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A human fetal monoclonal DNA-binding antibody shares idiotypes with fetal and adult murine monoclonal DNA-binding antibodies

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SUMMARY

A human DNA-binding monoclonal antibody was produced by fusing the hepatocytes from a 12-week-old human fetus with the lymphoblastoid cell line GM 4672 using polyethylene glycol. This antibody, designated BEG 2, binds to single-stranded (ss) DNA but also binds to double-stranded (ds) DNA, poly(dT), polyI and poly(ADP-ribose), but not to RNA, cardiolipin or K-30. The binding of BEG 2 to these polynucleotides can be inhibited by incubation with polynucleotides in the fluid phase. A rabbit polyclonal anti-idiotype was raised, and using this reagent it was shown that the BEG 2 idiotype is present in normal human serum (7%), systemic lupus erythematosus (SLE) sera (8%) and rheumatoid arthritis sera (23%).The extent of idiotypic sharing between BEG 2 and murine monoclonal DNA-binding antibodies, in particular monoclonal antibody (mAb) 423 (derived from a 15-day-old fetal MRL/Mp-*lpr*/*lpr* mouse) and mAb 402 (derived from an adult MRL/*lpr* mouse), was also investigated. Using a competition ELISA, it was shown that preincubation of BEG 2 with rabbit anti-423 and rabbit anti-402 inhibits the binding of BEG 2 to DNA, and the binding of 402 to DNA by anti-BEG 2 and anti-423. These data suggest that mAb BEG 2, 423 and 402 share common idiotypes, that autoreactivity is present in early fetal life, and that autoantibodies may be encoded for by germline genes, which have been conserved through evolution.

INTRODUCTION

Human systemic lupus erythematosus and murine lupus are autoimmune diseases characterized by the production of autoantibodies against a wide range of autoantigens, including single-stranded (ss) DNA, double-stranded (ds) DNA, RNA, phospholipids, histones, Sm, Ro, La and ribosomal RNP (Tan, 1989). Although such autoantibodies are usually associated with disease, natural antibodies against some of these antigens, but especially DNA, may be detected in the serum of normal healthy individuals (Guilbert, Digherio & Avrameas, 1982), but the relationship between the pathological and natural antibodies is not totally understood.

The genetic and immunochemical analysis of human and murine DNA-binding mAb has, however, been particularly revealing. It has been shown, for example, that DNA-binding antibodies carrying the idiotype (Id) 16/6 are encoded directly by the conserved VH26 gene without somatic mutation (Chen *et al.*, 1988). Similarly, in mice it has been shown that a VH gene (VH130) from a DNA-binding antibody (H130) derived from an MRL-*lpr*/*lpr* mouse is identical to the H18VH gene in normal BALB/c mice, and the other VH genes homologous to VH130 occur in many strains of mice (Trepicchio, Maruya & Barrett, 1987). These data show that V genes encoding autoantibodies are conserved in both mice and humans, and may also be expressed in healthy individuals.

Public Id, identified originally on DNA-binding antibodies, have been detected on a variety of other immunoglobulins, such as those in the sera of normal individuals, lupus patients and their healthy relatives (Isenberg *et al.*, 1988), a Waldenström immunoglobulin (Ig) recognizing the Klebsiella K30 antigen (Atkinson *et al.*, 1985), Ig in normal mouse serum (Mayus & Pisetsky, 1985), and in supernatants of cultures of normal mouse splenic cells (Datta, Stollar & Schwartz, 1983). This distribution suggests that these Id are not uniquely expressed by DNA-binding Ig.

Furthermore, it has been shown that human and murine DNA-binding antibodies can share Id (Eilat, Fischel & Zlotnik, 1985); for example, the human monoclonal DNA-binding antibody 16/6 shares an Id with several murine monoclonal DNA-binding antibodies (Morgan *et al.*, 1985b). The cross-species sharing of Id extends also to anti-Sm antibodies. Dang *et*

Abbreviations: Bic buffer, 0·05 M bicarbonate buffer, pH 9·6; HAT, hypoxanthine, adenine, thymidine; Id, idiotype; Ig, immunoglobulin; mAb, monoclonal antibody; PBS-T, PBS-0·1% Tween 20; poly(dT), polydeoxythymidilic acid; polyI, poly inosinic acid; SLE, systemic lupus erythematosus.

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al. (1988) reported that approximately half the lupus patients they tested expressed an idiotype, Y2, first identified on a murine anti-Sm antibody derived from the spleen of a MRL-*lpr*/*lpr* mouse. These data are compatible with the idea that autoantibodies of these specificities may be encoded by closely related genes which have been conserved through evolution.

In order to investigate further the nature of DNA-binding Ig, a DNA-binding mAb was prepared from normal human fetal hepatic lymphocytes, and it is demonstrated here that it carries a common Id BEG 2 and that this antibody is idiotypically related to several murine DNA-binding antibodies, including one derived from a fetal lupus mouse.

MATERIALS AND METHODS

Human monoclonal antibodies (BEG 2 and VAR 4)

Fetal liver. The liver was obtained from a normal 12-week-old human fetus following elective suction termination of pregnancy. A single-cell suspension was prepared and the cells were washed in RPMI-1640 medium before fusion.

Peripheral blood lymphocytes. Peripheral blood was obtained from a patient with polymyositis and lymphocytes separated by Ficoll density gradient centrifugation using standard techniques.

Cell fusion. Somatic cell hybridization was performed following the method of Shoenfeld *et al.* (1982), using the human lymphoblastoid cell line GM 4672 (Wistar Institute, Philadelphia, PA). The cells were distributed in 96-well microtitre plates at 3×10^5 cells/well and cultured in HAT-containing medium (Sigma, Poole, Dorset) for 3 weeks. Hybrids were identified macroscopically and transferred to 24-well culture plates. Supernatants were screened for Ig production and binding to ssDNA and dsDNA using a direct-binding ELISA. Hybrids were selected for further growth on the basis of the binding profile of their antibodies and were subcloned twice by limiting dilution. Selected clones were transferred to serum-free medium (Northumbria Biologicals, Cramlington, Northumberland) for bulk culture. Culture supernatants were centrifuged to remove cells and concentrated using a stirred cell concentrator and YM 30 membrane (Amicon, Stonehouse, Glos.). Monoclonal antibody was purified from the concentrate on a goat anti-human IgM–Sepharose 4B affinity column (Pharmacia, Milton Keynes, Bucks.) and the eluate reconcentrated using the cell concentrator.

The preparation, purification and properties of mAb PR4, 3F9, 601, 604, 100-1, 15/4, 134 have been described previously (Shoenfeld *et al.*, 1982; Rauch, Massicote & Tannenbaum, 1985; Lockniskar *et al.*, 1988). These are all human mAb that bind ssDNA; some, like PR4 and 134, also bind dsDNA and synthetic polynucleotides.

Production and purification of polyclonal anti-idiotype. On the basis of its binding specificity, a mAb designated BEG 2 was selected for production of anti-idiotype antibodies. A rabbit (NZWxhalf-lop hybrid) was immunized by multiple intradermal injection with a total dose of 100 μg affinity-purified mAb BEG 2 emulsified in complete Freund's adjuvant. A control rabbit received only adjuvant. A booster dose of immunogen in incomplete Freund's adjuvant was administered on Day 36. Sera were obtained before immunization on Day 53, and tested for activity against mAb BEG 2 and human IgM in a direct-binding ELISA.

Polyclonal anti-idiotype serum was rendered Id-specific by repeated passage through an immunoaffinity absorbent column containing pooled human Ig linked to Sepharose 4B and by further positive purification on a BEG 2 mAB–Sepharose 4B column, eluting the bound anti-Id-specific antibodies with 0·1 M glycine HCl (pH 2·3). The rabbit anti-Id reagent was shown to be Id specific using a direct binding ELISA (Shoenfeld *et al.*, 1983).

Murine monoclonal antibodies. The preparation, affinity purification and properties of murine hybridoma antibodies 402, 228, 423 are described elsewhere (Morgan *et al.*, 1985a; C. T. Ravirajan and N. Staines, manuscript in preparation). Monoclonal antibody 423 was derived from a 15-day-old fetal MRL/Mp-*lpr*/*lpr* mouse and mAb 402 and 228 were derived from adult MRL/*lpr* mice. Polyclonal rabbit anti-idiotypic sera against the mouse mAb were prepared in a manner comparable to that described above.

Patients

Sera were obtained from 74 patients with systemic lupus erythematosus (SLE), each of whom met four or more of the American Rheumatism Association's (ARA) revised criteria for the classification of the disease (Tan *et al.*, 1982), and from 22 patients with rheumatoid arthritis each of whom met the revised ARA criteria for rheumatoid arthritis (Arnett *et al.*, 1983). Normal sera were obtained from 29 normal individuals with no personal or family history of autoimmune disease.

Immunoassays

ELISA for detecting and quantifying immunoglobulin. Human Ig production was detected using a previously described capture ELISA (Watts *et al.*, 1989). A similar ELISA was used to quantify rabbit Ig.

ELISAs for determination of mAb binding specificity. Monoclonal antibody to ssDNA, dsDNA, poly(dT), polyI, ribosomal RNA and poly(ADP-ribose) was determined using previously described direct-binding ELISAs(Le Page *et al.*, 1989; Dudenev *et al.*, 1986). Poly(dT) acid (Sigma), polyI(Sigma) and ribosomal RNA (Sigma) were coated onto ELISA plates at a concentration of 10 μg/ml, and poly(ADP-ribose) (gift of Professor S. Shall, University of Sussex) at 20 μg/ml. Binding to cardiolipin (Sigma) and the Klebsiella K30 cell wall Ag (a gift of Dr R. Feldman, University College and Middlesex School of Medicine) was determined using previously published methods (Isenberg *et al.*, 1988, 1987b). Rheumatoid factor activity was determined using a published ELISA (Watts *et al.*, 1989). All supernatants were checked for non-specific binding to control wells lacking Ag. Supernatants were initially screened and subsequently a binding curve was established using affinity-purified mAb. The ability of mAb to bind to subcellular components was determined in indirect immunofluorescence assays using *Crithidia luciliae* (Biodiagnostics) (Isenberg *et al.*, 1987a) and Hep 2 cells (Biodiagnostics) (Morgan *et al.*, 1985b).

Competition of BEG 2 binding to polynucleotides. The ability of mAb BEG 2 to bind to polynucleotides in the fluid phase was determined by competition ELISA. Polynucleotides were coated onto immunoassay plates as described above. The concentration of mAb giving 50% maximal binding to polynucleotide in the direct-binding ELISA was established (35 μg/ml). Equal volumes of mAb and competing polynucleotide (10-fold dilutions between 100 μg/ml and 0·05 μg/ml in PBS-T) were

incubated together for 1 hr at 37° before transfer to wells in antigen-coated plates. The assay was completed as described for the DNA-binding ELISA.

ELISA for determining specificity of rabbit anti-idiotypic serum. A previously described direct-binding ELISA (Shoenfeld *et al.*, 1983) was used to determine the specificity of polyclonal rabbit anti-idiotype serum. Monoclonal antibodies BEG 2 and VAR 4 were coated onto ELISA plates at a concentration of 10 μg/ml, together with pooled normal human sera at an equivalent Ig concentration. Rabbit serum was used at a dilution of 1:10 or 1:100.

Inhibition ELISA for idiotype expression. Using a previously described method (Shoenfeld *et al.*, 1983), rabbit anti-Id BEG 2 (0·1 μg/ml, corresponding to 50% maximal binding of anti-Id BEG 2 to BEG 2) was preincubated with potential inhibitor mAb (3F9, PR4, 601, 604, 15/4, 134, 601, BEG 2) (2·5–0·02 μg/ml in PBS-T) for 2 hr at room temperature. Reaction mixtures were then added to mAb BEG 2 (1 μg/ml)-coated wells. After 1 hr at room temperature, wells were washed, goat anti-rabbit IgG whole molecule alkaline phosphatase (Sigma) conjugate added and the assay completed.

Antigen competition ELISA for binding-site idiotype expression. Immunoplates (pre-coated with poly-L-lysine) were coated with ssDNA and dsDNA (5 μg/ml and 10 μg/ml, respectively) overnight at 4°. Reaction mixtures of mAb and anti-Id were incubated for 2 hr at room temperature before being added to antigen-coated wells for 1 hr at room temperature. Bound mAb was detected by goat anti-human IgM alkaline phosphatase conjugate (Sigma) or goat anti-mouse IgG (γ-chain specific) horseradish peroxidase conjugate (Sigma).

Capture ELISA for detection of Id BEG 2 on Ig in human sera. Half the wells of polystyrene 96-well ELISA plates (Nunc, Gibco, Paisley, Renfrewshire) were coated overnight at 4° with rabbit anti-BEG 2 (0·1 μg/ml in 0·05 M borate buffer, pH 9·6), and the others were coated with rabbit IgG (0·1 μg/ml in borate buffer). Non-specific binding was blocked with 2% BSA. Samples of human sera at a dilution of 1:1600 in PBS-T/1% BSA were added in duplicate to wells in both halves of the plates and incubated for 1 hr at 37°. This dilution had been shown in preliminary experiments to give the best discrimination between the BEG 2 and the control halves of the plates. Bound human immunoglobulin was detected using goat anti-human IgG $F(ab')_2$ and IgM $F(ab')_2$ alkaline phosphatase conjugate (Sigma; used at 1/500), which had previously been passed through a normal rabbit serum Sepharose 4B column, and the assay completed in the usual way. The OD values obtained on the IgG side were subtracted from those on the anti-BEG 2 side and the results were expressed as a percentage of a known positive control (serum NR). Sera were considered positive when the calculated percentage value fell outside the ninetieth percentile.

SDS–PAGE and Western blotting. To determine the location of the Id BEG 2 on the Ig molecule, SDS–PAGE followed by immunoblotting and immunostaining was carried out as described previously (Williams *et al.*, 1988).

Ethical committee

The approval of the Bloomsbury District Health Authority Ethical Committee was sought and obtained for the use of fetal and adult human tissue in these experiments.

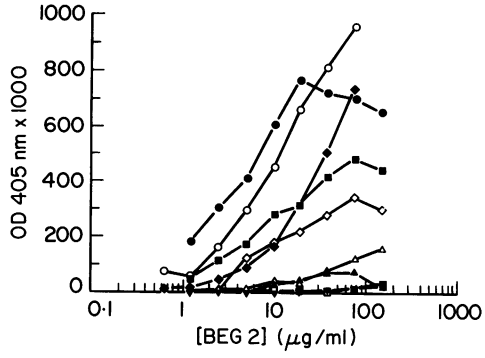

Figure 1. Antigen-binding specificity of mAb BEG 2. (○) ssDNA, (◆) ds DNA, (●) polyI, (◇) poly(dT), (■) poly (ADP-ribose), (□) K30, (▲) RNA, (△) cardiolipin, (▼) binding mAb VAR-4 to ssDNA.

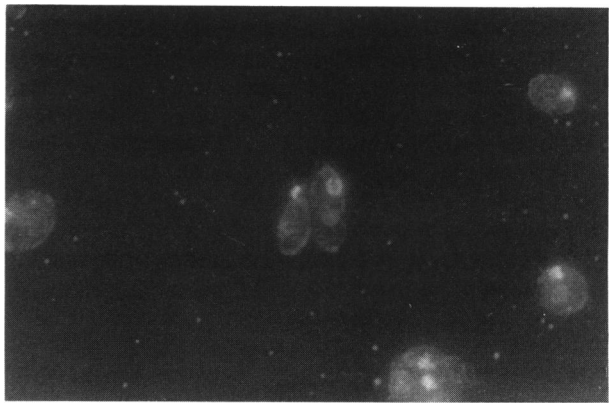

Figure 2. Indirect immunofluorescence of *Crithidia* showing staining of kinetoplast (× 70).

RESULTS

Characterization of mAb BEG 2

The fetal liver yielded a single-cell suspension containing $1·9 \times 10^7$ cells which were fused with GM 4672 cells at a 1:1 ratio. Clones were macroscopically visible at 4 weeks after fusion in 3/120 wells. Two clones secreted IgM, as determined by ELISA, one of which had DNA-binding activity, and this was designated BEG 2 and was selected for further study. The third clone did not secrete Ig. Monoclonal BEG 2 is an IgMλ. A second IgMλ mAb, VAR 4 (derived from the peripheral blood lymphocytes of a patient with polymyositis), which did not bind to ssDNA, was selected as a control. In a direct-binding ELISA, mAb BEG 2 preferentially bound to ssDNA, but also to dsDNA, poly I, poly(dT) and poly(ADP-ribose). It did not bind detectably to RNA, cardiolipin or the Klebsiella K-30 cell wall antigen (Fig. 1), or possess rheumatoid factor activity (data not shown). Monoclonal antibody BEG 2 stained kinetoplast structures of *Crithidia luciliae* (Fig. 2) and nucleolar structures of Hep 2 cells (Fig. 3). Binding of mAb BEG 2 to ssDNA in the solid phase was inhibited by incubation with ssDNA, dsDNA, poly I and poly(dT) but not by poly(ADP-ribose) or RNA (Fig. 4). Binding to dsDNA, poly(dT) and poly I in the solid phase

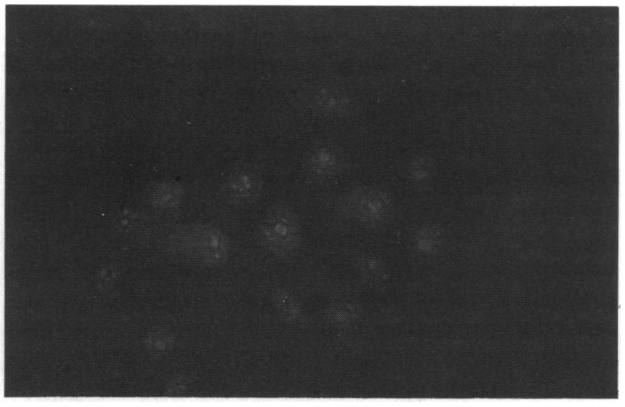

Figure 3. Indirect immunofluorescence of Hep 2 cells showing staining of nucleolar structures (×24).

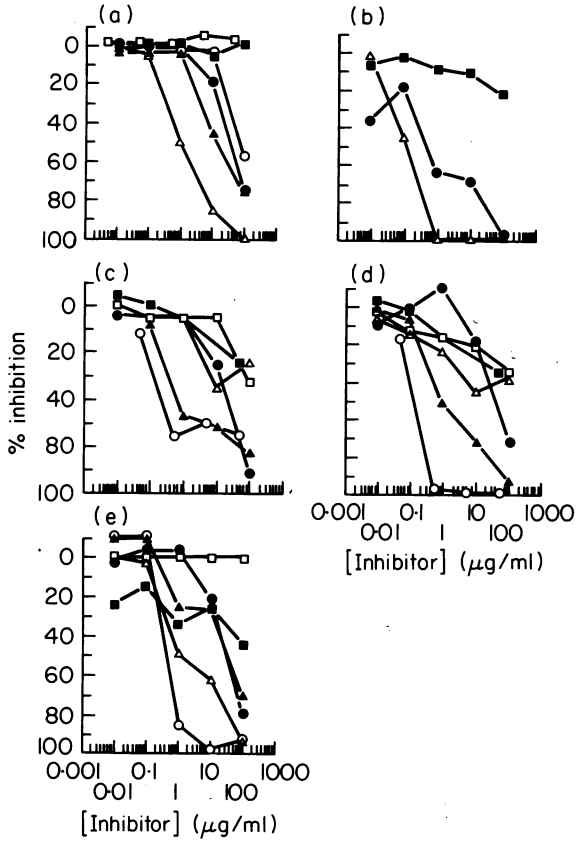

Figure 4. Inhibition of binding of mAb BEG -2 to polynucleotides by fluid-phase polynucleotides. Antigen (a) ssDNA, (b) dsDNA, (c) poly I, (d) poly(dT), (e) poly (ADP-ribose). Inhibitor: (△) ssDNA, (●) dsDNA, (▲) polyI, (○) poly(dT), (■) poly (ADP-ribose), (□) RNA.

was also inhibited by incubation in the fluid phase (Fig. 4). However, poly (ADP-ribose) in the fluid phase did not inhibit binding of mAb BEG 2 to poly (ADP-ribose) in the solid phase, suggesting that immobilized poly (ADP-ribose) presents different epitopes to those presented by antigen in the fluid phase. These inhibition experiments confirm the specificity of mAb BEG 2 seen in the direct binding assays.

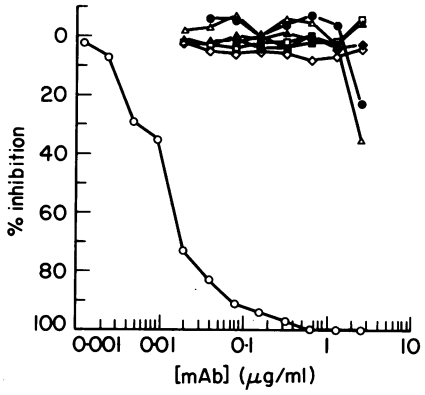

Figure 5. Inhibitor binding rabbit anti-Id BEG 2 to mAb BEG 2 by mAb. (○) BEG-2, (◆) PR4, (●) 601, (◇) 134, (■) 15/14, (□) 604, (▲) 100-1, (△) 3F9.

Characterization of polyclonal rabbit anti-idiotype serum

A polyclonal rabbit anti-idiotypic serum was produced and purified as described above. Rabbit serum obtained before immunization did not react with mAb BEG 2, mAb VAR 4 or normal human sera; following immunization rabbit anti-serum reacted equally against mAb BEG 2 and normal human serum. After affinity purification the putative anti-idiotype reacted 32 times more strongly with mAb BEG 2 than with normal human serum. No reactivity against mAb VAR 4 could be detected. Serum obtained from the control rabbit showed no anti-human Ig or anti-idiotypic activity.

The ability of rabbit anti-Id BEG 2 to bind to mAb BEG 2 in the solid phase could be completely inhibited by preincubation with mAb BEG 2 in the fluid phase (Fig. 5), with 50% inhibition occurring at a concentration of mAb of 0·01 μg/ml. Monoclonal antibodies 3F9 and 601 weakly inhibited this reaction, but at concentrations 250 times and 600 times (respectively) greater than that of mAb BEG 2, making it unlikely that mAb 3F9 and 601 share major idiotypic determinants with mAb BEG 2. mAb PR4, 100-1, 604, 15/4 and 134 did not compete with mAb BEG 2 in this system (Fig. 5).

Using a similar competition assay, the extent of sharing of idiotypic structures between human (BEG 2) and three murine DNA-binding mAb (423, 402, 228) was investigated. It was found that binding of mAb BEG 2 to DNA was inhibited by anti-Id 402, anti-Id 423 as well as by its homologous anti-Id (Fig. 6). Similarly, binding of mAb 402 to DNA could be inhibited by anti-Id 423 and anti-Id BEG 2 (Fig. 6), and binding of mAb 423 to DNA by anti-Id 402 (Fig. 6). Anti-Id 228 was unable to inhibit the binding of mAb BEG 2, 402 or 423 (Fig. 6). These data suggest that mAb BEG 2, 402 and 423 share idiotypic determinants. Since inhibition of binding was not complete in these systems, it is probable that some idiotopes recognized by the anti-Id reagents are restricted to their homologous antibodies.

Location of Id BEG 2 on IgM molecule

Figure 7 shows the immunostained Western blot following SDS–PAGE of denatured mAb BEG 2, and demonstrates that the Id identified by rabbit anti-Id BEG 2 is present on the λ light chain.

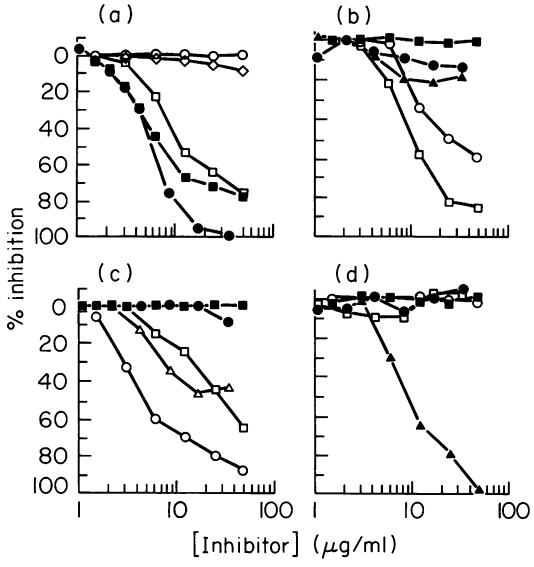

Figure 6. Inhibition of binding of mAb BEG 2 to DNA by anti-Id. (a) Inhibition binding mAb BEG 2 to DNA by anti-Id (□) mAb BEG 2+anti-Id 423;(○) mAb BEG 2+rabbit IgG; (●) mAb BEG 2+anti-Id BEG 2; (◇) mAb BEG 2+anti-Id 228; (■) mAb BEG 2+anti-Id 402. (b) Inhibition of binding of mAb 423 to ssDNA by anti-Id. (□) mAb 423+anti-Id 423; (●) mAb 423+rabbit IgG; (▲) mAb 423+anti-Id BEG 2; (○) mAb 423+anti-Id 402; (■) mAb 423+anti-Id 228. (c) Inhibition of binding of mAb 402 to dsDNA by anti-Id. (△) mAb 402+anti-Id BEG 2; (●) mAb 402+rabbit IgG; (○) mAb 402+anti-Id 402; (□) mAb 402+anti-Id 423; (■) mAb 402+anti-Id 228. (d) Inhibition of binding of mAb 228 to ssDNA by anti-Id. (□) mAb 228+anti-Id BEG 2; (●) mAb 228+rabbit IgG; (▲) mAb 228+anti-Id 228; (○) mAb 228+anti-Id 423; (■) mAb 228+anti-Id 402.

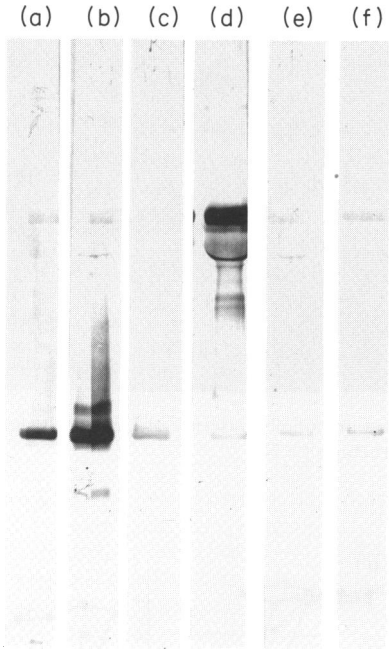

Figure 7. Photograph of immunostained Western blot. mAb was run on each lane at 3 mg/ml and immunostained. (a) rabbit anti-Id BEG 2; (b) rabbit anti-human λ light chain; (c) rabbit anti-human κ light chain; (d) rabbit anti-human μ heavy chain; (e) rabbit anti-human γ chain; (f) normal rabbit serum.

Detection of Id BEG 2 in human serum

The Id BEG 2 could be detected in 7% of normal sera, 8% of lupus sera and 23% of rheumatoid arthritis sera, at levels up to three times the upper limit of normal.

DISCUSSION

This report is, to the best of the authors' knowledge, the first to describe the immunochemical specificity and idiotypic properties of DNA-binding mAb derived from human fetal tissue.

There is considerable evidence to suggest that B cells with the capability of secreting autoantibodies are components of the normal immune system. Autoantibodies have been found in normal human sera with binding specificities including tubulin, actin, thyroglobulin, transferrin, cytochrome-c, collagen (Guibert *et al.*, 1982) and neurofilaments (Stefansson *et al.*, 1985). Monoclonal antibodies which bind to ssDNA have been prepared from normal peripheral blood lymphocytes (Rauch *et al.*, 1985; Hoch, Schur & Schwaber, 1983) and from normal human tonsillar lymphocytes (Cairns, Block & Bell, 1984). Furthermore, mAb with specificities against autoantigens have been obtained in high frequency from spleen cells of newborn non-autoimmune mice (Digherio *et al.*, 1985).

The spectrum of reactivity of the murine pre-immune B-cell repertoire tends toward autoreactivity, with many antibodies binding ssDNA, anti-nuclear antibody (ANA), keratin, nuclear DNA and small (Sm)RNP (Souroujan *et al.*, 1988). Similarly, after EBV stimulation, many human umbilical cord and fetal B cells secrete polyreactive IgM antibodies which bind to autoantigens (Lydyard *et al.*, 1989). The mAb BEG 2 described here has similar properties emphasizing that virgin B cells expressing polyreactive IgM autoantibodies exist in the fetal and perinatal repertoires.

Monoclonal antibody BEG 2 binds to DNA and other synthetic polynucleotides in ELISA as well as to the DNA contained within the kinetoplast of *Crithidia luciliae*. The nucleolar pattern seen on indirect immunofluorescent staining of HEP-2 cells by mAb BEG 2 was unexpected. Other studies have shown that mAb which bind to DNA in ELISAs or radioimmunoassay may demonstrate a variety of staining patterns on HEP-2 cells (M. Weigert, personal communication).

It is demonstrated here that the Id BEG 2, as defined by polyclonal rabbit anti-idiotype, is carried on the λ light chain of the IgM molecule. Because the binding of mAb 2 to ssDNA and dsDNA was totally inhibited by rabbit anti-Id BEG 2, it appears that the recognized Id is located at or near the antigen binding site. Although the peptide or gene sequence of the antibody is not yet known, it is likely that mAb BEG 2 is encoded for by an unmutated germline V gene given that it was derived from a 12-week-old fetus.

The Id BEG 2 is present in the serum of both normal individuals (7%), patients with SLE (8%) and those with rheumatoid arthritis (23%) In a recent international collaborative study involving 11 laboratories, the expression of 19 idiotypes in a set of coded sera was compared and all but five of the idiotypes could be detected in at least some (0–13%) normal sera and all could be detected in sera from patients with autoimmune rheumatic diseases (Isenberg *et al.*, 1989). Thus, Id BEG 2, like these other Id, is not disease specific, though, unlike

some of the Id, it could not be detected in the sera of patients with primary Sjögrens syndrome or myositis.

The present data suggest that the idiotype of DNA-binding autoantibodies is relatively limited in its heterogeneity. Using competition assays it has been shown that binding of mAb BEG 2 to rabbit anti-Id BEG 2 could only be weakly inhibited by mAb 3F9 and 601, and not at all by mAb PR4, 100-1, 604, 15/14 and 134. Idiotypic sharing did, however, extend across the human–mouse species barrier, confirming and extending previous work (Morgan *et al.*, 1985b; Eilat *et al.*, 1985; Dang *et al.*, 1988). Thus, it has been shown that binding of the antibodies BEG 2, 423 or 402 to DNA may be competitively cross-inhibited by any or each of their respective rabbit anti-idiotype reagents. This suggests that the cross-reactive Id expressed on each antibody are located at the antigen-binding site, and implies that the encoding genes have been conserved during evolution and are present in both the fetal and adult B-cell repertoires of both species. Idiotypes of mAb BEG 2 and 423, of fetal mouse origin, and 402, from an adult mouse, belong to one idiotype group that does not include 228 and the human antibodies examined. Currently under investigation is the question of isotype switching of the BEG 2 Id, since clearly IgG rather than IgM antibodies tend to be those associated with immunopathology, especially in SLE. The clinical expression of autoimmune disease, however, depends upon a combination of factors, of which expression of a particular Id is just one.

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