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Monoclonal anti-DNA antibodies: An approach to studying SLE nephritis

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SUMMARY

Several data suggest that the glomerular deposits of DNA and anti-DNA antibodies observed in SLE result from complex formation *in situ*. The aim of this study was to investigate this hypothesis in normal C57BL/6 mice by using monoclonal anti-DNA antibodies (mAb). Renal localization of intravenously introduced ds DNA was demonstrated in mice injected intraperitoneally with LPS 48 h before. Then, a single IgG2b or a mixture of IgG2a and IgG2b anti-ds DNA mAb were given with the aim of forming DNA: anti-DNA complexes at the glomerular level. No immunoglobulin deposits were observed regardless of the antibody dose used. The mAb used may possess some of the qualitative properties suspected to be nephritogenic. Thus, the limiting factors in the induction of a passive nephritis could be either the absence of glomerular DNA deposits or the inability by using a single antigen–antibody system, to recreate the pathophysiological conditions seen in SLE, where a high number of antigen-antibody systems is implicated in the genesis, of glomerular lesions.

Keywords lupus nephritis-DNA anti-DNA complexes *in situ*

INTRODUCTION

Human and murine systemic lupus erythematosus (SLE) are characterized by the existence of hypergammaglobulinaemia, the deposition of immunoglobulin (Ig) and complement tissue components in the kidney, and the production of autoantibodies directed against various nuclear components (Agnello, Koffler & Kunkel, 1973; Koffler *et al.*, 1969; Steinberg & Reinertsen, 1977–1978).

Many studies have implicated anti-deoxyribonucleic acid (DNA) antibodies in the pathogenesis of SLE glomerulonephritis through the tissue deposition of DNA: anti-DNA complexes. However, one may wonder whether the renal localization of DNA and anti-DNA antibodies results directly from the deposit of circulating DNA:anti-DNA complexes or whether other mechanisms are involved. Circulating DNA:anti-DNA complexes have been found in only a minority of SLE patients (Barnett *et al.*, 1979; Bruneau & Benveniste, 1979; Harbeck *et al.*, 1973; Tron *et al.*, 1982). The *in vitro* demonstration of a particularly high affinity of DNA for the glomerular basement membrane (GBM) and experimental evidence for an *in vivo* binding of single-stranded DNA (ss-DNA) to renal structures (Izui, Lambert & Miescher, 1976), as well as the results obtained from experimental glomerulonephritis (Izui *et al.*, 1977) have led to the hypothesis that DNA:anti-DNA complexes could form *in situ*, after the binding of the antigen to the GBM.

Recently, we reported the production of anti-DNA monoclonal antibodies (mAb) following fusion between a non-secreting myeloma cell line and (NZB × NZW)F_1 (B/W) spleen cells (Tron *et*

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al., 1980). Such anti-DNA mAb could contribute to the elucidation of the pathogenetic role of anti-DNA antibodies and to the determination of the respective roles of DNA:anti-DNA complexes formed *in situ* or in the circulation. The aim of the present study is to investigate the hypothesis of *in situ* glomerular formation of DNA:anti-DNA complexes by inducing a passive glomerulonephritis in normal mice injected successively with DNA and anti-DNA mAb.

MATERIALS AND METHODS

Mice. 5-week-old female C57BL/6 mice (18–20 g) were obtained from CSEAL–CNRS (Orléans, La Source, France).

Lipopolysaccharides. Bacterial lipopolysaccharides (LPS) B from *Salmonella typhimurium* were obtained from DIFCO Laboratories (Detroit, Michigan, USA). The lyophilized material was dissolved in saline, diluted to the concentration of 500 μg/ml and a final volume of 200 μl was injected intraperitoneally (i.p.) into LPS primed mice.

In vivo *localization of injected ^{3}H-double-stranded DNA in the kidneys.* 48 h after an i.p. injection of 100 μg LPS, C57BL/6 mice were injected intravenously (i.v.) with 100,000 ct/min (6 μg) of ^{3}H-bacteriophage Lambda double-stranded DNA (dsDNAλ) (Miles, Elkhart, Indiana, USA) (specific activity: 5·6 μCi/mole) (LPS-DNA treated mice). Mice were sacrificed by cervical dislocation either 4 h or 30 h later and immediately perfused with iced Tyrode's solution by a needle inserted into the left ventricle. Kidneys were removed, decapsulated and weighed. The renal cortex was isolated, cut into pieces, heated for 20 min at 37°C and mixed with 200 μl of Soluene 350 (Packard). The suspension was maintained for 5 days at 37°C. After complete kidney dissolution, 10 ml of PCS were added and the solution was neutralized with acetic acid. The radioactivity of the samples was evaluated in a liquid scintillation counter. The results were expressed as ct/min/g of organ weight and in nanograms of dsDNA/g of organ weight. Control mice were injected with either LPS and borate buffered saline (BBS) or only ^{3}H-dsDNA. Quenching was studied by evaluating the number of ct/min emitted by a known quantity of ^{3}H-dsDNAλ added to a mouse kidney processed as described above.

Clearance of ^{3}H-dsDNA in mice primed with LPS. 48 h after an i.p. injection of LPS, 190,000 cpm of ^{3}H-dsDNAλ were injected i.v. Serial orbital punctures were performed with heparin-coated graduated pipettes. The plasma was separated by centrifugation and precipitated with 10% trichloracetic acid (TCA). The supernatant was removed and the precipitate dissolved in 0·1 N NaOH. The dissolved precipitate and the supernatant were counted. Normal mouse plasma and mouse plasma containing a known quantity of ^{3}H-dsDNA were processed as described above and used as controls.

DNA binding capacity. The DNA binding capacities of mouse sera primed with LPS were measured using the cellulose ester filter radioimmunoassay with the ^{14}C-dsDNA from *Escherichia coli* (Amersham, Le Vésinet, France) (Ginsberg & Keiser, 1973).

DNA. dsDNAλ (Miles) was diluted in phosphate buffered saline (PBS) to a final concentration of 30 μg/ml. 6 μg were injected into the tail vein 48 h after LPS injection.

Murine monoclonal anti-DNA antibodies (mAb). The hybridomas secreting anti-DNA antibodies were isolated following fusions between a non-secreting myeloma cell line (P3 × 63–Ag 8·653) and B/W spleen cells. The selection of the hybrids, cloning and subcloning of the lines, purification of the antibodies, characterization of their classes and subclasses and their specificity studies were all described in detail previously (Tron *et al.*, 1980; Jacob & Tron, 1982). Double immunodiffusion analysis showed that PME77 and PMF10 are IgG2b, kappa chains, PME11, MA16 and MA512 are IgG2a, kappa chains. These mAb are all specific for DNA and directed against a conformational antigenic determinant of dsDNA (Jacob & Tron, 1982).

Protocols for injection of LPS, dsDNA and anti-DNA mAb (Table I). C57BL/6 mice were injected i.p. with 100 μg of LPS followed by an i.v. injection of 6 μg of dsDNA 48 h later. Anti-DNA mAb injections started 4 h after dsDNA λ. In the first series of experiments, LPS–DNA-treated mice were given IgG2b PME77 mAb. Group 1 received 50 μg (eight mice) or 100 μg (eight mice) of PME77 in a single injection. Group 2 (four mice) received one injection of 80 μg mAb every 2 h (total dose 240

Table 1. Protocol for anti-DNA mAb injections into LPS-DNA treated C57BL/6 mice

Group	No. of mice	Anti-DNA mAb*	Dose/mouse
1 a	8	PME77	1 × 50 μg
b	8	PME77	1 × 100 μg
2	4	PME77	3 × 80 μg (240 μg)
3	4	PME77	3 × 150 μg (1050 μg)
4	4	PME77+PMF10+PME11+ MA16+MA512	5 × 80 μg (500 μg)

* PME77 and PMF10 are IgG2b mAb. PME11, MA16 and MA512 are IgG2a mAb.

μg). Group 3 (four mice) received one injection of 350 μg mAb daily for 3 days (total dose 1050 μg).

In the second series of experiments, a polyclonal system was reconstituted by mixing IgG2b (PME77 and PMF10) and IgG2a (MA16, MA512 and PME11) purified anti-DNA mAb. Group 4 (four mice) received one injection of 80 μg of each of five mAb (total dose 500 μg) every 2 h. Before each i.v. injection, aggregates were removed by centrifugation at 25 psi for 10 min in a Beckman Airfuge t.m. ultracentrifuge.

Detection of circulating DNA:anti-DNA complexes. Analysis was performed by a 10–37% (weight/vol) sucrose density gradient centrifugation in tris-buffered saline 0·07 M, pH 7·4 (TBS) or in glycine-HCl buffer 0·12 M, pH 2·8, as described by Benveniste & Bruneau (1979). Positive controls consisted of DNA:anti-DNA complexes formed *in vitro* using PME77 anti-DNA mAb and dsDNA.

Studies of renal tissues. Kidneys were studied by direct immunofluorescence using FITC rabbit anti-mouse IgG, IgA, IgM, C3 (Cappel, Cochranville, USA).

RESULTS

In vivo *renal localization of injected ^{3}H-dsDNA in LPS-primed and normal mice*

LPS-primed mice were sacrificed 4 h after the i.v. injection of ^{3}H-dsDNAλ. The mean radioactivity (±s.d.) per gram of kidney weight was 6288 ± 1993 ct/min (377 ± 119 ng of ^{3}H-dsDNA/g of organ weight). Comparable results were observed when mice were sacrificed 30 h after ^{3}H-dsDNAλ injection. The mean radioactivity of control kidneys for the four mice injected with BBS (and also LPS-stimulated) and for the four mice injected with ^{3}H-dsDNA, without LPS priming is given in Table 2. The results are similar to those reported by Izui *et al.* (1976) who injected mice with ^{125}I-calf

Table 2. Localization in the kidney of ^{3}H-dsDNA injected i.v. into LPS-treated and normal mice

Group*	^{3}H-dsDNA† ct/min/gram of organ weight (mean ± 1 s.d.)	^{3}H-dsDNA† ng/gram of organ weight (mean ± 1 s.d.)	BBS ct/min/gram of organ weight
LPS‡	6888 ± 1993	377 ± 119	1341 ± 216
Normal	1996 ± 252	120 ± 15	—

* 5-week-old C57BL/6 female mice (four mice in each group).

† 6·0 μg of radiolabelled preparations were injected intravenously.

‡ 100 μg of *Salmonella typhimurium* LPS were injected intraperitoneally on day 0. Radiolabelled preparations were given on day 2. Mice were sacrificed 4 h later.

Table 3. Clearance of ^{3}H-dsDNA injected into LPS-treated C57BL/6 mice

Group	No. of mice	Dose/mouse	Time (h)	Plasma:TCA 10%*
BBS	2	—	2	94
^{3}H-dsDNA	4	190,000 ct min	2	138·55 ± 12
			4	144·5 ± 23

* Results are expressed as ct/min/100 μl of precipitated plasma (mean ± 1 s.d.) Similar amounts were found in supernatants (results not shown). Absence of quenching was verified.

thymus ssDNA. In any case, the amount of dsDNA bound to the kidney of LPS-dsDNA treated mice appears sufficient to indicate that the dsDNA: anti-dsDNA system may be used to evaluate the hypothesis of a glomerulonephritis induction by *in situ* formation of immune complexes.

Clearance of ^{3}H-dsDNAλ in mice treated with LPS

Since the *in vivo* formation of circulating DNA:anti-DNA complexes could result from the binding of injected anti-DNA mAb to DNA remaining in the circulation of LPS-DNA treated mice, the time required for the disappearance from the plasma of i.v. introduced ^{3}H-dsDNA was studied in these mice. 2 and 4 h after the injection of ^{3}H-dsDNA the radioactivity contained in plasma samples was 138·55 ± 12 cpm and 144·5 ± 23 cpm, respectively. These values were similar to those observed with the plasma samples of control mice (Table 3). Thus, the absence of DNA remaining in the circulation of LPS-DNA treated mice allowed the i.v. administration of anti-DNA mAb without the risk of formation of contaminating DNA–anti-DNA complexes in the circulation.

Injections of mice with LPS, DNA and anti-DNA mAb (Table 1)

C57BL/6 mice were injected i.p. with LPS and 48 h later with cold dsDNA. When anti-DNA mAb (PME77) was then injected either in a single dose or in repeated doses (up to 1 mg) in LPS-DNA treated mice, no glomerular deposits of immunoglobulin were observed in direct immunofluorescent studies of kidney specimen. Similarly, no glomerular deposits were observed when a polyclonal system obtained by mixing IgG2b and IgG2a anti-DNA mAbs was used. No circulating DNA:anti-DNA complexes could be detected in the plasma of LPS-DNA treated mice injected with anti-DNA mAb using the sucrose gradient technique.

DISCUSSION

Several data suggest that the glomerular deposits of DNA and anti-DNA antibodies observed in SLE patients and B/W mice result from complex formation *in situ* (Izui *et al.*, 1976, 1977). The report by Izui *et al.* of a significant quantity of injected DNA bound to the kidneys of LPS-primed mice (Izui *et al.*, 1976) and the availability of purified murine anti-DNA mAb prompted us to ask whether the *in situ* glomerular formation of DNA:anti-DNA complexes could be induced experimentally.

Renal localization of DNA was achieved according to the protocol described by Izui *et al.* (1976). In spite of the *in vitro* demonstration of a higher affinity of GBM for ssDNA, as compared to dsDNA (Izui *et al.*, 1976), we observed that the entrapment by the renal cortex of ^{3}H-dsDNA injected i.v. into LPS-treated C57BL/6 mice was higher than that reported by Izui *et al.* (1976) for i.v. introduced ^{125}I-ssDNA.

Anti-DNA mAb were given i.v. with the aim of forming DNA:anti-DNA complexes at the glomerular level. The demonstration of the rapid clearance of ^{3}H-dsDNA and the low DNA-binding capacities of LPS treated mouse sera made both the simultaneous presence of DNA and

anti-DNA mAb in the circulation and the *in vivo* formation of circulating DNA:anti-DNA complexes unlikely. If present, these complexes would probably have been removed by the mononuclear phagocyte system which would have prevented the glomerular deposition of anti-DNA mAb. In any case, the absence of DNA:anti-DNA complexes in the plasma of LPS-treated mice that received multiple injections of dsDNA and anti-DNA mAb was confirmed by sucrose gradient analysis.

No immunoglobulin deposits were observed in the glomeruli of LPS-DNA treated mice injected with a single IgG2b anti-DNA mAb (PME77), regardless of the antibody dose used. To reproduce a more physiological condition, a polyclonal situation was recreated by injecting a mixture of several mAbs with different subclasses (IgG2b and IgG2a) and isoelectric focusing characteristics. Again, no immunoglobulin deposits could be demonstrated in glomeruli.

These observations raised the question of whether the renal topography of DNA or the mAb properties could account for the failure to induce immunoglobulin deposits. So far, the morphological distribution of the kidney-bound DNA in LPS-treated mice has not been defined. In this regard, experiments are under way in our laboratory.

In any case, it has been reported that a single i.p. injection of LPS into normal C57BL/6 mice leads to the release of DNA into the general circulation and, DNA deposition at the glomerular level (Izui *et al.*, 1977). Accordingly, in the present study, the failure to induce complex formation *in situ* might be explained by either quantitative or qualitative properties of the anti-DNA mAb or by their inability to permeate the capillary wall. Up to 1 mg of purified anti-DNA mAb was injected i.v. The experimental passive nephritis studied by Couser *et al.* (1978) and Feenstra *et al.* (1975) were induced by the injection of 1 mg/20 g rat of a partially purified IgG fraction of a heterologous antiserum directed against F × IA. This represents an amount of specific antibody lower than that used in most protocols of the present study. Moreover, Miller *et al.* (1960), have shown that the minimal dose of antibodies required to induce a passive nephritis was much lower when homologous antibodies were used. Therefore, in this investigation, it is unlikely that an insufficient quantity of injected mAb accounts for the failure to induce complex formation *in situ*.

It is generally accepted that only subpopulations of anti-DNA antibodies are pathogenic for the kidney. Ebling & Hahn (1980) and Dang & Harbeck (1981) reported that anti-DNA antibodies eluted from B/W mouse kidneys have alkaline isoelectric points (7·5 to 9·2). The IgG2b and IgG2a anti-DNA mAb used in this study have isoelectric points ranging from 7·5 to 8·5 (Tron, Jacob & Bach, 1983) and thus may possess some of the qualitative properties suspected to be nephritogenic.

Transcapillary passage of a molecule depends upon the composition of GBM itself. The concentration and distribution of anionic sites in the lamina rara externa and on the epithelial aspect of GBM may be decisive factors since they regulate transcapillary passage and the selective retardation of circulating molecules (Renke, Cotran & Venkatachelem 1975). Indeed, the subepithelial fixation of perfused cationic protamine provides strong evidence for the importance of charge in membrane permeability (Seiler, Venkatachelem & Cotran, 1975a,b). As for IgG molecules, Ryan, Hein & Karnowsky (1976) have shown that free IgG can penetrate the GBM *in vivo* when the renal blood flow is reduced. LPS is a bacterial endotoxin reported to be able to damage capillary endothelium and to modify renal blood flow (McGrath & Stewart, 1969). It seems likely that LPS pretreatment provided the ideal conditions for membranary penetration by the cationic anti-DNA IgG employed in our experiments. In addition, the anti-DNA mAb injections repeated for 3 days should have allowed a sufficiently prolonged contact with the glomerular capillary wall. This contact did not result in the deposition of immune complexes in the kidney. Finally, the limiting factor in the induction of passive nephritis attempted in this study could be the absence of DNA deposits at the glomerular level.

One may also hypothesize that SLE is a disease implicating a high number of antigen–antibody systems in terms of antigenic determinant (Andres *et al.*, 1970); (Koffler, Schur & Kumbel, 1967; Krishnan & Koplan, 1967; Maddison & Reichlin, 1979; Panem *et al.*, 1976). It is possible that by using a single or a restricted number of such determinants in the case of mAb mixing, one does not recreate the pathophysiological conditions seen in patients. These reservations being made, it should be appreciated that by using a well-defined experimental model we could not confirm the planted antigen hypothesis in a disease where it had been suggested to play a role.

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