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A monoclonal DNA-binding autoantibody causes a deterioration in renal function in MRL mice with lupus disease

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SUMMARY

Two DNA-binding monoclonal antibodies (MoAb) derived from lupus mice were examined for their effects on kidney function. Antibody IV-228, reactive with single-stranded DNA (ssDNA) but not double-stranded DNA (dsDNA) significantly altered kidney function in some MRL/Mp−*lpr/lpr* (MRL/lpr) mice with lupus glomemerulonephritis. Antibody II-410, preferentially reactive with dsDNA, did not modify kidney function in MRL/lpr mice. Neither antibody affected normal healthy MRL/Mp− +/+ (MRL/n) mice. Not all MRL/lpr mice were equally affected by IV-228 and in some animals with clinical disease it was without effect even when high circulating antibody levels were maintained by five sequential daily injections. Its affects upon kidney function appeared to be related to its ability to localize *in vivo* in glomeruli. It is assumed that epitopes on trapped immune complexes are immunologically available to circulating antibodies, and in those animals where injected antibody is not captured this is because the antigen epitope is either absent or is masked by the animals' own auto-antibodies. We conclude that antibodies which bind to ssDNA contribute to lupus nephritis; that individual mice make DNA-binding antibodies of different fine specificity; and that kidney disease is not always due to the same balance of antibodies in members of a genetically homogeneous stock.

Keywords DNA monoclonal antibody glomerulonephritis SLE

INTRODUCTION

The nosology of kidney diseases reveals a close association between autoantibodies, especially those reactive with double-stranded DNA (dsDNA) and glomerulonephritis in lupus disease in humans and in mice (Koffler *et al.*, 1967). Support for a pathological role for these antibodies can be drawn from the facts that disease and autoantibody levels can be suppressed in parallel by idiotypic manipulation (Hahn & Ebling, 1983; 1984) and that passively transferred serum autoantibodies localize in kidneys of diseased mice (Dang & Harbeck, 1984). In humans anti-DNA antibody idiotypes have been identified on tissue bound immunoglobulins in renal biopsies of patients with systemic lupus erythematosus (Isenberg & Collins, 1985). But in spite of these compelling associations, the definitive experimental evidence proving that DNA-binding antibodies cause glomerulonephritis does not exist.

It is to be expected that immunochemically defined monoclonal antibodies (MoAb) might be better than serum antibodies as subjects of study in this regard, but while Ben-Chetrit *et al.* (1985) showed that one particular MoAb would localize in kidneys of normal and lupus mice they did not provide evidence that it modified kidney function. Other studies have shown that DNA-binding MoAb do not have any affinity for glomeruli in healthy mice (Cukier & Tron, 1985; Lake & Staines, 1986). Collectively these studies reinforce the findings that antibodies alone do not cause kidney damage and that other factors are important: antigen must be available and this may localize in kidneys in the absence of antibodies (Izui, Lambert & Meisicher, 1976; Lake *et al.*, 1985); non-specific agents such as fibronectin may augment glomerular deposition of immune complexes (Lake *et al.*, 1985); and the physiological activity of phagocytic cells may alter in disease and compromise immune complex degradation (Kavai *et al.*, 1986; Roberts, Isenberg & Segal, 1987).

In this paper we examine the pathogenic properties of two DNA-binding antibodies. We show that one of them can, in some individuals with established disease, contribute to renal failure.

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MATERIALS AND METHODS

Animals

Inbred mice of the MRL/Mp−*lpr/lpr* (MRL/lpr) and MRL/Mp− +/+(MRL/n) strains were bred at, and obtained

from, The Kennedy Institute (Hammersmith) and outbred mice of the LACA strain were obtained from Tuck & Sons (Raleigh, Essex).

Assessment of disease

Experimental animals were weighed daily and serum and urine samples collected as required. Blood samples were taken from the tail vein and at the end of experiments animals were exsanguinated by cardiac puncture. Serum creatinine was measured by the indirect Jaffe reaction (Cook, 1974); blood urea nitrogen (BUN) was determined by the Berthelot reaction using a commercial kit (Sigma, Poole, Dorset) according to the manufacturers' instructions; albumin levels in urine were measured by rocket electrophoresis using a rabbit-anti-mouse serum albumin antiserum according to the procedure of Devey & Steward (1980).

Monoclonal antibodies (MoAb)

The preparation and characterization of these MRL/lpr antibodies have been described elsewhere (Morgan *et al.*, 1985a,b). Both antibodies are of the IgG2aK type; antibody II-410 reacts with dsDNA and ssDNA and gives homogeneous nuclear staining of mammalian cells, whereas antibody IV-228 reacts only with ssDNA giving weak nucleolar and cytoplasmic staining in immunofluorescence assay.

Monoclonal antibodies were conjugated to fluorescein isothiocyanate (FITC) according to the general procedure described by Hudson & Hay (1981). Antibodies were prepared in phosphate-buffered saline (PBS), IV-228 at 4 mg/ml with a fluorescein to protein ratio (F:P) of 2·4:1 and II-410 at 2 mg/ml with an F:P of 2·7:1. Antibodies were conjugated to horse radish peroxidase (HRP) at an HRP:MoAb ratio of 4:1 by periodate linkage according to Nakane & Kawaoi (1974).

DNA-binding antibody levels

An enzyme linked immunosorbent assay (ELISA) as described by Morgan *et al.* (1985) was followed in all cases. Where binding of enzyme-labelled MoAb was to be assessed, the stage of adding a developing anti-immunoglobulin reagent was omitted. Results are expressed as antibody binding titres (the reciprocal of the interpolated dilution giving an optical density reading of 1·0).

Table 1. Clinical changes in 90-day-old male MRL mice after five daily intraperitoneal injections of DNA-binding monoclonal antibodies

		Weight (g)		Albuminuria (μg/ml)		BUN (mg/dL)		Creatinine (mg/dL)		DNA-binding titre			
										ss		ds	
Group	No*	pre	post	pre	post	pre	post	pre	post	pre	post	pre	post
(A)	1	40·4	41·3	28	21	24·1	25·6	0·59	1·13	750	2240	57	996
MRL/lpr	2	41·9	42·8	21	12	22·6	22·9	NT	1·01	1330	1824	55	591
410	3	38·7	39·2	9	7	19·5	23·8	NT	0·82	366	1240	39	604
	4	43·8	44·3	15	8	34·8	24·1	0·63	0·99	408	2650	49	1150
	5	43·8	44·1	7	21	24·7	21·8	0·67	0·99	1010	2100	67	1190
(B)	6	41·4	41·7	8	13	15·8	25·9	NT	0·97	439	1340	150	845
MRL/n	7	41·5	42·6	9	8	20·9	24·8	0·57	0·95	168	1473	106	2690
410	8	40·2	39·7	8	8	18·5	23·7	NT	0·90	304	313	74	317
	9	48·3	48·2	8	7	17·1	19·7	0·52	0·92	147	2650	78	740
	10	39·6	39·5	8	7	21·9	19·4	NT	0·99	109	3650	38	849
(C)	11	43·5	42·8	11	8	22·5	17·5	0·58	0·93	768	12300	38	76
MRL/lpr	12	40·7	36·8	18	>50	35·5	47·8	0·78	NT	2950	3640	71	84
228	13	40·5	37·3	8	>50	18·0	57·9	1·10	1·38	1670	14800	164	99
	14	40·3	39·1	15	32	23·8	22·0	0·85	0·91	1320	36100	54	59
	15	39·6	34·2	19	NA	32·6	NA	0·87	NA	673	NA	59	NA
(D)	16	42·1	43·9	8	11	17·8	16·5	1·00	0·81	106	21800	107	63
MRL/n	17	42·9	41·7	9	15	17·8	16·0	0·92	0·78	147	16600	109	72
228	18	44·6	44·2	7	7	16·6	16·7	0·89	0·77	131	52100	106	66
	19	41·2	41·1	7	7	19·0	19·2	0·91	0·89	152	107000	117	66
	20	48·7	49·0	9	7	19·4	19·2	0·85	0·76	106	19800	100	69
(E)	21	42·8	43·7	16	11	21·7	17·6	0·97	0·74	823	699	73	77
MRL/lpr	22	44·1	41·7	16	11	22·4	24·4	1·07	0·86	2060	2070	125	168
Saline	23	52·2	45·3	17	21	20·3	49·9	1·05	1·29	801	1340	62	65
	24	42·2	41·9	15	7	24·4	20·6	0·87	0·76	6200	5440	95	88
	25	44·6	43·9	17	7	20·5	19·9	0·91	1·37	2036	1673	64	81

* No 12 Died on day 4 (serum and urine collected day 3); No 13 Killed on day 4 (serum and urine collected day 4); No 15 Died on day 2.

NA, data not available; NT, not tested.

BUN, blood urea nitrogen.

Table 2. Clinical changes in 120-day-old MRL/lpr mice after five daily intraperitoneal injections of DNA-binding MoAb IV-228

No*	Weight change%	Albuminuria (μg/mL) pre	Albuminuria (μg/mL) post	BUN (mg/dL) pre	BUN (mg/dL) post	Creatinine (mg/dL) pre	Creatinine (mg/dL) post	DNA-binding titre ss pre	DNA-binding titre ss post	DNA-binding titre ds pre	DNA-binding titre ds post
Animals receiving IV-228											
1	5·6	110	150	18·6	14·7	0·89	0·80	2030	16300	211	257
2	NA	95	NA	10·8	NA	0·77	NA	2680	NA	225	NA
3	5·6	170	150	19·8	18·2	0·83	0·76	1760	10800	210	214
4	6·8	105	140	15·6	28·0	0·72	0·68	5600	7200	246	99
5	2·4	100	100	22·7	21·0	0·74	0·55	780	18700	10	7
6	12·4	80	150	19·6	23·0	0·97	0·82	3560	17600	1230	1660
7	NA	55	NA	16·3	NA	0·91	NA	2890	NA	338	NA
8	6·0	25	90	19·1	20·9	0·86	0·77	1540	11900	192	207
9	−1·9	180	190	22·6	31·9	0·74	0·77	2674	9700	184	180
10	−1·0	60	100	14·2	17·8	0·79	0·76	3200	14400	298	381
Animals receiving ascites from the P3 myeloma											
11	10·5	40	< 10	19·9	20·8	0·74	0·58	430	420	59	26
12	9·4	25	75	19·3	22·7	0·69	0·75	1920	2180	168	189
13	13·2	75	20	19·8	24·2	0·83	0·91	3750	4800	121	208
14	11·0	80	45	24·5	22·5	0·78	0·84	907	1800	7	110
15	13·1	100	90	21·8	20·7	0·81	0·86	2385	2970	162	248

* No 2 died day 2; no 7 died day 3.
NA, data not available.

Immunofluorescence assays

To identify material localized in tissues these were removed at autopsy and fragments mounted on cork boards in Tissuetek (Raymond Lamb, North Acton, London.), snap-frozen in n-hexane (stored in liquid nitrogen if required) and sectioned in a cryotome. To identify the localization of injected fluoresceinated antibodies, sections were fixed (60 s in methanol followed by 30 s in acetone at −20°C) then observed using a Zeiss Universal Microscope under epi-illumination. Indirect assays employed an FITC-conjugated anti-immunoglobulin reagent or an anti-C3c reagent (Nordic, Tilburg, The Netherlands) diluted 1/50 and 1/25 respectively in PBS containing 5% normal rabbit serum. All specimens were mounted in Cityfluor retardant (Department of Chemistry, City University, London). Fluorescent material in the kidney was assessed by scanning at least 100 glomeruli and grading fluorescence intensity on a scale of − to +++.

RESULTS

Clinical changes induced in healthy mice and lupus mice by DNA-binding MoAb

Monoclonal antibodies were injected for 5 consecutive days into the peritoneal cavities of 90-day-old male MRL/n and MRL/lpr mice. Experimental animals received 0·5 ml daily of either ascites fluid or saline. Two antibodies were studied; II-410 (2 mg/ml) which binds to both ssDNA and dsDNA and IV-228 (3 mg/ml) which binds only to ssDNA. Surviving animals were killed 24 h after the last (intraperitoneal) injection and their tissues and sera collected for analysis.

From the data summarized in Table 1, it can be seen that antibody II-410 had no obvious effects on kidney function in either MRL/n (healthy) or MRL/lpr (diseased) animals. In both types of mice the average values for albuminurea, BUN and serum creatinine were unaltered by exposing them to this antibody. In contrast, antibody IV-228, while not affecting the healthy MRL/n mice, did cause increases in protein in the urine (3/5 animals), serum creatinine (1/3) and BUN (2/4) values in MRL/lpr mice. In addition 2/5 animals lost weight and 3/5 became clinically sick or died during the experimental period. One animal (No. 15) in group C died on the second day of the experiment and another (No. 12) on the fourth day; a third animal (No. 13) was killed at the same stage because it was clearly ill.

Comparisons between these different groups of animals provide, to a certain extent, internal controls for the experiment. To control for the fact that the MRL/lpr mice had demonstrable signs of clinical disease at the start of the experiment, some of them were injected with saline: mean values for the clinical parameters measured were, in general, not altered. One control animal (No.23), was much heavier than the others and suffered a big weight loss and an increase in BUN over the 5 days of observation.

The data are complex in the sense that the clinical parameters (malaise/death, albuminuria, BUN, creatinine and body weight) changed independently in individual mice: not all animals had the same exacerbated symptoms of kidney disease. By comparing all changes from mean pretreatment values for all MRL/lpr mice, it was found that, in mice treated with IV-228, there were 11 changes in the 22 observations made which fall outside 2 s.d. from the starting mean values. By contrast there

Table 3. Fluorochromated DNA binding antibody is sequestered within the renal glomeruli of some MRL/lpr mice

No	Sex	Wt (g)	Age (days)	BUN (mg/dL)	Albuminuria (μg/ml)	Glomerular Fluorescence	DNA-binding titre ss	DNA-binding titre ds
Animals receiving IV-228-FITC								
1		43·5	120	22·5	< 10	−	2956	140
2		40·9	120	23·8	32	++	3040	195
3		38·6	140	19·6	80	−	3650	1230
4		42·9	140	19·1	75	−	1540	192
5		43·1	140	22·6	180	++	2674	184
6		41·4	140	14·2	95	−	3200	298
7		38·7	150	13·9	150	−	9680	121
8		39·4	150	25·2	210	+	6200	501
9		42·9	150	21·3	110	−	2250	37
10		43·1	150	20·3	< 10	+	2780	136
11		41·2	150	22·0	50	−	3190	35
12		39·3	170	18·6	125	−	2030	211
13		40·8	170	19·8	110	−	1770	210
14		40·9	170	15·6	115	−	5600	246
15		41·3	170	22·7	95	−	780	10
16		40·7	270	23·1	210	−	720	5
Animals receiving 410-FITC								
17		40·9	120	25·4	40	+	1300	130
18		42·1	150	24·7	20	−	3790	23
19		40·9	150	19·7	25	−	5830	325
20		44·2	150	24·9	15	−	8540	188
21		39·7	150	20·2	< 10	−	2910	51
22		38·7	150	17·5	< 10	−	7430	926
23		39·2	150	17·2	> 200	−	5240	461
24		40·7	150	17·5	> 200	−	8260	130

Fluorescence staining: −, No staining over background; +, > 50% of glomeruli show weak staining; ++, < 80% heavily stained, rest weakly so; +++, all glomeruli heavily stained.

were only five such changes in 50 observations in the animals in groups A and E combined. These frequencies are significantly different (χ^2, $P < 0{\cdot}001$) and this implies that antibody IV-228 contributes directly to renal disfunction in mice with kidney disease. It is without effect in otherwise healthy MRL/n mice. Antibody II-410, in comparison, is innocuous.

Animals treated repeatedly with MoAb had elevated levels of DNA-binding antibodies in their sera at the end of the experiment. Mice of both strains that received II-410 had similar antibody titres, irrespective of their disease state, but the increments were numerically greater in the MRL/n mice because their pre-treatment antibody levels were lower than those in MRL/lpr mice. After treatment with IV-228, residual titres were much higher in MRL/n than in MRL/lpr mice which may indicate that in the diseased mice antibody was absorbed by existing tissue deposits of antigen, thereby contributing to the lupus pathology. This is consistent with the other clinical findings.

The relative titres of antibodies reacting with ssDNA and dsDNA in both strains of mice treated with II-410 or IV-228 were of the order of 2:1 or 200–1000:1 respectively which are condordant with the known immunochemical properties of the antibodies. These results show that exposure to an MoAb does not induce the synthesis or release of significant amounts of antibody in the host.

Both antibodies were without effect in healthy MRL/n mice and this finding accords well with our previous observations (Lake & Staines, 1986). To expand some of the findings shown in Table 1, older MRL/lpr mice (120 days) were treated as before with antibody IV-228 and assessed in the same way (Table 2). Control mice were treated with 0·5 ml of P3 myeloma ascites fluid (the line of origin of the NS-1 myeloma used to prepare the hybridomas) to control for any non-specific effects of ascites fluid. These animals showed no significant clinical changes. In the experimental group of 10 animals, however, two mice died after two and three injections respectively of MoAb IV-228. BUN, serum creatinine and protein levels in the urine did not alter in the other animals. This shows that older mice, with more advanced disease, may be no more sensitive to the nephrotoxic effects of the antibody than are younger, 90-day-old, mice.

The fate of injected antibodies

Direct evidence that injected antibodies can localize in the kidney was obtained by injecting 100–200 μl of fluorochromated

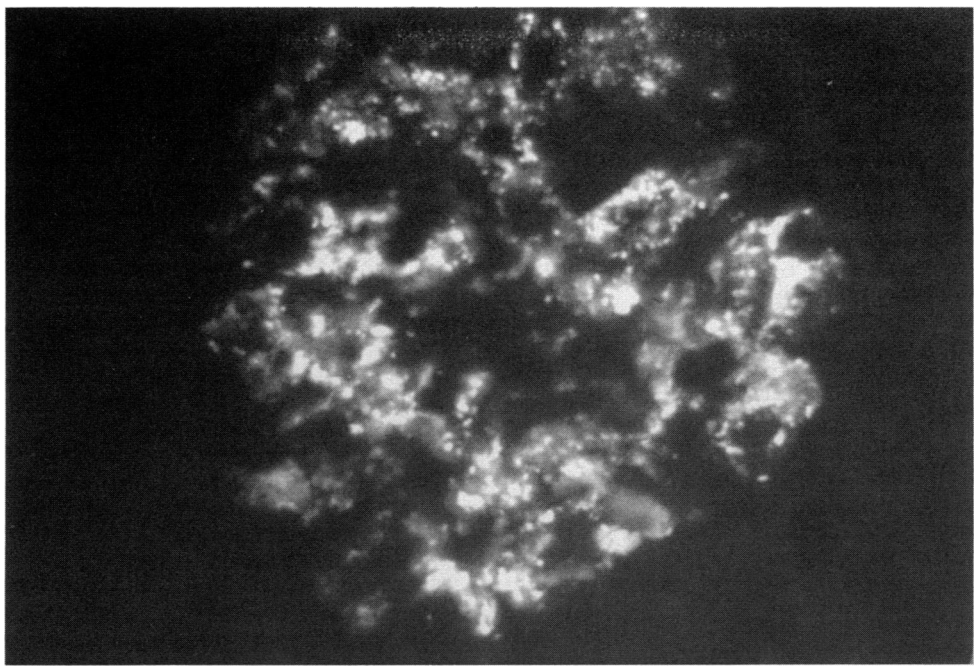

Fig. 1. Direct fluorescence staining of a glomerulus in the kidney of an MRL/lpr mouse injected 1 h previously with FITC-labelled monoclonal antibody IV-228. Localization of antibody is predominantly mesangial.

MoAb intravenously into MRL/lpr and MRL/n mice. One hour later the animals were killed, their tissues snap-frozen, sectioned and examined by fluorescence microscopy.

Fluorescent material was detectable in the livers and spleens of all mice examined but was found in the kidneys of only MRL/lpr mice (Table 3); but of these only 4/16 animals receiving IV-228 and 1/8 receiving II-410 showed glomerular fluorescence. The typical pattern of localization is shown in Fig. 1 and represents a predominantly mesangial localization of antibody, captured presumably by antigen in immune complexes sequestered in the kidney. Injected, free FITC did not localize in glomeruli of mice of either strain.

All MRL/lpr kidneys had immunoglobulin in their glomeruli, revealed by indirect immunofluorescence, in a pattern similar to that in Fig. 1. Likewise, C3 was detectable in the glomeruli of some MRL/lpr kidneys which did not bind the fluorescent monoclonal antibody. Hence the presence of immunoglobulin or C3 did not relate to the ability of individual kidneys to bind either of the monoclonal antibodies.

These experiments indicate that although all the MRL/lpr kidneys contained immune complexes it was only some of them that displayed epitopes appropriate for the capture of individual MoAb. In those in which the injected antibody did not localize it must be assumed that the relevant epitopes were either absent or masked by pre-existing antibodies. In either case these results may explain why individual antibodies do not have consistent physiological effects in all mice *in vivo*. Further, we infer that different antibodies have different pathological significance in individual mice of a genetically homogenous stock. There was also a trend (Table 3) for antibody to localize less readily in glomeruli of older mice with more progressive disease. The finding that antibody capture was a feature of only some kidneys was confirmed *in vitro* by exposing sections of kidneys from untreated MRL/lpr mice to fluorochromated antibody IV-228: only one out of four bound significant amounts of antibody and although background interstitial and tubular fluorescence was seen, the pattern of *glomerular* localization was the same as that after exposure to antibody *in vivo*. Results with antibody II-410 could not be interpreted because it gave vivid nuclear fluorescence. Neither antibody localized in the glomeruli of MRL/n mice.

Serological interactions between antibodies of different specificities and between antibodies and other serum factors

The experiments above implied that pre-existing DNA-binding antibodies influence the potential of an infused antibody to contribute to pathology. The experimental system did not allow an analysis of the inhibitory, additive, or synergistic effects that can occur when antibodies are mixed. Several pieces of serological evidence indicate that such effects do occur and these may help to explain the results from the experiments *in vivo*.

First, addition of normal serum (from LACA mice) to the diluent in ELISA caused an elevation of titre in proportion to the amount of normal serum present, Figures 2a and 2b show examples in which the titres of antibodies II-410 and IV-228 against ssDNA and dsDNA were elevated.

Second, such normal serum promoted an interaction between IV-228 and dsDNA. In the absence of serum, antibody IV-228 did not bind to S_1 nuclease-treated dsDNA (Fig. 2b). Further, antibodies in MRL/lpr, but not MRL/n sera, inhibited the binding of MoAb to DNA of both physical forms (Fig. 2c and 2d), but in reacting with dsDNA, antibody IV-228 was most easily inhibited implying that this interaction was of low affinity (Fig. 2d). This is an example where the ligand-binding profile of an antibody can be altered by the presence of other antibodies. Similar effects would be expected to occur *in vivo*.

Third, the two MoAb when co-titrated were additive in their reaction with ssDNA but not with dsDNA (Fig. 2a and 2b). We

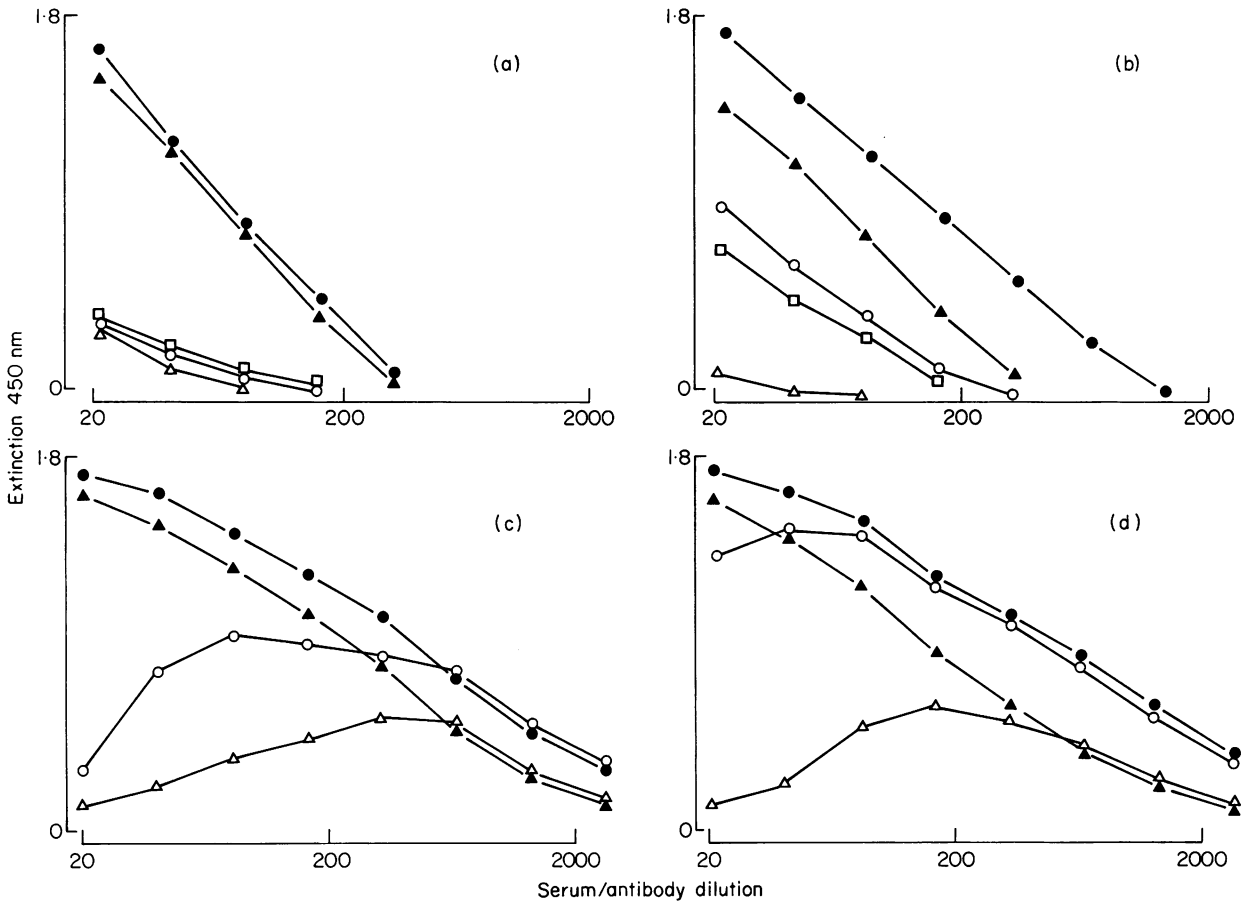

Fig. 2. Reactions of HRP-labelled MoAb II-410 (○ ●), IV-228 (△ ▲), and combined (□), and their interactions with serum antibodies and normal serum in ELISA against (a, c) ssDNA and (b, d) dsDNA. In (a) and (b) the antibodies are titrated individually (○ △) and combined (□) in buffer or are co-titrated with LACA normal serum (● ▲). In (c) and (d) the antibodies are co-titrated in buffer containing a 1/40 dilution of normal serum with either MRL/lpr serum (○ △) or MRL/n serum (● ▲).

conclude that antibodies of different specificity modify the binding of each other to antigen and these interactions are influenced by normal serum.

DISCUSSION

DNA-binding MoAb infused into MRL/lpr mice with established disease can modify kidney function and lead to the death of some animals during a 5-day course of treatment. This is in clear contrast to the effects of much larger amounts of the same antibodies in healthy mice, reported previously (Lake & Staines, 1986). Since these antibodies, and those studied by others (Ben-Chetrit *et al.*, 1985; Cukier & Tron, 1985; Jones, Pisetsky & Kurlander, 1986) are without pathophysiological effect in healthy animals, we have been obliged to study them in mice with clinical disease. This has undoubtedly complicated the interpretation of the data and made it difficult to select animals which, as controls, show only minimal changes during the brief experimental period.

Mortality curves for MRL/lpr mice reflect considerable variation in the progress of the disease even though the strain is inbred. We ascribe the variations in the response to antibody infusion by individual mice to the underlying heterogeneity in the disease, in particular to the range and fine specificity of autoantibodies made by different mice.

The effects of IV-228, which reacts with ssDNA, were more pronounced than those of II-410, which reacts with both dsDNA and ssDNA. The direct binding of the former to glomeruli could be demonstrated in a higher proportion of individuals and morbidity and mortality associated with its infusion were greater. Admittedly about 30% less II-410 was administered on a weight basis, but in both ELISA against dsDNA and immunofluorescence assays this antibody had titres very much higher than antibody IV-228. These antibodies have such different properties (Morgan *et al.*, 1985a,b) that a rational basis for designing comparable single dosing regimes is not obvious to us. We conclude that antibodies that react with ssDNA will, under appropriate circumstances, contribute to kidney failure. This, and the fact that a particular antibody does not do this in all individuals, has implications for understanding the significance of serological data to the disease process.

The changes in kidney function are likely to be a direct result of the binding of infused antibody to antigen (DNA) in the glomeruli. The experiments are short and kidney function changes so rapidly that the effects are unlikely to be an indirect result of the injected antibody inducing, through idiotypic

networks, the production of other pathogenic autoantibodies of similar or unrelated specificity (Migliorini *et al.*, 1987).

The binding of antibody to glomerular structures shows that tissue fixed antigen is immunologically available to circulating antibody. We interpret these observations to mean that, in individuals where MoAb capture does occur, competing antibodies of the same specificity are absent, or that injected antibodies displace existing antibodies from sequestered complexes. The data do not allow us to decide between these alternatives but whichever is correct, it is clear that individual mice do not all make the same amounts of the various DNA-binding antibodies. Whether these variations contribute to the heterogeneity of disease is unknown but it is of particular importance in understanding the selective pressures on the expression of somatic variants of antibody germ-line genes. Published data imply an important role for the process of somatic diversification superimposed upon the expression of a restricted number of germ-line gene elements (Eilat *et al.*, 1984; Diamond & Scharff, 1984; Koffler *et al.*, 1985; Naparstek *et al.*, 1986).

There is a dynamic equilibrium between circulating and bound antibodies in diseased animals and this complicates the analysis of the pathogenic potential of individual MoAb. No matter how precisely the MoAb have been defined in controlled immunochemical assays, one cannot predict how they will combine, synergise or cross-inhibit other antibodies in the microenvironment of the glomerulus. The serological data presented in this paper reveal some features of antibody interactions that may be relevant to the biological activity of the antibodies. Notably, the presence of normal serum in an ELISA system promotes the binding of antibody IV-288 to dsDNA with which it does not normally interact. Further, the ability of DNA-binding antibodies in sera from MRL/lpr mice (but not MRL/n) to compete with defined MoAb for antigen again, is only easily demonstrable in the presence of added normal serum. The components of normal serum responsible for these effects have not been identified, but we have found that normal serum immunoglobulins that bind to staphylococcal protein A are not involved. It may be that low affinity natural IgM antibodies, possibly working in concert with other serum proteins, synergise with the MoAb in this serological system. In general though, MoAb directed to different epitopes on DNA do not synergise but will, in some cases, show simple additive effects. In consequence we would be cautious in predicting the activity of an antibody *in vivo* from its ligand-binding characteristics determined *in vitro*.

This study does not support the notion that reactivity with antigens expressed *solely* on dsDNA is the hallmark of antibodies that cause lupus glomerulonephritis. On the contrary, antibody IV-228 which by accepted criteria binds exclusively to ssDNA can contribute significantly to kidney damage. In view of the ubiquity of antibodies reactive with ssDNA this study may point to a widespread involvement of ssDNA-binding antibodies in other nephritides, especially those associated with parasite infections, where they are an unexplained correlate of the disease process.

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