COMBINING SITE SPECIFICITIES OF MOUSE HYBRIDOMA ANTIBODIES TO DEXTRAN B1355S*

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Dextrans are naturally occurring high molecular weight homopolysaccharides of D-glucose residues linked predominantly $\alpha(1 \rightarrow 6)$ (1). Class I dextrans are considered to have a comblike structure with a linear backbone of $\alpha(1 \rightarrow 6)$-linked glucoses, and short branches of one or two glucoses linked $\alpha(1 \rightarrow 2)$, $\alpha(1 \rightarrow 3)$, or $\alpha(1 \rightarrow 4)$ (1–3). Class II dextrans have sequences of alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ glucoses, and side chains of $\alpha(1 \rightarrow 6)$-linked glucoses (4) or a more ramified structure with longer branches of alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$-linked residues (5).

Immunochemical studies of antibodies from human and rabbit antisera, mouse myeloma proteins, and mouse hybridoma antibodies to dextran B512, a class I dextran with 95% $\alpha(1 \rightarrow 6)$ linkages and 5% $\alpha(1 \rightarrow 3)$ linkages at branch points, have yielded valuable insights into the size, shape, and specificity of antibody-combining sites, essentially because of the simple, well-defined structure of this dextran (6–9).

Dextran B1355S is a more complex class II dextran, with 54% $\alpha(1 \rightarrow 6)$ and 46% $\alpha(1 \rightarrow 3)$ linkages, with 11% of the $\alpha(1 \rightarrow 3)$ at branch points (3), and uncertainties remain as to its precise structure and therefore as to the nature of its antigenic determinants. Immunochemical characterization of the combining sites of myeloma proteins binding dextran B1355S, CAL20 TEPC1035 (IgG2bκ), J558 (IgAλ), and MOPC104E (IgMλ) have previously been reported (10–12), but because specific myeloma proteins are found fortuitously by screening pristane-induced plasmacytomas (13), they do not provide insight into the repertoire of the response to B1355S. A set of 10 mouse hybridoma antibodies to dextran B1355S has been studied, and amino acid sequences of their heavy chain V-regions (14) as well as their private and cross-reactive idiotypes have been determined (15). This information is considered with the results presented here of an immunochemical study of the combining site specificities of some of these and additional hybridoma antibodies.

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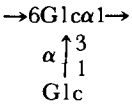
Materials and Methods

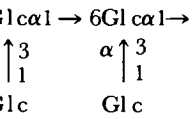
Immunization. BALB/c or (BAB14 × BRVR)F_1 mice were immunized by two methods. Primary immunization consisted of an injection of 100 μg of B1355S in complete Freund's adjuvant. Hyperimmunization involved primary immunization followed by three injections of 2×10^9 *Escherichia coli* at 2-d intervals 1 mo later. Fusions were performed 5–7 d after final immunization.

Production of Hybridomas. The procedure used was essentially that of Galfré et al. (16). Briefly, ~10^8 spleen cells from dextran-immunized mice were fused with at least 10^7 HGPRT$^-$ MPC11 (Hdex 1 and 3), NS1 (Hdex 2, 6, 9 and 31), or Sp2/O-Ag14 (Hdex 11, 12, 14, 24, 25, and 36) cells using polyethylene glycol (Carbowax 1500 [1,500 mol wt]; Fisher Scientific Co., Pittsburgh, PA). The cell lines were generously supplied by Dr. M. Scharff (Albert Einstein College of Medicine, New York), Dr. C. Milstein (Medical Research Council, Cambridge, England), and Dr. G. Köhler (Basel Institute for Immunology, Basel, Switzerland) (17–19), respectively. After fusion, cells were grown in hypoxanthine, aminopterin, thymidine selection media (20) in 24-well culture plates. Dextran-binding proteins were detected either by isoelectric focusing or by radioimmunoassay. Hybrids were cloned in soft agar over 3T3 feeder layers (21) and grown in BALB/c mice as ascites tumours.

Dextrans. Native dextrans have been described previously (1–5) and were obtained from Northern Regional Research Center, U. S. Dept. of Agriculture, Peoria, IL. Synthetic dextran V39 was provided by Dr. C. Schuerch, State University of New York, Syracuse, NY. The molecule has a linear backbone of about 213 α(1 → 6)-linked D-glucopyranosyl units with ~27 single glucose (Glc)[1] side chains linked (1 → 3) in a ratio of 95% α/5% β linkages. About 15%, by weight, consists of units



4% of units



and <1% of units with three branch points in a row. Unbranched segments vary widely in length.

Quantitative Precipitin Assay. The procedure has been previously outlined (22). A constant amount of antibody, 4–7.3 μg antibody nitrogen (AbN), was added to varying quantities, 0–60 μg, of dextran; the total volume was 280 or 400 μl. After incubation for 1 h at 37°C, and 5–7 d at 4°C with daily mixing, AbN in the washed precipitates was determined by Ninhydrin assay. The precipitin patterns and the classification into groups were not affected by the total volume or amount of AbN chosen, and lower amounts of AbN permitted use of smaller quantities of scarce oligosaccharides in inhibition assays.

Amino Acid Sequences. V_H amino acid sequences of MOPC104E, J558, and Hdex 1 through 10 have been reported previously (14, 23, 24). The sequences of Hdex 11, 12, 14, 24, 25, 31, and 36 will be published elsewhere.[2] Amino acid positions are denoted by sequential and homologized numbering (24) in square brackets [ ] to facilitate comparisons with other V_H chains.

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[1] *Abbreviations used in this paper*: CDR, complementarity determining region; FR, framework region; Glc, glucose IdI, individual idiotype; IdX, cross-reactive idiotype. Amino acid code: A, Ala; R, Arg; N, Asn; D, Asp; C, Cys; Q, Gln; E, Glu; G, Gly; H, His; I, Ile; L, Leu; K, Lys; M, Met; F, Phe; P, Pro; S, Ser; T, Thr; W, Trp; Y, Tyr; and V, Val.

[2] J. Schilling, B. Clevinger, M. Bond, J. M. Davie, and L. E. Hood. Manuscript in preparation.

Results

Quantitative Precipitin Assays. The results in Table I and Fig. 1 show the relationships among the hybridoma (Hdex) antibodies from quantitative precipitin assays with numerous dextrans. The maximum precipitation of any dextran (− to ++++) and the amount required for 50% of maximum precipitation relative to B1355S are given. When the antibodies are arranged in order of increasing cross reactivity, five groups are seen. Previously characterized mouse myeloma proteins CAL20 TEPC1035, J558, and MOPC104E fit into groups 1, 3, and 5, respectively (10–12).

From Fig. 1, it is apparent that all hybridoma antibodies react maximally and to similar extents with class II dextrans, B1355S, B1498S, and B1501S; the classification into groups therefore is based on the varying extents to which cross reactions with class I dextrans occur.

In group 1, CAL20 TEPC1035, and Hdex 12, 25, and 31, react with none of the class I dextrans. However, beginning with group 2, once a class I dextran shows some cross-reactivity, it continues to precipitate in all succeeding groups. Hdex 36 is precipitated by B742S, but this curve enters the antigen excess zone sooner than is seen with other antibodies binding this dextran. This is probably due to Hdex 36 being IgG3λ, whereas all other Hdex antibodies are IgMλ. The three members of group 3, Hdex 6, 9, and J558, also react with B742S. In addition, proteins in this group cross-react somewhat with three or four other dextrans: Hdex 6 and 9 with B1299S, B1355L, and B1255, and J558 (10, 11) with B1299S, B1255, B1375, and B742L.

Group 4, Hdex 1, 3, and 24 are precipitated to greater extents by B742S, B1299S, and B1355L. With Hdex 3 and 24, B1255 reacts almost as well; B1375, B1254S, and B742L react less strongly. With Hdex 1, B1255 behaves more like B742L and B1254S, and B1375 is only slightly active.

Group 5 precipitates with all dextrans reactive in group 4, and is distinguished from group 4 primarily by the greater precipitating power of dextrans B742S, B1299S, B1355L, and B1255. This is seen most strikingly with Hdex 11 and 14; B1255 and B1355L appear somewhat less active with Hdex 2. Less strongly reacting dextrans include B1375 and B1254S, which behave similarly, followed in decreasing order of activity by B742L, B1141, and possibly B1501L.

The remaining class I dextrans B1498L, B1351S, B512, B1399, B1425, B1142, B1424, and the synthetic dextran V39 do not react with any of the Hdex antibodies with which they were tested.

Dextran Structure and Reactivity. The structural compositions of these dextrans to the extent that they are currently known may be found in refs. 1–5. The three maximally reacting class II dextrans, B1355S, B1498S, and B1501S, are similar in linkages and degrees of branching: 54–67% $\alpha(1 \rightarrow 6)$ and 33–46% $\alpha(1 \rightarrow 3)$, with 9–11% of the $\alpha(1 \rightarrow 3)$ at branch points (3); all are proposed to have an alternating $\alpha(1 \rightarrow 3)$, $\alpha(1 \rightarrow 6)$ sequence (4, 5).

Two strongly cross-reactive class I dextrans in groups 4 and 5, B1299S and B742S both have highly branched structures with 35% $\alpha(1 \rightarrow 2)$ and 50% $\alpha(1 \rightarrow 3)$ linkages at branch points, respectively (3). However, two other strongly reactive dextrans, B1355L and B1255, have infrequent branching (2, 3). B1355L, with ~5% $\alpha(1 \rightarrow 3)$ branch points (3), appears similar to B512, which is nonreactive in all precipitin groups, and thus its reactivity is puzzling. It is known that B1355L is synthesized

TABLE I

Precipitating Powers of Dextrans with Hybridoma Antibodies to Dextran B1355S and with Myeloma Proteins that Bind Dextran B1355S

Dextran	Symbol	Group 1				Group 2	Group 3			Group 4			Group 5			
		CAL 20 TEPC1035* IgG2bκ	Hdex 12 IgMλ (H)‡	Hdex 25 IgMλ (H)	Hdex 31 IgMλ (P)	Hdex 36 IgG3λ (P)	Hdex 6 IgMλ (P)	Hdex 9 IgMλ (P)	J558* IgAλ	Hdex 1 IgMλ (H)	Hdex 24 IgMλ (H)	Hdex 3 IgMλ (H)	MOPC 104E* IgMλ	Hdex 11 IgMλ (H)	Hdex 14 IgMλ (H)	Hdex 2 IgMλ (H)
		μg antibody N used/analysis														
		7.0	7.0	4.4	5.8	4.3	4.0	4.3	6.5	7.3	6.4	6.3	5.3	6.7	6.5	4.5
B1355S	◫	++++§ 1.0‖	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0	++++ 1.0
B1498S	▲	+++ 1.2	++++ 1.0	+++ 1.3	++++ 1.1	++++ 1.0	++++ 0.8	+++ 1.0	++++ 1.0	++++ 0.8	++++ 0.7	+++ 1.6	++++ 0.6	++++ 0.8	++++ 1.0	+++ 0.8
B1501S	▽	+++ 1.2	++++ 1.7	+++ 1.6	++++ 1.5	++++ 1.0	++++ 1.2	++++ 1.0	++++ 1.0	+++ 1.6	++++ 1.4	++++ 1.5	++++ 1.1	++++ 1.3	++++ 1.6	+++ 1.3
B742S	◧	–	–	–	–	++ 3.2	++ 7.2	++ 4.8	++ 8.4	++ 5.7	++ 5.1	++ 3.6	++ 2.7	++++ 2.9	+++ 2.6	+++ 2.5
B1299S	⊠	–	–	–	–	–	+	+	+	++ 4.6	++ 7.1	++ 8.5	++++ 1.8	+++ 3.6	+++ 2.3	+++ 4.5
B1355L	⊟	–	–	–	–	–	+	+	–	++	++ 4.2	++ 8.4	++ 3.8	+++ 2.9	+++ 2.6	++ 5.1
B1255	○	–	–	–	–	+	+	+	+	+	++	++	++ 4.1	+++ 3.3	+++ 3.2	++ —¶
B1254S**	●	–	–	–	–	–	–	+	–	+	+	++	++ 7.8	++ 13.4	++ 10.0	++ 6.4
B1375	△	–	–	–	–	–	–	–	+	–	+	+	++	++ 11.5	++ 10.5	++ 13.6
B742L	⊗	–	–	–	–	–	–	–	+	+	–	+	++	+	+	++ 15.5
B1141	◕	–	–	–	–	–	–	–	–	–	–		–	–	+	++ 16.8
B1498L	▼	–	–		–				–	–	–	–	–	–	–	–
B1501L	▽	–	–		–				–	–	–	–	–	–	–	–
B1351S	⧗	–	–	–	–	–	–	–	–	–	–	–	–	–		–
B512	◹	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
B1399	⋈	–	–		–		–	–	–	–	–	–	–	–	–	–
B1425	◨	–	–	–	–	–	–	–	–	–	–	–	–	–		–
B1142	◘	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
B1424	◔		–		–							–			–	
V39	■		–		–							–				

* Data for CAL20 TEPC1035 (10), J558 (10, 11), and MOPC104E, (S. Sugii, unpublished observation).

‡ H, hyperimmunization; P, primary immunization.

§ Percent of maximum precipitation with any dextran relative to maximum precipitation with dextran B1355S.
–, 0–20%; +, 21–40%; ++, 41–75%; +++, 76–95%; ++++, 96–100%.

‖ μg of dextran giving 50% of maximum precipitation relative to μg of dextran B1355S giving 50% of maximum precipitation.

¶ B1255 does not reach 50% of maximum precipitation with Hdex 2.

** B1254S refers to the fraction also designated B1254S [*Y*].

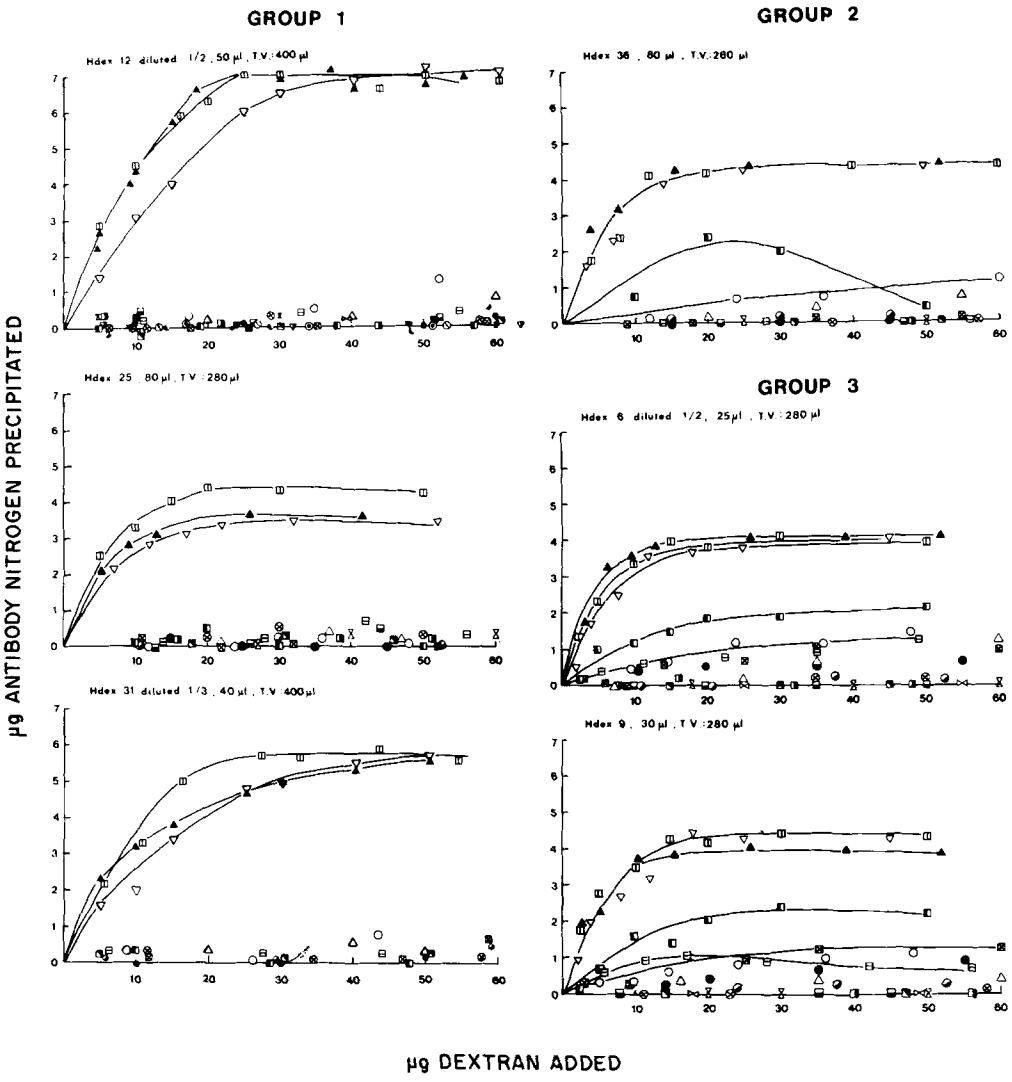


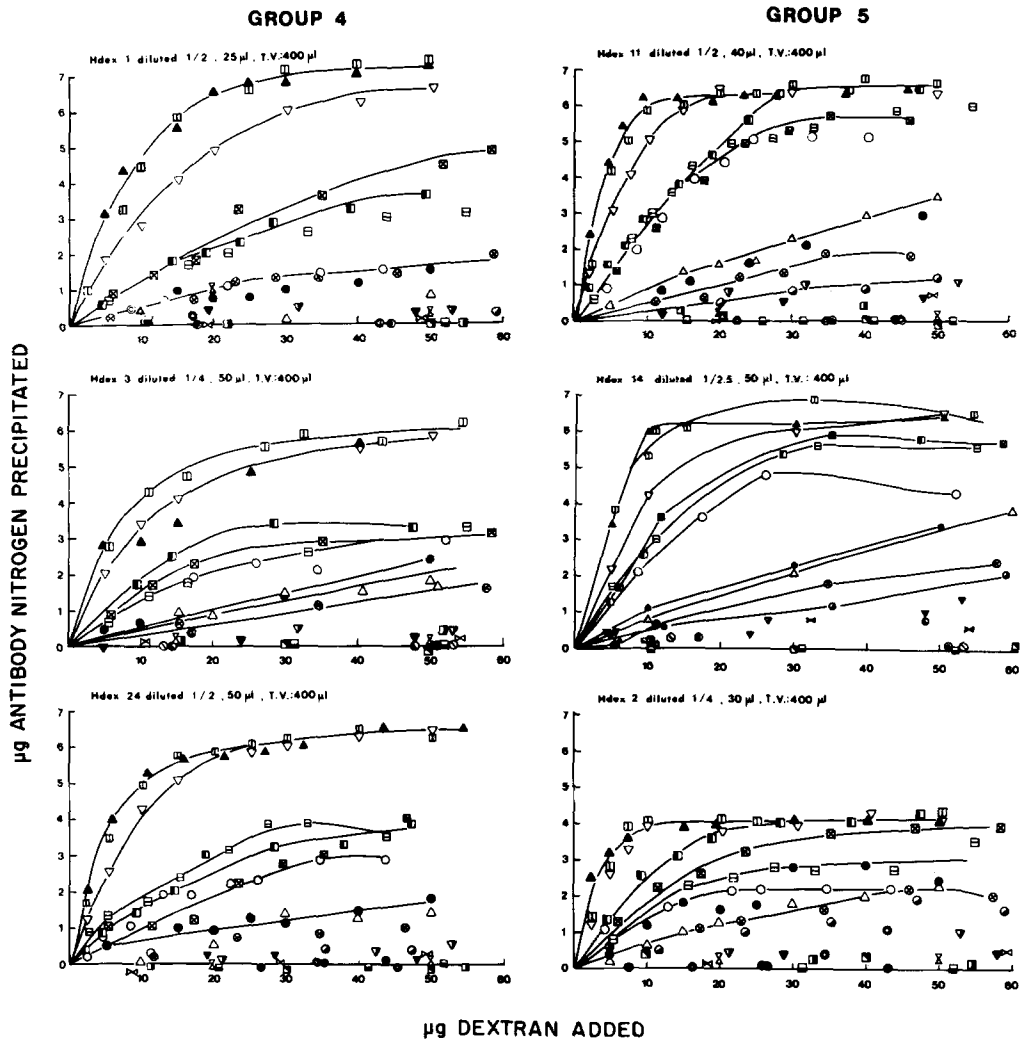
FIG. 1. Quantitative precipitin analysis of the Hdex antibodies.

simultaneously with B1355S in the same culture containing an enzyme capable of forming alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ linkages (25), and perhaps it contains a small percentage of these alternating sequences.

B1254S, with 20% $\alpha(1 \rightarrow 4)$ (3), and B1375, with 26% $\alpha(1 \rightarrow 3)$ (1) branch point linkages, respectively, are both moderately cross reactive in groups 4 and 5.

The very weakly reactive and nonreactive dextrans generally have low percentages of branched residues with the exception of B1142 (3); its failure to react has been observed previously (26) and is not understood.

Little is known about the distribution of the side chains, i.e., the spacing of the "teeth" on the proposed comblike class I dextrans, which would provide the multivalent determinants for the cross reactions. Some of the apparent inconsistencies in



the correlation of dextran composition with the precipitin assays may be resolved by further structural study of these dextrans.

V_H Amino Acid Sequences and Idiotypes. The V_H regions of all dextran-binding proteins except CAL20 TEPC1035 and Hdex 36 have been sequenced (14, 23)[2] and their idiotypic determinants characterized (15).[2] The sequences of the heavy chains follow a pattern of homology consistent with their origin from three gene segments, V_H, D_H, and J_H. The proteins studied show four V segments, V_1, V_1', V_3, and V_4 (Table II), which correspond to amino acid positions 1-99 [1-95]. V_1 is found in 10 of 13 proteins. The other V segments differing from V_1 by 1, 4, and 2 amino acids, respectively, are each represented once. Eight different pairs of amino acids are found at positions 100, 101 [96, 97] which are encoded by D_H gene segments; four J_H regions are seen. Table II shows that individual gene segments appear to assemble in an unrestricted manner with other segments.

The idiotypes of the dextran-binding proteins have been localized to particular

TABLE II

Association of Specificity, Sequence, and Idiotype

Specificity		Sequence				Idiotype		
		V_H 1–99 [1–95]*	D_H 100, 101 [96, 97]	J_H 102–117 [98–113]		IdX	IdI (J558)	IdI (MOPC104E)
Group 1	Hdex 12	1′	GN	Y R – A Y – – Q – – – V – – – –	J_3	++	–	–
	Hdex 25	1	SY	– – – – – – – – – – – – – – – –	J_1	++	–	–
	Hdex 31	1	RY	Y A M – Y – – Q – – S – – – – –	J_4	++	++	–
Group 2	Hdex 36	?	?	?		++	++	–
Group 3	Hdex 6	1	SH	– – – – – – – – – – – – – – – –	J_1	++	–	–
	Hdex 9	3	RY	– – – – – – – – – – – – – – – –	J_1	++	++	–
	J558	1	RY	– – – – – – – – – – – – – – – –	J_1	++	++	–
Group 4	Hdex 1	1	NY	H – – – – V – – – – – – – – – –	J_1'	++	+	–
	Hdex 24	1	SS	Y – – – Y – – Q – – – L – – – –	J_2	++	–	–
	Hdex 3	1	RD	– – – – – – – – – – – – – – – –	J_3	++	–	–
Group 5	MOPC104E	1	YD	W Y F D V W G A G T T V T V S S	J_1	++	–	++
	Hdex 14	4	YD	– – – – – – – – – – – – – – – –	J_1	– –	–	++
	Hdex 11	1	YD	F – – – Y – – Q – – – L – – – –	J_2	++	–	++
	Hdex 2	1	NY	– – – – – – – – – – – – – – – –	J_1	++	+	–

* The V_H1 sequence of MOPC104E is reported in refs. 23, 24. V_H1' of Hdex 12 differs with N at position 63 [62] instead of K; V_H3 of Hdex 9 has R, H, N, and F at positions 63 [62], 73 [72], 77[76], and 80[79], respectively, instead of K, D, S, and Y; V_H4 of Hdex 14 has K, K at positions 54, 55 [53, 54] instead of N, N.

regions of the heavy chain. Cross-reactive idiotype (IdX) determinants are correlated with amino acid residues 54, 55 [53, 54] in the second hypervariable region (CDR2), and only in V_4 (Hdex 14) are KK substituted for NN. Both individual idiotype (IdI)(MOPC104E) and IdI(J558) determinants are associated with D_H residues 100, 101 [96, 97] in the third hypervariable region (CDR3); IdI(J558) is correlated with RY and IdI(MOPC104E) with YD at these positions (Table II).

V_H and J_H segments do not appear to be restricted to any precipitin group. Similarly, IdX determinants are found on molecules in all groups. However, D_H residues and IdI determinants may correlate, although imperfectly, with binding specificities. In groups 1 and 3, two out of five Hdex antibodies and J558 carry IdI(J558) determinants and have RY D_H segments; the other three antibodies, with GN, SY, and SH, lack IdI(J558). The only antibody in group 2, Hdex 36, expresses an IdI(J558) determinant, although its D_H residues are still unknown. Thus four of seven proteins in groups 1, 2, and 3 express IdI(J558). Two of seven proteins in groups 4 and 5 partially express IdI(J558) and both have NY at 100, 101 [96, 97]. A better correlation is found in group 5 in which two of three Hdex antibodies and MOPC104E have IdI(MOPC104E) determinants and YD D_H segments. Five proteins lacking both IdI(J558) and IdI(MOPC104E) fall into three groups; each differs at amino acid positions 100, 101 [96, 97], suggesting that they might represent additional IdI specificities.

Discussion

Quantitative precipitin studies, similar to those with blood group substances and monoclonal anti-I and anti-i sera (27), reveal significant differences in combining site specificities among 12 mouse hybridoma antibodies to dextran B1355S. The spectrum appears greater than that seen in 12 mouse hybridomas to dextran B512 (9), reflecting the greater structural complexity of B1355S. However, immunization, fusion, screening, subcloning, and culturing procedures for obtaining hybridomas may affect the findings by favoring certain clones, perhaps those growing faster or secreting immu-

noglobulins of higher affinity. Such factors could be responsible for the failure on immunization of mice with B1355S to obtain hybridoma antibodies cross reacting with B512, which have been seen in humans immunized with this dextran (6, 28, 29). Thus, the five-group classification may not include the entire repertoire (30).

The precipitation of all of the Hdex antibodies with class II dextrans suggests that the sequence of alternating $\alpha(1 \rightarrow 6)$ and $\alpha(1 \rightarrow 3)$-linked glucoses is the preferred determinant. In rabbits injected with B1355S, two distinct populations of antibodies specific for the alternating sequence were seen, with the $\alpha(1 \rightarrow 6)$ or the $\alpha(1 \rightarrow 3)$ linkage being immunodominant (31). Quantitative precipitin inhibition studies (unpublished) show that tri- and tetrasaccharides with alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$-linked glucoses are the best inhibitors thus far found of the precipitation of the Hdex antibodies with B1355S. They are better than nigerosyl $\alpha(1 \rightarrow 3)$-linked oligosaccharides of the same size; the $\alpha(1 \rightarrow 6)$-linked isomaltosyl series does not inhibit. Larger oligosaccharides, as well as alternating oligosaccharides with side chains, are needed for a more complete definition of the combining sites. CAL20 TEPC1035 (group 1) is inhibited best by panose, Glcα1 $\rightarrow$ 6Glcα1 $\rightarrow$ 4Glc (10), which does not inhibit any of the Hdex antibodies or MOPC104E. It was previously suggested (10) that the combining site of CAL20 TEPC1035 might be most specific for an unknown molecule containing a panose-like determinant with which class II dextrans cross react. Therefore, the CAL20 TEPC1035 combining site specificity differs from the group 1 Hdex antibody sites, although the precipitin patterns appear identical.

Inclusion within a group therefore implies similarity but not necessarily identity of binding specificity. Indeed, with the exception of group 1, in which the precipitation pattern of all proteins is indistinguishable, no other proteins show such identical binding specificities. Although one could legitimately consider each protein as a separate group, it may be more useful to cluster them into five groups of gradually increasing cross-reactivity patterns, if only to determine which structural features of the variable regions may be responsible for binding specificity.

As seen in Table II, the group 1 precipitin pattern is shared by three Hdex antibodies with very different amino acid sequences in the third hypervariable region (CDR3) and FR4. From the inhibition data, the combining sites are specific for the alternating $\alpha(1 \rightarrow 3)$ and $\alpha(1 \rightarrow 6)$ linkages, but precipitin analyses show that they cannot accommodate cross reacting determinants on class I dextrans; the other groups of Hdex antibodies also have sites specific for the alternating sequence with perhaps, a subsite that accommodates class I determinants. Conceivably, access to this subsite might be blocked by side chains of one or more amino acid substitutions through steric hindrance, conformational changes, or charge effects. For example, R 103 [99] in Hdex 12 (group 1), with a long positively charged side chain, replaces Y 103 [99], found in all of the cross reactive Hdex antibodies. It is of interest that in sites of the serine proteases, the presence in elastase of valine and threonine instead of two glycines in chymotrypsin obstructs and thereby prevents hydrolysis of chymotrypsin substrates having bulky side chains (32).

There are several instances in which proteins in different groups differ by only a single amino acid. Thus Hdex25 (group 1), J558 (group 3), and Hdex2 (group 5) have S, R, and N, respectively, at position 100 [96]; Hdex25 (group 1) and Hdex6 (group 3) differ at position 101 [97] with Y and H, respectively. Whereas these residues

are obviously of importance to specificity, it is difficult to imagine how the group classification could depend exclusively on single amino acid substitutions in V_H. The light chains, although λ, most probably contribute to blocking part of the site responsible for the various cross reactivities, but final conclusions must await sequencing of these chains.

All proteins with RY at positions 100, 101 [96, 97] are in groups 1 and 3, and all those with YD are in group 5. These residues are associated with IdI(J558) and IdI(MOPC104E) determinants and binding specificity appears to correlate somewhat with these markers. That not every protein within a group shares the same D_H-encoded residues suggests that similar binding specificities can be generated in more than one way, rather than that these residues are unimportant.

Some variable region structures, however, do seem unimportant to the binding specificity. For example, NN, at positions 54, 55 [53, 54] with an associated carbohydrate side chain (14, 15) on N 55 [54], is found on all proteins except Hdex 14 which has KK at these positions. The heavy chain of MOPC104E differs from Hdex 14 only at these residues, and yet both proteins are in group 5. J_H regions also seem unrelated to binding patterns: J_1 is found in four groups, J_2 in two groups, and J_3 and J_4 occur only once each.

These findings clearly establish that relatively minor changes in V_H amino acid sequence are associated with major differences in binding specificity. The data presented here put in perspective the gaps in our understanding of the relationship of diversity to antibody site specificity and complementarity (33).

Summary

The combining sites of 12 mouse hybridoma antibodies to dextran B1355S have been characterized by quantitative precipitin assay. All antibodies preferentially bind the immunizing antigen B1355S and two other class II dextrans, B1498S and B1501S, but show substantial differences in the extents to which they cross react with class I dextrans, suggesting their clustering into five groups. Three myeloma proteins, CAL20 TEPC1035, J558, and MOPC104E, which bind dextran B1355S, each fall into a different group. There appears to be a substantial, but imperfect, correlation of D_H region structure and individual idiotypic determinants with dextran binding patterns. Proteins with RY D_H segments and IdI (J558) idiotypes are in groups 1 or 3, and proteins with YD D_H segments and IdI (MOPC104E) idiotypes are exclusively in group 5. However, identical patterns of precipitin curves accompany very different sequences in CDR3. Antibodies of group 1, which react only with class II dextrans, differ the most in primary sequence, a finding suggesting that subsites responsible for cross reactivity with class I dextrans may be blocked and that this may be effected by side chains of different amino acids. This finding delineates a new aspect of the relationship of variability in amino acid sequence to antibody complementarity.

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