Idiotypic Markers of Polyclonal B Cell Activation

Public Idiotypes Shared by Monoclonal Antibodies Derived from Patients with Systemic Lupus Erythematosus or Leprosy

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Abstract

We investigated idiotypic markers of monoclonal antibodies derived from patients with polyclonal B-cell activation. Four monoclonal antibodies with different ligand binding specificities derived from a patient with lepromatous leprosy and three monoclonal anti-DNA antibodies from two patients with SLE were studied. Three new public idiotopes, which were common to monoclonal antibodies from all three patients, were defined by five polyclonal rabbit antiidiotypes, two monoclonal mouse antiidiotopes, and a monoclonal mouse antibody against a synthetic peptide that contains residues of the heavy chain CDR-1 of a monoclonal lupus anti-DNA antibody. The antibody against the synthetic idiotype was found to react with native immunoglobulins in solution. One idiotope was found to be consistently immunogenic in all animals tested. Since the three patients are of different ethnic origins, these shared idiotypes are probably encoded by germline V genes. These genes may be recurrently expressed in states of polyclonal B-cell activation, regardless of etiology. The results suggest that some autoantibodies arise by expansion of a pool of precursors in the normal antibody repertoire.

Introduction

The immunoglobulin variable region contains antigenic determinants, termed idiotypes, which can be recognized by serological techniques. In a typical case, an idiotype of a monoclonal human antibody is defined by an antiidiotypic antiserum raised in a rabbit. If the defining serological reagent is itself a monoclonal antibody, then the variable region antigen is termed an idiotope. The structure that determines the serological specificity of an idiotype may reside in either the heavy or light chain of the variable region (1–3), or it may consist of an antigenic surface made up of both chains (4–6). An idiotype, therefore, is a serologically defined product of immunoglobulin V genes (7). Idiotypes are of considerable interest because they are recognition elements in regulatory networks of B cells and T cells (8); and, when used as serological markers, they can trace the origins of idiotypically related antibodies.

This paper deals with idiotypic markers of monoclonal antibodies derived from patients with leprosy or systemic lupus erythematosus (SLE).[1] In both disorders there is polyclonal B cell activation, a common event in many infectious and autoimmune diseases; and in both conditions there are autoantibodies against diverse, ubiquitous autoantigens like DNA (9, 10). The particular question we addressed was whether antibodies produced in leprosy and SLE share common idiotypic markers, despite the different causes and predisposing factors of these two diseases (11, 12). We found that some leprosy- and lupus-derived monoclonal antibodies bear closely related, probably highly conserved, idiotypes. Our results support the hypothesis that certain idiotypic markers are recurrently expressed in polyclonal B cell activation, regardless of its cause.

Methods

Human monoclonal antibodies. As previously described (13), peripheral lymphocytes from patients with SLE or leprosy were fused with cells of the GM4672 cell line. Before fusion the lymphocytes were stimulated with pokeweed mitogen (Gibco Laboratories, Grand Island, NY), and also, in the case of those from the patient with leprosy, with staphylococcal protein A (The Enzyme Center, Inc., Boston, MA). Immunoglobulin-producing clones were selected, subcloned, and expanded, and the immunoglobulins were purified from culture supernatants on goat anti-human IgM (Cappel Laboratories, Cochranville, PA) linked to Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ). Seven monoclonal antibodies were used in the present study. All were of the IgM isotype. Four of them (8E7, 4D5, 4G7, and TH9) were derived from the peripheral lymphocytes of a male from Thailand with borderline lepromatous leprosy (Duggan, D., et al., manuscript in preparation); one (16/6) was derived from the peripheral lymphocytes of an American male of Irish/Scottish descent with SLE (13); the remaining two (1/13 and 21/28) were derived from the peripheral and splenic lymphocytes, respectively, of an Iranian woman with SLE (13). The ligands identified for these antibodies are summarized in Table I.

Polyclonal rabbit antisera. Rabbit polyclonal anti-idiotypic sera were raised against the four leprosy monoclonals 8E7, 4D5, 4G7 and TH9 and the lupus monoclonal 16/6. (The antiserum against 16/6 was distinct from that generated in a previous study [16]). New Zealand White rabbits (Jackson Laboratories, Bar Harbor, ME) were immunized intramuscularly and subcutaneously with the purified monoclonal weekly or fortnightly. Complete and incomplete Freund's adjuvant (Difco Laboratories, Detroit, MI) were used for the first and second injections; all remaining injections were given with phosphate-buffered saline (PBS) only. After five injections aliquots of rabbit serum were tested for the presence of antibodies to human IgM by Ouchterlony double immunodiffusion. Rabbits with positive sera were then bled; those with negative sera received further injections of monoclonal antibody before subsequent retesting. Positive sera were absorbed extensively on normal human IgG and IgM

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1. *Abbreviations used in this paper:* ALP, alkaline phosphatase; CDR, complementarity-determining region; HAT, hypoxanthine, aminopterin, and thymidine; KLH, keyhole limpet hemocyanin; NHIgM, normal human IgM (pooled myeloma protein); PMSF, phenylmethylsulphonylfluoride; RT, room temperature; SLE, systemic lupus erythematosus.

Table I. Antigen-binding Specificities of Human Monoclonal Antibodies

Patient/diagnosis	Racial origin	Antibody	Ligand	Antiidiotype raised: Rabbit (polyclonal)	Antiidiotype raised: Mouse (monoclonal)
Leprosy	Thai	8E7	Mitochondria	RId8E7	MId8E7
		4G7	ssDNA; *M. leprae*	RId4G7	
		TH9	$\alpha\alpha$ACh receptor*	RIdTH9	
		4D5	?	RId4D5	
Lupus (i)	Celtic	16/6	ssDNA	RId16/6	MId16/6 MD‡
Lupus (ii)	Iranian	1/13	ssDNA		
		21/28	ssDNA; poly(I) (14)		

Antigen-binding specificities of human monoclonal antibodies, and antiidiotype reagents raised against them. ssDNA: single-stranded DNA. poly(I): polyinosine. *M. leprae* refers to a sonicate of the whole organism treated with DNAase and RNAase. * Mouse monoclonal antibody AH21, which recognizes a cross-reactive idiotope on antiacetylcholine receptor antibodies from patients with myasthenia gravis (15). No ligand has so far been identified for 4D5. ‡ This monoclonal antibody was raised against a synthetic peptide (D) which contains residues of the heavy chain 1st CDR of 16/6.

(pooled myeloma protein, Cappel Laboratories) linked to Sepharose 4B until no optical density (OD) peak was detected on elution with 0.1 M glycine/HCl pH 2.5. The absorbed sera were then tested by an enzyme-linked immunosorbent assay (ELISA) for binding to their respective immunizing monoclonal antibody, and for the absence of binding to normal human IgM (NHIgM); this was performed by both direct-binding and competition assays. For isolation of the immunoglobulin from rabbit serum, the absorbed serum was passed over a column of Sepharose 4B linked to goat anti-rabbit IgG (Cappel Laboratories). The antibodies were eluted with 0.1 M glycine/HCl (Fisher Scientific, Fair Lawn, NJ) pH 2.5, dialyzed against PBS/0.02% azide (Sigma Chemical Co., St. Louis, MO), and stored at −20°C until use.

Mouse monoclonal antiidiotypes. The generation of mouse monoclonal anti-16/6 has been previously described (17). In order to generate a mouse monoclonal antibody against the human leprosy-derived monoclonal 8E7, a 12-wk old BALB/c mouse (Jackson Laboratories) was immunized with purified 8E7. Complete and incomplete Freund's adjuvant were used for the first and second injections; these were given intraperitoneally. Four subsequent injections of 8E7 in PBS only were given intravenously and intraperitoneally, the last three being administered 6, 3, and 1 d before fusion. Splenocytes from the mouse were fused with cells from the NS1 myeloma cell line using 44% polyethylene glycol (J. J. Baker Chemical Co., Phillipsburg, NJ) according to a standard protocol (18). The fused cells were plated, selected in medium containing hypoxanthine, aminopterin, and thymidine (HAT), and subcloned as previously described (18). Culture supernatants from wells showing hybridoma growth were tested for ELISA for immunoglobulin production and anti-idiotype activity against 8E7. The cells from selected wells were subcloned at 0.5 cells per well and retested. After five such subcloning procedures a stable antiidiotype-producing line was isolated. These cells were then grown in bulk, and the supernatant harvested and stored in 0.02% azide and 2 mM phenylmethylsulphonylfluoride (PMSF; Sigma Chemical Co.) at 4°C.

Synthetic peptides. The sequences of the first 40 amino-acids of the amino terminal portion of the heavy chains of 16/6 and 1/13 have been determined in a previous study (19). A 12 amino acid peptide (peptide "D") was synthesized which contains residues of the first complementarity determining region (CDR-1) of the 16/6 heavy chain, together with seven flanking amino acids. The sequence of this peptide begins at amino acid residue 27, and is as follows: Phe-Ser-Phe-Ser-Pro-Ser-Ala-Met-Ser-Trp-Val-Arg. An irrelevant peptide ("F", Leu-Ile-Tyr-Gly-Ala-Thr-Ser-Leu-Gln-Ser-Gly-Val-Pro) was used as a control. The synthesis of these peptides was performed by Dr. S. Wood, University Hospital, Boston University Medical Center. The peptides were linked to keyhole limpet hemocyanin (KLH, Sigma Chemical Co.) by an adaptation of a previously described method (20). A mixture of equal weight (2–5 mg) of lyophilized KLH and the lyophilized peptide was prepared in a final volume of 0.7 ml of 0.25 M K_2PO_4, pH 7.2. 4 μl of 25% glutaraldehyde (Fisher Scientific Co.) was added to the solution, the mixture was vortexed and then allowed to stand at room temperature (RT) for 30 min. It was then dialyzed against three changes of PBS/0.02% azide, and stored at −20°C until use. Linking of peptide D to bovine serum albumin (BSA) (Fisher Scientific Co.) was carried out using the same method. As a control, BSA was treated in the same way in the absence of peptide.

Generation of monoclonal antibody against peptide D. A 12-wk-old BALB/c mouse (Jackson Laboratories) was injected intraperitoneally with peptide D-KLH conjugate (500 μg of KLH) in complete Freund's adjuvant, followed by a further injection (250 μg of KLH) intravenously/intraperitoneally in PBS 2 wk later. 5 d after the second injection splenocytes from the mouse were fused with NS1 cells. The methods for cell fusion, plating, HAT selection and subcloning were the same as those described above. Clones were selected by ELISA for the production of immunoglobulin which bound specifically to peptide D linked to KLH (D-KLH) or (D-BSA) and not to peptide F linked to BSA (F-BSA), or to BSA alone, glutaraldehyde-treated or untreated. After three subcloning procedures a stable antipeptide cell line was isolated. This was grown in bulk. The harvesting and storage of culture supernatant were performed as above.

Determination of immunoglobulin concentration. A standard ELISA method was used. Microtiter plates (Immulon 1, Dynatech Laboratories, Alexandria, VA) were coated with the relevant antiserum (e.g., goat anti-human IgM) at 1 μg/ml. The plates were blocked with 3% BSA and the test samples applied in serial dilutions (2 h at RT). Myeloma immunoglobulins (IgM or IgG; Cappel Laboratories) were used to generate control curves. Alkaline phosphatase (ALP)-linked second step antibodies (e.g., [ALP]-linked goat anti-human IgM [Sigma Chemical Co.]) were then added, followed by the developing substrate.

Detection of antiidiotypes. Two methods, direct binding and liquid-phase competition, were used for detection and quantitation of cross-reacting idiotypes. In the direct method, microtiter plates were coated with human monoclonal antibody or as a negative control, with NHIgM at 0.75 g/ml. The plates were blocked with 3% BSA and the antiidiotype reagent was then added in serial dilutions, e.g., rabbit antiserum, mouse hybridoma culture fluid or purified mouse monoclonal antibody (2 h at RT); negative controls included normal rabbit serum (extensively absorbed on normal human IgG and IgM) and TEPC 183 or UPC 10 (Litton Bionetics, Kensington, MD) in NS1 culture supernatant or in PBS/0.1% Tween (Fisher Scientific Co.). The appropriate second-step

ALP-linked antibody was then added (e.g., goat anti-rabbit IgG, goat anti-mouse IgM or IgG), followed by developing substrate. Curves generated by such direct binding assays were used to determine the optimum dilution of antiidiotype for use in competition experiments. For rabbit anti-16/6, -8E7, -4G7, -4D5, and -TH9 these were 1:500, 1:1,000, 1:2,000, 1:1,000, and 1:20,000, respectively; for culture fluids of mouse anti-16/6, -8E7, and -D they were 1:20, 1:2, and 1:4, respectively. For purified mouse anti-16/6 the optimum concentration was 0.75 μg/ml.

For competition assays the human monoclonal antibody bearing the idiotype under examination was coated on the solid phase at a concentration determined by direct binding experiments (usually 0.75 μg/ml); NHIgM was used as a negative control. The plates were blocked with 3% BSA for 1 h at RT. A mixture of rabbit or mouse antiidiotype and competitor was then added. The dilution or concentration of antiidiotype was previously determined by direct binding assays. Competitors consisted of purified human, mouse or rabbit immunoglobulin. The antiidiotypes and the competitors were incubated together in their final dilutions or concentrations for 1 h at 37°C followed by 1 h at 4°C before being put on the plate (2 h at RT). The appropriate ALP-linked second step antibody (directed against the antiidiotype, i.e., goat anti-rabbit IgG, or goat anti-mouse IgM or IgG) was then added (overnight at RT), followed by developing substrate.

Immunoabsorption of rabbit antisera. In order to examine different subpopulations of rabbit antiidiotype antibodies, we passed individual rabbit antisera over Sepharose-4B linked to the human monoclonal antibody 16/6. The column was eluted with 0.1 M glycine/HCl pH 2.5, and the eluate dialyzed against PBS/0.02% azide. The eluate and the flow-through from the column were used in direct binding and competition assays as described above.

Results

Nomenclature. The following notations will be used for the reagents and assay systems described in this paper. The four monoclonal antibodies derived from the leprosy patient are designated 8E7, 4D5, 4G7, and TH9; the three monoclonal antibodies from the two lupus patients are 16/6 (from one patient) and 1/13 and 21/28 (from the other patient). The rabbit antiidiotypes against these monoclonal antibodies are: RId8E7, RId4D5, RId4G7, RIdTH9, and RId16/6. The monoclonal mouse antiidiotopes are designated MId8E7 and MId16/6; the monoclonal antibody against peptide D is termed MD. For designating competition assays, the antibody in the liquid phase is noted first, followed by the antibody on the solid phase; e.g., RId8E7-8E7.

Idiotypes of monoclonal leprosy antibodies. When examined in homologous idiotype competition assays, i.e., with the immunizing monoclonal antibody on the solid phase and the corresponding rabbit antiidiotype in the liquid phase, the four leprosy antibodies exhibited little or no idiotypic cross-reactivity. In the assay RId8E7-8E7, shown in Table II, the concentration in nanograms per milliliter of 8E7 that produced 50% inhibition was 120, compared to 1,200 and 8,000 of 4G7 and 4D5; 10,000 ng/ml of TH9 did not inhibit this reaction. In the RId4D5-4D5 assay, illustrated in Fig. 1 *b*, 50% inhibition was produced by 26 ng/ml 4D5 and 2,000 ng/ml TH9; 10,000 ng/ml of 8E7 or 4G7 caused < 20% inhibition. Similar specificity was found for the immunizing monoclonal antibody in the RIdTH9-TH9 (Fig. 1 *a*), and RId4G7-4G7 assays (Table II). We can conclude, therefore, that each of the polyclonal rabbit antisera contained a population of antibodies that was relatively specific for a unique idiotypic determinant on the corresponding immunizing monoclonal antibody.

Monoclonal leprosy and lupus antibodies share idiotypes. By contrast with the preceding, a substantial degree of cross-reactivity was found when the leprosy and lupus antibodies were tested in cross-idiotype assays. All seven monoclonal antibodies were cross-reactive when the assay RId8E7-1/13 was employed. The order of cross-reactivity was: 4D5 (50 ng/ml) > 1/13 (60 ng/ml) = 4G7 (60 ng/ml) > 8E7 (100 ng/ml) > 16/6 (160 ng/ml) > 21/28 (1,100 ng/ml) > TH9 (1,700 ng/ml) [concentrations in parentheses gave 50% inhibition; Table II]. With a rabbit antiserum against the lupus antibody 16/6 in the liquid phase and the leprosy antibody 8E7 on the solid phase (RId16/6-8E7), cross-reactions were also found, in the order: 21/28 (60 ng/ml) > 16/6 (200 ng/ml) > 4D5 (350 ng/ml) = 1/13 (350 ng/ml) > 8E7 (450 ng/ml) > 4G7 (1,200 ng/ml) > TH9 (5,000 ng/ml). A notable feature of these results, shown in Fig. 2, is that the inhibition curves are generally similar, indicating that the most cross-reactive monoclonal antibodies bear closely related idiotypic determinants.

An idiotypic relationship between the leprosy and lupus monoclonal antibodies was also seen in an assay with 1/13 on the solid phase and RId16/6 in the liquid phase (Table II). In this case the monoclonal antibody on the plate is structurally related to the monoclonal antibody against which the rabbit antiserum was raised (19). Notably, the leprosy antibody 4D5 showed strong cross-reactivity in this assay; the order of inhibition was: 1/13 (120 ng/ml) = 16/6 (120 ng/ml) > 21/28 (200 ng/ml) > 4D5 (240 ng/ml) > 4G7 (800 ng/ml) > 8E7 (1,400 ng/ml) > TH9 (3,200 ng/ml). Fig. 3 shows the inhibition curves for this assay.

The relative activities of the rabbit anti-8E7 reagent against both 8E7 and 1/13 were compared by examining the binding of serial dilutions of RId8E7 to constant concentrations of 8E7 and 1/13 on the solid phase: RId8E7 bound to 8E7 at sevenfold higher dilutions than were required for similar binding to 1/13 in this assay, while normal rabbit serum did not bind to 8E7 or 1/13 at any dilution (data not shown). In view of the similar inhibition curves in the RId8E7-1/13 competition assay (Fig. 2), this result suggests that a relatively small fraction of the RId8E7 antibodies binds to 1/13.

To confirm that the polyclonal antiidiotype against the leprosy antibody 8E7 contained antiidiotypes against the lupus antibody 16/6, we purified RId8E7 by absorption on a 16/6-Sepharose column. Tests of the eluate and flow-through fractions demonstrated that the column removed all of the anti-1/13 ac-

Table II. Inhibition of Binding of Rabbit Antisera to Human Monoclonal Antibodies: ng/ml of Competitor Required for 50% Inhibition

Antiserum:	8E7		4D5	4G7	TH9	16/6	
Binding to: / Inhibitor	8E7	1/13	4D5	4G7	TH9	8E7	1/13
1/13	2,000	60	NI	NI	NI	350	120
16/6	NI	160	*	*	*	200	120
21/28	NI	1,100	*	*	*	60	200
8E7	120	100	NI	NI	NI	450	1,400
4D5	8,000	50	26	NI	NI	350	240
4G7	1,200	60	NI	75	NI	1,200	800
TH9	NI	1,700	2,000	NI	70	5,000	3,200
NHIgM	NI	NI	NI	NI	NI	NI	NI

NI, not inhibited by amounts of competitor up to 10,000 ng/ml.
* Not tested.

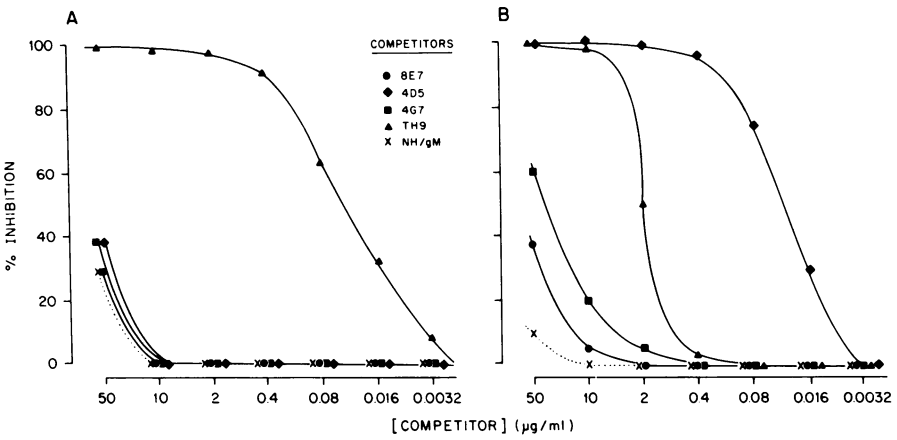

Figure 1. Inhibition by leprosy-derived monoclonals in homologous competition assays: (A) binding of RIdTH9 to TH9; (B) binding of RId4D5 to 4D5.

tivity of the antiserum (Fig. 4). The eluate and flow-through fractions were tested in liquid phase competition assays with 1/13 or 8E7 on the solid phase (Table III). The results for the eluate were almost identical to those for the whole RId8E7 serum (Fig. 5). By contrast, the only monoclonal antibody which produced any inhibition of the binding of the RId8E7 flow-through fraction to 8E7 was 8E7 itself (Fig. 6). This confirms that RId8E7 contains at least two populations of antiidiotypes: one that detects a cross-reactive idiotype shared by leprosy and lupus antibodies and another more specific to 8E7, the immunizing leprosy monoclonal antibody.

Similar results were obtained with eluate and flow-through fractions of RId4D5 and RId4G7 when these sera were absorbed on a 16/6-Sepharose column (Table III). RIdTH9 was not absorbed in this way, since it showed only weak binding to 16/6 in direct binding assays.

Cross-reactive idiotopes on monoclonal leprosy and lupus antibodies. Monoclonal mouse antiidiotopes against 16/6 (MId16/6) and 8E7 (MId8E7) were used to probe further the relationships between leprosy and lupus antibodies. In direct binding assays, MId16/6 bound strongly to all of the lupus- and leprosy-derived monoclonals, with the exception of TH9, and MId8E7 bound strongly to all of the antibodies except 4G7 and TH9 (data not shown). These results were confirmed by cross-idiotope competition assays, shown in Fig. 7. For MId16/6-8E7 the order of 50% inhibition was: 1/13 > 4D5 > 16/6 > 4G7 > 8E7 > 21/28; and for MId8E7-1/13 the order of 50% inhibition was: 1/13 = 21/28 > 8E7 > 4D5 (Table IV). The two monoclonal antiidiotopes thus recognize different determinants, both of which are shared by lupus- and leprosy-derived antibodies. We shall refer to these cross-reactive idiotopes as IdLL^a (detected by MId16/6) and IdLL^b (detected by MId8E7).

To investigate whether the various rabbit and mouse antiidiotypes bind to the same or related sites, inhibition studies were performed. Immunoglobulin from RId8E7 was purified on a goat anti-rabbit IgG Sepharose column. This immunoglobulin, and the RId8E7 eluate of the 16/6 column, were used as competitors of the binding of MId8E7 to the panel of monoclonal antibodies. Neither antiidiotype preparation produced significant inhibition. However, MId16/6 completely inhibited the binding of RId8E7 to 1/13 and 21/28 and RId8E7 eluate to 1/13, 21/28, and 8E7, whereas UPC 10, a mouse monoclonal antibody of the same isotype and subclass, did not (representative graphs are shown in Fig. 8). Similar results were obtained with RId4D5, RId4G7, and RIdTH9. Therefore, IdLL^a, the idiotope recognized on the lupus and leprosy antibodies by MId16/6, was also recognized by a population of antibodies in RId8E7. This variable region determinant, which is shared by all four leprosy antibodies as well as by all three lupus antibodies, thus elicited antibodies in four rabbits and the mouse from which MId16/6 was derived.

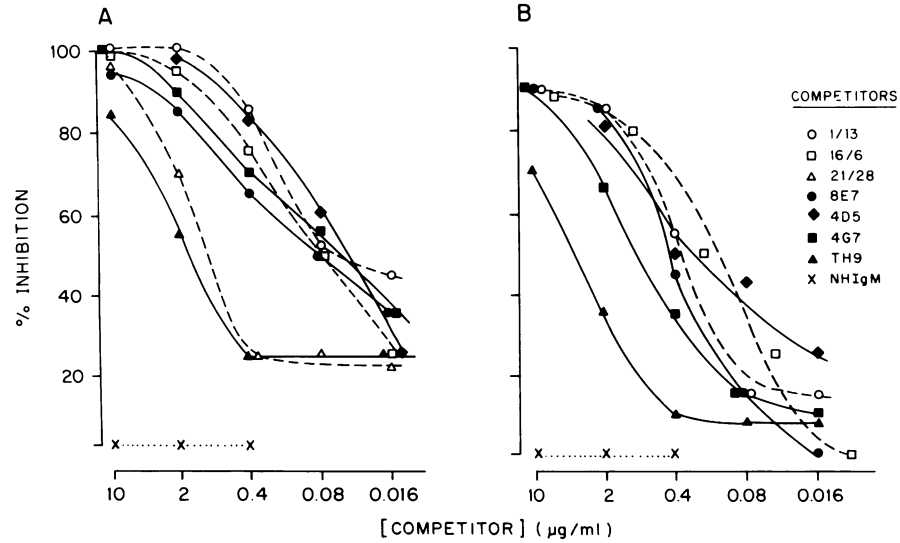

Figure 2. Inhibition by leprosy- and lupus-derived monoclonals in cross-idiotype competition assays: (A) binding of RId8E7 to 1/13; (B) binding of RId16/6 to 8E7.

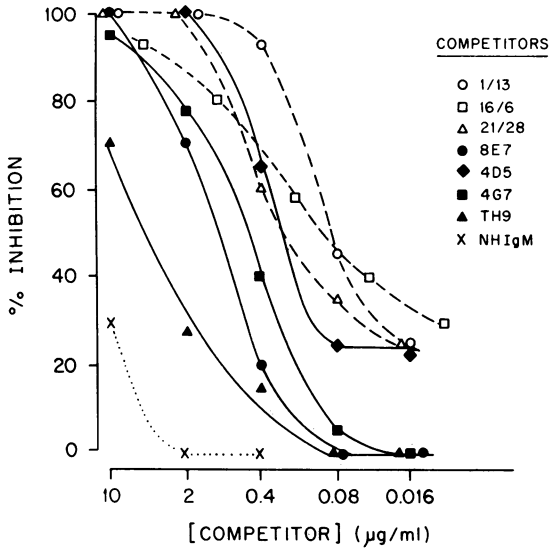

Figure 3. Inhibition by leprosy- and lupus-derived monoclonals of the binding of RId16/6 to 1/13.

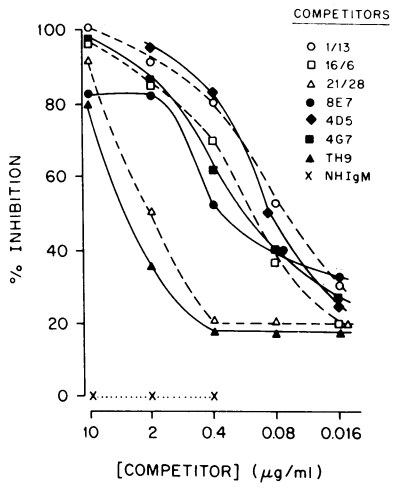

Figure 5. Inhibition by leprosy- and lupus-derived monoclonals of the binding of RId8E7 eluate of 16/6 column to 1/13.

That the inhibition in these assays was caused by the binding of MId16/6 to the human monoclonals and not to the rabbit antiidiotypes was confirmed by the failure of MId16/6 to bind to purified immunoglobulin from RId8E7 (data not shown).

Mouse monoclonal anti-peptide D. Thus far, we have seen that two classes of idiotypes occur on the lupus and leprosy monoclonal antibodies under investigation: four distinct "private" idiotypes detected in homologous inhibition assays (Fig. 1), and two distinct "public" idiotopes ($IdLL^a$ and $IdLL^b$). To determine if a defined variable region sequence contributes to these public variable region markers, we used a monoclonal antibody against peptide D, which contains amino acid residues of the CDR-1 of the heavy chain of 16/6. This antibody, MD, bound to both D-BSA and D-KLH, but not to F-BSA or to F-KLH (see Methods). In competition assays the binding of MD to D-BSA on the solid phase was specifically inhibited in solution by D-BSA but not by F-BSA or glutaraldehyde-treated BSA. In direct binding assays, MD bound to all of the human monoclonals with the exception of 21/28 and TH9 (data not shown). In competition assays all of the human monoclonals except 21/28 and TH9 produced inhibition of the binding of MD to both 1/13 and 8E7 (Fig. 9, Table IV). The strong inhibition of the binding of MD to 1/13 by 4D5 is notable, especially when compared with 16/6, from which peptide D was derived. A similar relationship to 4D5 was found in the assay MD-8E7. These results suggest that the sequence of peptide D (or similar structure) is more exposed to the fluid phase on 4D5 than on the other monoclonal antibodies. Since the cross-reactive idiotope recognized by MD appears distinctive, we refer to it as $IdLL^c$.

Discussion

One of the assumptions underlying our studies is that polyclonal B-cell activation, regardless of its cause, involves the expression of particular sets of germline V genes. Immunoglobulin molecules encoded by such genes could be expected to occur in unrelated patients with different diseases, all united by the common feature of polyclonal B-cell activation. These kinds of recurrent immunoglubulins might be identified by public idiotypes, which, due to the combinatorial mechanisms that generate immunoglobulin variation (21), are independent of the antigen binding specificities of the antibodies that bear them (22). Idiotypic markers, it should be stressed, do not identify V genes, but rather conformation in native immunoglobulins or short amino acid sequences that may occur in different antibodies. A given idiotype, therefore, might originate from different V genes. How-

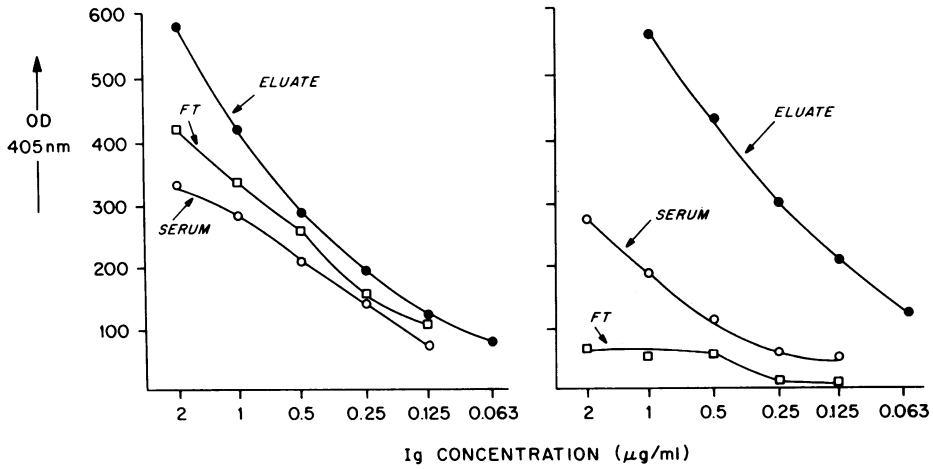

Figure 4. Direct binding of RId8E7 ("serum") and the eluate and flow-through ("FT") of RId8E7 from a 16/6 column to (a) 8E7 and (b) 1/13.

Table III. Inhibition of Binding to Human Monoclonal Antibodies of Rabbit Antisera after Purification on a 16/6 Column: ng per ml of Competitor Required for 50% Inhibition

Antiidiotype preparation	Eluate of 16/6 column				Flow-through of 16/6 column		
Antiidiotype:	8E7		4D5	4G7	8E7	4D5	4G7
Binding to: / Inhibitor	8E7	1/13	4D5	4G7	8E7	4D5	4G7
1/13	2,000	55	1,000	2,500	NI	NI	NI
16/6	NI	170	*	*	NI	*	*
21/28	NI	1,900	*	*	NI	*	*
8E7	310	240	1,200	1,100	300	NI	NI
4D5	NI	50	2,000	2,000	NI	340	NI
4G7	500	105	1,800	NI	NI	NI	200
TH9	NI	3,400	NI	NI	NI	NI	NI
NHIgM	NI	NI	NI	NI	NI	NI	NI

NI, not inhibited by amounts of competitor up to 10,000 ng/ml.
* Not tested.

ever, if it occurs repetitively in immunoglobulins from unrelated patients it could provide a marker of sets of recurrently expressed germline V genes.

To test these ideas, we selected a panel of monoclonal antibodies from patients with leprosy and SLE, diseases characterized by marked polyclonal B-cell activation (9, 10). The seven monoclonal antibodies were derived from three patients of different racial origin and they have different ligand binding properties (Table I). Five of them are autoantibodies against DNA or mitochondria; one binds to a monoclonal antibody bearing an internal image of the acetylcholine receptor (15), and may therefore also be an autoantibody; the binding specificity of the seventh is unknown. Of the six autoantibodies, 4G7, derived from a patient with lepromatous leprosy is of considerable interest. It binds to both *Mycobacterium leprae* and single-stranded DNA, which might indicate that the stimulus for the production of anti-DNA antibodies in leprosy is the infecting organism (Duggan, D., et al., manuscript in preparation). Four of the monoclonal antibodies bind to DNA, but the fine specificities differ in each case (14).

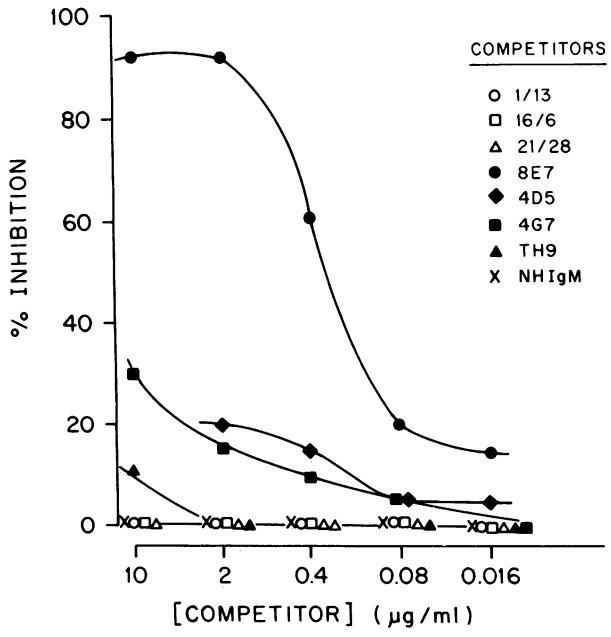

Figure 6. Inhibition by leprosy- and lupus-derived monoclonals of the binding of RId8E7 flow-through of 16/6 column to 8E7.

Both private and public idiotypes were found in the seven monoclonal antibodies. The cross-idiotype inhibition assay system that we used to identify the public idiotypes was first described with monoclonal human antibodies by Kunkel et al. (23), and by Kindt et al. (24) with monoclonal rabbit antibodies. They emphasized the sensitivity of the method for detecting idiotypes shared by different antibodies. Our results with two antiidiotypes, RId16/6 (prepared by immunizing a rabbit with a monoclonal lupus antibody) and RId8E7 (prepared from a monoclonal leprosy antibody), showed strong idiotypic cross reactions with a small subpopulation of antiidiotypes present in the polyclonal reagents. Further evidence for the presence of idiotypically related structures on these antibodies was obtained with monoclonal mouse antiidiotopes. The results were especially clear with a monoclonal antibody (MId8E7) against an idiotope on the leprosy antibody 8E7 ($IdLL^b$); MId8E7 actually bound more avidly to 1/13 and 21/28 (two independently derived lupus antibodies) than to 8E7 itself.

Our results illustrate that idiotypic markers are a complex group of antigenic determinants. This is to be expected in view of the structure of the immunoglobulin variable region. Idiotypes can be serological markers of either the heavy or light chains of antibodies, or of an antigenic surface made up of portions of both chains (1–6). Hence, two antibodies with the same heavy chain but unrelated light chains, may, by different folding of the pairs of chains express different antigenic determinants (5). Moreover, the identical heavy chain amino acid sequence may, in the context of different light chains, be more or less exposed to the surface of the molecule, and thus to an antiidiotype. These considerations can explain our results with MD, a monoclonal antibody against a synthetic peptide derived from the first hypervariable region, and its flanking amino acids, of the heavy chain of 16/6. The lupus antibodies 1/13 and 16/6 have nearly identical sequences in that region (19), and their binding to MD was the same. Antibody 21/28, also from a lupus patient, but with a different CDR-1 (Dersimonian, H; unpublished data), did not bind to MD. Judging by the competitive inhibition assay shown in Fig. 8 (MD-1/13), MD was 10-fold more avid for 4D5, a leprosy antibody, than for 1/13. It would appear, therefore, that peptide "D" is more exposed on the surface of 4D5 than on 1/13, or, alternatively, that a distortion brought about by adhering 1/13 to the solid phase most closely resembles the conformation of peptide "D" in 4D5. The actual sequence recognized by MD is presently unknown. We suppose that the antigenic determinant of the peptide resides in its carboxy-terminal region, which would constitute its exposed terminus after conjugation of its NH_2-terminal phenylalanines to KLH with glutaraldehyde.

The similarities between the behavior in cross-idiotype systems of RId8E7, RId4D5, RId4G7, and the reactions of MId16/6 are of particular interest. Our data indicate that subpopulations of antibodies in these sera from three different rabbits bind to the same determinant ($IdLL^a$) as MId16/6. There is also evidence that RId16/6 binds to $IdLL^a$ (Naparstek, Y., unpublished observations). Taken together, these findings indicate that $IdLL^a$ is highly immunogenic. It is therefore likely to be exposed

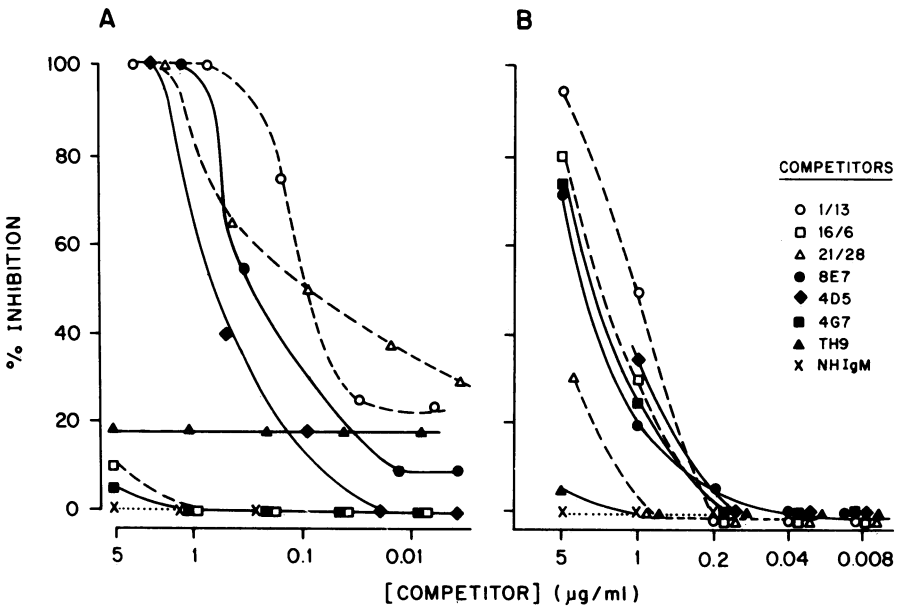

Figure 7. Inhibition by leprosy- and lupus-derived monoclonals of the binding of (A) MId8E7 to 1/13 and (B) MId16/6 to 8E7.

on the surface of the variable region, and thus available for interactions in idiotypic networks in vivo.

These structural aspects of immunoglobulin variable regions can account for the various kinds of idiotypic reactions we and others (8, 16) have previously observed. The original descriptions of the serological properties of immunoglobulin variable regions (25, 26) defined unique markers of individual antibodies, hence the term *idiotype*. Such "private" markers were also observed in the present experiments. TH9 is a notable example. Its reactions with all of the antiidiotopes except RIdTH9 were either undetectable or weak at best. It is of particular interest that this monoclonal antibody shares a high frequency public idiotope with antiacetylcholine antibodies present in myasthenia gravis serum (15). Therefore, the idiotypic network to which TH9 belongs may be distinct from the network(s) that gave rise to the other antibodies in this study.

It can be argued that in vitro polyclonal B cell activation, or even B cell hybridization, which were used in developing the monoclonal antibodies we studied, merely selects for antibodies that are present in the normal B cell repertoire. That hybridomas derived from lymphocytes of normal subjects (27, 28), or, as we have shown, from a leprosy patient produce antibodies with the ligand binding properties of monoclonal antibodies derived from lupus patients seems to support this view. However, a different way of stating this possibility is that the normal repertoire of "natural" antibodies is indeed the source of lupus autoantibodies. Previous investigations have abundantly demonstrated that a set of shared idiotypic markers is a common thread uniting lupus autoantibodies with the normal immune repertoire. The 16/6 idiotype was originally identified in a monoclonal lupus autoantibody (16) (by an antiserum from a different rabbit which produced the RId16/6 used in this study). It occurs in the serum of patients with active SLE and, in some cases, it fluctuates concordantly with the activity of the disease (29). It has also been found deposited in the renal (30) and skin (31) lesions of the disease. Some antibodies bearing 16/6 idiotype may therefore be relevant to the pathogenesis of SLE. Yet up to 1% of the total immunoglobulins produced in vitro by polyclonally activated B cells from normal subjects also bear this idiotype (32). Another public idiotype, termed 3I, defined by a monoclonal antibody against affinity purified serum anti-DNA antibodies, has remarkably similar properties (33–35). We can surmise from these studies of the 3I and 16/6 idiotypes that at least a portion of lupus autoantibodies originates from antibodies derived from the normal immune repertoire. Investigations of shared idiotypes on monoclonal antibodies derived from patients with rheumatoid arthritis (36), normal human (37), and normal (38) or autoimmune (39) mice, as well as the results reported here, support this conclusion. The essential point, therefore, is not whether lymphocytes from normal subjects can produce lupus autoantibodies, but whether lupus autoantibodies originate from unscheduled expression of the normal repertoire.

It is interesting to compare our findings with those of others who have used synthetic peptides to study the structural basis of idiotypy. Andrews and Capra (40) demonstrated marked sequence homology between light (but not heavy) chains of two

Table IV. Inhibition of Mouse Monoclonal Antiidiotypes to Human Monoclonal Antibodies: ng/ml of Competitor Required for 50% Inhibition

Antiidiotype	MId8E7	MId16/6	MD	
Binding to: / Inhibitor	1/13	8E7	1/13	8E7
1/13	90	1,000	2,000	2,300
16/6	*	2,300	3,000	8,000
21/28	90	8,000	NI	NI
8E7	300	3,100	1,200	2,100
4D5	620	1,900	200	1,500
4G7	NI	2,700	4,300	5,000
TH9	NI	NI	NI	NI
NHIgM	NI	NI	NI	NI

NI, not inhibited by amounts of competitor up to 10,00 ng/ml.
* Not tested.

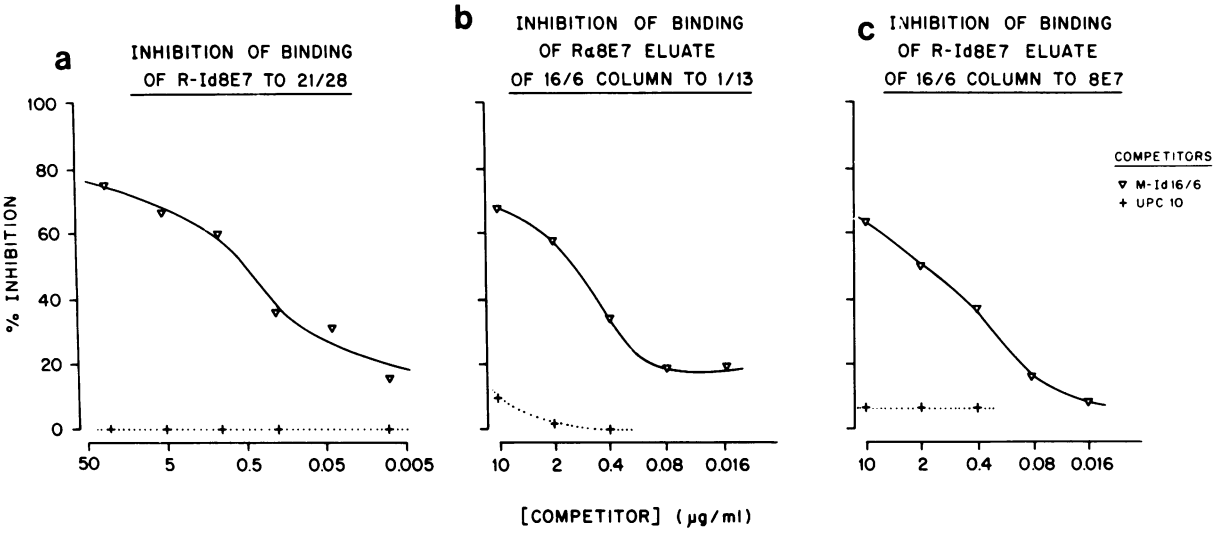

Figure 8. Inhibition by MId16/6 of binding of: (a) RId8E7 to 21/28; and RId8E7 eluate of 16/6 column to (b) 1/13 and (c) 8E7.

monoclonal IgM rheumatoid factors which were derived from patients with Waldenstrom's macroglobulinemia, and which share a cross-reactive idiotype (23). Using antibodies against particular peptide sequences, Carson and co-workers (41) showed that idiotypic cross-reactions among a group of such IgM rheumatoid factors may be attributable to shared sequences in the light chain second CDR. By contrast, antibodies against heavy chain third CDR sequences appear to recognize private idiotopes (42), a finding confirmed in a murine system by McMillan et al. (43). Seiden et al. (44) have demonstrated that many different determinants may be found within a stretch of 16 amino acids in the region of the JH segment, suggesting that the multiplicity of conformational differences in this region is responsible for the "privacy" of such idiotopes. We examined the serology of only the heavy chain CDR-1, but found evidence for a shared amino acid sequence at this site among our monoclonal antibodies.

The present experiments extend the scope of the anti-DNA antibody network defined by the 16/6 idiotype to other antibodies from a different source and with different ligand binding properties. Altogether, our various antiidiotype reagents appear to recognize at least three distinct determinants which are common to monoclonal antibodies from three unrelated patients. The fact that these three individuals have widely different ethnic and geographic origins suggests that the structures recognized by our antiidiotypes are conserved in the germline. Indeed, the gene segment encoding the VH region of 16/6 is highly homologous to that of the human germline gene VH26 (45). The present results therefore suggest that the antibody repertoire expressed during polyclonal B cell activation may, in some respects, be stereotyped. It may consist, at least in part, of immunoglobulins bearing a recurrent subgroup of germline-encoded idiotypes. The reagents we developed, which do not react with normal serum, could therefore be of particular value for analysis of the expression of germline V genes during in vivo polyclonal B cell activation.

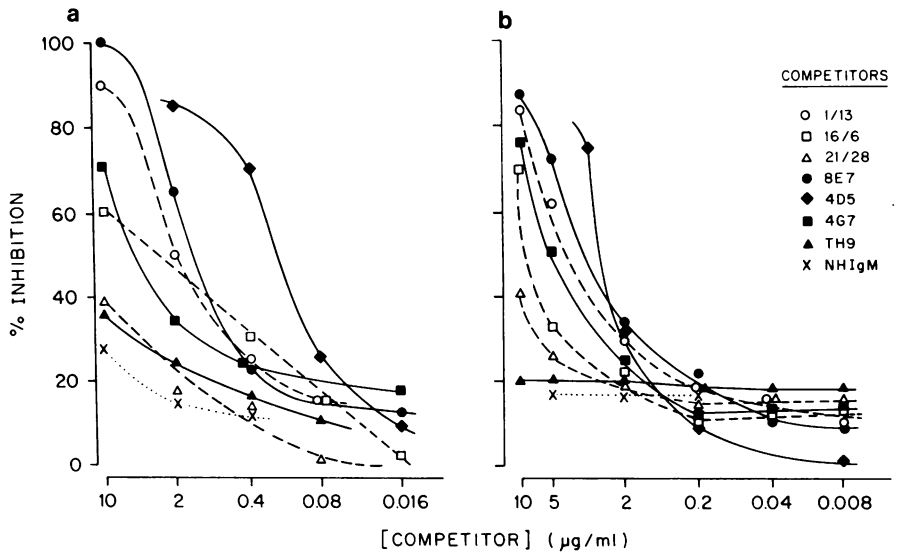

Figure 9. Inhibition by leprosy- and lupus-derived monoclonals of binding of monoclonal anti-peptide D (MD) to (a) 1/13 and (b) 8E7.

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