, 0.001 , 0.03 respectively, by χ^2 test with Yates's correction).

absence of drug induced lupus, or the drug involved. The heterogeneity of antihistone specificity may reflect mixtures of antibodies to individual histones or antigenic cross reactivities between these proteins. The important point is that individuals differ, but within strict bounds, in their autoimmune response to a particular agent.

Some differences in autoimmune response may be drug related. Rubin *et al* emphasised a switch from IgM to IgG class antihistone antibody at the time procainamide induced lupus appears,²⁶ but we could not confirm this with hydralazine. Antihistone antibody was chiefly IgM in one of four hydralazine-lupus cases, and our previous studies showed over 60% of hydralazine induced ANA were a mixture of IgG and IgM whether or not the patient had lupus.²⁷

A further difference from procainamide is that the ANA induced by hydralazine is usually not detectable on tissues extracted with acid and reconstituted with histones. This lack of reactivity with histone reconstituted tissues is now well established⁶⁻⁸ (M Fritzlter, personal communication) and indicates a distinction between the ANA specificities induced by hydralazine and procainamide (made apparent because the nucleosome is not reconstituted in native form²⁸). The amounts of antihistone antibody and total ANA are not always correlated,²⁹ and our present results emphasise that additional antibody-antigen reactions are involved. A monoclonal antibody to poly(adenosine diphosphate-ribose) gives homogeneous nuclear staining,³⁰ and in the present study there is a close correlation between ANA titre and the Farr assay for antibody to poly(adenosine diphosphate-ribose). Antibody to double stranded DNA may also rise a little,²³ but cross reactivity between poly(adenosine diphosphate-ribose) and DNA is minimal.³¹ Cross reactivity between poly(adenosine diphosphate-ribose) and histones has not been excluded by absorption studies but seems unlikely because there was no correlation between the amounts of antibody to these dissimilar antigens.

Histones, poly(adenosine diphosphate-ribose), and DNA are all components of chromatin, and the question arises as to how drugs induce autoimmunity to these macromolecules. Immunisation of animals with hydralazine conjugated to histones or albumin induces antibodies to the drug³² and to single stranded DNA,³³ but no antihistone antibodies arise.³² Stollar and Ward raised antihistone antibodies by immunisation of rabbits with histone-RNA complexes,³⁴ and it may be relevant, therefore, that hydralazine and procainamide can bind to DNA.³⁵⁻³⁶ Such altered DNA might overcome T cell tolerance,³⁷ permitting an antihapten response to associated macromolecules. It may be relevant both

that poly(adenosine diphosphate-ribose) is thought to play a part in DNA repair,³⁸ and that high titres of antihistone antibodies have been associated with photosensitivity in SLE.¹³ Hardin and his colleagues have shown that the antigenic regions on histones, as detected by immunoblotting using histone fragments, are located mainly on the surface of the nucleosome, suggesting that this whole particle is the immunogen.^{12, 39} The induction of antilymphocyte antibodies^{40, 41} and the expression of DNA⁴² and histones⁴³ on lymphocyte surfaces raise the possibility that cell surface material rather than nuclear chromatin is rendered immunogenic by interaction with drugs.

The frequency of ANA induction can be as high as 50–90% depending on the drug, the dose, and the duration of therapy, yet drug induced lupus supervenes less often.^{1–4} Female sex, polymorphic drug metabolism (slow acetylation), and HLA phenotype (DR4) have been implicated as risk factors for the disease,^{1–4, 44} and a further important factor may be the ability of complement to clear immune complexes.⁴⁵ Null and non-functioning allotypes of C4 appear to be increased in drug induced lupus,⁴⁶ as also in patients with idiopathic SLE^{27, 47} and their asymptomatic relatives with autoantibodies.⁴⁸ Several lupus inducing drugs block the binding site of activated C4, with some allotypes perhaps more susceptible than others.⁴⁹ Reidenberg pointed out previously that 'pharmacological' rather than 'immunological' drug concentrations are required for the development of drug induced lupus.⁵⁰ Hence the development of drug induced lupus may depend on a two pronged assault by the drug on chromatin and complement.

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