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Human monoclonal antibodies to phenolic glycolipid-I derived from patients with leprosy, and production of specific anti-idiotypes

M. LOCNISKAR, A. ZUMLA, D. W. MUDD, D. A. ISENBERG,* W. WILLIAMS* & K. P. W. J. MCADAM
*Department of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine and *The Bloomsbury Rheumatology Department/Rheumatology Research Department, Middlesex Hospital Medical School, London*

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SUMMARY

Human monoclonal antibodies (mAb) were produced by hybridomas derived from fusion of the GM4672 lymphoblastoid cell line and peripheral blood mononuclear cells from leprosy patients. Hybridoma supernatants were screened for immunoglobulin (Ig) secretion, binding to *Mycobacterium leprae*, phenolic glycolipid-I (Phen GL-I), the unique *M. leprae* glycolipid and single-stranded(ss)DNA by ELISA. On the basis of direct-binding ELISAs, two IgMk mAb (PR4 and TH3) were selected for characterization. PR4 and TH3 bound to *M. leprae*, Phen GL-I and ssDNA; PR4 also bound to *M. avium* and *M. kansasii* and TH3 to *M. kansasii*. Inhibition assays demonstrated that these antibodies did not bind to the terminal disaccharide of Phen GL-I. In addition, both PR4 and TH3 bound to several autoantigens: ssDNA, double-stranded(ds)DNA and poly(ADP-ribose) but not RNA. PR4 and TH3 were used for preparation of rabbit anti-idiotype antisera. Inhibition studies demonstrated that the affinity purified rabbit anti-idiotype antisera were specific for their respective idiotype and that both Phen GL-I and ssDNA inhibited binding of idiotype to its anti-idiotype. PR4, but not TH3, was found to be similar but not identical to the 16/6 idiotype originally identified on a human monoclonal anti-DNA antibody derived from a patient with systemic lupus erythematosus (SLE).

INTRODUCTION

Patients with lepromatous leprosy (LL) lack a *Mycobacterium leprae*-specific cell-mediated immune response, yet their humoral response is intact and they often exhibit hypergammaglobulinaemia (Almeida, 1970). Although high titres of antibodies to *M. leprae* (Almeida, 1970; Harboe *et al.*, 1978) and phenolic glycolipid I (Phen GL-I), the unique *M. leprae* glycolipid, are present in lepromatous patients (Young *et al.*, 1984), these antibodies are ineffective at killing the organism in its protected site within host cells. In addition to *M. leprae*-specific antibodies, multiple autoantibodies have been detected in the serum of patients with leprosy (reviewed by Turk & Bryceson, 1971), but the origin and significance of these autoantibodies in leprosy is not understood. In contrast to many of the classical autoimmune diseases, such as systemic lupus erythematosus (SLE), whose etiology is still unknown, infection with *M. leprae* is clearly the cause of leprosy. The production of monoclonal antibodies (mAb) by lymphocytes from patients with leprosy provides an opportunity to investigate the relatedness and functional significance of antibodies directed to *M. leprae* and to autoantigens. Here we report on the production of human mAb which bind to *M. leprae*-specific determinants as well as autoantigens, and the preparation of rabbit anti-idiotypic sera directed to these idiotypes.

Abbreviations: BB, borderline leprosy; BL, borderline lepromatous leprosy; BT, borderline tuberculoid leprosy; CBB, carbonate-bicarbonate buffer; CLF, clofazamine; DDS, dapsone; dsDNA, double-stranded DNA; ELISA, enzyme-linked immunosorbant assay(s); ENL, erythema nodosum leprosum; IFA, incomplete Freund's adjuvant; Ig, immunoglobulin; LL, lepromatous leprosy; LPS, lipopolysaccharide; mAb, monoclonal antibody(ies); *M. tb*, *Mycobacterium tuberculosis*; ND, not done; NPP, *P*-nitrophenyl phosphate; OD, optical density; PBS, phosphate-buffered saline; PBS-BSA, phosphate buffered saline with bovine serum albumin; PBS-T, phosphate-buffered saline with Tween-20; Phen GL-I, phenolic glycolipid-I; RFP, rifampin; ssDNA, single-stranded DNA; SLE, systemic lupus erythematosus.

Correspondence: Professor K. P. W. J. McAdam, Dept. of Clinical Tropical Medicine, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K.

MATERIALS AND METHODS

Patients

Blood was obtained from leprosy patients attending the Georgetown Hospital Public Health Clinic, Georgetown, Guyana through Dr Patricia Rose, and the Hospital for Tropical Diseases, London, through Dr Anthony Bryceson. Whole blood specimens were flown to London from Guyana on the

same day of bleeding and the mononuclear cells were isolated within 24 hr. The clinical diagnosis of each patient is given in Table 1. Patients had been treated with dapsone and rifampin, with or without clofazamine, for 1·5 months to 23 years at the time blood was drawn. According to the method described below, a serum sample from each patient was found to have detectable antibody to Phen GL-I.

Production and purification of human mAb

Human mAb were produced by hybridomas derived from the polyethylene glycol (PEG)-mediated fusion of the GM4672 (kindly provided by Dr R. Schwartz, Tufts University School of Medicine, Boston, MA)(Croce *et al.*, 1980) or UC729-6 (Glassy *et al.*, 1983) lymphoblastoid fusion partners and peripheral blood mononuclear cells (PBMC) obtained from leprosy patients. The hybridomas produced in our laboratory from the lymphocytes of patient TH have been described in another publication (D. B. Duggan, C. Mackworth-Young, A. K. Lefvert, J. Andre-Schwartz, D. Mudd, K. P. W. J. McAdam and R. S. Schwartz, manuscript submitted for publication). Fusions using the lymphocytes of patients PR, IS, FB, GMc and LA were performed according to the method of Shoenfeld *et al.* (1982) with the following modifications. Pokeweed mitogen (PWM, Gibco-Biocult, Paisley, Renfrewshire; 1:100 for 24 hr for PR, FB, GMc, LA; 1:1000 for 5 days for IS)-transformed lymphocytes and the GM4672 cells were co-pelleted and 0·5 ml 44% PEG (1540 MW; Baker Chemical Co., Phillipsberg, NJ) added while the pellet was gently resuspended. The cells were pelleted immediately, washed twice and cultured in growth medium (containing RPMI-1640, 15% fetal bovine serum, 10 mM HEPES, 200 mM L-glutamine, 100 U penicillin, 100 μg streptomycin, 10 mM sodium pyruvate and 4% non-essential amino acids; Gibco-Biocult) overnight. The cells were resuspended in growth medium containing $2{\cdot}5 \times 10^{-5}$ M hypoxanthine, 1×10^{-7} M aminopterin and 4×10^{-6} M thymidine (HAT; Sigma Chemical Co Ltd, Poole, Dorset) and seeded (2×10^5 cells/well) into wells of 96-well, flat-bottomed culture plates (Nunc, Roskilde, Denmark). Four to eight weeks later, macroscopically visible colonies were monitored for antibody production by testing the supernatants in the assays described below.

The hybridomas derived from lymphocytes of the patient RM were prepared with either the GM4672 fusion partner as above or the UC729-6 lymphoblastoid cell line. In brief, PWM-transformed lymphocytes and UC729-6 cells were co-pelleted in a ratio of 5:1. The pellet was gently resuspended, incubated with 0·5 ml 45% PEG (1500 MW; Boehringer-Mannheim, FRG) for 1 min and RPMI-1640 then added slowly. After centrifugation, the cells were resuspended in 50 ml growth medium and seeded into two 24-well tissue culture plates containing BALB/c mouse peritoneal macrophages. Four to six weeks later colonies were screened for Ig production.

Selected antibody-positive colonies were cloned by limiting dilution, seeding the cells in growth medium at a concentration of 0·2 cells/well. Selected clones were expanded to bulk cultures and mAb were purified from culture supernatants on a goat anti-human IgM–Sepharose 4B (Pharmacia, Uppsala, Sweden) affinity column and concentrated using a Stirred Cell Concentrator and YM300 membrane (Amicon, Stonehouse, Gloucestershire).

Production and purification of polyclonal anti-idiotype antiserum (rabbit anti-PR4 and rabbit anti-TH3)

On the basis of their binding specificities, two mAb, PR4 and TH3, were selected for the production of anti-idiotypic antibodies. New Zealand White rabbits (Foxfield Rabbit Co., Foxfield, Hants) were immunized with 250 μg of affinity-purified monoclonal PR4 or TH3 emulsified in incomplete Freund's adjuvant (IFA) (Sigma) s.c. and intramuscularly at several sites. Control rabbits received IFA only, at similar sites. Booster doses of the immunogen in IFA were given on Days 14, 28, 35 and 42. Rabbit sera were tested for reactivity to human IgM in direct-binding ELISAs.

Polyclonal anti-idiotype serum was rendered idiotype specific by extensive absorption over a human IgG/IgM–Sepharose 4B affinity column until no anti-human Ig activity was detectable by an Ig ELISA. The anti-idiotype serum was purified further on an idiotype–Sepharose 4B column, eluting anti-idiotype-specific antibody with 0·1 M glycine hydrochloride (pH 2·3).

Immunoassays

Solid-phase enzyme-linked immunosorbant assays (ELISA) were employed for the screening of primary hybrid colonies, clones, purified mAb and anti-idiotype antisera. An initial screen of hybridoma supernatants for Ig production and antibody class was followed by direct-binding ELISA for the antigens of interest.

Competition ELISA in fluid phase were performed to verify binding specificities. A dilution giving 50% of maximal binding for each antibody was used for competition assays and the test antibody was mixed with dilutions of the inhibitor antigens for 1 hr at 37° before adding to antigen-coated wells.

Ig ELISA. Affinity-purified goat anti-human IgG or IgM (Sigma Chemical Co. Ltd) at 5 μg/ml in 0·05 M sodium borate buffer (pH 8·6), was absorbed to half of the wells of Immulon 2 plates (Dynatech Laboratories Inc., Alexandria, VA) and wells of the other half received buffer only. Plates were incubated overnight at 4°, washed with PBS (pH 7·4), containing 0·1% Tween 20 (PBS-T), culture supernatants added and plates incubated at room temperature for 90 min. The samples were aspirated, plates washed with PBS-T and alkaline phosphatase-conjugated affinity-purified goat anti-human IgM (mu-chain specific) or anti-human IgG (gamma-chain specific) (Sigma Chemical Co. Ltd) added and incubated overnight at room temperature. The substrate, *P*-nitrophenyl phosphate (NPP) (Sigma), was added to the wells to detect bound alkaline phosphatase, and the optical density (OD) of each well determined using a MicroElisa MR600 spectrophotometer (Dynatech Laboratories Inc.) at 405 nm wavelength. OD readings of control wells were subtracted from test values.

Mycobacterial ELISA. *M. leprae* [isolated from infected nine-banded armadillos (Draper, 1976)], *M. vaccae*, *M. kansasii*, *M. avium*, BCG and *M. tuberculosis* H37 (*M. tb*) were diluted to 1 mg dry weight per ml PBS, and sonicated in 5-min bursts on ice for a total of 20 min. The suspensions were spun for 1 hr at 100,000 *g*. The supernatant was then treated, successively, with micrococcal S1 nuclease, DNAse and RNAse (Sigma Chemical Co.), with high-speed (65,000 *g*) centrifugation and dialysis (3,500 MW pore size) after each treatment, in order to eliminate possible nucleic acid contaminants from the soluble mycobacterial preparations.

Immulon 1 plates were coated with mycobacterial antigens (5 μg/ml) in carbonate-bicarbonate buffer (CBB; pH 9·6) overnight at 4°. Wells were washed with PBS-T and culture supernatants diluted in PBS-T were added and incubated 90 min

at room temperature. Wells were again washed with PBS-T, alkaline-phosphatase-coupled goat anti-human IgG and IgM added and incubated overnight at room temperature. The plates were washed, NPP added and absorbance of each well determined as above.

Phen GL-I ELISA. Purified Phen GL-I was suspended in CBB (2 μg/ml) by sonication, as described by Cho *et al.* (1986), and 100 μl aliquots added to wells of Immulon 1 plates. The plates were incubated at 37° overnight, wells washed with PBS and PBS containing 2% bovine serum albumin (PBS-BSA) (Sigma) added for 1 hr. Culture supernatant samples diluted in PBS-BSA were added to designated wells and incubated at 37° for 1 hr. The wells were washed, peroxidase-conjugated goat anti-human IgM (Sigma Chemical Co. Ltd) in PBS–BSA added and allowed to react for 2 hr at room temperature. After washing, the substrate O-phenylenediamine (OPD) (Pitman Moore, Washington Crossing, NJ) was added to the wells and the OD of each well read at 490 nm.

Disaccharide ELISA. The terminal disaccharide of Phen GL-I conjugated to bovine serum albumin (disaccharide-BSA; Gigg *et al.*, 1986) was used to coat (2 μg/ml in CBB) half the wells of an Immulon 1 plate and the other half received buffer only. Plates were incubated overnight at room temperature, washed with PBS-T and non-specific binding sites blocked with PBS-BSA at room temperature for 1 hr. After washing, supernatant samples diluted in PBS-BSA added, incubated for 2 hr at room temperature and the assay completed as described for Phen GL-I.

Anti-ssDNA ELISA. Hybridoma supernatants were screened for their binding reactivity to ssDNA (Worthington, Freeland, NJ) employing the method described by Shoenfeld *et al.* (1982).

Anti-poly(ADP-ribose) ELISA. Screening of hybridoma supernatants and purified mAb for reactivity to poly(ADP-ribose) has been described elsewhere (Dudeney *et al.*, 1986).

Reactivity of anti-idiotype antisera to IgM and mAb. Wells of Immulon 1 plates were coated with pooled IgM or the mAb (PR4, TH3 and LA6) at 2·5 μg IgM per ml CBB. Control wells were coated with CBB or culture medium only and plates incubated overnight at 4°. Anti-idiotype antisera were diluted and added to wells. After 1 hr incubation, wells were washed and goat anti-rabbit IgG alkaline phosphatase conjugate added and the assay completed by adding NPP substrate and measuring absorbance at 405 nm.

Reactivity of anti-idiotype antisera to Waldenstrom's macroglobulinaemia sera. PR4 or TH3 anti-idiotype antisera (2·5 μg IgM per ml CBB) were adsorbed to the wells of microtitre plates. Serum samples from 13 patients with Waldenstrom's macroglobulinaemia (8 IgM K and 5 IgM L) were diluted 1:50 and incubated in coated wells followed by goat anti-human IgM alkaline phosphatase conjugate and substrate.

Inhibition of mAb PR4 and TH3 idiotypes binding to their respective anti-idiotypes by Phen GL-I, LPS and polynucleotides. Immulon 1 plates were coated with the anti-idiotype overnight at 4°, washed with PBS and incubated with PBS-BSA for 1 hr at room temperature. Log-fold dilutions in PBS of the competitors Phen GL-I, LPS (List Biochemicals Inc., Campbell, CA) ssDNA, dsDNA, poly(dT) (Sigma), poly(ADP-ribose) were pre-incubated in tubes with the mAb at 37° for 1 hr. The reference sample contained mAb in PBS. Reaction mixtures were then added to the anti-idiotype-coated and uncoated wells and incubated at 37° for 1 hr. The plates were washed with PBS-T and goat anti-human IgM alkaline phosphatase conjugate added, and the assay completed as described above.

Competition of PR4, TH3 and LA6 to Phen GL-I by constituents of Phen GL-I. MAb PR4, TH3 and LA6 were incubated for 1 hr with log-fold dilutions of the inhibitors: phenyl-trisaccharide, propyl-trisaccharide, disaccharide-BSA, unconjugated disaccharide (Gigg *et al.*, 1986) and Phen GL-I. The reaction mixtures were added to Phen GL-I-coated wells (described above). After 1 hr, goat anti-human IgM alkaline phosphatase conjugate (Sigma) was added and the assay completed as described above.

Competition of rabbit anti-16/6 binding to 16/6 by mAb PR4, TH3 and 16/6. As described by Shoenfeld *et al.* (1983a), rabbit anti-16/6 was pre-incubated with potential inhibitors PR4, TH3 and 16/6. Reaction mixtures were then added to mAb 16/6-coated wells. After 1 hr at 37°, wells were washed, goat anti-rabbit IgG [$F(ab')_2$] alkaline phosphatase conjugate (Sigma) added and the assay completed.

A similar assay was performed coating wells with the mAb PR4 and pre-incubating the purified rabbit anti-PR4 antiserum with the potential inhibitors PR4, TH3 and 16/6.

RESULTS

Fusions

Data from each of the eight fusions are shown in Table 1. The fusion between PBMC from TH and the GM4672 cell line yielded 171 Ig-secreting wells, 22% reactive to *M. leprae*, 13% to ssDNA and 10% to both *M. leprae* and ssDNA (Duggan *et al.*, manuscript submitted for publication). The fusion between lymphocytes from PR and GM4672 resulted in 81 wells secreting Ig out of 336 seeded wells. Supernatant from only one well demonstrated binding to *M. leprae*, Phen GL-I and ssDNA. Fusions with PBMC from IS, FB and GMc failed to produce any colonies secreting Ig. The fusion with lymphocytes from LA resulted in five Ig-secreting colonies out of 240 wells seeded; however, none of these reacted with *M. leprae* or Phen GL-I. Finally, PBMC from RM were fused with both the GM4672 and the UC729-6 fusion partners. Fusion with GM4672 cell line produced no colonies, whereas fusion with the UC729-6 cell line produced 89 Ig-secreting colonies. Supernatants from colonies producing IgG bound to *M. leprae* only, and five supernatants producing IgM bound to ssDNA. None reacted with Phen GL-I (Table 1).

Binding specificities of mAb

Two colonies, one from the PR fusion, PR4 (originally named PR4A2), and one from the TH fusion (Duggan *et al.*, manuscript submitted for publication), TH3 (originally named 3F96E10), were chosen for cloning on the basis of their binding reactivity to *M. leprae*, Phen GL-I and ssDNA. One colony from the LA fusion, LA6, and one from the TH fusion, TH1, secreted Ig but did not bind to the antigens tested and were also chosen for cloning and used as negative controls. Clones which had the same binding specificities as the parent hybridomas were selected, grown in bulk culture and the purified mAb were then characterized further. Chromosomal analysis of the cloned hybridomas has demonstrated that PR4 and TH3 are tetraploid.

The binding reactivities of the mAb to the various mycobac-

Table 1. Incidence and binding specificities of hybridomas derived from fusions with lymphocytes from leprosy patients

Patient	Diagnosis*	Treatment†	Duration of treatment	Serum Phen GL-I	Fusion partner‡	Growth frequency§	Ig^+	IgG	IgM	IgG+M	*M. leprae*	ssDNA	PGL-I
TH¶	BL	—	—	+	GM	206/672	171	9	162	0	37	21	NT
PR	LL (ENL)	DDS, RFP, CLF	3 months	+	GM	162/336	81	0	68	0	1	1	1
IS	BT	RFP	6 months	+	GM	0/240							
FB	BB	DDS, RFP	4 years	+	GM	0/300							
GMc	BL	RFP, CLF	23 years	+	GM	0/480							
LA	LL	DDS, RFP	4 months	+	GM	21/240	5	1	4	0	0	NT	0
RM	LL	DDS, RFP, CLF	1·5 months	+	GM	0/240							
					UC	96/192	89	12	71	6	7	5	0

* LL, Lepromatous leprosy; BL, borderline lepromatous leprosy; BB, borderline leprosy; BT, borderline tuberculoid leprosy; ENL, erythema nodosum leprosum.

† DDS, Dapsone; RFP, rifampicin; CLF, clofazimine.

‡ GM, GM4672, UC, UC729-6.

§ Growth frequency is expressed as the number of wells with cell growth divided by the number of wells seeded.

¶ Duggan *et al.*, manuscript submitted for publication.

NT, not tested.

Table 2. Binding of human monoclonal antibodies to various mycobacterial antigens and autoantigens by ELISA

Clone	Ig class	*M. leprae*	PGL-I	*M. vaccae*	*M. avium*	BCG	*M. tb*	*M. kansasii*	ssDNA	dsDNA	Poly(ADP)	RNA
TH3	M (k)	+	+	−	−	−	−	+	+	+	+	−
PR4	M (k)	+	+	−	+	−	−	+	+	+	+	−
TH1	M (k)	−	−	−	−	−	−	−	−	−	−	−

teria and other antigens are displayed in Table 2. PR4 bound to *M. leprae*, *M. avium* and *M. kansasii*, whereas TH3 bound to *M. leprae* and *M. kansasii* and neither bound to *M. vaccae*, BCG or *M. tb*. Both, PR4 and TH3 bound to Phen GL-I (Table 2) but not to disaccharide-BSA (data not shown). In addition, they both bound to several polynucleotide autoantigens: ssDNA, dsDNA and poly(ADP-ribose) but not to RNA.

Competition assays were performed to identify the epitope on Phen GL-I bound by the mAb (Fig. 1). Phen GL-I was bound to the solid phase while phenol-trisaccharide, propyl-trisaccharide, disaccharide-BSA, unconjugated disaccharide and Phen GL-I were used as competitive inhibitors to the binding of the mAb to Phen GL-I. For PR4, 7, 10, and 0·5 μg/ml, respectively of the inhibitors phenol-trisaccharide, propyl-trisaccharide and Phen GL-I were required for 50% inhibition, whereas, for TH3, 1·0, 3·0 and 4·0 mcg/ml of the inhibitors were

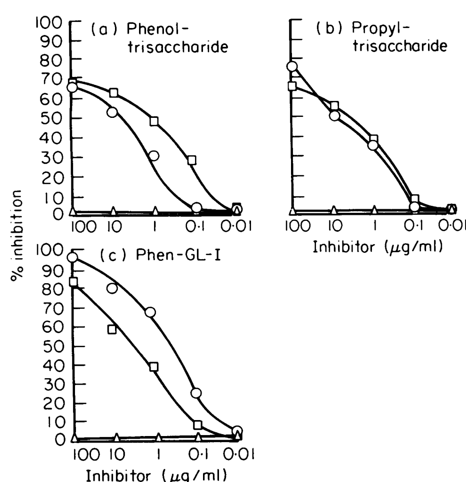


Figure 1. Inhibition of binding of affinity-purified monoclonal antibodies PR4 (○), TH3 (□) and LA6 (△) to Phen GL-I by (a) phenol trisaccharide, (b) propyl trisaccharide and (c) Phen GL-I. Results are expressed as percentage inhibition for log-fold dilutions of inhibitor.

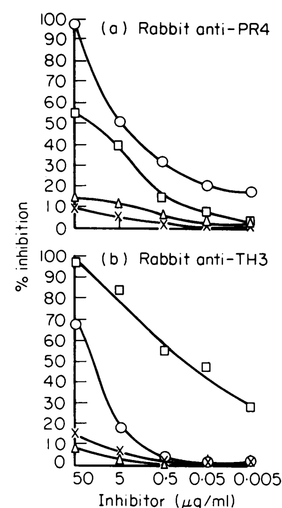


Figure 2. Inhibition of binding of rabbit (a) anti-PR4 antiserum and (b) anti-TH3 antiserum to their respective idiotype by PR4 (○), TH3 (□), LA6 (△) and pooled human IgM (×). Results are expressed as percentage inhibition for each concentration of inhibitor.

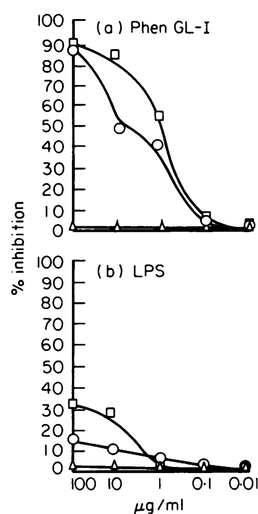


Figure 3. Competition of binding of idiotype PR4 (○), TH3 (□) and LA6 (△) to their respective rabbit anti-idiotype by inhibitors (a) PGL-I and (b) LPS. Data are expressed as percentage inhibition for each concentration of inhibitor. LA6 was used as a negative control for binding to anti-PR4 or anti-TH3.

required. The unconjugated disaccharide and disaccharide-BSA were not inhibitory (data not shown), indicating the binding site of the mAb was not the terminal disaccharide.

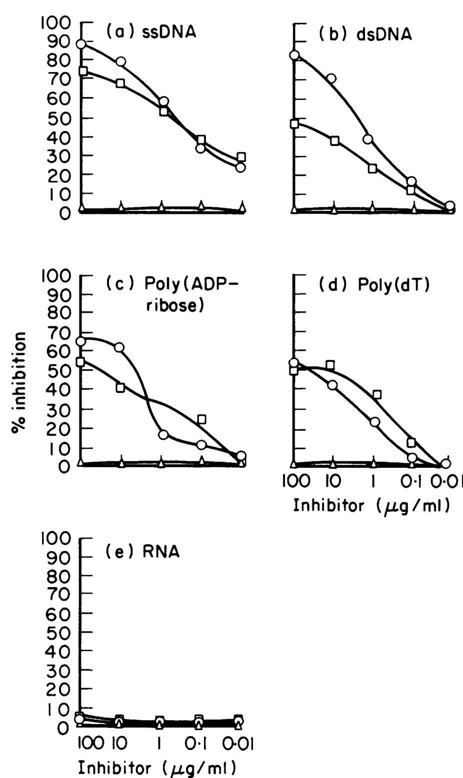
Binding specificity of the anti-idiotype antisera

After extensive absorption on a human IgG/IgM column, the purity and specificity of the anti-idiotype sera, anti-PR4 and anti-TH3, were confirmed. Neither antibody bound to a panel of human mAb, pooled human IgM or serum from patients with Waldenstrom's macroglobulinaemia (data not shown). Preimmune rabbit sera contained no reactivity to the mAb.

Inhibition studies were performed to define further specificities of the anti-idiotype reagents. PR4 inhibited binding of anti-PR4 to PR4-coated plates, whereas 10 times higher concentration of TH3 was required to inhibit binding of anti-PR4 to PR4 (Fig. 2a). Conversely, 0·8 μg/ml TH3 inhibited binding of anti-TH3 to TH3 but a 100-fold increase in PR4 was necessary for inhibition (Fig. 2b). Unrelated mAb, such as LA6 and pooled IgM, did not inhibit binding of either idiotype. These results indicate that the PR4 idiotype and TH3 idiotype have different though related binding specificities.

Binding of PR4 and TH3 to their respective anti-idiotypes were also found to be inhibited by 10 μg/ml and 1 μg/ml respectively of Phen GL-I (Fig. 3a) but not by LPS (Fig. 3b). Since PR4 and TH3 also bound to ssDNA and poly(ADP-ribose), competition assays were performed using ssDNA, dsDNA, poly(ADP-ribose), poly(dT) and RNA, to inhibit binding of idiotypes to their respective anti-idiotypes. As displayed in Fig. 4, less than 1 μg/ml ssDNA is required for 50% inhibition of anti-idiotype binding to its respective idiotype. For PR4, 2 μg/ml dsDNA and 4 μg/ml poly(ADP-ribose) inhibited 50% binding. Neither dsDNA, poly(ADP-ribose) nor poly(dT) effectively inhibited TH3 binding to anti-TH3. RNA did not inhibit binding of either mAb to its anti-idiotype.

Since the idiotypes PR4 and TH3 bound to autoantigens, they were compared to another autoreactive idiotype/anti-



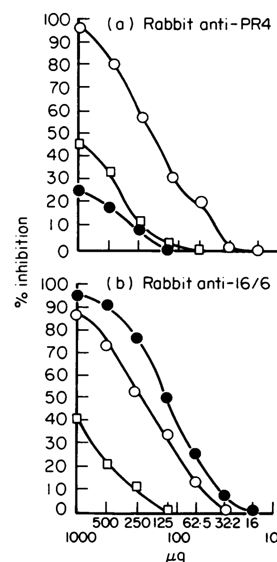


Figure 4. Competition of binding of idiotype PR4 (○) and TH3 (□) to their respective anti-idiotype antisera by the inhibitors (a) ssDNA, (b) dsDNA, (c) poly(ADP-ribose), (d) poly(dT) and (e) RNA. LA6 (△) was used as a negative control for binding to anti-PR4 or anti-TH3 in the presence or absence of inhibitors.

Figure 5. Inhibition of binding of (a) rabbit anti-PR4 to idiotype PR4 by PR4 (○), TH3 (□) and 16/6 (●) and (b) rabbit anti-16/6 antisera to idiotype antibody 16/6 by PR4 (○), TH3 (□) and 16/6 (●).

idiotype system, 16/6. Rabbit anti-PR4 was inhibited from binding to mAb PR4 by PR4 but much less by TH3 or 16/6 (Fig. 5a). Conversely, rabbit anti-16/6 was inhibited from binding to 16/6 by 16/6 and PR4 but considerably less by TH3 (Fig. 5b).

DISCUSSION

We have shown that human mAb derived from patients with leprosy bind not only to *M. leprae*-specific antigens (i.e. *M. leprae*-soluble extract and Phen GL-I) but also to autoantigens such as DNA and poly(ADP-ribose). Previous studies using human hybridoma technology have focused mainly on autoimmune diseases such as SLE (Shoenfeld *et al.*, 1982) and rheumatoid arthritis (Rauch, Massicotte & Tannebaum, 1985) and have described mAb binding to several antigens, including DNA, synthetic polypeptides (Shoenfeld *et al.*, 1983a, b), platelet membranes (Shoenfeld *et al.*, 1982), vimentin (Andre-Schwartz *et al.*, 1984) and Raji cells (Shoenfeld *et al.*, 1985). These observations have been used as evidence that an individual antibody may possess a binding site capable of binding to more than one antigen. This study extends these observations, demonstrating antibodies can bind to mycobacterial antigens such as Phen GL-I as well as to DNA and poly(ADP-ribose). The human mAb we produced are of the IgM isotype. It is well recognized that attachment of antigen to a solid phase can amplify binding of IgM (Eilat, 1986) but we have shown that these antibodies bind to antigen in fluid phase as well. In addition, we have shown that the binding of these human mAb is not due simply to low affinity IgM antibody reacting with charged sites, since competition assays demonstrated specific inhibition with DNA but not with RNA.

Peripheral blood lymphocytes from seven patients were used to produce human–human hybridomas. Several experimental parameters could explain the variability in fusion frequency but it is our prejudice that the clinical status of the patient is the most important. Patients IS, FB and GMc had been treated for 6 months, 4 years and 23 years, respectively (Table 1). In contrast, the two most successful fusions were derived from an untreated patient (TH) and a patient under multi-drug therapy for less than 3 months (PR) who, in addition, had a history of erythema nodosum leprosum (ENL) reaction 2 months before bleeding. The fusion frequencies reported here are much lower than has been reported by Atlaw, Kozbor & Roder (1985) also using lymphocytes derived from patients with leprosy. They, however, produced human–human hybridomas using Epstein–Barr virus-transformed lymphocytes for fusion. They too produced a majority of IgM antibodies, suggesting that antibodies to Phen GL-I are mainly of the IgM class, an observation supported by the serological studies of Young *et al.* (1984) who detected predominantly IgM antibodies to Phen GL-I in serum of patients with lepromatous leprosy. An alternative but speculative explanation for the predominance of IgM antibodies is that there is selection of IgM B cells in the fusion process.

Anti-idiotypic sera were generated using the two mAb PR4 and TH3. Although PR4 and TH3 share similar specificities in direct binding assays, they do not share identical antigen-binding sites as demonstrated in the competition assays. Idiotype analysis has also demonstrated that the mAb PR4 shares some of the binding characteristics of 16/6, an idiotype identified in SLE (Shoenfeld *et al.*, 1983), but they are not identical. Our observations add to that of others who have identified idiotypes on anti-DNA antibodies from patients with SLE (Solomon *et al.*, 1983; Zouali & Eyuem, 1984) and support those published by Mackworth-Young, Sabbaga & Schwartz (1987), who have analysed idiotypes from SLE and leprosy patients, suggesting that the expression of these idiotypes is more closely related to polyclonal B-cell activation rather than to a specific disease. We are using these anti-idiotypic reagents to determine to what extent these leprosy-derived idiotypes are involved in the immunopathogenesis of the disease.

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