*Clin. exp. Immunol.* (1984) **58**, 29–36.

# Presence of Z-DNA specific antibodies in Crohn's disease, polyradiculoneuritis and amyotrophic lateral sclerosis

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*(Accepted for publication 11 April 1984)*

## SUMMARY

Two modified polynucleotides having the Z-DNA conformation (poly [dG-dC] dien Pt and poly [dG-br$^5$dC]·poly [dG-br$^5$dC]) have been used for determination of antibodies to Z-DNA. Such antibodies were found in sera of patients with systemic lupus erythematosus and with Crohn's disease. They were scarcely observed in polyradiculoneuritis and in amyotrophic lateral sclerosis. In Crohn's disease sera, no antibodies to B-DNA were ever found but presence of two different families of antibodies to Z-DNA was demonstrated

**Keywords** Z-DNA antibodies Crohn's disease polyradiculoneuritis amyotrophic lateral sclerosis multiple sclerosis

## INTRODUCTION

The discovery that alternating d (CpG) DNA fragments crystallize as a left handed double helical molecule (Wang *et al.*, 1979; Drew *et al.*, 1980) raises the question of whether this form, called Z-DNA, exists in solution. Numerous studies have shown that synthetic polynucleotides and segments of natural DNA can adopt the Z conformation (Rich, 1983; Leng, 1983). Up to now almost all, if not all, the assays to elicit in rabbits antibodies to right handed double stranded DNA (B form) by immunization with native DNA have failed (Stollar, 1973). On the other hand, two recent studies (Lafer *et al.*, 1981; Malfoy & Leng, 1981) have shown that the antisera of rabbits immunized with left handed double stranded poly (dG-dC)·poly (dG-dC) (Z form) gave a strong positive reaction with Z-DNA. Moreover, the presence of antibodies to Z-DNA in human systemic lupus erythematosus (SLE) sera was demonstrated by Lafer *et al.* (1983). A small number of sera from patients with rheumatoid arthritis, scleroderma and juvenile rheumatoid arthritis gave a reaction above background.

The presence of antibodies to double stranded DNA (B form) was demonstrated in the cerebrospinal fluid (CSF) of some patients with infectious (viral) processes of the central nervous system (Allinquant *et al.*, 1982). The present study is an attempt to search for antibodies specific to Z-DNA especially in diseases where an autoimmune process is suspected.

Poly (dG-dC)·poly (dG-dC) has the Z conformation in high salt concentration (Pohl & Jovin, 1972). Several chemical modifications of base residues can stabilize the Z conformation as the fixation of monofunctional platinum derivative (dien Pt) on the N7 of guanine residues (Malfoy, Hartman & Leng, 1981), methylation of cytosine residues (Behe & Felsenfeld, 1981) bromination of cytosine and guanine residues (Malfoy, Rousseau & Leng, 1982; Lafer *et al.*, 1981). These modified polynucleotides have been used in this work. As will be shown, sera from SLE react with

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poly(dG-dC) dien Pt and poly (dG-br$^5$dC)·poly (dG-br$^5$dC) while some sera from Crohn's diseases react with one of the two polynucleotides but not with both of them.

## MATERIALS AND METHODS

*Population investigated.* (a) Thirteen sera from 13 healthy donors (20–40 years old); (b) 15 sera from 15 patients with SLE; (c) neurological diseases: 72 matched sera and CSF and two single sera from 72 patients: 35 polyradicyloneuritis (PRN) 13 degenerative processes (including seven amyotrophic lateral sclerosis (ALS), one Alzheimer disease, one Parkinson and four Behçet syndromes), 11 multiple sclerosis, eight infectious processes (including five subacute sclerosing panencephalitis, two B-hepatitis with neurological complications and one schistosomiasis of the central nervous system) and five miscellaneous and (d) 15 sera from 10 patients with Crohn's disease.

*Chemicals.* Poly (dG-dC)·poly (dG-dC), poly (dG-m$^5$dC)·poly (dG-m$^5$dC) and poly (dT) were from PL Biochemicals (St Goar, Federal Republic of Germany). $PM_2$ DNA was bought from Boehringer (Mannheim, Federal Republic of Germany), and poly (I)·poly (C) was purchased from Choay (Paris, France). $^3$H-poly (dG-dC)·poly (dG-dC) and $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC) ($0{\cdot}5 \times 10^6$ ct/min/$\mu$g) were synthesized with *Escherichia coli* DNA polymerase large fragment (Boehringer) in presence of $^3$H-dGTP (New England Nuclear, Boston, Massachusetts, USA) and unlabelled dCTP or br$^5$dCTP (PL Biochemicals) (Malfoy *et al.*, 1982). Other nucleotides were purchased from Sigma (St Louis, Missouri, USA). S1 nuclease treated *E. coli* $^{14}$C-DNA was from Amersham (UK).

The chemical modification of poly (dG-dC)·poly (dG-dC) and poly (dG-m$^5$dC)·poly (dG-m$^5$dC) by chlorodiethylenetriamine platinum (II) chloride (dien Pt) has been previously described (Malfoy *et al.*, 1981). We write poly(dG-dC) dien Pt (0·12) or poly (dG-m$^5$dC) dien Pt (0·12) for poly (dG-dC)·poly(dG-dC) or poly (dG-m$^5$dC)·poly (dG-m$^5$dC) modified by dien Pt on 12% of the bases. In order to achieve conformational equilibrium all solutions were heated at 50°C for 10 min and then cooled at room temperature before use.

Bromination of poly (dG-dC)·poly (dG-dC) was prepared as described by Lafer *et al.* (1981): we shall write poly br (dG-dC)·poly br (dG-dC).

*Radioimmunoassays.* Antibodies to Z-DNA have been screened with two tracers: $^3$H-poly (dG-dC) dien Pt (0·12) and $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC). In the conditions used (0·2 M NaCl, 5 mM Tris-HCl, pH7·3, 1 mM $MgCl_2$) the modified polynucleotides are in Z conformation (Malfoy *et al.*, 1981, 1982). Decompleted serum (1, 2·5, 5, 10 $\mu$l) in a final volume of 50 $\mu$l was incubated for 16 h at 4°C in presence of the tracer (0·3 $\mu$M). Then a specific anti-human Fc IgG (from goat) was added and after 2 h at 4°C the tubes were centrifuged. The radioactivity of the supernatant and of the pellet was counted. Each serum was tested in triplicate. The binding obtained with the highest serum concentration (10 $\mu$l) was taken into account for result. In CSF investigations the same methodology was used except that each CSF sample was concentrated up to eight times before assay.

Antibodies to B-DNA have been screened with $^{14}$C-DNA in a similar way. The concentration of the tracer was 0·25 $\mu$M in 0·1 M NaCl, 10 mM borate, 0·1mM EDTA, pH 7·6.

Competitive experiments have been performed with the following polynucleotides:

Z form: Poly (dG-dC) dien Pt (0·12);
Poly (dG-m$^5$dC) dien Pt (0·12);
Poly (dG-br$^5$dC)·poly (dG-br$^5$dC);
Poly br (dG-dC)·poly (dG-dC).
B form: $PM_2$ DNA;
Calf thymus DNA purified by methyl albumin kieselgühr column (Sueoka & Cheng, 1967);
Poly (dG-dC)·poly (dG-dC).
A form: Poly (I)·poly (C).
Single stranded form: poly (dT).

*Separation of IgG.* It was obtained by DEAE cellulose according to Peterson & Sober (1962) or by protein A–sepharose (Miller & Stone, 1978).

## RESULTS

*Binding to $^3$H-poly (dG-dC) dien Pt*

The binding average in normal sera was $11{\cdot}2 \pm 2{\cdot}1\%$ (mean $\pm$s.d.): so the top limit of the normal background was 16% (mean +2 s.d.), as indicated in Fig. 1 with a dotted line. Significantly increased amounts of binding to the tracer were found in 13 patients with SLE. The average binding was $39{\cdot}7 \pm 5{\cdot}5\%$ (mean $\pm$s.e., $P < 0{\cdot}001$).

In the 72 neurological patients, an abnormal binding was observed in four patients only (three patients with PRN among 35 and one patient with ALS among seven). This binding was never found in the CSF.

Significantly increased amounts of binding to the tracer were also found in four patients with Crohn's disease among 10. The average binding was $20{\cdot}9 \pm 3{\cdot}9\%$ ($P < 0{\cdot}02$). Similar binding curves were obtained with IgG purified by chromatography (see Materials and Methods) and with the whole sera.

Patients with an abnormal binding (above 16%) were, when possible, examined some months later. Two patients (among three) with PRN had always a similarly increased binding 1 month later for the first, and 6 months later for the second. The ALS patient had an increase of this abnormal binding 6 months later.

Among the four patients with Crohn's disease and increased IgG binding to $^3$H-poly (dG-dC) dien Pt, three patients have been studied 3 months later. In the first patient such antibodies were detected during an acute phase and no reaction could be observed 3 months later during a relapse phase. IgG binding very close to the initial test (40% instead of 50%) was found in the second patient. Normal IgG binding was detected in the third patient 3 months later.

*Binding to $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC)*

Among the patients' population previously analysed with $^3$H-poly (dG-dC) dien Pt, 23 sera from 17

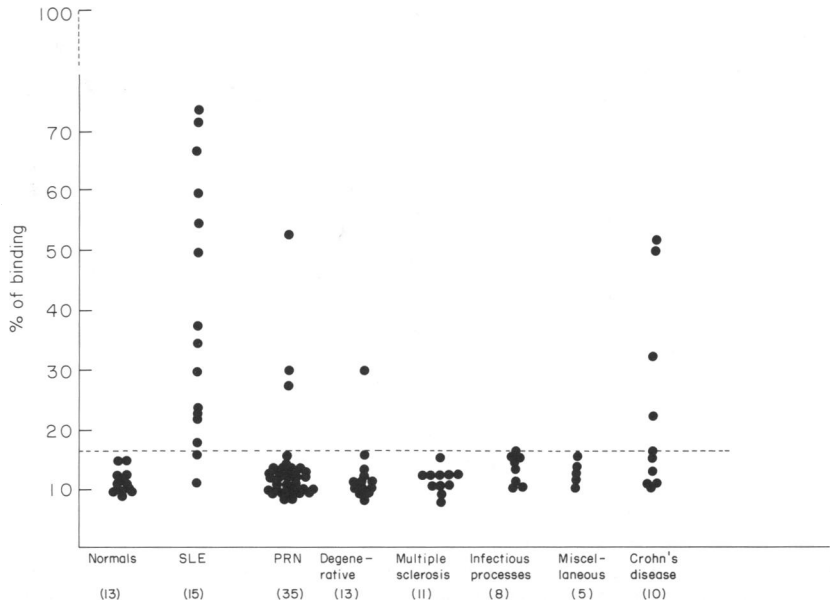


**Fig. 1.** Direct binding radioimmunoassay of 1/5 dilution of each serum reacting with $^3$H-poly (dG-dC) dien Pt.

**Table 1.** Differences in IgG binding to $^3$H-poly (dG-dC) dien Pt and to $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC)

| Diseases (number of sera) | Reactivity with* | |
|---|---|---|
| | $^3$H-poly (dG-dC) dien Pt | $^3$H-poly (dG-br$^5$dC). poly (dG-br$^5$dC) |
| SLE (5) | + | + (5) |
| PRN (3) | + | 0 (1) |
| | + | + (2) |
| ALS (1) | + | + (1) |
| Crohn (14) | 0 | 0 (7) |
| | + | 0 (2) |
| | 0 | + (3) |
| | + | + (2) |

* Binding reactivity: positive (+) if above normal background, i.e.: 16% with $^3$H-poly (dG-dC) dien Pt; 20% with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC).

patients (five SLE, three sera from two PRN, one serum from ALS, 14 sera from nine Crohn's patients) were tested with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC).

The binding average with the tracer in five normal sera was $16{\cdot}3 \pm 1{\cdot}8$ (mean $\pm$s.d.). So the top limit of the normal background is 20% (mean +2 s.d.).

Sera from 10 patients (five SLE, two PRN, one ALS and two Crohn) had both an increase of IgG binding to $^3$H-poly (dG-dC) dien Pt and to $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC). Differences were found in six sera (one PRN serum and five Crohn's sera) (Table 1). In these six sera IgG

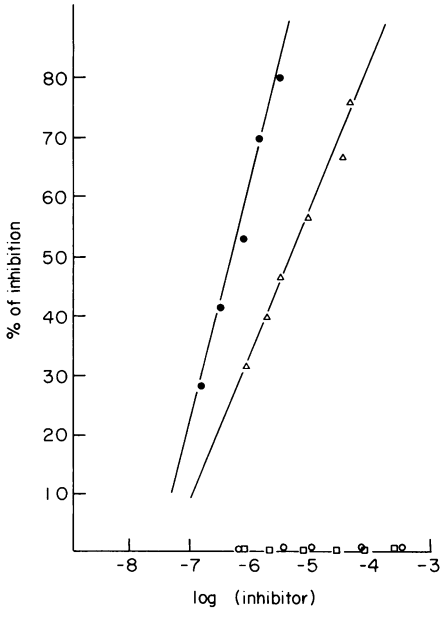


**Fig. 2.** Inhibition of $^3$H-poly (dG-dC) dien Pt binding as a function of the logarithm of the inhibition concentrations (expressed in moles of nucleotides). Binding of Crohn's serum reacting with $^3$H-poly (dG-dC) dien Pt and not with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC). ● = Poly (dG-dC) dien Pt; △ = poly br (dG-dC)·poly br (dG-dC); □ = poly (dG-m$^5$dC) dien Pt; ○ = poly (dG-br$^5$dC)·poly (dG-br$^5$dC)

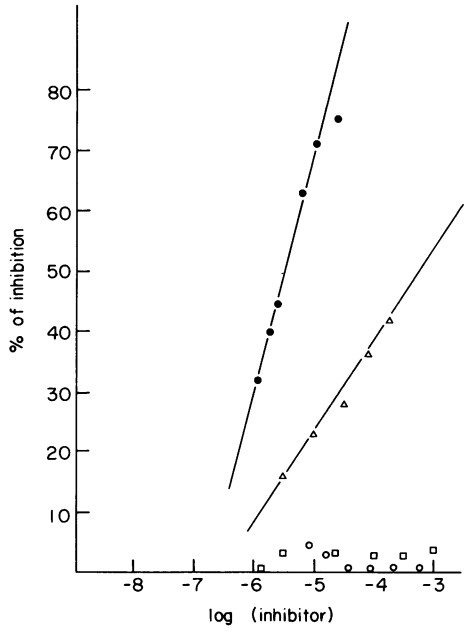


**Fig. 3.** Inhibition of $^{3}$H-poly (dG-br$^{5}$dC)·poly (dG-br$^{5}$dC) binding as a function of the logarithm of the inhibition concentrations (expressed in moles of nucleotides). Binding of Crohn's serum reacting with $^{3}$H-poly (dG-br$^{5}$dC)·poly (dG-br$^{5}$dC) and not with $^{3}$H-poly (dG-dC) dien Pt. ● = Poly (dG-br$^{5}$dC)·poly (dG-br$^{5}$dC); △ = poly br (dG-dC)·poly br (dG-dC); □ = poly (dG-m$^{5}$dC) dien Pt; ○ = poly (dG-dC) dien Pt.

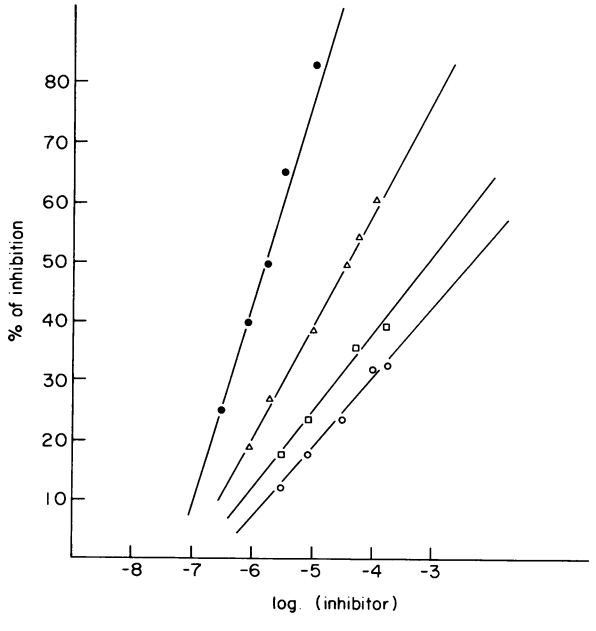


**Fig. 4.** Inhibition of $^{3}$H-poly (dG-dC) dien Pt binding as a function of the logarithm of the inhibition concentrations (expressed in moles of nucleotides). Binding of ALS serum reacting with $^{3}$H-poly (dG-dC) dien Pt and with $^{3}$H-poly (dG-br$^{5}$dC)·poly (dG-br$^{5}$dC). ● = Poly (dG-dC) dien Pt; △ = poly br (dG-dC)·poly br (dG-dC); □ = poly (dG-m$^{5}$dC) dien Pt; ○ = poly (dG-br$^{5}$dC)·poly (dG-br$^{5}$dC).

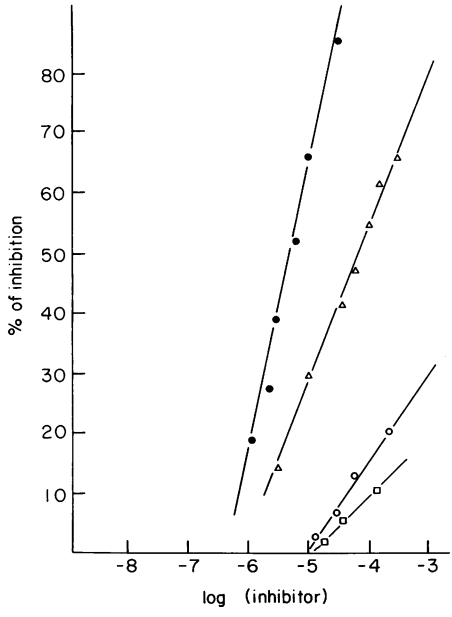


**Fig. 5.** Inhibition of $^3$H-poly (dG-br$^5$dC)·poly (dG)br$^5$dC) binding as a function of the logarithm of the inhibition concentrations (expressed in moles of nucleotides). Binding of the ALS serum reacting with $^3$H-poly (dG-dC) dien Pt and with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC). ● = Poly (dG-br$^5$dC)·poly(dG-br$^5$dC); △ = poly br (dG-dC)·poly br (dG-dC); □ = poly (dG-m$^5$dC) dien Pt; ○ = poly (dG-dC) dien Pt.

binding Z-DNA form was restricted to one type of Z-DNA only. Three sera from three patients with Crohn's disease had antibodies to $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC) only. For two of them, the binding of IgG was just above normal background.

*Competition experiments*

These assays were performed in sera of patients with Crohn's disease reacting with one of the two tracers studied and in the ALS serum reacting with both. Competition experiments with different forms of Z-DNA are given in Figs 2–5. A Crohn's disease serum reacting with $^3$H-poly (dG-dC) dien Pt was inhibited by poly (dG-dC) dien Pt and by poly br (dG-dC)·poly br (dG-dC) but not by poly (dG-br$^5$dC)·poly (dG-br$^5$dC) nor by poly (dG-m$^5$dC) dien Pt (Fig. 2). Another Crohn's serum reacting with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC) was inhibited by poly (dG-br$^5$dC)·poly (dG-br$^5$dC) and by poly br (dG-dC)·poly br (dG-dC), but not by poly (dG-dC) dien Pt nor poly (dG-m$^5$dC) dien Pt (Fig. 3).

On the other hand, the binding of the ALS serum reacting with $^3$H-poly (dG-dC) dien Pt and with $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC) was inhibited by the four Z-DNA used (Figs 4 & 5).

No inhibition of IgG binding to $^3$H-poly (dG-dC) dien Pt or to $^3$H-poly (dG-br$^5$dC)·poly (dG-br$^5$dC) was obtained using a B form or an A form or single stranded DNA or mononucleotides, suggesting their specificity for a Z-DNA form.

*Binding to B-DNA*

The whole population studied was also investigated for anti-B-DNA antibodies. These were found in 10 lupus and one PRN sera only, but also in the five CSF from the five patients with subacute sclerosing panencephalitis. In the patients' sera from Crohn's disease, no antibodies to B-DNA were ever found, but presence of antibodies to Z-DNA form was observed in seven patients out of 10. As in Crohn's disease in the ALS patient with antibodies to Z-DNA no antibodies to B-DNA and no increase of whole serum IgG (unshown results) were observed contrary to SLE patients.

## DISCUSSION

The presence of antibodies reacting with Z-DNA was recently reported in SLE (Lafer *et al.*, 1983). In the present work, IgG type antibodies reacting with Z-DNA were found in seven patients with Crohn's disease out of 10 patients and in four neurological patients.

About the Crohn's disease, two points seem to be of importance. There are no antibodies to B-DNA and there are at least two different families of antibodies to Z-DNA, suggested by inhibition experiments. It is known that there is a polymorphism of Z-DNA as observed on oligonucleotide crystals (Wang *et al.*, 1979; Drew *et al.*, 1980) and by the affinity of the antibodies to Z-DNA towards several polynucleotides having the Z conformation (Malfoy *et al.*, 1982). On the other hand, it is surprising that poly br (dG-dC)·poly br (dG-dC) is recognized by the two families of antibodies to Z-DNA in the sera of patients having a Crohn's disease. A detailed characterization of these antibodies is in progress.

The presence of these specific anti Z-DNA antibodies remains unexplained, but some facts would show they are a form of a possible autoimmune reaction. This hypothesis was clearly discussed by Lafer *et al.* (1983) in SLE patients and it is conceivable that a different antigen might stimulate formation of antibodies that cross-react with nucleic acids. A Crohn's disease is a chronic inflammatory disorder involving the gut. It's aetiology is not known but Crohn's patients have serum antibodies indistinguishable from those of ulcerative colitis patients which react with the polysaccharide antigen from colonic mucosal cells and with *E. coli* 0:14 antigen (Perlman *et al.*, 1967). Lymphocytotoxic antibodies are also present in sera of such patients as in SLE sera. Many other observations suggest that T cell-mediated immunity is altered in Crohn's disease as in SLE.

On the other hand, Latov *et al.* (1980, 1981) reported the presence of an anti-PN myelin IgM antibody reacting with a myelin associated glycoprotein in peripheral neuropathies, a fact establishing clearly an autoimmune reaction in such diseases. At least, Bradley & Krasin (1982) recently proposed a fascinating hypothesis of the aetiology of ALS: a deficiency of DNA repair with accumulation of abnormal DNA (probably related to a deficiency of an isozyme of one of the DNA repair enzyme) may be implicated directly in the motoneuronal degeneration observed. Considering the high immunogenicity of Z forms, the production of specific anti-Z DNA antibodies may be explained by such accumulation in ALS patients.

In conclusion, an autoimmune processus may be suspected in all clinical conditions where specific anti-Z-DNA antibodies are found, but for their investigation larger populations are needed to prove such immunological hypothesis.

We thank Professor J.Emerit for his helpful collaboration, and M. Josien for the presentation of this text. This research was supported by University Paris VI (grant No. 75.76 REC), DRET (grant No. 82/352) and the Association pour la Recherche sur la Sclérose en Plaques.

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