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In vivo clearance and tissue uptake of an anti-DNA monoclonal antibody and its complexes with DNA

E. BEN CHETRIT, E.H. DUNSKY,* SHULAMIT WOLLNER & D. EILAT
Department of Medicine A, Hadassah University Hospital and the Hebrew University Hadassah Medical School, Jerusalem, Israel

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SUMMARY

In vivo clearance and tissue localization of a purified mouse anti-DNA monoclonal antibody (MoAb) (A52 IgG2b) and its complexes with DNA were studied in normal BALB/c and autoimmune NZB/NZW mice. The plasma half-life of the autoantibody in both mouse strains was significantly shorter ($T_{\frac{1}{2}} = 10$–15 min), compared with that of purified NZB myeloma proteins ($T_{\frac{1}{2}} \geq 180$ min). DNA antigen and DNA-A52 IgG complexes in antibody excess were cleared very rapidly ($T_{\frac{1}{2}} = 4$–8 min), while complexes formed in antigen excess persisted in the circulation much longer ($T_{\frac{1}{2}} = 60$ min). Organ studies showed that the anti-DNA MoAb was transiently retained by the liver and the spleen but demonstrated a particular affinity for the kidney tissue. We suggest that tissue damage in SLE glomerulonephritis may be facilitated by direct interaction of anti-DNA antibodies with glomerular components.

Keywords anti-DNA lupus glomerulonephritis NZB/NZW mice immune complexes

INTRODUCTION

Anti-DNA antibodies and their complexes with DNA are believed to play a major role in the pathogenesis of tissue injury in systemic lupus erythematosus (SLE) both in humans (Koffler, Schur & Kunkel, 1967) and in mouse models (Lambert & Dixon, 1968). Anti-DNA antibodies and complement components are found at the sites of inflammatory lesions and material with the properties of immune complexes often circulates in the blood in patients and mice with SLE (Agnello *et al.*, 1971). These findings and the general correlation of the level of antibodies to DNA with disease activity (Koffler *et al.*, 1971) have led to a formulation of a model, in which DNA released into the circulation combines with anti-DNA antibodies to form immune complexes. Immune complexes that are not removed by the reticuloendothelial system (RES) are deposited in the kidney and in other tissues, activate complement and cause inflammation. This model has been refined by many investigators to include the possibility that only certain populations of high avidity (Winfield, Faiferman & Koffler, 1977) or high isoelectric point (Hahn, 1982) anti-DNA antibodies participate in the disease process. Furthermore, evidence has been presented to indicate a direct binding of DNA to glomerular basement membranes (GBM). This observation raised the possibility that pathogenic immune complexes are locally formed at the target organs (Izui, Lambert & Meischer, 1976).

* Present address: Department of Paediatrics, The Hahnemann University, 230 North Broad Street, Philadelphia, Pennsylvania 19102, USA.

Correspondence: Dr Dan Eilat, Department of Medicine A, Hadassah University Hospital, Jerusalem, Israel.

However, many features of the proposed mechanisms remain obscure. For example, DNA, regardless of its size or strandedness, is cleared from the circulation extremely rapidly (Emlen & Mannik, 1983) and careful studies by counter immunoelectrophoresis have indicated that the majority of SLE patients fail to exhibit DNA in their plasma and do not differ in this regard from normal subjects (Steinman, 1984). Reports on the presence of circulating DNA–anti-DNA immune complexes have been conflicting (Bruneau & Benveniste, 1979; Izui, Lambert & Meischer, 1977). Furthermore, although the clearance of IgG coated erythrocytes, a measure of immune reticuloendothelial function, was found to be abnormal in patients with active SLE (Frank *et al.*, 1983), the clearance of soluble complexes was unimpaired in SLE mice (NZB/NZW F_1) at a stage when clinical disease was present (Finbloom & Plotz, 1979).

Very recently, the glomerular deposition of polyclonal mouse anti-DNA antibodies (Dang & Harbeck, 1984) and the clearance of polyclonal human anti-DNA complexes with DNA (Emlen & Mannik, 1982) were studied in autoimmune and normal mice, respectively. We have chosen to study the *in vivo* behaviour of well characterized monoclonal autoantibodies to nuclear components, produced by the hybridoma technique (Kohler & Milstein, 1976). These monoclonal antibodies (MoAb) can be purified to homogeneity and their structure and fine specificities can be studied in detail. Although it may be argued that the individual antibodies do not necessarily represent a polyclonal immune reaction, the MoAb to nucleic acids are capable of forming large size complexes with their multivalent antigens due to the repeating antigenic sites on the nucleic acid polymer (Eilat, 1982). Several laboratories have prepared MoAb to DNA from SLE mice and human patients (Andrzejewski *et al.*, 1980; Hahn *et al.*, 1980; Tron *et al.*, 1981; Lee *et al.*, 1981; Klotz *et al.*, 1981; Marion *et al.*, 1982; Koike *et al.*, 1982; Pisetsky & Caster, 1982; Ballard & Voss, 1982; Shoenfeld *et al.*, 1982). In this report we describe some biological properties of MoAb to nucleic acids that were prepared in our laboratory from autoimmune NZB/NZW F_1 mice (Eilat *et al.*, 1982, 1984). In particular, the serum clearance and tissue deposition of a well characterized anti-DNA antibody (A52 IgG2b) in normal (BALB/c) and autoimmune (NZB/NZW) mice have been studied in detail.

MATERIALS AND METHODS

Purification of hybridoma and myeloma antibodies. The anti-DNA A52 (IgG2b,k) and anti-RNA D44 (IgG3,k) hybridoma antibodies were produced by fusion of the BALB/c NSI/1 myeloma cell line with spleen cells of unimmunized, female NZB/NZW mice. The A52 IgG was purified from serum free culture medium by protein A–Sepharose affinity chromatography (Eilat *et al.*, 1984). The D44 IgG was purified from mouse ascitic fluid by a similar procedure (Eilat *et al.*, 1982). Ascitic fluids of the NZB myeloma proteins 6657 (IgG2a), 7074 (IgG2b) and 8224 (IgG3) were a gift from Dr Herbert Morse, NIH, Bethesda, Maryland, USA. The purified myeloma and hybridoma immunoglobulins were obtained by ammonium sulphate fractionation and protein A–Sepharose chromatography. All preparations were shown to be pure by Ouchterlony double diffusion in agar and SDS-polyacrylamide slab gel electrophoresis in the presence of 2-mercaptoethanol.

Preparation of human aggregated IgG. Purified human IgG (80 mg) (Cohn fraction II, Sigma, USA) were dissolved in PBS pH 7·2 (4 ml) and heated to 63°C for 20 min. The aggregated IgG was instantly cooled to 4°C (2 h), centrifuged to remove insoluble material and separated by size on a column of Sephacryl S-300 (Pharmacia, Sweden) and the high molecular weight complexes that eluted in the void volume were collected, aliquoted and frozen at −20°C. Insoluble aggregates were again removed by centrifugation immediately before use.

Preparation of DNA. High molecular weight mouse DNA was prepared from myloma solid tumours by the method of Blin & Stafford (1976). Closed circular plasmid DNA (pBR 322) was prepared by the method of Birnboim & Doly (1979). The purity of the DNA preparations was ascertained by agarose gel electrophoresis, by u.v. 260/280 nm absorbance ratio and by digestion with restriction enzymes.

Antigen binding capacity of anti-DNA and anti-RNA MoAb. DNA and RNA binding of the

monoclonal autoantibodies was measured by the nitrocellulose filter assay as described before (Eilat *et al.*, 1984).

Radioactive labelling of proteins and nucleic acids. MoAb and aggregated IgG were radioactively labelled with N-succinimidyl 3-(4-hydroxy 5-[^{125}I] iodophenyl) propionate (3000 Ci/mmol, Amersham, UK) by the method of Bolton & Hunter (1973). The labelled proteins were separated from free reagent by Sephadex G-50 column chromatography followed by extensive dialysis. The specific activities of the iodinated proteins was 3×10^5 to 1×10^6 ct/min/μg. The biological activity of the radioactively labelled A52 anti-DNA was tested in the enzyme linked immunosorbent assay (ELISA), where the binding of unlabelled and ^{125}I-labelled A52 IgG to immobilized DNA (Pisetsky & Peters, 1981) were compared. Goat anti-mouse–peroxidase conjugate (Sigma) served as a second antibody and the intensity of the colour reaction was measured at 488 nm. All *in vivo* experiments with ^{125}I-A52 IgG were performed within 2 weeks of the labelling reaction.

DNA was radioactively labelled with ^{32}P-NTP (6000 Ci/mmol, New England Nuclear, USA) by nick translation with DNA polymerase I (Rigby *et al.*, 1977) to a specific activity of 10^8 ct/min/μg. Unlabelled DNA was added to the ^{32}P-labelled DNA to give a final specific activity of 1×10^6 ct/min/μg.

Preparation of DNA–anti-DNA immune complexes. Immune complexes in either antibody excess (weight ratio of IgG to DNA = 100:1) or antigen excess (weight ratio of IgG to DNA = 1:100) were prepared by mixing 1 μg of radioactively labelled component (DNA or IgG) and 100 μg of unlabelled component (DNA or IgG) in a final volume of 0·2 ml borate-buffered saline (BBS) pH 8·0. The mixture was incubated for 1 h at 37°C and for an additional 1 h at 4°C. The samples were briefly warmed to room temperature before injecting them to the recipient mice.

Animal studies. All experiments were performed on 3–4 month old female BALB/c mice or 8–9 month old autoimmune NZB/NZW female mice (obtained from Dr Alfred Steinberg, NIH). Antibodies (in 0·2 ml PBS pH 7.2), DNA (in 0·2 ml PBS pH 7·2) or antibody–DNA complexes (in 0·2 ml BBS pH 8·0) were injected into the lateral tail veins of triplicate mice. Clearance studies were carried out by collecting blood (10 μl) from the retro-orbital venous plexus at different times. The ^{125}I-labelled proteins were counted directly in an auto gamma spectrometer. The ^{32}P-labelled DNA was precipitated on Whatman No. 3 filter paper discs with 5% trichloracetic acid (TCA). The filters were washed three times with 5% TCA, one with 1:1 mixture of acetone-ether and once with ether. The dried filters were counted in toluene-based scintillation liquid.

Organ uptake experiments were performed as described above except that three animals were sacrificed at each time point by cervical dislocation and the liver, spleen and kidneys were removed and counted for ^{125}I or ^{32}P radioactivity. The radioactivity measurements were normalized to give counts/min/mg tissue.

RESULTS

Clearance of DNA and anti-DNA from the circulation of BALB/c and NZB/NZW mice.
The NZB/NZW monoclonal A52 anti-DNA (IgG2b, k) has been purified to homogeneity and the amino terminal sequences of its heavy and light chains were determined (Eilat *et al.*, 1984). This antibody binds both ssDNA and dsDNA with a preference for the single stranded conformation. Furthermore, the A52 autoantibody has been shown to possess a major cross-reactive idiotype in the immune response to DNA in both humans and mice. Several procedures were attempted in order to label the purified IgG with radioactive iodine. The chloramine-T method (Hunter & Greenwood, 1962) resulted in a very poor specific radioactivity. The IODO-GEN method (Fraker & Speck, 1978) yielded a highly radioactive protein, but the biological activity of the labelled antibody, as measured by DNA binding in ELISA was completely lost. In contrast, the method of Bolton & Hunter (1973) which introduces the radioactive label into lysine residues rather than tyrosine, resulted in a moderately radioactive molecule ($3\text{–}4 \times 10^5$ ct/min/μg) that retained full biological activity (not shown). Furthermore, the iodinated A52 IgG could be fully retained on a protein A-Sepharose column (95% of input radioactivity) and showed a pattern of undegraded heavy and light chains in autoradiography of SDS-polyacrylamide slab gel electrophoresis. It could

also be demonstrated by column chromatography on Ultrogel ACA 34 that no dimers or higher aggregates were formed as a result of the labelling reaction (not shown). Consequently, all *in vivo* experiments were carried out with iodinated immunoglobulins that had been labelled by the Bolton–Hunter procedure.

Figure 1 shows the relative clearance rates of A52 IgG2b anti-DNA, NZB IgG2b myeloma protein (7074) and DNA from the circulation of young (3 month old) female BALB/c mice and premorbid (8 month old) female NZB/NZW mice. Mice were injected i.v. with trace amounts (1 μg) of radioactive IgG or DNA and blood samples were collected and counted at different times. The radioactivity measured at 1 min after injection served as a reference point (100 per cent) for subsequent measurements of plasma half-life ($T_{\frac{1}{2}}$). It is immediately clear from Fig. 1 that the clearance rates of all three molecular species were strikingly similar in the autoimmune and non-autoimmune mouse strains. No significant differences were observed in several such experiments. However, a major, reproducible difference was observed between the plasma half-life of A52 IgG2b ($T_{\frac{1}{2}}$ = 10–15 min) and that of NZB IgG2b myeloma ($T_{\frac{1}{2}}$ > 240 min) in both strains of mice. A NZB IgG2a myeloma protein (6657) had a clearance rate which was similar to that of 7074 (not shown). In addition, anti-RNA IgG3 monoclonal antibodies (D44) derived from NZB/NZW hybridoma (Eilat *et al.*, 1982) cleared from the circulation of NZB/NZW and BALB/c mice at the same rate ($T_{\frac{1}{2}} \simeq$ 240 min) as did an IgG3 myeloma protein (8224) of NZB origin (not shown). Figure 1 also shows that DNA was removed from the blood of both mouse strains extremely rapidly ($T_{\frac{1}{2}}$ = 3–5 min). There was no difference whether the ^{32}P radioactivity was first precipitated by TCA or directly counted. Also, very high molecular weight mouse DNA (3 × 10^9 daltons) and a bacterial plasmid DNA (pBR 322; molecular weight = 2·8 × 10^6 daltons) cleared from the mouse circulation at the same rate.

Fig. 2 is a dose–response curve which describes the relative clearance rates of high and low dose A52 IgG and DNA. Again, there was no difference in the capacity of young BALB/c and old NZB/NZW mice to clear high doses (100 μg) of antibody and DNA. However, while DNA clearance was unaffected by increasing the dose in the range of 1–100 μg, the removal of A52 IgG from the mouse circulation was dose-dependent and the plasma half-life of 100 μg anti-DNA antibody was significantly prolonged ($T_{\frac{1}{2}} \simeq$ 45 min) compared to that of a trace (1 μg) amount of the immunoglobulin.

The clearance of DNA–anti-DNA immune complexes in normal and autoimmune mice was studied by preparing two types of complexes in the test tube: one complex was formed by incubating

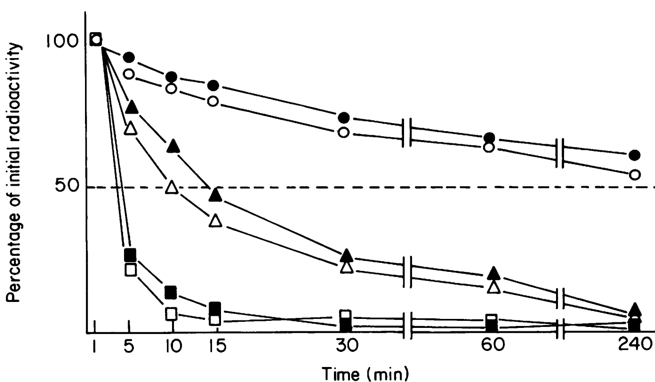

Fig. 1. The clearance of ^{125}I-labelled NZB/NZW IgG2b anti-DNA (△), (▲); ^{125}I-labelled 7074 NZB IgG2b myeloma (○), (●); and (□), (■) ^{32}P-labelled mouse DNA from the plasma of BALB/c (solid characters) and NZB/NZW F_1 (open characters) mice. Blood samples (10 μl) were taken at different times (three mice for each time point) after i.v. injection of 1 μg (5 × 10^5 ct/min) of proteins or DNA and were processed as described in Materials and Methods. The difference between measurements in each time point did not exceed 10 per cent. The initial levels of radioactivity (100%) represent an average of 1·3 × 10^5 ct/min A52 IgG, 1·5 × 10^5 ct/min 7074 IgG and 10^5 ct/min of TCA precipitable DNA, respectively, in 1 ml of blood, 1 min after injection.

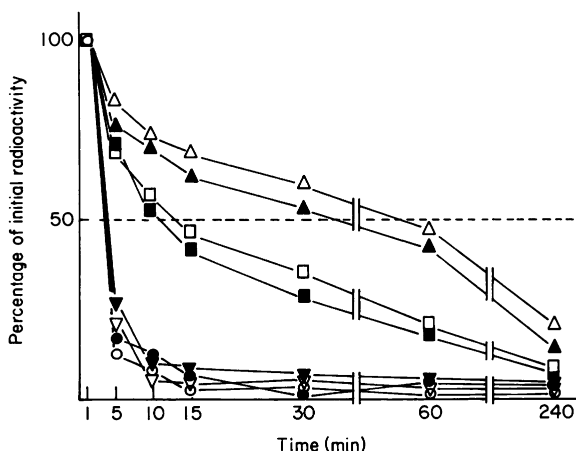

Fig. 2. The clearance of high dose and low dose DNA and A52 IgG anti-DNA from the plasma of BALB/c (solid characters) and NZB/NZW F_1 (open characters) mice. (▼), (▽) high dose (100 μg) DNA; (●), (○) low dose (1 μg) DNA; (▲), (△) high dose (100 μg) A52 IgG; (■), (□) low dose (1 μg) A52 IgG. Initial levels of radioactivity and standard deviations were similar to those specified in the legend to Fig. 1.

a trace amount (1 μg) of radioactive DNA with a large excess (100 μg) of anti-DNA MoAb; the second complex was prepared by mixing 1 μg of radioactive A52 IgG with a large excess (100 μg) of high molecular weight DNA. The preformed complexes were injected i.v. to BALB/c and NZB/NZW mice and their relative clearance rates were determined as described for the isolated antibody and antigen. Note that in each type of complex only the tracer component was radioactively labelled in order to increase the probability of following the clearance of complexed molecules. Fig. 3 shows that the half-life of ^{32}P-DNA ($T_{\frac{1}{2}}$ = 6–8 min) after its administration with excess antibody was very rapid compared with that of the free antibody but was slightly prolonged relative to the clearance of the free antigen (Figs 1 & 2). In contrast, the removal of ^{125}I-A52 IgG following its administration with excess DNA was very much delayed ($T_{\frac{1}{2}} \simeq 60$ min) relative to that of both free antibody or free antigen. Again, the clearance rates of the two types of complexes were strikingly similar in both the normal and the autoimmune animals. A control experiment in which

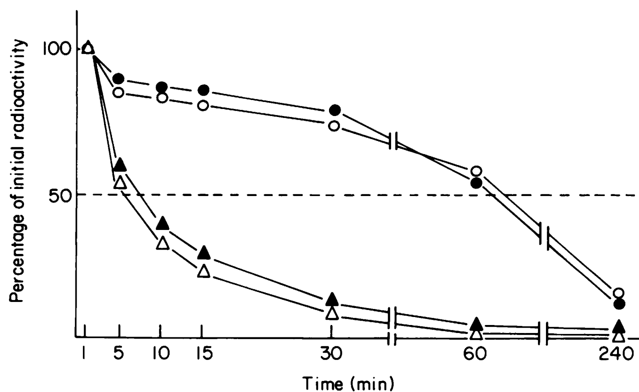

Fig. 3. The clearance of DNA–anti-DNA immune complexes from the plasma of BALB/c (solid characters) and NZB/NZW F_1 (open characters) mice. (▲), (△) ^{32}P-labelled mouse DNA (1 μg) in complex with excess unlabelled A52 IgG (100 μg); (●), (○) ^{125}I-labelled A52 IgG (1 μg) in complex with excess unlabelled mouse DNA (100 μg). The initial levels of radioactivity represent an average of $1{\cdot}9 \times 10^5$ ct/min A52 IgG and $2{\cdot}1 \times 10^5$ ct/min of TCA precipitable DNA, respectively, in 1 ml of blood, 1 min after injection.

1 μg of ^{125}I-labelled NZB IgG2b was mixed with 100 μg of DNA and injected into mice showed that excess DNA had no effect on the clearance rate of the myeloma protein.

In another control experiment (not shown), the clearance of model complexes prepared by heat aggregation of human IgG (HAG) was measured. These large soluble complexes were cleared from the circulation extremely rapidly and only a minute fraction of the administered material could be found in the mouse circulation at the shortest time measured (1 min).

The tissue uptake of the various components was studied by injecting triplicate mice with DNA, monoclonal anti-DNA and DNA–anti-DNA immune complexes and counting the radioactivity in the liver, spleen and kidneys at different time points. Only BALB/c mice were used in this study because, (a) it required a large mumber of test animals, and (b) no differences were found between the half lives of the various components in the clearance experiments (Figs 1–3). Fig. 4a shows that the amount of radioactivity which was measured in the different tissues after injection of NZB ^{125}I-labelled IgG2b myeloma remained constant for a period of 180 min and was roughly equal in the liver, spleen and kidney (calculated as ct/min/mg of tissue). This finding is compatible with the long half-life of this molecule in the mouse plasma; the measured radioactivity probably reflects the continued circulation of the IgG myeloma in blood vessels, with little or no uptake by the tissues. The ^{125}I-labelled NZB myeloma could therefore serve as an internal blood-vessel marker for subsequent experiments. In contrast, the monoclonal anti-DNA A52 IgG2b, showed a significant retention by mouse tissues, with a particular affinity for the kidney (Fig. 4A). The position of the observed radioactive peak was roughly parallel to the measured half-life of the A52 IgG in the circulation. The radioactivity, then, slowly declined to a background level.

The tissue uptake of preformed DNA–anti-DNA immune complexes in excess antigen is shown in Fig. 4b. Here, again, only the radioactive tracer component of the complex was followed with time. The MoAb in complex with excess DNA (antigen:antibody ratio = 100:1) showed a tissue distribution pattern, similar to that exhibited by the isolated A52 IgG with a very striking affinity for the kidney tissue. However, the peak of ^{125}I radioactivity of the complexed antibody was markedly shifted to a longer time period ($\simeq$60 min) probably reflecting the longer half-life (Fig. 3) of this type of complex in the mouse circulation.

A different situation was observed with ^{32}P-labelled, high molecular weight mouse DNA (Fig. 5A). Here, too, there was a transient peak of radioactive DNA in the kidney, which roughly coincided with the half-life of this molecule in the circulation; however, this peak declined rapidly

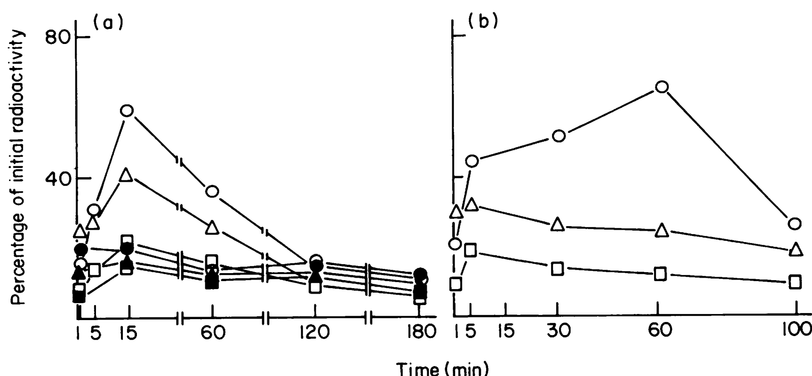

Fig. 4. Tissue deposition of ^{125}I-labelled A52 IgG (A) and its complex with DNA (B) in BALB/c mice. Three mice were sacrificed in each time point. The difference between measurements in each time point did not exceed 15%. The initial level of radioactivity (100%) represents an average of 2×10^6 ct/min A52 IgG (open characters) or control NZB IgG myeloma (solid characters) in 1 ml of blood, 1 min after injection. Experimental points were calculated as percentage of initial radioactivity in 1 mg of tissue. (△), (▲) liver; (□), (■) spleen; (○), (●) kidney, The DNA–anti-DNA immune complex was prepared in antigen excess by incubating 1 μg of ^{125}I-A52 IgG with 100 μg of unlabelled mouse DNA.

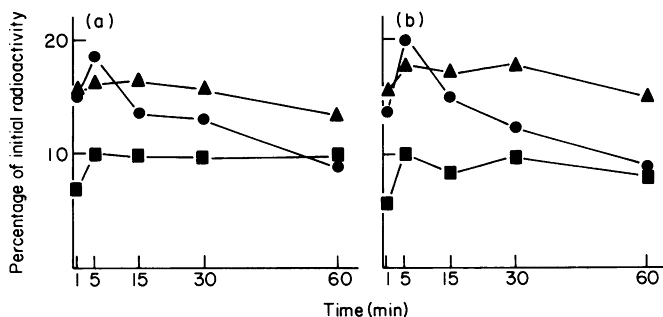

Fig. 5. Tissue deposition of ^{32}P-labelled DNA (A) and its complexes with A52 IgG (B) in BALB/c mice. The initial level of radioactivity (100%) represents an average of TCA precipitable, 2×10^5 ct/min DA in 1 ml of blood, 1 min after injection. Experimental points were calculated as percentage of initial radioactivity in 1 mg of tissue. (▲) liver; (■) spleen; (●) kidney. The DNA–anti-DNA immune complex was prepared in antibody excess by incubating 1 μg of ^{32}P-DNA with 100 μg of unlabelled A52 IgG.

while most of the DNA accumulated in the liver (and some in the spleen) and remained there at roughly constant levels during the course of the experiment. When administered in complex with excess antibody (antigen: antibody ratio = 1 : 100) the DNA showed a very similar pattern of tissue distribution to that observed with DNA alone (Fig. 5b).

In a control experiment (not shown), the deposition of heat-aggregated human IgG was also followed in the various tissues. Here, most of the ^{125}I radioactivity accumulated in the liver and peaked between 5–15 min. A smaller radioactive peak was observed in the spleen, while relatively little AHG was found in the kidney.

DISCUSSION

This report describes an attempt to investigate several *in vivo* properties of a monoclonal autoantibody to DNA (A52), its specific antigen (DNA) and two types of DNA–anti-DNA immune complexes in normal (BALB/c) and autoimmune (NZB/NZW) mice. One of the most important conclusions of these experiments is that no differences were observed between the normal and autoimmune mice with regard to plasma clearance of antibody, antigen or immune complexes. This finding supports previous studies of Finbloom & Plotz (1979) who showed that the clearance and uptake of model immune complexes were unimpaired in autoimmune NZB/NZW mice. In agreement with other investigators (Chused, Steinberg & Talal, 1972; Emlen & Mannik, 1984) we found that DNA was cleared from the circulation of mice rapidly and efficiently and that the liver was the major organ of DNA uptake. Furthermore, we found that no saturation of DNA clearance was observed in the range of 1–100 μg and that no degradation products of DNA were circulating in the blood (by comparing direct counting to TCA precipitable radioactivity) suggesting that a specific cellular mechanism and not nucleolytic activity is responsible for the rapid removal of this nucleic acid.

The most significant result of this study was the finding that the anti-DNA MoAb had a different clearance rate from that of other NZB myeloma proteins including an IgG2b myeloma which belongs to the same specific class and subclass and shares the same $IgCH^e$ allotype specificities. The shorter half-life of the A52 IgG correlated with a specific retention of this autoantibody by the kidney and to a lesser extent by other tissues. This specific uptake was transient and its peak was roughly correlated with the plasma half-life, suggesting that the antibody was not retained by the kidney for an extended period of time and was not returned to circulating blood within the duration of the experiment. It follows that an additional processing mechanism must be present. The affinity of the anti-DNA antibody to the kidney tissue may be explained on the basis of opposing electric charges: since DNA has a very acidic sugar-phosphate backbone, the antibody combining site must contain basic (cationic) residues. There is now an overwhelming evidence

indicating that cationic molecules such as lysozyme (Caufield & Farquhar, 1976) cationized ferritin (Rennke, Cotran & Veukatachlam, 1975; Batsford, Takamiya & Vogt, 1980) or cationized albumin (Border *et al.*, 1982) have a specific affinity to glomerular capillary wall and bind to anionic sites that are present throughout the basment membrane. These anionic sites have been postulated to consist of glycosaminoglycans such as heparan sulfate (Kanwar & Farquhar, 1979). The anti-DNA antibody could bind to these anionic sites either by charge interaction or by cross-reactivity with glycosaminoglycans. Such cross-reactivity has been recently demonstrated by Faaber *et al.* (1984) who suggested that tissue damage in SLE could possibly be initiated by direct binding of antibodies to fixed antigenic structures in the target organs. A supporting experimental evidence to such mechanism may come from the studies of Hahn (1982) and Dang & Harbeck (1984) who showed that anti-DNA antibodies which bind to kidney are restricted to a more alkaline pI range.

In our experiments, DNA itself also showed some affinity for kidney tissues (Fig. 5) which is in agreement with the experiments of Izui *et al.* (1977) and with the model of *in situ* immune complex formation. However, the absence of DNA in plasma of most SLE patients (Steinman, 1984), the extremely efficient removal mechanism of DNA from the circulation by the liver and the very weak evidence for the presence of significant amounts of DNA in diseased glomeruli make it unlikely that DNA–anti-DNA complexes are responsible for kidney damage in SLE.

We have prepared two types of DNA–anti-DNA immune complexes: a complex in excess antibody which presumably forms a large lattice and a complex in excess antigen which is known to give small size complexes. In each case only one of the components (antibody or DNA) which was in a trace amount could be used as a radioactive label in order to follow the clearance and tissue deposition of fully complexed material. The half-life of ^{32}P-DNA in complex with excess antibody was only slightly prolonged as compared with DNA alone and it was rapidly removed by the liver. This is in agreement with the experiments of Emlen & Mannik (1982) who studied preformed complexes of DNA with polyclonal human SLE anti-DNA; but it is difficult to tell whether the DNA in our case was removed as a complex or as free antigen because both species are rapidly cleared by the liver. In contrast, the markedly increased half-life of the A52 IgG in complex with excess DNA (Fig. 3) strongly suggests that the antibody was circulating as an immune complex, because the excess DNA which had been used to form the complex must have been removed immediately by the non-immune DNA clearance mechanism (Fig. 2); Yet these presumably small immune complexes were not removed by the liver, but showed the same affinity for the kidney tissue with a shift to a longer retention time, a finding which is compatible with their longer half-life.

The experiments described in this paper suggest, therefore, that DNA–anti-DNA immune complexes whether formed *in situ* or deposited from the circulation may not play a major role in the pathogenesis of tissue damage in SLE glomerulonephritis. A certain class of autoantibodies with specificity for acidic antigens such as DNA or proteoglycans could bind directly to glomerular tissue and trigger with mediators of inflammation. Such mechanism would be compatible with the majority of observations described to date, but more experiments with monoclonal and polyclonal anti-DNA antibodies will be necessary to substantiate its validity.

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