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A cross-reactive idiotype on anti-DNA and lymphocytotoxic antibodies

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SUMMARY

The possibility that shared idiotypes may be present on antibodies of different specificities was explored using sera from 13 patients with systemic lupus erythematosus. Eleven sera had anti-DNA and seven carried the 16/6 idiotype (ID). Two of the sera with 16/6 ID also had lymphocytotoxic antibodies, the activity of which was blocked by antibody to the 16/6 ID. Our studies reveal that 16/6 ID is present on two antibodies with different binding specificities.

Keywords cross-reactive idiotype 16/6 idiotype lymphocyte antibodies anti-DNA SLE

INTRODUCTION

Cross-reactive idiotypes (CRI) have been shown to be present on both antibodies to foreign antigens (Kuettner, Wang & Nisonoff, 1972; Altevogt & Wigzell, 1983; Kennedy & Dreesman, 1983) and autoantibodies in a number of autoimmune diseases (Kunkel *et al.*, 1973; Lefvert *et al.*, 1982; Delves & Roitt, 1984). Idiotype (ID) expression is regulated by both T cells and antibodies (Greene *et al.*, 1982; Bottomly *et al.*, 1984) and it has been proposed that autoantibodies may arise not only through a defect in specific suppression but also ID-specific suppression (Cooke, Lydyard & Roitt, 1984). Since autoreactive cells exist in normal individuals and the idiotypic network forms a major regulatory system, defective ID-specific suppression could lead to production of autoantibodies against different epitopes which share a CRI (Cooke & Lydyard, 1981).

In systemic lupus erythematosus (SLE), autoantibodies can be found with specificity for DNA which carry a CRI (16/6 ID). Up to 50% of lupus patients have 16/6 ID detectable in their sera (Isenberg *et al.*, 1984). Not all the 16/6 ID is associated with anti-DNA (Isenberg *et al.*, 1986) and autoantibodies directed towards other antigens also arise in these patients. In this study we have examined the possibility that anti-DNA and lymphocytotoxic antibodies (LCA) in SLE patients sera which are directed against different epitopes may carry the CRI 16/6.

We present evidence that at least some of the lymphocytotoxic antibodies directed to determinants other than those with DNA specificities carry 16/6 idiotype.

MATERIALS AND METHODS

Patients and sera

As part of an initial screen, sera from 13 patients with SLE, each of whom met four or more of the American Rheumatism Associations revised criteria for the classification of SLE, were studied (Tan

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et al., 1982). These patients demonstrated the broad clinical diversity of SLE. Their sera were stored at −20°C. Sera from laboratory and paramedical personel served as controls.

Anti-idiotype antibodies

Antibodies to the cross-reactive idiotype (CRI) 16/6 were obtained by immunizing a rabbit with human monoclonal anti-DNA antibodies (clone 16/6; Shoenfeld *et al.*, 1983a). This clone was obtained by fusing blood lymphocytes from an SLE patient with GM 4672 parent cells (Shoenfeld *et al.*, 1983b). Anti 32/15 ID was prepared in a similar way.

16/6 ID was detected in serum as previously described (Isenberg *et al.*, 1984). Briefly ELISA plates were incubated with 1/25,000 sera and then washed. Anti 16/6 was added and this was followed by the enzyme linked anti rabbit immunoglobulins. The substrate was added and the OD read on a microelisa reader after 1 h incubation.

16/6 ID was detected on DNA antibodies as previously described (Schoenfeld *et al.*, 1985).

Since the 16/6 ID was first identified on a molecule with strong activity against ssDNA but very little activity towards dsDNA our experiments have focussed on ssDNA activity. Anti-ssDNA antibodies were measured in an ELISA as described elsewhere (Dudeney *et al.*, 1986).

Rheumatoid factor was detected by the slide latex test.

Lymphocytotoxicity assay

Tonsils were obtained from routine tonsillectomy operation and kindly provided by the ENT Hospital, Golden Square, London. Cell suspensions were prepared by gentle teasing of the tissue and centrifugation over Ficoll-Hypaque to remove dead cells. Tonsils from donors < 20 years were used for the cytotoxicity experiments since they proved to be the most susceptible 'targets'. Triplicate microwells containing 5×10^5 tonsil cells in 25 μl were incubated with 25 μl of serially diluted SLE or normal serum at 37°C for 60 min. The cells were washed once and 50 μl of rabbit serum preabsorbed with tonsil cells was added at 1/10 final dilution (as a source of complement) and incubated for a further 30 min at 37°C. Fifty microlitres of a mixture of acridine orange and ethidium bromide (Lee, Singh & Taylor, 1975) was then added and the percentages of dead cells scored under a microscope using UV optics. Using this assay, triplicate samples showed less than 10% variation.

Blocking of LCA by anti-16/6 ID

SLE sera were titrated for LCA activity and a dilution chosen on the sensitive portion of the dose response curve for testing the inhibition of anti-ID. Twenty-five microlitres of rabbit anti 16/6 at different dilutions was incubated with SLE serum (1/4) for 30 min at 37°C, then 60 min at 4°C. The mixture was then added to the tonsil cells and incubated for a further 60 min at 37°C. After washing, the lymphocytotoxicity was determined as above.

Absorption of sera with ssDNA or tonsil cells

ssDNA. Serum-diluted 1/2 was passed through a column containing 5 mg ssDNA coupled to cellulose (Sigma) and concentrated back to the original volume.

Tonsil cells. Serum was absorbed twice with 50% packed tonsil cells (prepared as above) incubated at room temperature for 30 min followed by 30 min on ice.

RESULTS

ssDNA antibodies, LCA and expression of 16/6 ID in SLE sera

Eight of eleven sera showed an increase in anti-ssDNA activity compared with control values (< 100 OD units; Table 1). Seven of thirteen also showed detectable 16/6 ID compared with controls (< 115 OD units). However, there appeared to be no clear correlation between these two parameters. Although it was notable that the two sera (PE and ML3) with the highest ssDNA levels also had high 16/6 ID levels. LCA were high in only two of the sera tested (RR and ML). Since both of these sera were 16/6 ID+ they were chosen for further study on the expression of 16/6 on LCA.

Table 1. Detection of 16/6 ID, % cytotoxicity and anti ssDNA antibodies in the sera of 13 SLE patients

Patient	ID 16/6 ($OD \times 10^3$)	% Cytotoxicity (1/8)*	RF	Anti-ssDNA ($OD \times 10^3$)
A.D.	216	1·6	−ve	105
T.H.	142	0·0	1:40	NT
A.M.	63	8·0	−ve	113
A.N.	63	3·6	−ve	80
P.H.	12	9·3	−ve	47
P.R.	147	6·7	−ve	47
C.R.	102	2·0	1:5, 120	273
P.E.	257	3·2	1:160	607
T.O.	179	0·0	−ve	155
S.E.	106	4·0	−ve	380
C.O.	83	0·4	1:32	NT
R.R.	311	42·6	−ve	119
M.L. (1)	185	50·7	1:28	181
(2)	95	10·0	—	141
(3)	170	35·8	—	398
	$n < 115$	$n = 2{\cdot}0–5{\cdot}3$		$n < 100$

Normal = (n) = Mean + 2 s.d.
* Normal value removed.

Expression of 16/6 ID on LCA

There was a dose dependent inhibition of LCA in both RR and ML sera examined (Fig. 1). Neither normal rabbit serum nor another anti ID-32/15 inhibited LCA under the same conditions. Inhibition by the anti 16/6 ID was not simply due to complement consumption since two washes were made between addition of the mixture of anti ID and SLE serum to the tonsil cells and addition of complement (see methods). Furthermore, the anti 16/6 ID did not interfere non-specifically with cytotoxicity. No inhibition was observed following incubation of 1/50 dilution of anti-16/6 ID with monoclonal pan T cell antibody (anti T3) and complement (data not shown).

Antibodies to ssDNA do not recognize the same epitope(s) as on lymphocytes in ML serum

Absorption of LCA leaves most of the anti-DNA activity intact. Absorption of both SLE sera with tonsil cells completely removed LCA from both sera (Table 2). Most of the anti-DNA activity remained intact in ML serum: RR serum did not have detectable anti-DNA before absorption.

Absorption of anti-DNA fails to inhibit LCA. To confirm further that LCA and anti-DNA were distinct antibodies in ML serum with different epitope recognition, the serum was absorbed with ssDNA. No effect on LCA was seen even though some anti-DNA activity was removed. Table 3 shows one of two experiments showing the same results.

16/6 ID is not expressed on ML DNA antibodies

Anti 16/6 ID does not block binding to ssDNA. Anti-16/6 ID showed a dose-dependent inhibition of binding of two SLE sera (FL and YB12) to ssDNA but failed to block binding of ML serum in three separate experiments. Data from one is shown in Table 4. Anti-ssDNA in RR serum was consistently at too low a level to give reliable data in these assays. Thus ML anti-ssDNA antibodies do not appear to carry 16/6 ID, at least in the binding site.

Lack of non-binding site related 16/6 ID on ML ssDNA antibodies. To exclude the possibility that 16/6 ID was present on ML anti-DNA but not in the antibody binding site, ML serum was added to DNA on an ELISA plate and anti 16/6 ID added. No specific binding of anti 16/6 ID was observed (Table 5) even though on the same plate ML serum directly bound to the plate showed specific binding of the anti-16/6 ID. This indicates that 16/6 ID is not present on ML ssDNA antibodies either inside or outside the antigen binding site.

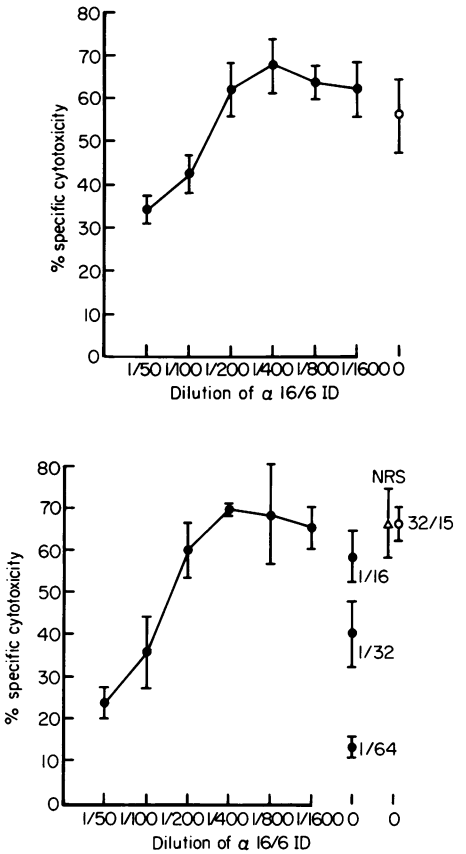

Fig. 1. Blocking of LCA by α 16/6 idiotype. α 16/6 idiotype was added at different concentrations to two SLE sera (a) ML and (b) RR before addition of tonsil cells and rabbit complement (see materials and methods). (a) There was a dose dependent inhibition of LCA by anti-16/6 ID (●) using 1/8 ML serum compared with cells incubated with 1/50 normal rabbit serum (○) and 1/8 ML serum. (b) Similar inhibition of LCA with 1/16 RR serum was seen with anti-16/6 idiotype (●). The percentage cytotoxicity of cells treated with 1/32 or 1/64 RR serum without addition of anti 16/6 idiotype is shown for comparison. Additional controls included normal rabbit serum (△) and another anti-idiotype, anti-32/15 (○).

Table 2. Removal of cytotoxicity leaves most anti-DNA activity intact in patient ML

Serum	Tonsil absorption	% Cytotoxicity	ssDNA (OD × 10^3)	
			IgM	IgG
Patient M.L.	—	66·3	370	908
	×2	8·3	233	622
Patient R.R.	—	46·3	NT	NT
	×2	8·4	NT	NT
NHS	—	7·0	NT	NT

NHS, Normal human serum.

Table 3. Reduction in anti-ssDNA antibody levels leaves cytotoxicity intact in serum from patient ML

Serum	DNA Absorption	% Cytotoxicity	ssDNA (OD × 10^3)	
			IgM	IgG
ML	—	60·8	148	394
	+	60·7	64	118

Table 4. Attempts to inhibit the binding of SLE sera to ssDNA by pre incubation with Rα16/6

		Serum plus Rα16/6 ID			Serum plus NRS
Patient serum*	Serum plus buffer	1/500	1/1000	1/5000	1/500
FL	682	391	257	870	545
YB12	907	321	247	592	844
RR	78	77	31	35	52
ML	320	296	275	300	340

NRS, Normal rabbit serum. Values given as OD × 10^3.

*† Patient sera diluted 1/200.

Values represent means of duplicates whose values differ by 10% except patient RR where low readings were associated with slightly higher variation; even though a decrease was observed with lower dilutions of 16/6. Values below 100 in this assay represent background.

Table 5. Attempts to demonstrate the relationship between DNA antibody binding site and 16/6 ID in various patients

Patients	Sera coated wells add, Rα16/6 then GαR Ig (OD × 10^3)	DNA coated wells add sera then Rα 16/6, and finally GαR Ig (OD × 10^3)
FW (known −ve DNA ab and −ve 16/6 id)	0	25
KH* (known +ve DNA ab and 16/6)	198	23
EM* (known +ve DNA ab and 16/6)	232	19
ML (DNA ab +ve with 16/6 id +ve but ? at different binding site)	318	28

* 16/6 ID has previously been shown to be at the DNA ab binding site.

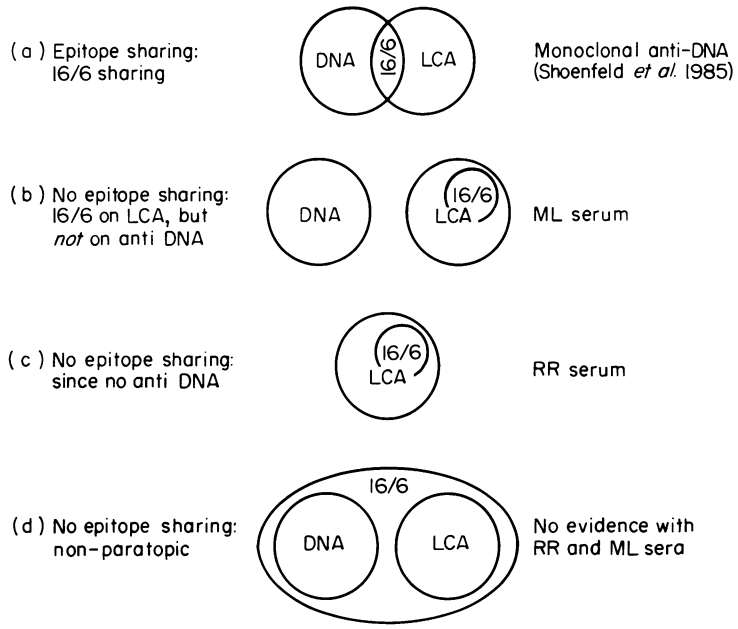

Fig. 2. Possible relationships between anti-DNA, LCA and 16/6 idiotype (see Discussion).

DISCUSSION

In this paper we confirm the presence of 16/6 ID in the serum of a number of patients with SLE (Isenberg *et al.*, 1984). There was no clear correlation between those sera possessing 16/6 ID, anti-ssDNA and LCA (Table 1), although a relationship with dsDNA cannot be excluded. However, it is evident that the 16/6 ID, when detectable in sera without DNA binding capacity, may be present as LCA.

There are a number of possible relationships which could exist between these autoantibodies in SLE patients (see Fig. 2). There is evidence that 1/22 monoclonals which bind DNA and which were prepared from blood lymphocytes in SLE patients (Shoenfeld *et al.*, 1985) are cytotoxic for human lymphocytes. This would suggest that some anti-DNA clones recognize shared epitopes on DNA and lymphocyte surfaces (Fig. 2a). We could find no evidence for epitope sharing with the two sera examined (RR and ML). Absorption of ML serum with tonsil cells had little effect on anti-ssDNA activity (Table 2) indicating that these autoantibodies belong to a different set from LCA. This was confirmed by the observation that there was no change in LCA following absorption with ssDNA (Table 3; Fig. 2b). In addition, in ML serum 16/6 ID was not associated with anti-DNA either inside or outside the binding site (Table 4), although present on much of the LCA (Fig. 1). Serum RR was similar to that of ML in respect of LCA but little anti ssDNA was detected (Fig. 2c).

In our experiments, autoantibodies to the two antigens ssDNA and lymphocyte surfaces did not cross-react and they did not share 16/6 ID. This indicates that at least for these two sera studied there is no evidence for them being related as members of a non-specific parallel set (see Fig. 2d; Jerne, 1974). However, we cannot exclude the possibility that they share other idiotopes or that autoantibodies in other SLE sera might bear 16/6 ID with distinct LCA and DNA binding activities.

It is not known why SLE patients have autoantibodies directed against a number of seemingly different antigens including ssDNA, vimentin and lymphocyte surfaces. The possession of 16/6 ID by these autoantibodies may shed some light on this. Malignant clones have been described which secrete 16/6+ve anti *Klebsiella* polysaccharide antibodies which also bind to ssDNA and polynucleotides (Naperstek *et al.*, 1985). Mutations at the level of anti-bacterial responses could

give rise to anti-DNA activities (Diamond & Scharf, 1984). Accumulation of mutations could give rise to LCA activity or vice versa. Epitope sharing by LCA and DNA antibodies may represent an intermediate stage between the two extremes which would be 16/6 ID on DNA antibodies in some patients and LCA in others.

In further studies we will examine the genetic relationships of expression of 16/6 ID and LCA by examining the family members of SLE patients, over 20% of whom have been found to express this idiotype (Isenberg *et al.*, 1985).

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