A MONOCLONAL ANTIBODY THAT DEFINES AN IDIOTOPE WITH TWO SUBSITES IN GALACTAN-BINDING MYELOMA PROTEINS

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Galactan-binding myeloma proteins (GalBMP)[1] of BALB/c mice form a family of closely related immunoglobulins characterized by VK4 L chains and VH1B H chains (see ref. 1 for classification). The V regions of four of these proteins have been completely sequenced (2–4). Three VK4 regions, XRPC24 (X24), XRPC44 (X44), and TEPC601 (T601), are identical up to position 95, whereas J539 VK4 differs at five positions. Each L chain has a different J_k region (4). In the V_H region, X44 and T601 are identical up to position 96, but X24 differs from both at three positions (37, 45, and 53). J539 has seven V_H differences from X24 and four each from X44 and T601. All four H chains differ in D and J region sequences (3).

In previous studies (5–7), we have shown that idiotypic antisera to individual GalBMP such as X24, X44, and J539 can be raised in strain A/J mice. We have also identified two cross-reacting idiotopes. T601, X24, and X44 proteins share a common idiotope, J539 and X44 share another. The availability of the primary structures of the four GalBMP makes it possible to identify the amino acids that determine these idiotopes. In addition, hypothetical three-dimensional models of GalBMP have been developed (8) that permit localization of the determining amino acids on the surface topography of the GalBMP.

Monoclonal anti-idiotypic antibodies enable us to further define the structure of these idiotopes, particularly when several determining amino acids are implicated. The interaction of homogeneous antibody with multiple sites on the antigen defines contact points for the antibody and outlines the topographical region of complementarity. Also, when the idiotope is associated with the antigen binding site of the antigenic molecule, hapten inhibition studies can be useful in localizing the idiotypic site. In the present study, we describe a monoclonal antibody, HyX24-14, produced by the Kohler-Milstein methodology (9) that binds different GalBMP with different affinities. Using amino acid sequence data and hypothetical topographical models, the binding site for this monoclonal antibody on the GalBMP can be partially outlined.

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DMEM, Dulbecco's minimal essential medium; GalBMP, galactan-binding myeloma protein; HBSS, Hanks' balanced salt solution; HT, hypoxanthine thymidine medium; PBS, phosphate-buffered saline; PEG, polyethylene glycol; RIA, radioimmunoassay.

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Materials and Methods

Animals. Strain A/J mice were used for immunization and (BALB/c × A/J)F_1 (CAF_1) mice were used for ascites production and as a source of thymocytes. Both strains were obtained from National Cancer Institute contract N01-CB-94326 at Litton Bionetics, Inc., Kensington, Md.

Immunizations. The production of anti-idiotypic antisera has been described previously (6). Briefly, strain A/J mice were injected with 400–600 μg NH_2SO_4 precipitate of XRPC24 ascites. In the first injection, the antigen was mixed with complete Freund's adjuvant, the second with incomplete Freund's adjuvant, and the third with saline, each given subcutaneously 5 d apart. Three more subcutaneous injections in saline were given 2 wk apart and the last boost of the antigen was injected intravenously in saline 6 wk later.

Fusion. 3 d after the last antigen injection, the mouse was bled out and its spleen cells were used for fusion with Sp 2/0-Ag cells (9, 10). Sp 2/0-Ag cells were grown in "full medium", i.e., Dulbecco's minimal essential medium (DMEM), 25 mM glucose, 25 mM Hepes buffer, 2 mM glutamine, 50 U/ml penicillin/streptomycin (all reagents from M. A. Bioproducts, Walkersville, Md.), 2×10^{-5} M 2-mercaptoethanol (Aldrich Chemical Co., Milwaukee, Wis.) and 10% newborn calf serum (Gibco Laboratories, Grand Island, N. Y.). 8-azaguanine, with a final concentration 1.3×10^{-4} M (Calbiochem-Behring Corp., San Diego, Calif.) was added to full medium for growth of the Sp 2/0-Ag tumor cells, to prevent revertants at the hypoxanthine-guanine-phosphoribosyl transferase (HGPRT) locus. After separately washing Sp 2/0-Ag and spleen cells twice in Hanks' balanced salt solution (HBSS) containing 10 mM Hepes (M. A. Bioproducts), cells were mixed in DMEM with 25 mM Hepes at a ratio of 6.5 nucleated spleen cells to one tumor cell, spun for 10 min at 190 *g*, and then fused at 37°C by gently resuspending the cell pellet in 0.5 ml autoclaved 45% (wt/vol) polyethylene glycol (PEG) 1,000 (J. T. Baker Chemical Co., Phillipsburg, N. J.) in HBSS. After 1 min, the cell PEG mixture was diluted by dropwise addition of 10 ml DMEM, 25 mM Hepes over the next 5 min. Cells were spun down and cultured overnight in 50 ml full medium plus 1.6×10^{-5} M hypoxanthine and 1×10^{-4} M thymidine (Sigma Chemical Co., St. Louis, Mo.) (HT medium) in a tissue culture flask (3150; Costar, Data Packaging, Cambridge, Mass.). The following day, 125 ml of HT medium and aminopterin (Sigma Chemical Co.) was added to a final concentration of 4×10^{-7} M. Cells were cultured in 24-well tissue culture plates (3524; Costar) at $\sim 1.25 \times 10^6$ spleen cells/1.25 ml culture. Cultures were fed at day 6 with 1 ml HT medium plus aminopterin. At day 11, 1 ml supernatant was exchanged for 1 ml HT medium.

Cloning. Cells of antibody-positive hybrid cultures were cloned by limiting dilution in suspension cultures in 96-well tissue culture plates (3596; Costar) in HT medium with 1×10^6/ml mouse thymocytes as the feeder layer. After 11 d, single growing clones were identified and marked under a dissecting microscope, clones were fed with 100 μl full medium, and the supernatants were analyzed for the presence of antibody 2–3 d later. Positive clones were transfered to 1-ml cultures with fresh thymocytes in full medium. One representative clone of each original hybrid culture was subcloned and afterwards maintained in continuous passaging in 1-ml cultures. Aliquots were frozen and kept in liquid nitrogen.

Ascites Production. After two cloning cycles, cells were grown up in tissue culture flasks and 1×10^5–1×10^6 washed cells were injected into 8–16-wk-old CAF_1 mice. Mice had been conditioned 4–8 wk earlier by intraperitoneal injection of 0.5 ml pristane (2,6,10,14-tetramethylpentadecane; Aldrich Chemical Co.). Rapidly growing intraperitoneal tumors developed after 12–20 d and 5–15 ml ascites fluid could be drained from each mouse.

Radioimmunoassays (RIA). All RIA were performed as solid-phase RIA in 96-well flexible, polyvinyl chloride microtiter plates (1-220-25; Dynatech Laboratories, Inc., Alexandria, Va.) in two variations of a previously described procedure (11, 12).

(*a*) For detecting antibody in tissue culture supernatants, a direct binding assay was applied. Microtiter plates were coated overnight with 100 μl/well of a mixture of rabbit anti-mouse immunoglobulin sera diluted in phosphate-buffered saline (PBS), pH 7.4. After emptying the plates, 50–100 μl of tissue culture supernatant was added and the plates were incubated for 1–2 h at 37°C, washed three times with 1% bovine serum albumin (BSA) in PBS, pH 7.4 (BSA-PBS), and then 100 μl of ^{125}I-labeled X44 IgA myeloma protein at 50,000 cpm diluted in BSA-PBS was added. After 1–2 h of incubation at 37°C and 10 washes in tap water, the wells were cut out and radioactivity was counted in a Beckman counter (Beckman Instruments, Inc.,

Fullerton, Calif.). Hybridoma culture fluids binding more than three times the counts per minute of control cultures without growing hybridomas were considered positive for mouse IgM or IgG with α-X44 activity. The heavy chain class of a given hybridoma antibody was determined by using the rabbit antisera individually.

(*b*) In competition assays, the plates were coated with 100 μl of diluted antiserum or ascites or 0.5 μg purified myeloma proteins and washed three times with 1% BSA. 20 μl of competitor diluted in 1% BSA-PBS (50,000 cpm) of ^{125}I-labeled myeloma or hybridoma protein was then added and incubated for 8–16 h at 37°C. Each competitor concentration was run in triplicate and the percent control binding was calculated as

$$\frac{\text{cpm competitor} - \text{cpm background}}{\text{cpm BSA} - \text{control} - \text{cpm background)}} \times 100.$$

The various competition systems are identified by first the name of the reagent coated to the plate and then the ^{125}I-labeled taget; e.g., the α-T601/X24 system used an α-T601 serum on the plate and ^{125}I-X24 as the target.

(*c*) Four rabbit antisera, each raised against a purified myeloma protein, (μ, κ), ($\gamma\kappa$), ($\gamma 2\kappa$), and ($\gamma 3\kappa$) were made immunoglobulin class specific by extensively absorbing them over two Sepharose-4B immunoadsorbent columns, one coupled with a Bence-Jones protein and the other with the myeloma protein X24 (IgA, k). Testing in direct binding RIA with ^{125}I-labeled myeloma proteins of various H chain classes showed them to be exclusively specific for individual mouse immunoglobulin heavy chain classes.

(*d*) Iodination of protein with ^{125}I was performed as previously described (12).

(*e*) Hapten inhibition was determined in microtiter wells coated with 100 μl of 5 μg/ml purified GalBMP in PBS solution. Haptens diluted in 0.1% BSA-PBS were used as competitors and ^{125}I-HyX24-14 was used as the target following the same procedure as described for the competition RIA. Cellobiose and *O*-methyl-β-D-galactopyranoside were both purchased from Sigma Chemical Co. β-1,6-D-galactodiose and β-1,6-D-galactotriose were synthesized by Apurba et al. (13) and kindly supplied to us by Dr. C. P. J. Glaudemans of the National Institute of Allergy and Infectious Disease.

(*f*) HyX24-14 was purified from crude ascites by affinity chromatography over an X24-Sepharose-4B column and elution with 3 M ammonium thiocyanate and rapid dialysis against PBS.

Results

Generation of HyX24-14. 1.75×10^8 spleen cells of a single A/J mouse hyperimmunized with X24 myeloma protein were fused with Sp 2/0 cells and grown in 141 individual cultures. 14 d after fusion, 115 cultures contained growing hybridoma cells, and ~1 out of 1.5×10^6 spleen cells had been successfully fused. To search for monoclonal antibodies with specificity for GalBMP, the supernatants of 45 growing cultures were screened for the presence of mouse IgM or IgG that would bind to ^{125}I-X44 in a solid-phase RIA. In this procedure, we selected only for cross-specific monoclonal antibodies.

17 (38%) of these supernatants had more than three times the binding (1,200 cpm) activity as compared with supernatants from cultures without growing hybridoma cells (400 cpm). Eight positive cultures were cloned by limiting dilution in suspension culture. All eight hybridomas gave rise to growing clones, but only from five were we able to derive clones with X44 binding activity in the supernatant. Further specificity testing of the cloned hybrids showed that four reacted with BALB/c IgA proteins. These hybridomas (HyX24-13, 16, 18, and 19), which recognize allotypic specificities, will be discussed in detail elsewhere. The fifth hybridoma antibody, HyX24-14, will be the subject of the remainder of this paper.

HyX24-14 was subcloned two more times. Only 1 out of 16 clones tested was

positive for X44 binding activity after the first subcloning, but after a second cloning cycle, 20 out of 20 clones tested were positive. Cells of one active subclone were grown up in mass culture and used for ascites production in pristane-conditioned CAF_1 mice. HyX24-14 was shown to be an IgG-1 protein by using individual heavy chain class-specific rabbit antisera.

Specificity of the HyX24-14 Monoclonal Antibody. In screening tests, a standard preparation of HyX24-14 ascites was prepared and used throughout. A dilution of HyX24-14 ascites was allowed to fix to the solid substratum and an ^{125}I target was added. The highest dilution that gave near-maximal counts bound was used. When ^{125}I-X24 was the target, a 1:50,000 dilution of the HyX24-14 ascites was used. Nine GalBMP and three myeloma proteins that did not bind galactan were tested for their ability to compete in the HyX24-14/X24 system (Table I, Fig. 1). 7.2 ng of cold X24 and 2,800 ng of X44 gave 50% control binding but no other protein competed in this system. HyX24-14 was most specific for X24 and appeared to bind to X24 with the highest affinity. X44 gave 50% control binding at ~400 times the concentration of X24.

Specificity of the HyX24-14 Monoclonal Antibody in the Hy-X24-14/X44 System. The binding of HyX24-14 to X44 was strong enough to permit the construction of a second RIA system in which the standard preparation of HyX24-14 was diluted 1:20,000 and ^{125}I-X44 was the target (Table I, Fig. 2). In this system, 0.75 ng of X24 and 9 ng of X44 gave 50% control binding; furthermore, S10, T191, T601, and T1084 weakly interacted. From these two RIA systems we conclude that the monoclonal antibody HyX24-14 has a specific idiotypic structures common to some of the GalBMP. The relative affinity is highest for X24, medium for X44, low for S10, T191, T601, and T1084, and nondetectable for J1, C4, J539, and three non-GalBMP.

TABLE I

Specificity of Monoclonal (HyX24-14) and Conventional Antibodies to X24 for Other GalBMP

Competitors		Competitor that gives 50% control binding		
		Monoclonal HyX24/X24 1:50,000	Monoclonal HyX24-14/X44 1:20,000	Conventional α-X24/X24 1:80,000
		nanograms per 20 liters		
GalBMP	J1	—‡	—	—
	C4	—	—	—
	S10	—	2,500	—
	X24	7.2*	0.75	9.5
	X44	2,800	9	—
	T191	—	2,900	—
	J539	—	—	—
	T601	—	1,100	12,000
	T1084	—	1,800	18,000
Non-GalBMP	E109	—	—	—
	M167	—	—	—
	T15	—	—	—

* Nanograms per 20 μl.

‡ No competition with 20,000 ng.

The data reported represent typical data selected from at least three experiments.

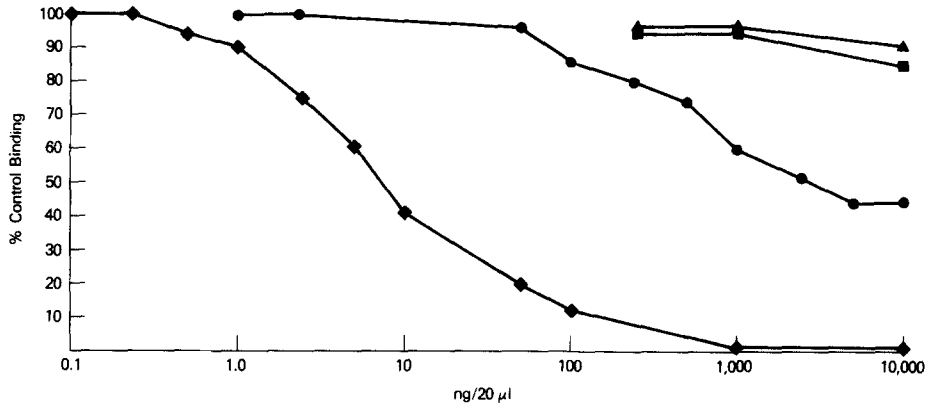

FIG. 1. Radioimmune competition of the HyX24-14 monoclonal antibody for the X24 target. The standard HyX24-14 ascites preparation was diluted 1:50,000. ◆, X24; ●, X44; ■, T539; ▲, T601.

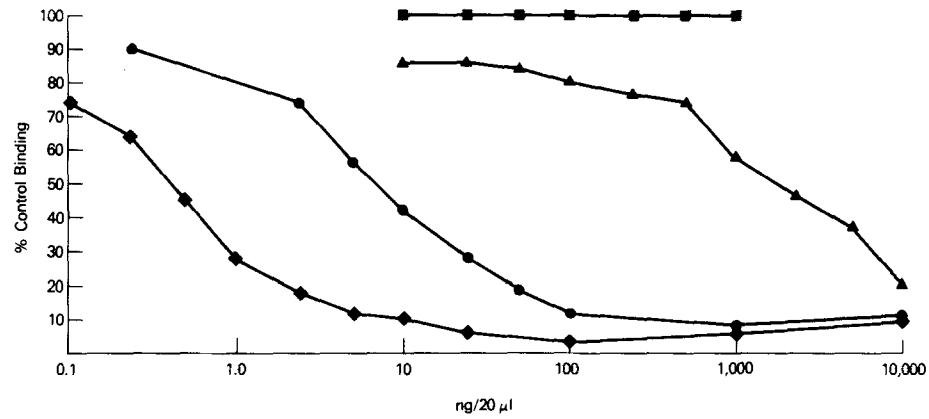

FIG. 2. Radioimmune competition of the HyX24-14 monoclonal antibody for the X44 target. The standard HyX24-14 ascites preparation was diluted 1:20,000. ◆, ×24; ●, X44; ■, J539; ▲, T601.

Hapten Inhibition Studies. Four oligosaccharides were tested for their ability to inhibit the binding of HyX24-14 to X44: α-methyl-β-D-galactoside (*O*-Me-Gal), β-1,6-D-galactodiose (Gal_2) and β-1,6-D-galactotriose (Gal_3) all bind to X24; cellobiose was used as a negative control. As can be seen in Fig. 3, the Gal_3 was the most potent inhibitor, Gal_2 was more effective than *O*-Me-Gal, and cellobiose was inactive. These results indicate that specific haptens, even the monosaccharide alone, block the ability of HyX24-14 to bind to its complementary idiotope.

Model Building. A hypothetical space-filled model of J539 has been described previously (8). This was constructed by modifying the coordinates of McPC603 determined by x-ray crystallography (14). The reconstruction process was done on an Evans & Sutherland (Salt Lake City, Utah) viewing screen in stereo. J539 (and all of the other completely sequenced GalBMP) are 11 residues shorter than McPC603 (2–4). These length differences occur at three locations, CDR-1 V_K, CDR-2, and CDR-3 V_H. The sites were located by sequence homology and the positions were deleted from the McPC603 structure. The open ends of the J539 structure were then rejoined by computer controls that permitted independent differential rotation of amide planes

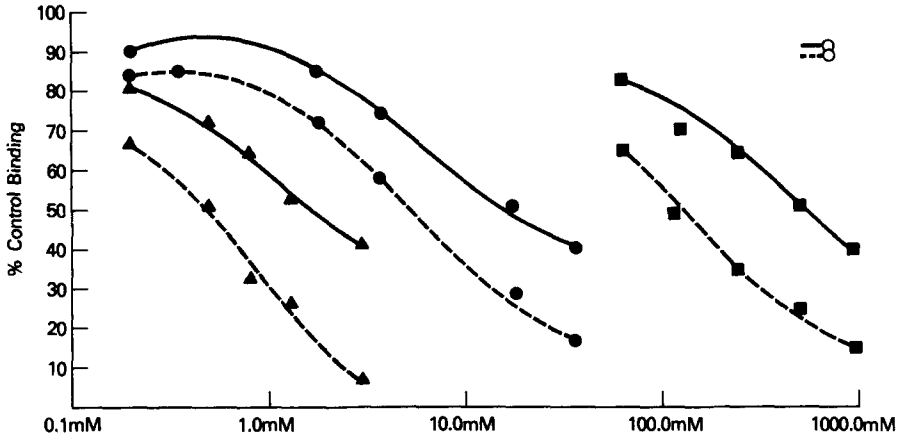

FIG. 3. Hapten inhibition of the binding of HyX24-14 to X24 (—) and X44 (---). ■, Gal_1; ●, Gal_2; ▲, Gal_3; O, cellobiose.

in upstream or downstream residues. The entire process is recorded. To build models of X24, X44, and T601, the amino acid substitutions and deletions were first changed in the corresponding McPC603 and positions. Second, the backbones were rejoined exactly as was done with J539, using the same rotational angles. The joined sites were visually inspected on the screen. After the rejoining process, the structure was refined by a computer program (ANNEAL) that searched the space around each amino acid side chain (one at a time) and placed the side chain in a location with no illegal contacts with other side chains. All but 12 side chains could be placed in a noncontacting location by this process, indicating that the molecule will have to be further refined. The hypothetical models, however, provide a plausible approximation of the relationship of side chains to each other, and a general contour map of the Fv surface.

The front views of J539, X24, X44, and T601 are shown in Fig. 4. The amino acids of CDR-2 V_H, the D region, and position 31 are shaded (Kabat number system) (15). The CDR-2 V_H region is formed by a bend-like structure that protrudes out on the front surface. There are three forms of this structure in the four GalBMP, as indicated by the amino acid sequence data (Fig. 5). X24 differs from the other GalBMP in position 53 by Gly instead of Asp. The negatively charged Asp 53 could prevent HyX24-14 from strong binding to CDR-2 V_H of X44, J539, and T601. The side chain of the D region amino acid position (96 His or Gly) is very close to position 31 (Arg or Lys). In X44, Arg 31 and His 96 form a unique pair not found in the other GalBMP (Fig. 4).

Discussion

In this study a monoclonal antibody (HyX24-14) was derived from an A/J mouse that had been immunized with the X24 GalBMP. The initial screening procedure used to detect active clones used the X44 myeloma protein and thus favored selection of monoclonal antibodies with cross-specific binding properties. When the HyX24-14 monoclonal antibody was tested for its ability to bind to a series of GalBMP whose V regions have been completely sequenced (2–4) (Fig. 5), it was found that the affinity of HyX24-14 was highest for X24, medium for X44, low for T601, and nondetectable for J539.

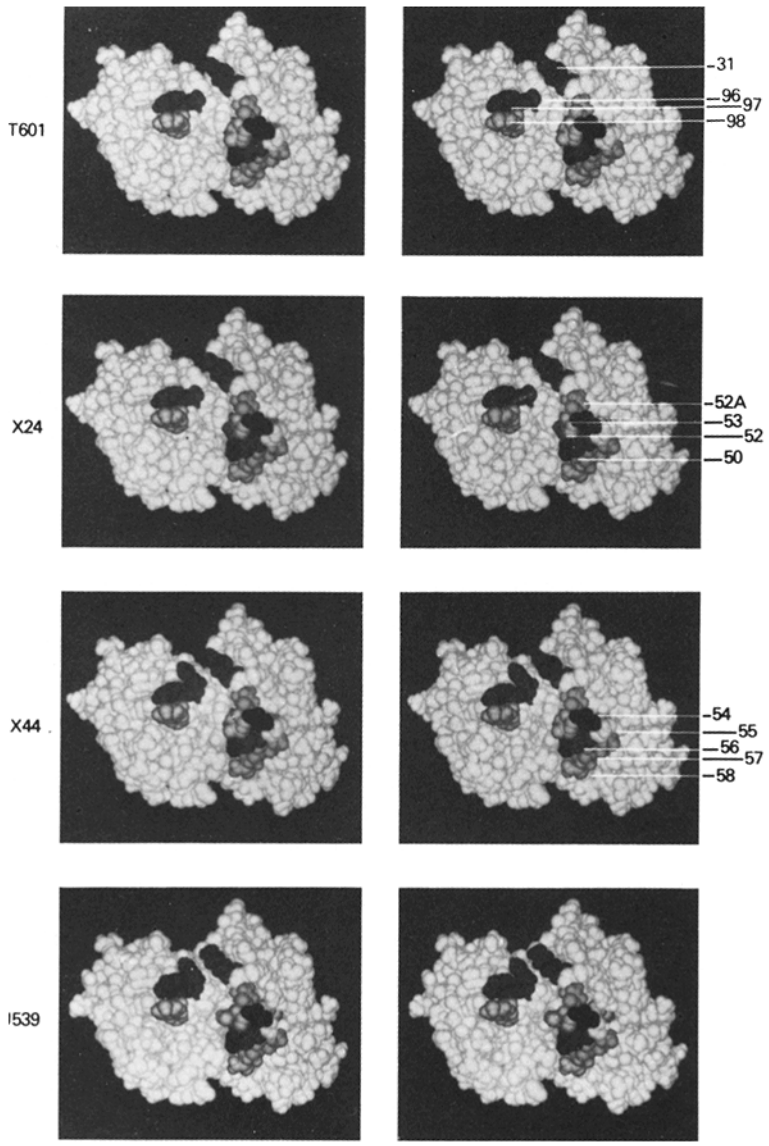

FIG. 4. Stereopictures of models of the Fv fragments of four GalBMP front view T601, X24, X44, and J539. The front surface faces the solvent and contains amino acid side chains that are implicated in hapten binding. The V_K domain occupies about the left half of the Fv and borders the V_H domain on an angle of about 45°. The shaded amino acids are: V_H amino acids belonging to CDR-2 V_H (Kabat numbers 50–58) and CDR-1 V_H (number 31). The CDR-3 V_H positions 96–97 are coded by the D region minigene. The pictures can be visualized in stereo with an inexpensive stereoviewer sold by Hubbard Scientific Co., Northbrook, Ill. Representative distances between selected atoms of X24 are NH_1 Arg31 → OH Tyr98 = 13.7 Å; NH_1 Arg31 → α C Gly 96 = 11.7 Å; NZ Arg31 → α C Gly 53= 13.0 Å; OH Tyr 98 → α C Gly 53 = 14.5 Å; CG Pro 52A → ND_2 Asn58 = 12.9 Å.

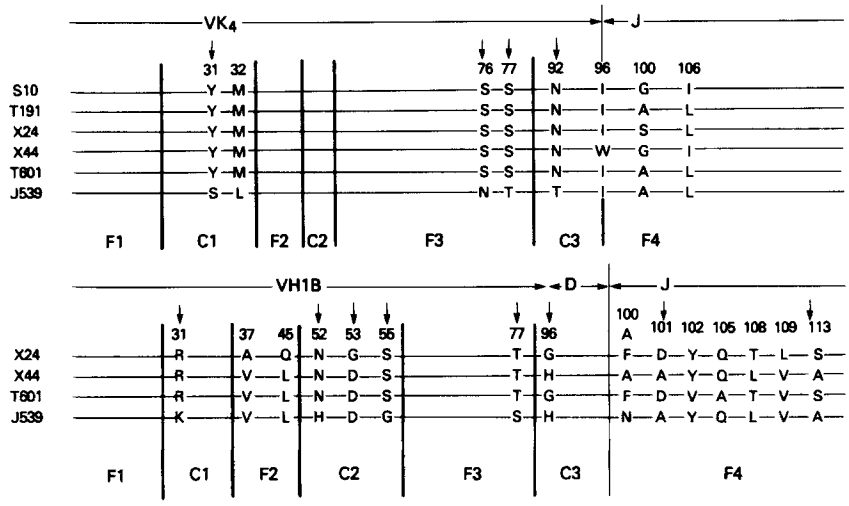

FIG. 5. Sequences of the GalBMP were determined by Rao et al. (2, 3) and Rudikoff et al. (4).

All of the binding studies were done by solid-phase competition assay systems. In this method, the antibody is fixed to the solid substratum and cold competitors are allowed to bind to the antibodies. The relative binding affinities are reflected in the amount of cold competitor that binds to the fixed antibody and competes with the labeled target. The smaller the amount needed of a given GalBMP to compete with the ^{125}I target antigen, the higher is the affinity to the antiidiotypic antibody. In the RIA system, where HyX24-14 was coated on the plate in the lowest concentration (1:50,000) and ^{125}I-X24 was the target, X24 had the highest affinity for HyX24-14 and the affinity of X44 was lower (400 times less potent). In a less specific assay system, where a higher concentration of HyX24-14 (1:20,000) was adhered to the plate and ^{125}I-X44 was used as the target, X24 bound again with the highest affinity, X44 was 10 times less potent, T601 was weak (1,500 times less potent), and binding of J539 was not detectable at all.

The availability of four GalBMP whose V regions have been completely sequenced and hypothetical molecular models that approximate the location of amino acids on the surface topography, enables us to propose a structural explanation for the observed binding activities of HyX24-14.

J_K region sequence differences should not directly cause the observed affinity difference for binding of the GalBMP to HyX24-14 because positions 96, 100, and 106 are inaccessible to the surface. Because X24 and T601 have identical L chains up to position 100L, sequence differences in V_H and D are probably responsible for the affinity differences. Essentially, we propose that HyX24-14 interacts with a relatively large surface of the GalBMP that includes both parts of CDR-2 and the CDR-3 (the D region) of the H chain. In the models, these regions project as two loop structures towards the solvent. These loops are separated by a depression (Fig. 4); thus the potential epitope can be divided into two subsites. The region contributing the larger part of the affinity for X24 is represented by the CDR-2 V_H subsite in the vicinity of 53H. X24 in this region has an important primary structural change, i.e., a glycine at position 53H instead of aspartic acid. This substitution changes the local secondary

structure of the CDR-2 loop as well as the electrostatic properties. Because T601 differs from X24 in V_H only at this position 53H (and two framework positions), we conclude that this region of the antibody surface is most important for the binding at X24 to HyX24-14.

The ability of HyX24-14 to bind X44 with moderate affinity, even though X44 also has the aspartic acid at position 53H, suggests the presence of a second subsite that contributes to binding. One possibility would be that there is a second subsite present in X24 and X44 but absent in T601 and J539. Surprisingly, the amino acid sequence data show that there are no shared primary structural similarities in X24 and X44 that are absent in T601. To explain this phenomenon, we postulate from the sequence data and the models that X44 has a unique structure in the region of this second subsite that binds HyX24-14 better than the corresponding structures in other GalBMP, including X24. The candidate amino acid combination for the second site is Arg 31H and His 96H in X44. The association of these two amino acids in X44 is unique among the GalBMP, and for this reason probably determines this second subsite. Because X44 was used as the selecting antigen during the isolation of the hybridoma, this is not an unreasonable explanation.

It is postulated that HyX24-14 is complementary to a general contour formed by all of the GalBMP. The contour of HyX24-14 would appear to have a projected structure that fits in the depression between CDR-2 V_H and the 31H-96H complex. Binding affinity is determined by complementarity forces between groups on amino acids side chains of idiotype and anti-idiotype. Distances between amino acids on the GalBMP are shown in Fig. 4 (see legend). The distances between the α carbon of Gly 53H, the β nitrogen of Arg 31H, and the α carbon of Gly 96H in X24 are all ~13–14 Å apart. The area of contact between idiotype and antiidiotype might even be larger. The hapten β-1,6-*O*-galactohexose was constructed and fitted to the frontal surface in such a way that the oxygens of the first, third, and fourth sugars made close contact with the hydrogen bond accepting amino acids in J539 (8). The binding site in J539 thus involves amino acids in CDR-2 V_H. The terminal galactose residue binds very near to Trp 33 V_H; thus the proposed CDR-2 V_H subsite is neighboring the proposed hapten binding site. Even smaller haptens, including the *O*-methyl-β-D-galactoside are proposed to bind very near to Trp 33 V_H and are therefore expected to inhibit the binding of X24 and X44 to HyX24-14. The hapten inhibition studies show that even the methylated monosaccharide can inhibit this binding. The presence of separate subsites, as suggested by the data, could provide two dissociated binding sites. Thus, a series of homogenous antibodies with the similar but not identical structure of HyX24-14 could bind to either epitope independently or, unlike HyX24-14, could bind to both subsites with equal energy. In antibody protein interactions, single amino acids can have profound effects. Thus, somatic mutations affecting such antibody sites could give rise to novel binding properties.

The concept of two subsites being recognized by HyX24-14 could also explain the apparent difference of the idiotype defined by conventional anti-idiotypic sera (α-X24-IdI) and the idiotype defined by the monoclonal anti-idiotypic antibody (HyX24-14) (Table I). The α-X24 serum contains specific antibodies from a pool of individual clones. These antibodies would all recognize the epitope subsite formed by CDR-2H (7). In addition, each clonal product would be able to bind some structures in the environment of this subsite. Antibodies of the HyX24-14 clonotype probably are also

present in the conventional α-X24 serum, but in such a low concentration that the RIA could detect neither direct binding of ^{125}I-X44 no competing activity of X44 in the α-X24/X24 system. However, in the HyX24-14/X24 system, all antibodies are of identical specificity, i.e., they bind the same part of the environment around CDR-2H in identical fashion. Thus, direct binding of ^{125}I-X44 and competing activity of X44 in the HyX24-14/X24 system could be detected.

Because the anti-idiotypic antibody can bind to an area larger than the epitope subsite, even small changes in the structure of the subsite environment could enhance or lower the affinity. In a conventional antiserum this would not be detectable because binding of only a small percentage would be influenced by this change, whereas the affinity of a monoclonal antibody could fully reflect this change when it covers this part of the subsite environment. This phenomenon might explain some of the unexpected findings when conventionally defined antigens were "redefined" with monoclonal antibodies (16).

The proposed subsites are determined by different gene elements, e.g, D region codons and V_H codons that are expressed independently. A series of clonotypes expressing VH1B and VK4 genes that elaborate β-1,6-D-galactan-binding specificity could manifest a variety of structures determined by J_K, J_H, and D exons that change the idiotypic antigenicity. Schilling et al. (17) have shown in the α-1,3 dextran system that a variety of α-1,3 dextran binding proteins with different D exons can be generated in the BALB/c mouse. The GalBMP reflect a similar pattern. Monoclonal idiotypic antibodies that enhance or suppress immune responses (18, 19) could modify some clonotypes in a given response without involving other closely related structures.

Much more information is needed on the binding specificities of antiidiotypic antibodies to provide a better basis for understanding and controlling biological actions of anti-idiotypic antibodies.

Summary

An IgG_1 monoclonal antibody HyX24-14 was derived from A/J mice that were immunized with the IgA XRPC24 (X24) galactan binding myeloma protein (GalBMP) of BALB/c origin by the Kohler-Milstein hybridoma technology. HyX24-14 specifically binds some but not all GalBMP. Different patterns of binding using a panel of nine GalBMP were found, depending upon the concentration of antibody and the antigenic target. From molecular models and amino acid sequence data, it was proposed that the idiotope defined by HyX24-14 had two subsites, each of which appeared to be able to bind independently to the antibody.

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