

LOSS OF AN INDIVIDUAL IDIOTYPE  
ON CHEMICAL MODIFICATION  
A Strategy for Assigning Idiotypic Determinants\*

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The existence of both cross-reactive and individual idiotypes on antibody molecules has provided a valuable means of following the inheritance of immunoglobulins and the generation of their diversity. In addition, the theory that the immune system can be regarded as a network of idiotypes and anti-idiotypes (1) has led to demonstrations of a major role for idiotypy in the regulation of the immune system.

Efforts to define the exact chemical nature of idiotypic determinants have employed both serological experiments and comparative sequence analyses of antibody molecules of defined idiotypy. The results of these efforts have led to a new appreciation of the diversity and the potential complexities of the immune system. For example, studies of mouse anti-*p*-azophenyl arsonate (2-4) and anti-phosphorylcholine antibodies (5-7) demonstrated that substantial percentages of antibodies of these specificities apparently always contain their respective cross-reactive idiotypes. Amino acid sequence analyses then showed that a cross-reactive idiotypy is actually a characteristic of a family of related molecules (8, 9). Further complexity and potential diversity were demonstrated with the finding of numerous normally rare noncross-reactive idiotypes (termed individual or private idiotypes) which arise in mice suppressed for the production of cross-reactive idiotypy before immunization (10, 11).

The existence of a cross-reactive idiotypy on a family of related antibodies is also illustrated by the myeloma and hybridoma proteins with dextran-binding activity (12-17). Sequence analysis of a series of these proteins permitted an indirect correlation between an individual idiotypy on protein J558 and the presence of an -Arg-Tyr-sequence at positions 100 and 101 of the J558 heavy (H)<sup>1</sup> chain sequence (17, 14). However additional information is needed to decide whether H chain positions 100 and 101 represent amino acid residues that are determinants directly bound by reagents directed against the individual J558 idiotypy. For example these amino acids could function, alternatively, by influencing the three-dimensional structure of J558 so that other amino acids would then serve as major determinants.

With the implication of a hierarchy of cross-reactive and individual idiotypes in immune regulation (1), the direct characterization of idiotypic determinants becomes important. It is necessary to determine if there are any structural overlaps between

\* Supported by grants AI-15907 and AI-15926 from the U. S. Public Health Service.

‡ Recipient of Research Career Development Award AI-00147.

<sup>1</sup> Abbreviations used in this paper: H, heavy; HPLC, high-performance liquid chromatography; IdI, individual idiotypy; IdX, cross-reactive idiotypy; L, light; SDS, sodium dodecyl sulfate.

the cross-reactive and individual idiotypes, or if they are indeed separate, as the sequence data for the J558 system suggest (17). This information may also aid in clarifying the genetic origins (i.e., V, D, and/or J gene segments) of individual and cross-reactive idiotypes as well as to elucidate the structural specificity of anti-idiotypic reagents.

In the absence of a detailed three-dimensional structure determined from crystals of the idiotypic-anti-idiotypic complex, we have adopted the approach of chemically modifying J558 and studying the effect on its idiotypic interactions. Although the rationale and many of the experiments are analogous to those previously used for labeling antibody-combining sites (18–22), recent advances in high performance liquid chromatography (HPLC) now also enable a direct test of chemically modified and unmodified antibody preparations for idiotypic reactivity. The results provide direct serological evidence for the participation of what we presume are tyrosyl residues in the interaction between J558 and a reagent directed against its individual idiotypic. As an adjunct to sequence analysis, chemical modification appears to be useful for assigning idiotypic determinants.

### Materials and Methods

**Antibodies.** Murine immunoglobulins J558 ( $\alpha, \lambda$ ), anti-individual idiotypic (anti-IdI) as the standardized monoclonal reagent EB3-7.2 and MOPC-21 ( $\gamma, \kappa$ ), have been previously described (14, 15). Protein Hdex 24 ( $\mu, \lambda$ ) is an anti-dextran binding hybridoma protein that bears the IdI idiotypic like J558, but differs in amino acid sequence from J558 in the D-J regions. Protein Hdex has been studied in unpublished work (J. Schilling, B. Clevinger, J. Davie, and L. Hood, manuscript in preparation).

**HPLC Assay for Idiotypic Reactivity.** Antibodies in phosphate-buffer saline or borate-buffered saline were used at concentrations of 0.5–1.5 mg/ml. Idiotypic reactions were carried out in microcentrifuge tubes at room temperature and were judged to be complete within 5 min; extending the incubation period did not increase the amount of complex formed. A typical idiotypic reaction used 25  $\mu$ l of J558 solution (40  $\mu$ g) and 50  $\mu$ l of anti-IdI reagent EB3-7.2 (40  $\mu$ g), but one-third of these amounts of protein was often used and assayed with no difficulty.

Analyses were performed on a Beckman model 322 liquid chromatograph using a Beckman TSK-3000SW steric exclusion column (0.75  $\times$  60 cm) (Beckman Instruments, Inc., Fullerton, Calif.). A Beckman Model 153 UV detector was used at 280 nm with a full-scale setting of 0.005 absorbance units. Data was plotted and integrated by a Hewlett-Packard model 3388A computer-integrator (Hewlett-Packard Co., Palo Alto, Calif.) which was also used to attenuate or amplify the signal from the detector. In this configuration, a full scale as low as  $5 \times 10^{-4}$  absorbance units was usable. In some experiments, samples were automatically applied to the column via a Waters autosampler (model 710B) (Waters Instruments, Inc., Rochester, Minn.). Retention times varied by <3% from one run to the next and were reproducible within 6% over the course of at least 1 yr.

The system was usually equilibrated with 0.1 M phosphate buffer at pH 7.0, but for experiments involving protection by hapten, the column buffer was brought to 0.1 M in 1-*O*-methyl- $\alpha$ -D-glucopyranoside.

**Chemical Modification.** Diazotization was carried out at pH 9.0 with 1- $^{14}$ C]-*p*-aminobenzoic acid diluted with unlabeled reagent so that the final specific activity was known. Radioactivity incorporated was measured with a Packard liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). Arginyl residues were modified by bringing the protein solution to 0.1 M 1,2-cyclohexanedione. The reaction was carried out in 0.25 M borate-buffered saline at pH 9.0 for 1 h at 30°C, after the method of Austen and Smith (23, 24). The number of arginyl residues modified was estimated after acid hydrolysis by comparing the amino acid analyses (25) of modified and unmodified proteins.

**Extent of Labeling of H vs. L Chains.** H and light (L) chains were prepared from samples (0.5 mg) of J558 or protein Hdex 24 that had been diazotized to various levels using 1- $^{14}$ C]-*p*-

aminobenzoate in the presence and absence of hapten. These preparations were reduced under  $N_2$  with 0.03 M dithiothreitol in 0.5 M Tris buffer that contained 0.003 M EDTA, pH 8.5 and then alkylated with 0.06 M iodoacetamide (26, 27). After dialysis against borate-saline buffer to remove excess reagents, the reduced and alkylated samples were incubated in 2% sodium dodecyl sulfate (SDS) for 1 h at 50°C and applied to the same steric exclusion column used for the assay of idiotypic reactivity. For these experiments the column was equilibrated with the 0.1 M phosphate buffer that contained 0.2% SDS (26).

Because the separation by HPLC of H and L chains was rapid, H and L chains were conveniently collected by hand in two separate tubes based on the output from the detector as monitored by the computer-integrator. Maximum absorbance of the peaks corresponding to H chains from J558 and Hdex 24 occurred at 14.7 and 13.6 min, respectively. L chains were well-separated from H chains and maximum absorbance of the peak representing L chains occurred at 17.8 min for both J558 and Hdex 24. These retention times are earlier than those expected in the absence of SDS because the samples were denatured and behaved as larger molecules. Nonetheless when the above retention times were compared with those of standards run in SDS, we were able to estimate that the molecular weights of the H chains from J558 and Hdex 24 were 61,000 and 76,000 respectively. Similarly L chains had a molecular weight of 23,000.

Isolated H and L chains were dialyzed against water, lyophilized, and assayed for radioactivity by liquid scintillation counting.

## Results

*HPLC Assay of the Reaction Between J558 and EB3-7.2.* We used a direct assay whenever possible in these studies for idiotypic reactivity that did not require any prior modification of either J558 or EB3-7.2, because such modification might have some effect on the interaction between the two proteins. We therefore developed an HPLC method based on detecting the changes in molecular weight that occur when the idio-anti-idiotypic complex is formed. Even though this method sacrifices much of the sensitivity attainable via radioimmunoassays, maximum and minimum binding of the anti-idiotypic reagent approach 100 and 0%, respectively. Moreover the levels of proteins required are still in the microgram range.

The HPLC column used contains a silica-based packing that resolves molecules on the same principles as classical gel filtration media. However the HPLC support is composed of particles that are on the average much smaller, more uniform in diameter ( $10 \mu\text{m} \pm 2 \mu\text{m}$ ), and that have shallower pores. These factors permit more rapid partitioning of the solute between the support and the solvent without as much dilution, and thus make possible the use of smaller columns at rather high flow rates (28). Practically, this means that in combination with an appropriate detection system sensitivity and speed are so improved over classical gel filtration as to make it possible to directly monitor and quantitate an idio-anti-idiotypic reaction using microgram amounts of each antibody.

Fig. 1 shows the application of this method to measure the interaction between the dextran-binding IgA protein, J558, and a monoclonal IgG reagent, EB3-7.2, directed against individual antigenic determinants on J558. The elution pattern of separate samples of J558 and of the anti-IdI reagent EB3-7.2 are represented in the figure. As a nonbinding control, the elution pattern of a mixture of J558 and an unrelated IgG antibody, MOPC 21, is also given. As can be seen from the figure, unbound J558 is composed of several different species that elute in positions whose molecular weights correspond to those of trimer or larger aggregates then dimer and then monomer. The monomeric form of J558 is well-separated from IgG as shown by the run that includes MOPC 21. This is designated as the nonbinding pattern in Fig. 1.

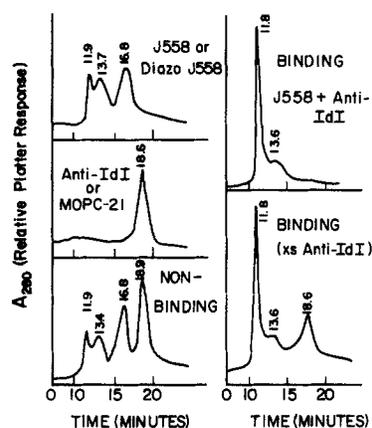


FIG. 1. Assay by HPLC for idiotypic reactivity. Elution patterns given are labeled in each panel with retention times (in minutes) given for each peak. The variation in retention times shown by corresponding peaks in each panel is the maximum variation we observed over a period of months. The plotter response shown was obtained by attenuating a full scale detector setting of 0.005 absorbance units by  $2^9$  so that full scale corresponds to  $\sim 0.02$  absorbance units. A flow rate of 0.8 ml/min was used throughout. The chart speed was 0.1 cm/min for the first 10 min and 0.4 cm/min thereafter. The nonbinding pattern was obtained from mixtures of J558 and MOPC 21 or from mixtures of J558 diazotized to contain 3.3 groups/subunit (see text) and EB3-7.2.

When the anti-IdI reagent EB3-7.2 is added to J558, the peak corresponding to unbound EB3-7.2 disappears and both EB3-7.2 and J558 elute as larger aggregates (designated as the binding pattern in Fig. 1). As excess anti-IdI reagent is added beyond the amount that can be bound to J558, the peak corresponding to unbound EB3-7.2 again appears (Fig. 1). In both cases a small amount of material eluting at 13.6 min remains. This corresponds in molecular weight either to unbound J558 dimer or to bound material composed to 1 mol of EB3-7.2/1 mol of J558 monomer.

The mixtures of J558 and EB3-7.2 were applied to the column shortly after mixing and the complex emerged 11–12 min later. The formation of a precipitate required overnight incubation but the entire assay was routinely completed so quickly that precipitation did not occur. This was verified from standard curves which showed that at least for the first few hours after mixing the amount of EB3-7.2 required for complete binding of J558 and the area under the peak representing the complex were directly proportional to the amount of J558 used over at least a fivefold range.

The HPLC assay was also used to demonstrate that the binding of EB3-7.2 to J558 involves the combining site of J558. The two proteins were mixed in the presence of 1 M 1-*O*-methyl- $\alpha$ -D-glucopyranoside, applied to the column, and eluted with phosphate buffer containing the pyranoside. Under these conditions, the nonbinding pattern (Fig. 1) was obtained indicating that virtually complete inhibition had occurred.

**Chemical Modification of Protein J558.** Our test of whether the -Arg-Tyr- sequence in J558 (positions 100 and 101) is a major IdI idiotypic determinant(s) was to react separate samples of protein J558 with reagents which would modify tyrosyl or arginyl groups. We then determined whether such modified preparations could still bind EB3-7.2. Although the choice was somewhat arbitrary, the diazonium derivative of *p*-aminobenzoic acid was selected to modify tyrosyl residues because of its relatively low

reactivity (22) and the ready availability of its isotopically labeled form. The reagent 1,2-cyclohexanedione was used for aginyl residues (23, 24).

Table I shows that when J558 is reacted with a twofold molar excess (over tyrosine) of diazotized 1-[<sup>14</sup>C]-*p*-aminobenzoate 3.3 groups/1 mol of J558 subunit were incorporated and most of this label was associated with the H chains. When diazotized J558 was mixed with the anti-IdI reagent EB3-7.2 and assayed by HPLC the nonbinding pattern (Fig. 1) was obtained indicating that the inhibition of reaction was essentially complete. Overnight incubation of modified J558 and EB3-7.2 gave the same result.

Lower levels of modification apparently had very little effect on the J558 idio type. When J558 was reacted with one-half the amount of diazonium reagent as above, 1.6 groups/1 mol of J558 subunit were incorporated but now most of the label was associated with the L chains (Table I). A mixture of this preparation and the anti-IdI reagent showed the binding pattern (Fig. 1) when assayed by HPLC.

In another experiment J558 was incubated with the hapten 1-*O*-methyl- $\alpha$ -D-glucopyranoside before reaction with diazonium reagent under conditions (first line of Table I) which were otherwise identical to those which abolished the IdI idio type. In this case 1.5 groups per subunit were found and again the label was primarily associated with the L chains (Table I). The binding pattern (Fig. 1) was obtained when this preparation was mixed with EB3-7.2 and assayed by HPLC. These observations probably indicate that the first one or two diazo groups are mainly incorporated on the L chain and do not significantly effect the J558 idio type. When subsequent incorporation of one or two additional groups occurs, it occurs on hypervariable amino acid residues contributed by the H chain to the hapten-binding site of J558 and the J558 idio type is lost.

Protein J558 and other dextran-binding myelomas share another variable region antigenic determinant denoted as the cross-reactive or (IdX) idio type (14). Diazo-J558 which contained 3.3 diazo groups per subunit and which was no longer reactive with the anti-IdI reagent was examined for the presence of the IdX idio type. Because the anti-IdX reagent was not available as a purified protein the reaction of diazo-J558 and IdX antiserum was tested by a solid-phase radioimmunoassay (14). The results showed that diazo-J558 was fully reactive with anti-IdX and confirmed that

TABLE I  
*Chemical Modification of IdI Idio type*

Sample	Diazo groups incorporated per subunit*	Groups on		Reaction with anti-IdI reagent
		H chain	L chain	
J558*	3.3	2.2	1.1	Inhibited
J558	1.6	0.5	1.1	Not inhibited
J558 + 1 M 1- <i>O</i> -methyl- $\alpha$ -D-glucose	1.5	0.5	1.0	Not inhibited
Hdex 24	2.3	ND‡		Inhibited
Hdex 24	1.5	1.0	0.5	Inhibited
Hdex 24 + 1 M 1- <i>O</i> -methyl- $\alpha$ -D-glucose	1.9	1.6	0.3	Inhibited

\* Molecular weights of 180,000 and 200,000 were used for J558 and Hdex 24 subunits, respectively.

‡ Not determined.

it was at least 100 times less reactive with anti-IdI reagent EB3-7.2 than unmodified J558.

Because replacement of Arg at position 100 with Asp results in a marked decrease in reactivity toward the anti-IdI reagent (14, 17), we investigated whether modification of arginyl residues would effect this interaction. A sample of J558 protein was reacted with cyclohexanedione and the extent of reaction was estimated by amino acid analysis as the loss in the number of Arg residues (23, 24). The results showed the disappearance of >20 Arg groups per subunit. In fact, the modifications were extensive enough to dissociate the larger aggregated forms of the molecule so that the monomeric form of J558 was then the major species present. Nonetheless, the modified protein still gave the binding pattern (Fig. 1) when mixed with EB3-7.2 and then assayed by HPLC.

*Chemical Modification of Protein Hdex 24.* A test of whether positions 100 and 101 always represent a major IdI determinant was to investigate the effect of diazotization on another anti-dextran myeloma protein, Hdex 24, which has a variable region which differs from J558 within the D and the J regions (Table II) (J. Schilling, B. Clevinger, J. Davie, and L. Hood, manuscript in preparation; and [17, 29-31]).

Because Hdex 24 is an IgM protein, it eluted as a single peak at the void volume of the HPLC column (11.3 min). Binding of Hdex 24 to the anti-IdI reagent was then taken as either the disappearance of the peak corresponding to EB3-7.2 or the increase in area under the peak eluting at 11.3 min. Nonbinding was when the peaks representing EB3-7.2 and Hdex 24 eluted separately with no change in area under either peak.

Table I summarizes the levels of incorporation of diazonium reagent for Hdex 24 and the effect on its binding to the anti-IdI reagent EB3-7.2 when Hdex 24 was subjected to experiments identical to those performed with J558. Differences in the results are obvious. Under conditions that resulted in the incorporation of 3.3 diazo groups per J558 subunit, only 2.3 groups were incorporated into Hdex 24; yet both diazotized proteins no longer reacted with the anti-IdI reagent EB3-7.2. After diazotization Hdex 24, like J558, was fully reactive with anti-IdX.

Reaction conditions employing one-half the concentration of diazonium reagent led to similar levels of incorporation for both Hdex 24 and J558 (1.5 groups vs. 1.6 groups, respectively). However under these conditions, modification occurred primarily on the H chain of Hdex 24 in contrast to J558 where most of the label was found on the L chain. Binding to EB3-7.2 was abolished for Hdex 24 but not for J558 (Table I).

The presence of 1 M hapten caused essentially complete inhibition of the interaction

TABLE II  
*D-J Regions of Proteins J558 and Hdex 24*

Protein	D region		J region															
	100	101	102	105				110				115		117				
J558	Arg	<i>Tyr</i>	<i>Trp</i>	<i>Tyr</i>	<i>Phe</i>	<i>Asp</i>	<i>Val</i>	<i>Trp</i>	<i>Gly</i>	<i>Ala</i>	<i>Gly</i>	<i>Thr</i>	<i>Thr</i>	<i>Val</i>	<i>Thr</i>	<i>Val</i>	<i>Ser</i>	<i>Ser</i>
Hdex 24	Ser	Ser	<i>Tyr</i>	<i>Tyr</i>	<i>Phe</i>	<i>Asp</i>	<i>Tyr</i>	<i>Trp</i>	<i>Gly</i>	<i>Gln</i>	<i>Gly</i>	<i>Thr</i>	<i>Thr</i>	<i>Leu</i>	<i>Val</i>	<i>Ser</i>	<i>Ser</i>	

Note that D and J regions are defined on the basis of amino acid sequences encoded by a mature V-D-J gene combination (17). Italicized residues represent amino acids encoded by proposed germ line J genes (30, 31).

between EB3-7.2 and J558 but had virtually no effect on the interaction between EB3-7.2 and Hdex 24. In addition hapten protected J558 but not Hdex 24 from the loss of the IdI idio type caused by diazotization. After diazotization in the presence of hapten, labeled diazo groups were found mainly on the L chain of J558 but again on the H chain of Hdex 24 (Table I).

### Discussion

To the extent that protein J558 and its interactions with reagents directed against its individual and shared idiotypes constitute a suitable model, a direct characterization of the idiotypic determinants on J558 and Hdex 24 may well provide insight into the details of the synthesis and control of a family of related antibody molecules. The use of chemical modification appears to be of value in assigning these determinants because it can provide a direct test of the involvement of particular groups and can furnish valuable coarse data for x-ray crystallographic analysis.

In our work, the existence of the IdX idio type provides a built-in control. Its preservation when the IdI idio type is abolished by diazotization of J558 and Hdex 24 provides evidence that the incorporation of diazo groups does not induce a major conformational change and emphasizes the independent nature of the IdX and IdI idiotypes. In addition, our data prove that residues from different locations in the three-dimensional structure of antibody molecules can form the same individual idiotypic determinant. This can occur despite differences in the amino acid sequences of the D and J segments. This is based on the finding that when hapten is present diazotized J558 retains the IdI idio type but when Hdex 24 is diazotized under the same conditions, the IdI idio type is lost. Thus the idiotypic determinant being modified in the case of J558 is in the hapten binding site but in the case of Hdex 24, the determinant is elsewhere in the molecule.

A disadvantage of chemical modification is that most reagents are not completely specific for one type of amino acid in all proteins. Diazotization of some proteins under some conditions has been reported to modify histidyl and lysyl residues in addition to tyrosyl residues (32, 33). However at pH 9, tyrosyl residues are highly susceptible to electrophilic attack with selective modification of histidine requiring a lower pH. In addition there are no histidines present in the hypervariable regions of J558 although one does occur outside the hypervariable regions (34). Lysine does not occur in the third hypervariable region of the H chains of J558 but it does occur throughout the remainder of the  $V_H$  region (34). However, lysine groups at pH 9 still retain a positive charge and modification of lysine normally requires a pH significantly  $>9$ . Thus, although it is difficult to exclude the possibility that groups other than tyrosine also reacted with the diazonium reagent we used, tyrosine is by far the most logical site of reaction.

For both J558 and Hdex 24, the reaction with the anti-IdI idio type reagent must be extremely precise because the addition of 1–3.3 relatively small diazonium groups per monomer is sufficient to abolish IdI idiotypic reactivity. This supports comparisons of amino acid sequences (17, 14, 35) which show that a substitution at H chain positions 100 or 101 can dramatically effect idiotypic reactivity. Even though the IdI idio type on J558 and Hdex 24 appears to require the participation of tyrosyl residues the interaction with the anti-IdI reagent must also depend on other factors. As evidence for this, we found that high concentrations of tyrosine or phenol had no

inhibitory effect on the binding of the IdI reagent. This indicates that other amino acid groups play a role in the expression of the IdI idio type. These additional groups could serve to position the crucial tyrosyl residues in the correct orientation and could also serve as additional determinants.

It appears unlikely that one such additional direct idiotypic determinant in J558 is Arg 100 on the H chains. This is based on the failure of extensive modification by cyclohexandione to abolish the IdI idio type. However this does not preclude some other role for Arg 100 in the expression of the IdI idio type. It is possible that antiserum raised in another animal could detect such an additional determinant.

The data in Table I lead to the conclusion that an individual J558 idiotypic determinant is located on the H chains of both J558 and Hdex 24. In all experiments with J558, the J558 L chains contained one diazonium label, regardless of whether or not the binding activity was retained. It was only when a significant number of groups was added to the J558 H chain that the IdI idio type was lost. Similarly, the L chain does not appear to be directly involved in expressing the same IdI determinant on Hdex 24. When 1.9 diazonium groups were put on Hdex 24 in the presence of hapten, the idio type was lost even though virtually none of the label was found on the L chains (Table I). Results of amino acid sequence analysis show that  $\lambda$ -type light chains are highly conserved (36) and also argue against their contributing residues that are directly involved in binding to anti-IdI reagents.

Because hapten prevents the loss of the IdI idio type when J558 is diazotized, we assume that the residue which reacts is within the J558 hypervariable regions. However in addition to the tyrosyl at position 101, other tyrosyl groups occur in hypervariable positions in the H chain sequence (34). These tyrosyls are conserved in other dextran-binding myelomas that lack the IdI idio type, but the possibility exists that a substitution at positions 100 and 101 causes a change in the orientation of one of the more conserved tyrosyls which then becomes a major idiotypic determinant. Thus existing data do not allow an unequivocal decision as to which tyrosyls in the H chains of J558 (and Hdex 24) are the groups whose diazotization abolishes the IdI idio type. Nonetheless, in the absence of establishing the positions of the labeled residues the idea that tyrosyl residues in the D-J region of each protein are the idiotypic determinants we modified remains as the simplest explanation.

Such locations in both an IgM and an IgG protein would raise the question of whether during class switching after antigenic stimulation the combining site on a B cell receptor can be altered somewhat whereas the individual idio type is retained and still subject to network regulation. In addition the nongermline origin of the D segment (29, 35) may well help account for the changes in the proportion of an antibody response bearing a given idio type after hyperimmunization (35) and perhaps even for the changes in antibody affinity observed (38, 39).

Jerne has proposed that the immune system can be regarded as a network of idio type-anti-idio type interactions which can either suppress or stimulate the production of particular antibodies. This seems to apply at both the B and the T cell levels (e.g., 40-44). Proteins J558 and Hdex 24 may well provide a laboratory model of the details of some of these interactions because the shared and the individual idiotypes clearly involve different determinants. Thus a whole series of anti-dextran antibodies could be controlled by factors which interact with the IdX (45). Finer, more-precise regulation of the response can then be accomplished by factors interacting with a

particular individual idiootype, which may also occur on a family of antibodies. All of these possibilities emphasize the interrelationships between antibody diversity and the regulation of the immune response.

### Summary

Two dextran-binding myeloma proteins, J558 and Hdex 24, which possess the same individual idiootype (IdI) were diazotized to low levels (1–3.3 groups per subunit) with 1-[<sup>14</sup>C]-*p*-aminobenzoate. Both proteins lost the IdI idiootype under these conditions with most of the label incorporated on the heavy chains of each protein. When the diazotization was carried out in the presence of the hapten 1-*O*-methyl- $\alpha$ -D-glucopyranoside the loss of idiotypic reactivity could be prevented for J558 but not for Hdex 24. Under these conditions most of the label was incorporated on the light chains of J558, but on the heavy chains of Hdex 24. For J558, these results show that a major determinant of the individual idiootype is within the hypervariable positions of the heavy chain. For Hdex 24 the determinant being modified is on the heavy chain but not involved in hapten binding. These results are consistent with previous work showing that J558 and Hdex 24 differ in amino acid sequence in the D and the J segments of the heavy chain and offer an alternative and complementary strategy for assigning idiotypic determinants.

We thank Dr. Ralph Reisfeld for his interest, his helpful discussions, and his encouragement of this work. We thank Dr. John Kearney for providing us with the EB3-7.2 monoclonal protein and Drs. James Schilling and Leroy Hood for allowing us to use the sequence of Hdex 24 before publication. We also offer special thanks to Dr. Joseph M. Davie for his helpful suggestions, comments, and invaluable advice.

*Received for publication 5 January 1981 and in revised form 17 February 1981.*

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