

IMMUNOCHEMICAL SIMILARITIES BETWEEN  
MONOCLONAL ANTIBACTERIAL WALDENSTROM'S  
MACROGLOBULINS AND MONOCLONAL ANTI-DNA LUPUS  
AUTOANTIBODIES

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Anti-DNA antibodies are an important and characteristic feature of systemic lupus erythematosus (SLE),<sup>1</sup> but little is known about the cause of their production. The origin of these autoantibodies has been sought by studies of immunoregulatory defects in the disease (1, 2), as well as by analyses of their antigen-binding properties (3), idiotypes (4, 5), and structure (6, reviewed in 7). In previous work, a human anti-DNA monoclonal autoantibody (mAb) termed 16/6 was found to bear an idiotypic determinant that also occurs in the serum of patients with active SLE (8), and in other anti-DNA mAb from unrelated patients (5). Four of these mAb have nearly identical reactions with an antiserum against the 16/6 idiotypic (5). Moreover, the first 40 light chain amino terminal residues of all four antibodies are identical (7). This light chain sequence also recurs, with the exception of a single hypervariable region residue, in the human macroglobulin WEA (9). The WEA IgM is of further interest because it has binding specificity for the *Klebsiella pneumoniae* polysaccharide K30 (10). We studied a panel of Waldenstrom macroglobulins with anti-*Klebsiella* activity and found that some of them not only shared idiotypic determinants with the lupus anti-DNA autoantibody 16/6 but also bound to nucleic acid antigens.

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<sup>1</sup> *Abbreviations used in this paper:* ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; nDNA, native DNA; PBS, phosphate-buffered saline; SLE, systemic lupus erythematosus; ssDNA, single-stranded DNA; TBS, Tris-buffered saline; TPBS, Tween-20-supplemented PBS; UV, ultraviolet; V, variable region of Ig.

### Materials and Methods

*Human mAb.* The human mAb 16/6 and 21/28 were produced by human-human hybridomas that were derived from two patients with SLE, as previously described (11). Human mAb WEA, NAE, ROS, MAY, TO, and CLE are IgM antibodies that were isolated from the plasma of patients with Waldenstrom's macroglobulinemia (12, 13). IgM FIS was isolated from a patient with polyneuropathy and IgM SON from a patient with mixed cryoglobulinemia. Relevant clinical data and the purification of the Ig have been described elsewhere (9, 11, 14, 15).

*Antigens.* The synthetic polynucleotides poly(I), poly(G), poly(A) and poly(dT) were purchased from P. L. Biochemicals, Milwaukee, WI, and Collaborative Research, Waltham, MA. Native DNA (nDNA), single-stranded DNA (ssDNA), and RNA were prepared as described previously (16). *Klebsiella* polysaccharides K30 (17) and K21 (18) were generous gifts from Professor B. Lindberg (Stockholm University, Stockholm, Sweden).

*Ligand-binding Assays.* mAb were tested for their ability to bind to *Klebsiella* polysaccharides or polynucleotides by means of an enzyme-linked immunosorbent assay (ELISA). *Klebsiella* polysaccharides were diluted to 1  $\mu\text{g/ml}$  in 0.05 M borate buffer, pH 8.4, and 150  $\mu\text{l}$  of the solution was added to the wells of polystyrene plates. After incubation overnight at 4°C, the plates were washed with phosphate buffered saline (PBS), at pH 7.4 and containing 1% Tween-20 (TPBS), then with PBS; 150  $\mu\text{l}$  of the antibody diluted in PBS containing 0.1% Tween-20 was added to the wells and incubated for 2 h at 23°C. After three washes with TPBS and PBS, 150  $\mu\text{l}$  of goat anti-human IgM conjugated to alkaline phosphatase was added, and the plates were incubated overnight at 23°C. Determination of bound alkaline phosphatase was performed as described previously (11).

A similar procedure was used to assay the binding of mAb to nucleic acids. The polynucleotides were diluted to 2.5  $\mu\text{g/ml}$  in Tris-buffered saline (TBS), and 150  $\mu\text{l}$  of the solution was added to a poly-L-lysine-coated plate; after 2 h the plate was washed three times with TBS. The plate was then blocked with poly-L-glutamate and stored at 4°C for at least 12 h. The wells were blocked with phosphate-EDTA buffer containing 1% bovine serum albumin for 30 min at 23°C before adding the antibody to the coated wells. The plates were washed with PBS containing 0.1% Tween-20, and the enzyme-linked goat anti-human IgM was diluted in TBS.

For competitive immunoassays, equal volumes of mAb and various dilutions of the inhibitor (*Klebsiella* polysaccharide or polynucleotide) were mixed and incubated for 1 h at 37°C, and then at 4°C overnight. The mixtures were then tested by ELISA for their residual binding to the antigen-coated plate.

*Idiotypic Assays.* A rabbit antiidiotypic serum against the anti-DNA mAb 16/6 was used for these experiments. The preparation of this antibody and its specificity have been described previously (5). Lupus mAb or the macroglobulins were diluted in 0.05 M borate buffer, pH 8.4, in a concentration of 1  $\mu\text{g/ml}$ , and 150  $\mu\text{l}$  was added to each well of a polystyrene plate and incubated overnight at 4°C.

For competitive inhibition, equal volumes of various dilutions of the antibodies were incubated with rabbit anti-16/6 antiserum for 1 h at 37°C, and then overnight at 4°C. The mixture was then transferred to the Ig-coated plate and incubated for 2 h at 23°C. After three washes with TPBS and PBS, 150  $\mu\text{l}$  of goat anti-rabbit Ig conjugated to alkaline phosphatase was added. The plates were incubated overnight at 23°C and the bound alkaline phosphatase was measured.

Plates coated with rabbit anti-16/6 serum (in borate buffer) were used in a solid-phase ELISA in which dilutions of the antigens were used as competitors for the reaction between the mAb and the antiidiotypic antibody.

### Results

*Binding of IgM mAb to Klebsiella Antigens.* The reactions of seven monoclonal macroglobulins, the monoclonal lupus autoantibody 16/6, and pooled normal human IgM with the *Klebsiella* polysaccharides K30 and K21 were examined by both direct binding to immobilized bacterial antigens and by competitive binding

in solution. Four macroglobulins (NAE, ROS, MAY, and WEA) bound to K30 in the direct binding assay (Table I). This reaction was inhibitable by preincubation of the macroglobulins with K30. Similar amounts of K30 (6–14 ng/ml) were needed for 50% inhibition of the binding of NAE, MAY, and WEA to K30, whereas a higher amount (4,000 ng/ml) was needed for 50% inhibition of ROS. The macroglobulins MAY and ROS also bound to K21; this reaction was inhibited by 6 ng/ml and 4,000 ng/ml of K21, respectively. MAY and ROS thus had similar quantitative reactions with both polysaccharides.

As shown in Fig. 1, the reaction of WEA and K30 was almost completely inhibited by 100 ng/ml of K30; in contrast, <10% inhibition was achieved by 20  $\mu$ g/ml of K21. The reaction of ROS and K30 was inhibited by both K30 and K21 (Fig. 1). 16/6 bound weakly to both K30 and K21 in a direct binding assay, but these reactions could not be inhibited by as much as 20  $\mu$ g/ml of the *Klebsiella* antigens (Table I). The macroglobulins TO, CLE, and FIS, and pooled IgM did not bind to either K30 or K21.

*Reactions of Waldenstrom's Macroglobulins with Polynucleotides.* Two macroglobulins were found to react with polynucleotides in the direct binding assay. WEA reacted with poly(G), poly(I) and ssDNA, but not with nDNA, poly(dT), or RNA (Fig. 2 and Table II). NAE reacted only with poly(G) (Table II). None of the other macroglobulins or pooled normal IgM reacted with any of the polynucleotides examined (Table II). The reaction of WEA with poly(G) could be inhibited by poly(G), poly(I), and ssDNA; 50% inhibition occurred with 80 ng/ml, 1,000 ng/ml and 3,500 ng/ml, respectively, of these polynucleotides. There was no inhibition by poly(dT) or poly(A). The reaction with NAE with poly(G) was inhibited by excess poly(G) (50% inhibition occurred with 200 ng/ml), but not by ssDNA, poly(dT), poly(I), or poly(A) (Table II).

*Inhibition of Binding of Macroglobulins to Polynucleotides by Klebsiella Anti-*

TABLE I  
Reaction of mAb with *Klebsiella* antigens K30 and K21

Antibody tested	Reaction with K30		Reaction with K21	
	Direct binding*	Competitive assay <sup>‡</sup>	Direct binding	Competitive assay
		ng/ml		ng/ml
NAE	0.7	<6	<0.01	ND <sup>§</sup>
ROS	0.25	4,000	0.2	4,000
MAY	0.6	6	1.0	<6
WEA	0.35	14	0.07	NI <sup>†</sup>
TO	<0.01	ND	<0.01	ND
CLE	<0.01	ND	<0.01	ND
FIS	<0.01	ND	<0.01	ND
16/6	0.1	NI	0.1	NI
pooled IgM	<0.01	ND	<0.01	ND

\* Optical density ( $A_{405}$ ) obtained by addition of 0.5  $\mu$ g/ml of antibody to wells coated with 1  $\mu$ g/ml of K30 or K21.

<sup>‡</sup> Concentration of soluble K30 or K21 required for 50% inhibition of the binding of the antibody to solid-phase K30 or K21, respectively.

<sup>§</sup> ND, not done (no direct binding).

<sup>†</sup> NI, not inhibited by 20  $\mu$ g/ml of antigens.

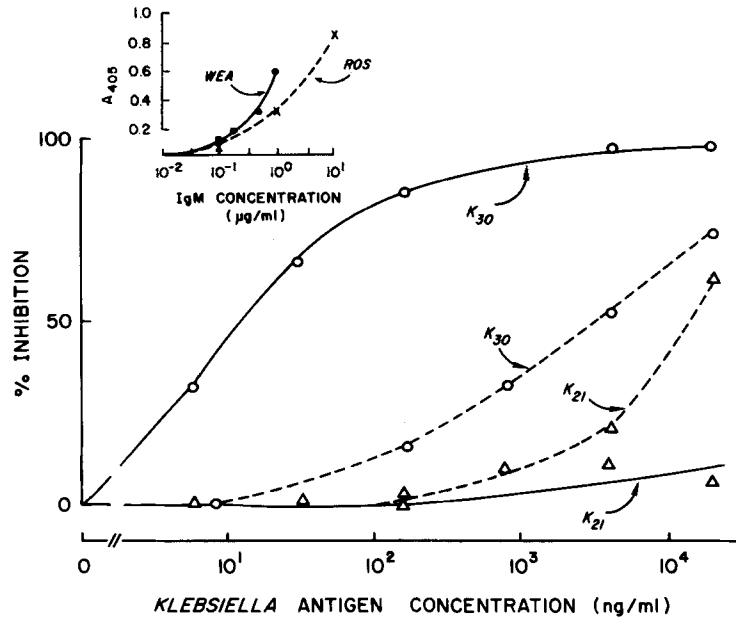


FIGURE 1. Inhibition of binding of IgM WEA and IgM ROS to K30 by *Klebsiella* antigens. Binding of 0.5 µg/ml IgM WEA (—) or IgM ROS (---) to K30 (2.5 µg/ml) on the solid phase was examined by ELISA after incubating the mAb with increasing amount of either K30 (○) or K21 (△). The binding of ROS to K30 was inhibited by K30 and K21, whereas only K30 inhibited the binding of WEA to K30. The inset shows the direct binding of WEA and ROS to K30.

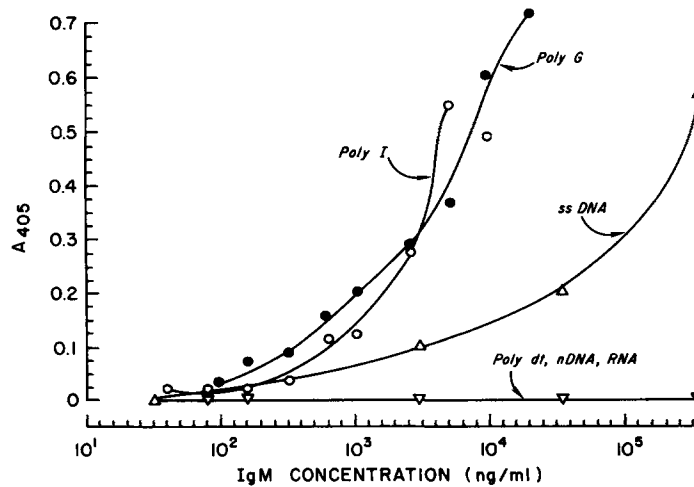


FIGURE 2. Binding of IgM WEA to polynucleotides. The binding of IgM WEA to the polynucleotides poly(G), poly(I), poly(dT), RNA, ssDNA, and nDNA was examined by ELISA. The plates were coated with 2.5 µg/ml of the polynucleotide and the reaction was tested with increasing amounts of IgM WEA. IgM WEA reacted with poly(I), poly(G), and ssDNA but not with poly(dT), RNA, or nDNA.

TABLE II  
*Binding of Macroglobulins to Polynucleotides*

Antibody tested*	Direct binding <sup>‡</sup>				Competitive assay <sup>§</sup>						
	ssDNA	poly(I)	poly(dT)	poly(G)	poly(G)	poly(I)	poly(dT)	poly(A)	ssDNA	K30	K21
NAE	— <sup>†</sup>	—	—	0.7	200	NI <sup>¶</sup>	NI	NI	NI	130	NI
ROS	—	—	—	—							
MAY	—	—	—	—							
WEA	0.15	0.55	—	0.6	80	1,000	NI	NI	3,500	2.5	NI
TO	—	—	—	—							
CLE	—	—	—	—							
FIS	—	—	—	—							
16/6	0.16	0.12	0.2	0.18							
Pooled IgM	—	—	—	—							

\* 10  $\mu\text{g/ml}$  (except 16/6, which was used at 2.5  $\mu\text{g/ml}$ ).

<sup>‡</sup> Optical density ( $A_{405}$ ) readings of ELISA of antibody binding directly to the polynucleotide (2.5  $\mu\text{g/ml}$ ) in the solid phase.

<sup>§</sup> Concentration of soluble polynucleotide or polysaccharide required for 50% inhibition of the binding of antibody to solid-phase poly(G).

<sup>†</sup> No binding.

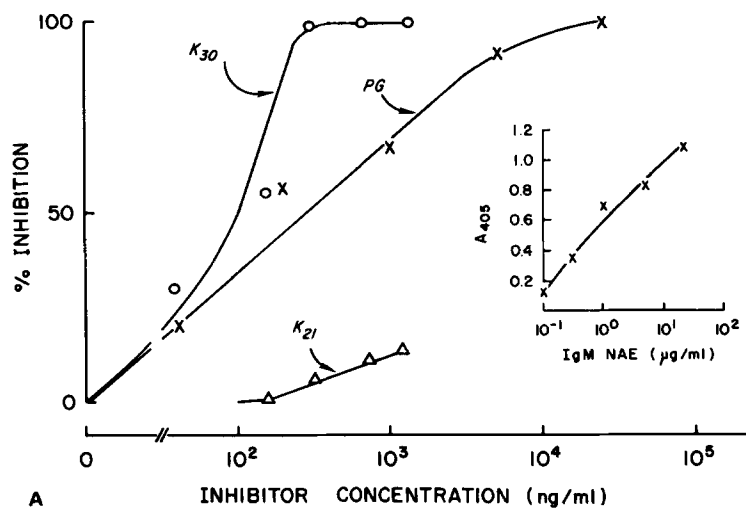
<sup>¶</sup> NI, not inhibited by 1  $\mu\text{g/ml}$  of the polysaccharide or 20  $\mu\text{g/ml}$  of the polynucleotide.

*gens.* To determine whether the reactions of the macroglobulins with both the *Klebsiella* antigens and the polynucleotides were related, we tested the ability of K30 and K21 to inhibit the binding of NAE and WEA to poly(G). As shown in Fig. 3, K30 inhibited the reactions of NAE and WEA with poly(G), and blocked the binding of WEA to ssDNA (Fig. 4). The specificity of the results was demonstrated by the failure of K21 to inhibit the binding of WEA and NAE to poly(G), and of WEA to ssDNA.

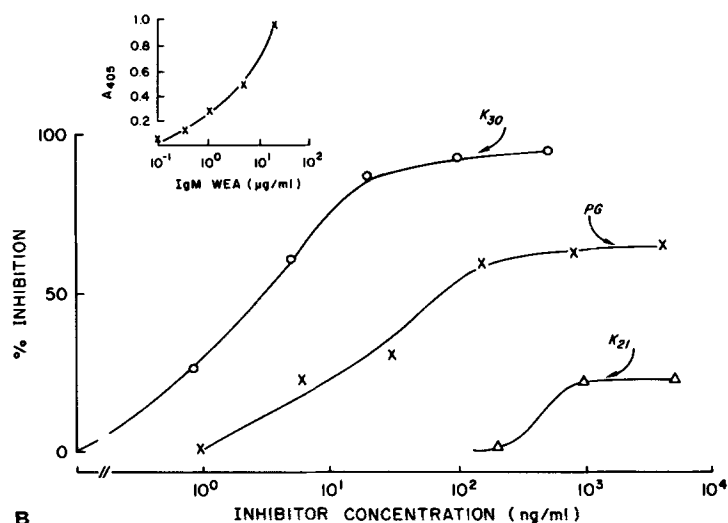
*Reactions of Waldenstrom's Macroglobulins With Anti-16/6 Antiidiotypic Serum.* The idiotypes of the macroglobulins were analyzed with a rabbit antiserum (anti-16/6) that has been shown (5) to react with a group of anti-DNA mAb from human-human hybridomas. NAE, ROS, MAY, WEA, and TO reacted with the antiidiotypic serum in a direct binding assay, but no reaction occurred with CLE, FIS, and pooled human IgM (Fig. 5). The specificity of the reaction was demonstrated by the failure of the macroglobulins to bind to normal rabbit serum, and by the ability of the 16/6 mAb to inhibit the reaction (Fig. 5).

The relative affinities of the reactions of the macroglobulins with the antiidiotypic antibody were tested by competitive inhibition assays, with WEA immobilized on the solid phase. As shown in Fig. 6, 16/6, WEA, NAE, MAY, and ROS inhibited the reaction almost completely, in the order listed, and in amounts that ranged from 400 ng/ml to 6  $\mu\text{g/ml}$ . Much less inhibition occurred with similar concentrations of TO, and no inhibition was found with the other antibodies tested.

To explore the relationship of the 16/6 idio type to the antigen-binding site, we tested the ability of K30 and K21 to inhibit binding of macroglobulins to the anti-16/6 serum. As shown in Fig. 7, the reactions of WEA and MAY with the anti-16/6 serum were totally inhibited by 50 ng/ml of K30. Reactions of antiidiotype with NAE and ROS were only partially inhibited by a much higher concentration of K30. The binding of TO to anti-16/6 was not inhibited by up to 25  $\mu\text{g/ml}$  of K30. Fig. 8 shows the results of a similar experiment with K21. This polysaccharide inhibited the binding of MAY and ROS to the anti-16/6



A



B

FIGURE 3. Inhibition of binding of IgM WEA and NAE to poly(G). The reactions of NAE (A) and WEA (B) (both used at  $0.5 \mu\text{g/ml}$ ) with poly(G) ( $2.5 \mu\text{g/ml}$ ) on the solid phase were examined by ELISA after preincubation of the mAb with increasing amounts of K30, K21, or poly(G). K30 and poly(G), but not K21 inhibited the binding of WEA and NAE to poly(G). The direct binding of WEA and NAE to poly(G) is shown in the inset.

serum, but the binding of NAE to anti-16/6 was only 30% inhibited by  $25 \mu\text{g/ml}$  of K21. No inhibition of the binding of WEA and TO to anti-16/6 serum was obtained with  $25 \mu\text{g/ml}$  of K21.

**Ultraviolet (UV) Light Absorbance of K30.** To detect possible contamination of the K30 polysaccharide with a nucleic acid antigen, we measured the UV light absorbance of this preparation. As shown in Fig. 9, there was no peak of light absorbance in the 260 nm region.

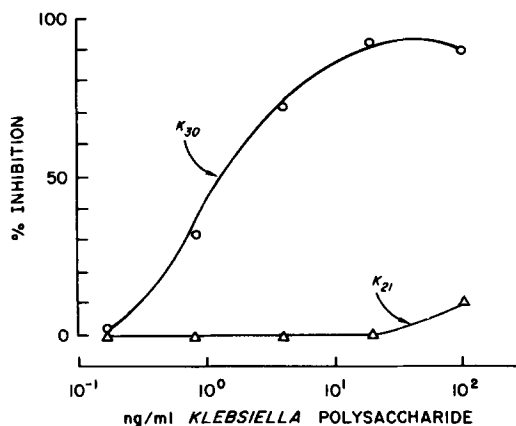


FIGURE 4. Inhibition of binding of IgM WEA to ssDNA. The reaction between WEA (0.5  $\mu\text{g/ml}$ ) and ssDNA (2.5  $\mu\text{g/ml}$ ) on the solid phase was examined by ELISA after preincubation of the mAb with increasing amounts of K30 or K21. K30, but not K21 inhibited the binding of WEA to ssDNA.

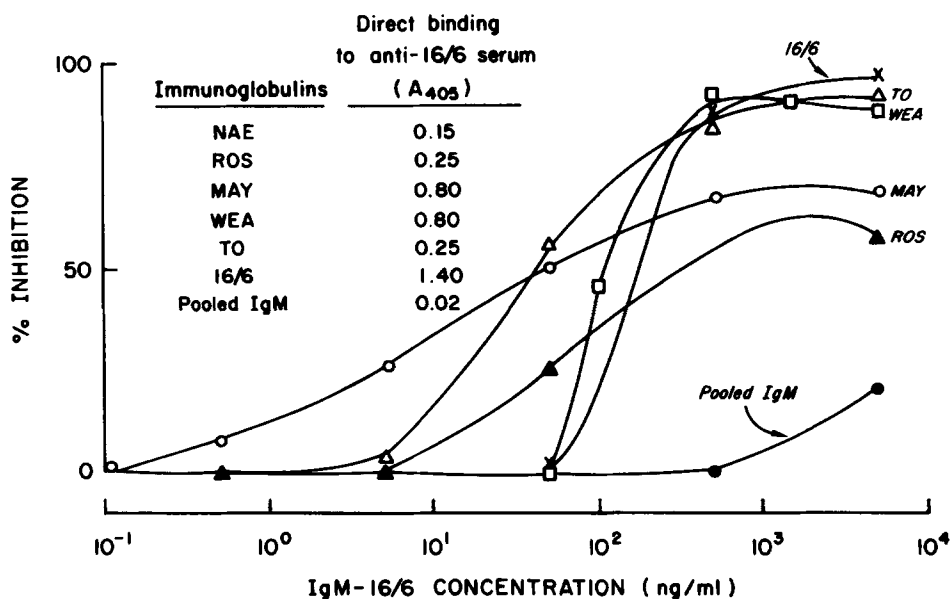


FIGURE 5. Inhibition of binding of anti-16/6 serum to mAb by the 16/6 antibody. The reaction between the anti-16/6 serum (1:8,000) and the mAb-coated plates (1  $\mu\text{g/ml}$ ) was examined by ELISA after preincubation of the serum with various dilutions of the 16/6 antibody. 16/6 inhibited the reaction between anti-16/6 and 16/6, WEA, MAY, ROS, and TO. The optical density ( $A_{405}$ ) of the binding of anti-16/6 serum to mAb before inhibition is shown in the inset.

### Discussion

The main conclusions from these studies are that certain *Klebsiella*-binding monoclonal Waldenstrom's macroglobulins share idiotypic determinants and polynucleotide-binding properties with a monoclonal lupus autoantibody (sum-

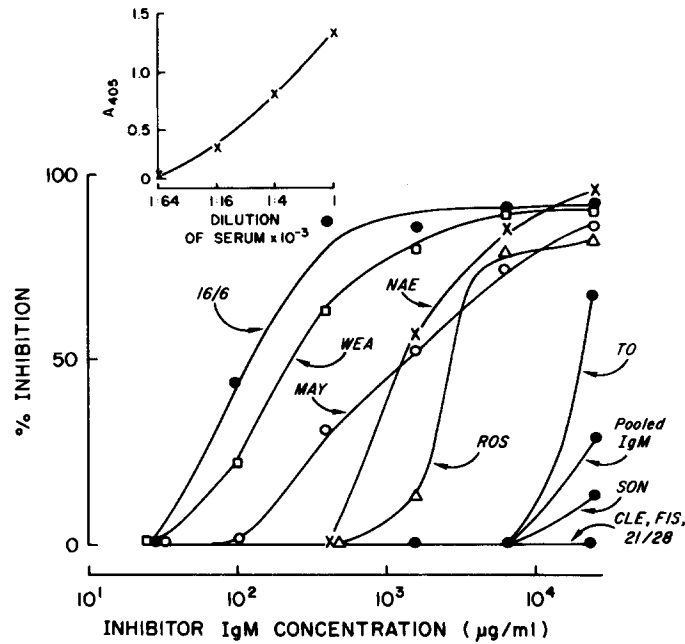


FIGURE 6. Inhibition of reaction between anti-16/6 serum and WEA by various IgM. The residual binding of anti-16/6 serum (1:4,000) to WEA (1 µg/ml on the solid phase) was assayed by ELISA after preincubation of the antiidiotypic serum with various human IgM. The reaction was almost completely inhibited by 16/6, WEA, NAE, MAY, and ROS, and partially inhibited by TO. No or very little inhibition was observed after preincubation with other IgM. Inset shows the direct binding of anti-16/6 serum to WEA.

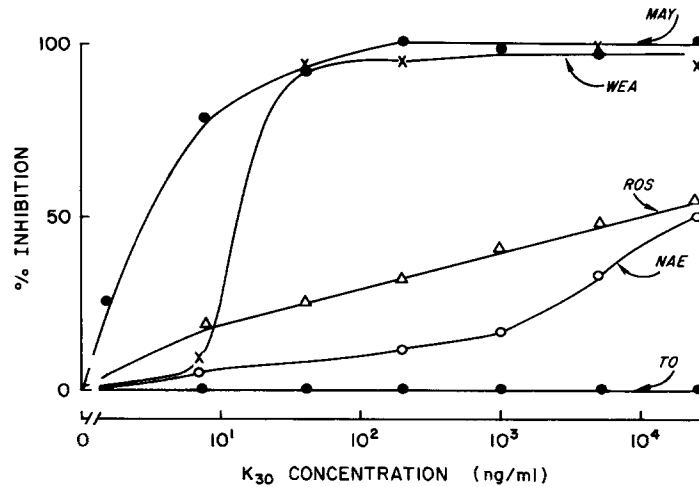


FIGURE 7. Inhibition of binding of macroglobulins to anti-16/6 by K30. The residual binding of macroglobulins to anti-16/6 serum (1:4,000)-coated plates was examined by ELISA after preincubation of macroglobulins with increasing amounts of K30. K30 completely inhibited the reaction with MAY and WEA, partially inhibited the reaction with ROS and NAE, and did not inhibit the reaction of TO with the anti-16/6 serum.



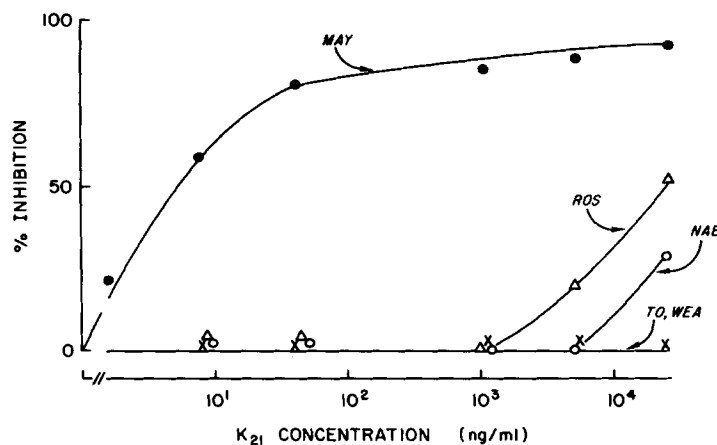


FIGURE 8. Inhibition of binding of macroglobulins to anti-16/6 by K21. Residual binding of macroglobulins to anti-16/6 serum (1:4,000)-coated plates was examined by ELISA after preincubation of the macroglobulins with increasing amounts of K21. K21 completely inhibited the reaction with ROS and MAY, partially inhibited the reaction with ROS and NAE, and did not inhibit the reactions of TO and WEA with the anti-16/6 serum.

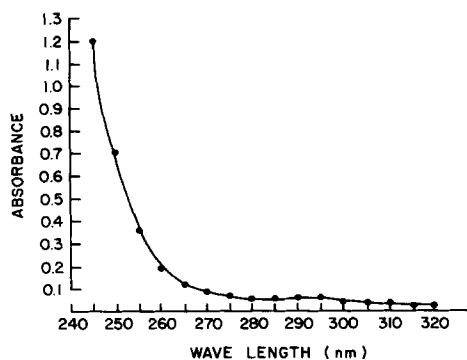


FIGURE 9. UV light absorbance spectrum of the K30 preparation. UV light absorbance of 100  $\mu$ g/ml of K30 was examined from 240 nm to 320 nm by a spectrophotometer. No peak of light absorbance was seen at 260 nm.

marized in Table III), and that these idiotypic determinants in the macroglobulins are closely related to their antigen-binding site.

Monoclonal macroglobulins with specificity for *Klebsiella* polysaccharides seem to occur in a relatively high frequency. In two studies, 7 of 270 monoclonal human IgM were found to react with *Klebsiella* polysaccharides (12, 19). Rao et al. (13) used quantitative immunoprecipitation assays and inhibition of precipitation by oligosaccharides to study the specificity of four of these seven antibodies, as well as two other macroglobulins that were also found to bind *Klebsiella* polysaccharides (13). We studied these six anti-*Klebsiella* macroglobulins for their reactions with the *Klebsiella* antigens K30 and K21 with an ELISA method. IgM NAE and IgM WEA reacted with K30, a *Klebsiella* polysaccharide containing 3,4-pyruvylated D-galactose. IgM ROS and IgM MAY reacted with K21, which contains 4,6-pyruvylated D-galactose, as well as with K30. IgM CLE and IgM

TABLE III  
*Summary of Antigen Binding and Idiotypic Determinants of mAb*

Antibody	Ligand binding					Cross reaction with 16/6 idiotype
	K30	K21	poly(G)	poly(I)	ssDNA	
NAE	+++	0	++	0	0	++
ROS	++	++	0	0	0	++
MAY	+++	+++	0	0	0	++
WEA	+++	0	++	++	++	++
TO	0	0	0	0	0	+
CLE	0	0	0	0	0	0
FIS	0	0	0	0	0	0
16/6	+	+	++	++	++	++
pooled IgM	0	0	0	0	0	0

For results of competitive inhibition by antigen: (0), no reaction in a direct binding assay; (+), no inhibition by 20  $\mu\text{g/ml}$  of antigen; (++) , 50% inhibition by <5  $\mu\text{g/ml}$  of antigen; (+++) , 50% inhibition by <20  $\text{ng/ml}$  of antigen. For results of crossreaction with 16/6 idiotype: (0), no reaction; (+), crossreacted with 16/6, but only weakly with WEA; (++) , 50% inhibition by <10  $\mu\text{g/ml}$ .

TO reacted with neither K30 or K21. Our results are similar to those obtained by Rao et al. (13). In their immunoprecipitation assays, the WEA and NAE proteins reacted with K21 as well as with K30 when examined at pH 4; at pH 7, however, there was no binding to K21, a result similar to ours in an ELISA carried out at pH 7.4. 16/6 reacted weakly with K30 and K21, whereas no reaction with either polysaccharide was found with the other mAb and pooled human IgM.

Two of the macroglobulins that reacted with *Klebsiella* antigens also reacted specifically with certain polynucleotides. WEA reacted with poly(G), poly(I), and ssDNA. NAE reacted with poly(G), but not with the other polynucleotides examined. The polyreactivity observed with these mAb was not simply a result of a high density of negative charges in the reactive antigens, as neither WEA nor NAE reacted with the similarly charged structures of RNA, poly(A), and poly(dT). Moreover, IgM FIS, with specificity for sulfated sugars (14), did not react with the polynucleotides. No contamination of K30 with polynucleotides could be detected by UV light absorbance in the 260 nm region.

Polyreactivity with nucleic acids, as well as with phospholipids, has been found with human and mouse anti-DNA autoantibodies (3, 20, 21). The immunodominant epitope that reacts with such anti-DNA antibodies may be a phosphodiester structure common to both phospholipids and polynucleotides (20). The reactivity of NAE and WEA, however, involved not only polynucleotides but polysaccharides. Previous immunochemical studies (13) showed that the binding of WEA, MAY, NAE, and ROS to *Klebsiella* capsular antigens involves a pyruvylated D-galactose in the polysaccharides, and that the carboxyl group of the pyruvate is crucial to the reaction. Polynucleotides do not contain carboxyl groups, but their backbone structure may have a conformational similarity to pyruvylated galactose polymers. Alternatively, these antibodies may possess one contact region for the bacterial antigen and another for the polynucleotides. The demonstration that K30 completely displaced poly(G) and ssDNA from WEA, and poly(G) from

NAE, suggests that only one binding site exists for these seemingly unrelated antigens.

Five of the six macroglobulins reacted with an antiidiotypic serum against 16/6. This idiotypic has been shown (5) to be in or near the binding site of 16/6 for poly(I). It is also in or near the antigen-combining site of the Waldenstrom's macroglobulins, because their reactions with the antiidiotypic serum was specifically inhibited by the appropriate *Klebsiella* antigens. Similar amounts of K30 or K21 were needed to inhibit the binding of ROS, MAY, and WEA to either the *Klebsiella* antigens or the anti-16/6 serum. In contrast, a much larger amount of K30 was required to inhibit the binding of NAE to the anti-16/6 serum than was needed to inhibit the reaction of NAE with K30 itself. The idiotypic in this antibody is thus less closely related to the polysaccharide-binding site than it is in ROS, MAY, and WEA.

The primary structure of IgM WEA has a striking similarity to that of four lupus mAb that share the 16/6 idiotypic; the first 40 N-terminal amino acids of the light chain of IgM WEA differ from those of the lupus mAb by only a single residue in the first hypervariable region (6, 9). The structural and idiotypic similarities between WEA and 16/6 suggest that at least one determinant of the 16/6 idiotypic on WEA might be located in the first light chain hypervariable region, but additional structural data are required for such a conclusion. Other idiotypic determinants have been correlated with primary structure and mapped to particular variable region sequences (22).

The 16/6 idiotypic is a frequent marker of anti-DNA autoantibodies. It has been found in anti-DNA mAb derived from unrelated lupus patients (5) and in the serum of a large group (40/74) of patients with active SLE (8). The presence of the same idiotypic marker in lupus autoantibodies and Waldenstrom's macroglobulins indicates its wide dispersion in man. In murine systems, recurrent idiotypes are based on the expression of a single or very few variable (V) heavy- and light chain germ line genes. This is the case in the murine response to arsonate (23),  $\alpha$ -1,3- $\alpha$ -1,6-dextran (24), phosphorylcholine (25), and the hapten nitrophenylacetyl (26). Our structural (6) and idiotypic analyses suggest that the recurrent human idiotypic 16/6 also derives from a single germ line V gene.

The sharing of an idiotypic by anti-DNA antibodies and monoclonal macroglobulins from patients with Waldenstrom's disease is reminiscent of the sharing of idiotypes by induced antibodies and myeloma proteins from mice. Antigen-induced anti-pneumococcal polysaccharide antibodies, for example, share idiotypic determinants with a large number of BALB/c myeloma proteins that bind phosphorylcholine (25). Similarly, antidinitrophenyl antibodies share idiotypic determinants with the myeloma protein MOPC 460 (27), and antibodies against  $\alpha$ -1,3- $\alpha$ -1,6-dextran show idiotypes common to myeloma proteins J558, M104, and U-102 (24, 28).

Another parallel between human and murine antibodies is the presence of the idiotypic of an anti-DNA antibody in five antibodies that react with *Klebsiella* antigens (NAE, WEA, ROS, MAY, and TO) (Table III). In the mouse, antiarsonate antibodies with the dominant arsonate-crossreactive idiotypic-positive idiotypic react with *Brucella abortus* (cited in 12), and antibodies with the 460 idiotypic (originally identified in antidinitrophenyl antibodies) react with *Pasteu-*

*rella pneumotropica* (27). The antibacterial properties of these antibodies, as well as those of the 16/6 family, may be a clue to their origin (29). It seems unlikely that the V genes of 16/6 have been conserved because they specify anti-DNA autoantibodies. It is more plausible that the original antigenic specificity of this prototypic autoantibody is not DNA, but a bacterial antigen.

Further evidence of a relationship between anti-DNA autoantibodies and antibacterial antibodies has been elucidated by structural studies of murine monoclonal anti-DNA autoantibodies. Eilat et al. (30) found that the amino acid sequence of the heavy chain of an mAb against nDNA (derived from an NZB/NZW mouse) closely resembles the sequence of an antiphosphorylcholine antibody from a CBA/J mouse. Antibodies of the latter type have been shown to protect mice against rough pneumococci and other bacteria (31). Diamond and Scharff (32) studied a somatic mutant of an antiphosphorylcholine mAb that differed from the original antibody by a single amino acid substitution in the first heavy chain hypervariable region. As a result of this change, the mutant antibody lost its ability to bind to phosphorylcholine, but it acquired reactivity against nDNA and cardiolipin; reactions typical of lupus autoantibodies. Diamond and Scharff (32) suggested that anti-DNA antibodies may arise by mutation of V genes that encode antibacterial antibodies. Alternatively, as shown here, antibacterial antibodies may crossreact with nucleic acid antigens, and thus behave like lupus autoantibodies. These two mechanisms are not mutually exclusive; antibodies generated by either mechanism could have the potential to cause autoimmune disease.

### Summary

Six monoclonal IgM from patients with Waldenstrom's macroglobulinemia that react with *Klebsiella* polysaccharides were tested for their ability to bind to nucleic acid antigens. One of the macroglobulins bound to the polynucleotide poly(G), and one bound to poly(G), poly(I), and single-stranded DNA. The reaction with the polynucleotides was specifically inhibited by the *Klebsiella* polysaccharide K30. A monoclonal lupus anti-DNA antibody (16/6) was found to react weakly with the *Klebsiella* polysaccharides K30 and K21. Five of the Waldenstrom macroglobulins shared an idiotypic determinant with the 16/6 anti-DNA antibody. The reaction between the macroglobulins and the antiidiotype serum was specifically inhibited by *Klebsiella* polysaccharides, an indication that the idiotypic marker was in the antigen-binding site of the macroglobulins. These results indicate the existence of widely dispersed conserved variable region genes that encode idiotypically related immunoglobulins with the capacity to bind to both bacterial polysaccharides and nucleic acids. Such genes can be expressed by patients with either Waldenstrom's macroglobulinemia or systemic lupus erythematosus.

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### References

1. Smith, H. R., and A. D. Steinberg. 1983. Autoimmunity—a perspective. *Ann. Rev. Immunol.* 1:175.

2. Miller, K. B., and R. S. Schwartz. 1979. Familial abnormalities of suppressor cell function in systemic lupus erythematosus. *N. Eng. J. Med.* 301:803.
3. Shoenfeld, Y., J. Rauch, H. Massicote, S. K. Datta, J. Andre-Schwartz, B. D. Stollar, and R. S. Schwartz. 1983. Polyspecificity of monoclonal lupus autoantibodies produced by human-human hybridomas. *N. Eng. J. Med.* 308:414.
4. Solomon, G., J. Schiffenbauer, H. D. Keiser, and B. Diamond. 1983. Use of monoclonal antibodies to identify shared idiotypes on human antibodies to native DNA from patients with SLE. *Proc. Natl. Acad. Sci. USA.* 80:850.
5. Shoenfeld, Y., D. A. Isenberg, J. Rauch, M. P. Madaio, B. D. Stollar, and R. S. Schwartz. 1983. Idiotypic cross-reactions of monoclonal human lupus autoantibodies. *J. Exp. Med.* 158:718.
6. Atkinson, P. M., G. Lampman, B. C. Furie, Y. Naparstek, R. S. Schwartz, B. D. Stollar, and B. Furie. 1985. Homology of the NH<sub>2</sub>-terminal amino acid sequence of the heavy and light chains of human monoclonal lupus autoantibodies containing the dominant 16/6 idio type. *J. Clin. Invest.* 75:1138.
7. Schwartz, R. S., D. B. Stollar. 1984. The origin of anti-DNA autoantibodies. *J. Clin. Invest.* 75:321.
8. Isenberg, D. A., Y. Shoenfeld, M. P. Madaio, J. Rauch, M. Reichlin, B. D. Stollar, and R. S. Schwartz. 1984. Measurement of anti-DNA antibody idiotypes to monitor the course of systemic lupus erythematosus. *Lancet.* 2:418.
9. Goni, F., and B. Frangione. 1983. Amino acid sequence of the Fv region of a human monoclonal IgM (protein WEA) with antibody activity against 3,4-pyruvylated galactose in Klebsiella polysaccharides K30 and K33. *Proc. Natl. Acad. Sci. USA.* 80:4832.
10. Kabat, E. A., J. Liao, H. Bretting, E. C. Franklin, D. Geltner, B. Frangione, M. E. Koshland, J. Shyong, and E. F. Osserman. 1980. Human monoclonal macroglobulins with specificity for *Klebsiella* K polysaccharides that contain 3,4-pyruvylated D-galactose and 4,6-pyruvylated D-galactose. *J. Exp. Med.* 152:979.
11. Shoenfeld, Y., S. C. Hsu-Lin, J. E. Gabriels, L. E. Silberstein, B. C. Furie, B. Furie, B. D. Stollar, and R. S. Schwartz. 1982. Production of autoantibodies by human-human hybridomas. *J. Clin. Invest.* 70:205.
12. Harboe, M., J. Deveril, and J. Eriksen. 1975. Capsular swelling and passive haemagglutination induced by monoclonal IgM reacting with acid polysaccharides with Klebsiella. *Acta Pathol. Microbiol. Immunol. Scand. Sect. C. Immunol.* 83:97.
13. Rao, A. S., J. Liao, E. A. Kabat, E. F., Osserman, M. Harboe, and W. Nimmich. 1984. Immunochemical studies on human monoclonal macroglobulins with specificities for 3,4-pyruvylated D-galactose and 4,6-pyruvylated D-glucose. *J. Biol. Chem.* 259:1018.
14. Kabat, E. A., J. Liao, W. H. Sherman, and E. F. Osserman. 1984. Immunochemical characterization of the specificities of two human monoclonal IgM's reacting with chondroitin sulphates. *Carbohydr. Res.* 130:289.
15. Ledford, D. K., F. Goni, M. Pizzolato, F. C. Franklin, A. Solomon, and B. Frangione. 1983. Preferential association of KIIIb light chains with monoclonal human IgMk autoantibodies. *J. Immunol.* 131:1322.
16. Stollar, B. D. 1977. Quantitative microcomplement fixation and radioactive antigen binding assays for measuring anti-DNA antibodies. *Ann. Rheum. Dis.* 36 (Suppl. 1):102.
17. Lindberg, B., F. Lindh, and J. Lonngren. 1979. Structural studies on the capsular polysaccharide of Klebsiella type 30. *Carbohydr. Res.* 70:135.
18. Choy, Y. M., and G. A. Dutton. 1973. The structure of the capsular polysaccharide from Klebsiella K-type 21. *Can. J. Chem.* 51:198.
19. Hannestad, K., J. Eriksen, T. Christenson, and M. Harboe. 1970. Multiple compo-

- nents in a single individual. I. The structural relationship of two serum Mk M-components as revealed by combining specificity and individual antigenic specificity. *Immunochemistry*. 7:899.
20. Lafer, E. M., J. Rauch, C. Andrzejewski, D. Mudd, B. Furie, R. S. Schwartz, and B. D. Stollar. 1981. Polyspecific monoclonal lupus autoantibodies reactive with the polynucleotides and phospholipids. *J. Exp. Med.* 153:897.
  21. Rauch, J., H. Tannebaum, B. D. Stollar, and R. S. Schwartz. 1984. Monoclonal anti-cardiolipin antibodies bind to DNA. *Eur. J. Immunol.* 14:529.
  22. Clevinger, B., J. Schilling, L. Hood, and J. M. Davie. 1980. Structural correlates of crossreactive and individual idiotypic determinants on murine antibodies to  $\alpha$ -(1  $\rightarrow$  3) dextran. *J. Exp. Med.* 151:1059.
  23. Margolies, M. N., E. C. Juszczak, R. Near, A. Marshak-Rothstein, T. L. Rothstein, V. L. Sato, M. Siekevitz, J. A. Smith, L. J. Wysocki, and M. L. Gefter. 1983. Structural Correlates of Idiotype in the Arsonate System. *Ann. N.Y. Acad. Sci.* 418:48.
  24. Hansburg, D., D. E. Briles, and J. M. Davie. 1977. Analysis of the diversity of the murine response to Dextran B 1355. II. Demonstration of multiple idiotopes with variable expression in several strains. *J. Immunol.* 119:1406.
  25. Lieberman, R., M. Potter, E. B. Mushinski, W. Humphrey, and S. Rudikoff. 1974. Genetics of a new IgV<sub>H</sub> (T15 idiotype) marker in the mouse regulating natural antibody to phosphorylcholine. *J. Exp. Med.* 139:983.
  26. Jack, R. S., T. Immanishi-Kari, and K. Rajewski. 1972. Idiotype analysis of the response of C57BL/6 mice to the 4-hydroxy-3-nitrophenyl acetyl group. *Eur. J. Immunol.* 7:559.
  27. Marion, T. N., E. A. Dzierzak, H.-S. Lee, R. L. Adams, and C. A. Janeway. Non-dinitrophenyl-binding immunoglobulin that bears a dominant idiotype (Id460) associated with antidinitrophenyl antibody is specific for an antigen on *Pasteurella pneumotropica*. *J. Exp. Med.* 159:221.
  28. Blomberg, B., W. R. Geckeler, and M. Weigert. 1972. Genetics of the antibody response to dextran in mice. *Science (Wash. DC)*. 177:178.
  29. Bottomly, K. 1984. All idiotypes are equal, but some are more equal than others. *Immunol. Rev.* 29:45.
  30. Eilat, D., M. Hochberg, J. Pumphrey, and S. Rudikoff. 1984. Monoclonal antibodies to DNA and RNA from NZB/NZW F<sub>1</sub> mice. Antigenic specificities and NH<sub>2</sub> terminal amino acid sequences. *J. Immunol.* 133:489.
  31. Briles, D. E., M. Nahm, K. Schroer, J. Davie, P. Baker, J. Kearny, and R. Barletta. 1981. Antiphosphorylcholine antibodies found in normal mouse serum are protective against intravenous infection with type 3 *Streptococcus pneumoniae*. *J. Exp. Med.* 153:694.
  32. Diamond, B. and M. D. Scharff. 1984. Somatic mutation of the T-15 heavy chain gives rise to an antibody with autoantibody specificity. *Proc. Natl. Acad. Sci. USA.* 81:5841.