A COMMON IDIOTYPE ON SJL AND C57BL/6 ANTI-(4-HYDROXY-3-NITROPHENYL) ACETYL ANTIBODIES AND ITS RELATIONSHIP WITH λ CHAIN PRODUCTION*

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C57BL mice, upon immunization with chicken γ-globulin conjugated with (4-hydroxy-3-nitrophenyl) acetyl (NP),[1] produced anti-NP antibodies having higher affinity for a cross-reactive analogue, (4-hydroxy-5-iodo-3-nitrophenyl) acetyl (NIP), than for NP itself (1). Furthermore, these "heteroclitic" antibodies share a set of common idiotypes, the predominant idiotype in primary B6 anti-NP antibodies (NP^b idiotype) that is composed of idiotypically related but nonidentical antibody molecules (2). Primary C57BL anti-NP antibodies expressing NP^b idiotype bear predominantly λ_1-light chain (3, 4). Both heteroclicity and NP^b idiotype were inherited in a simple Mendelian fashion and were controlled by *Igh-V* genes linked to the $Igh\text{-}1^b$ allele of the heavy chain linkage group (1, 3, 4). However, $Igh\text{-}1^b$-bearing SJL mice, which have a genetic defect in the ability to produce normal levels of λ_1 chain (5), produce either extremely low or undetectable levels of NP^b idiotype (3, 4). SJL mice on the other hand produce normal levels of λ_2 chain (6). The gene controlling λ_2-chain synthesis is demonstrated to be on the same chromosome that regulates λ_1-chain production (7). This suggests that the expression of a particular idiotype requires synthesis of a particular λ chain.

There is evidence that expression of λ-chain-bearing molecules requires the presence of the appropriate *Igh-V* gene or vice versa (5). Furthermore, amino acid sequences between the λ_1 chain variable region ($V\lambda_1$) and $V\lambda_2$ exhibit greater homology than between Vλ and the κ chain variable region (Vκ) (8). Only eight framework residue differences were observed between the available $V\lambda_1$ and $V\lambda_2$ sequences. Moreover, the strong homology of $V\lambda_1$ and $V\lambda_2$ genes was demonstrated by DNA sequence analyses (9, 10). This raises the possibility that SJL mice that possess the appropriate $Igh\text{-}Np^b$ gene(s) (3) may produce λ_2-bearing anti-NP antibody molecules idiotypically related to NP^b idiotypes. Studies of the idiotypes of λ_2-bearing anti-NP antibodies are, however, limited by the small quantity of such antibodies produced in SJL and C57BL/6 (B6) mice (3).

The present study focused on the idiotype (NP-1 idiotype) of an IgM anti-NP

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[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; BSA, bovine serum albumin; Cλ, lamda chain constant region; NIP, (4-hydroxy-5-iodo-3-nitrophenyl) acetyl; NP, (3-nitro-4-hydroxyphenyl) acetyl; NP^b, predominant idiotype in primary B6 anti-NP antibodies; PBS, phosphate-buffered saline, pH 7.2; V_H, heavy chain variable region; Vκ, κ chain variable region; Vλ, λ chain variable region.

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hybridoma antibody bearing a λ_2 (or λ_2-like) light chain.[2] In contrast to the strain distribution pattern of the NP^b idiotype on λ_2-bearing anti-NP antibodies that are either undetectable or present in very low levels in SJL and A/J immune sera, the expression of the NP-1 idiotype is clearly demonstrable in immune sera from SJL and A/J mice. In addition, we demonstrated that B6 mice can utilize both λ_1 and λ_2 chains to generate antibodies with NP-1 idiotype, whereas SJL mice utilize mainly λ_2 chains to construct the NP-1 idiotype. The data are discussed with respect to the influence of genes that regulate light chain production on the expression of defined idiotypes of anti-NP antibody molecules.

Materials and Methods

Animals. Inbred strains of mice (2–8 mo of age) were either purchased from The Jackson Laboratory, Bar Harbor, Maine, or were obtained from the breeding colonies maintained at Harvard Medical School, Boston, Mass. All recombinant inbred strains of mice were kindly provided by Dr. Benjamin A. Taylor of The Jackson Laboratory, Bar Harbor, Maine. Random bred guinea pigs (200 g) and rabbits (3–4 kg) were also obtained from Harvard Medical School. They were maintained with laboratory chow and chlorinated water ad lib.

Reagents. The succinimide ester and caproate derivatives of NP and NIP were purchased from Biosearch, Inc., San Rafael, Calif. High pressure liquid chromatography analyses indicates >99.9% purity of each compound. Conjugates of NP and NIP to bovine γ-globulin (BGG), bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.), and *N*-(2-aminoethyl) carbamylmethylated-Ficoll (Biosearch Inc.) were prepared by reacting the corresponding succinimide derivatives with the carrier at pH 9.0 at 4°C overnight, followed by extensive dialysis against phosphate-buffered saline (PBS) at pH 7.2. The number of haptenic groups conjugated per molecule of protein was measured spectrophotometrically. Class-specific antisera were purchased from Gateway Immune-Sera Co., Inc., St. Louis, Mo. and Bionetics Laboratory Products, Litton Bionetics Inc., Kensington, Md. Purified MOPC 104E (μ, λ_1) protein was obtained from Litton Bionetics Inc. MOPC 104E ascitic fluid was generated in pristane primed BALB/c mice with MOPC 104E myeloma cells kindly provided by Dr. M. Potter, National Institutes of Health. $(B6 \times DBA/2)F_1$ ascitic fluids were induced by the method of Tung et al. (12). Proteins were conjugated to cyanogen bromide-activated Sepharose 4B beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) according to the manufacturer's procedure.

Hybridoma Cell Line. Two SJL mice were immunized with 30 μg NP-Ficoll i.p. in PBS and boosted 42 d later with 10 μg NP-Ficoll. Splenic leukocytes were fused with the SP2/0 cell line by polyethyleneglycol 4,000 containing 5% dimethylsulfoxide, according to the method described by Galfre et al. (13). Three hybridoma cell lines (N-hybridoma, 4C2, and 4E3) were obtained. Only N-hybridoma antibody (μ, λ_2) exhibited weak NP^b idiotype and was analyzed in detail in this study. Ascitic fluids from other hybridoma cell lines (6,100 series) were similarly obtained from the fusion of NP-Fowl γ-globulin-immuned C.B-17 splenocytes and the x63-Ag8.653 cell line, as described elsewhere (14). Heavy and light chain subclasses were determined by Ouchterlony analyses.

Immunization. All mouse strains were immunized intraperitoneally with a mixture containing 100 μg of antigen (NP_{12}-BGG and NIP_{10}-BGG) and 25% (vol/vol) pertussis vaccine suspension. Blood was obtained by retroorbital bleeding. Mice were boosted at day 18 and bled 7 d later. In some experiments, mice were boosted at day 42 and bled 10 d later. These sera were stored at −20°C until use.

To prepare specifically purified antibodies to NP, groups of B6 mice and SJL mice were immunized as described, and were bled at days 12, 15, 18, 21, and 24 after immunization. Pooled sera (9 vol) were mixed with 0.1 M ethylenediaminetetraacetate, pH 8.0 (1 vol), and passed through columns conjugated with NP-BSA. After washing the columns extensively with

[2] Azuma et al. (11) recently demonstrated a third type of mouse λ light chain (λ_3) that is serologically cross-reactive with anti-λ_2 antiserum. This raises the possibility that N-hybridoma antibody may bear the λ_3 light chain.

PBS, the bound antibodies were eluted with 0.2 M glycine-HCl, pH 2.33. The eluted fractions (0.9 ml) were collected in tubes containing 0.1 ml of 2 M Tris-HCl, pH 7.9. N-hybridoma antibody from culture supernate and other serum anti-NP antibodies were purified by the same method.

Preparation of Anti-Idiotypic Antisera. Guinea pigs were immunized with 100 μg of specifically purified B6, SJL, or N-hybridoma anti-NP antibodies emulsified in complete Freund's adjuvant at 2-wk intervals. The first immunization was administered into four footpads, the second and third immunizations were administered intramuscularly and subcutaneously. 2 wk after the last immunization, blood was obtained by retroorbital bleeding, and sera were extensively adsorbed with columns conjugated with γ-globulin fractions of MOPC 104E and (B6 × DBA/2)F_1 ascitic fluids. The adsorbed antisera did not react with IgM, IgG, κ, and λ chains by Ouchterlony analysis.

Radiolabeling of Proteins. Proteins were radiolabeled with ^{125}I-Na (New England Nuclear, Boston, Mass.) by the chloramine-T method (15). The ^{125}I-labeled proteins were purified by Sephadex G25 columns.

Radioimmunoassays. A modified Farr binding assay was used to determine antibody activity. Various quantities of antisera were mixed with ^{125}I-labeled NIP-BSA. The bound antigen was precipitated with an equal volume of 95% $(NH_4)_2SO_4$, buffered at pH 8.0. A standard curve was established with specifically purified anti-NP antibodies to measure antibody activity in every binding assay. The effect of affinity on the assay has been discussed by Jack et al. (4).

Quantitative idiotype binding assays were carried out with double antibody methods. Various quantities of guinea pig anti-idiotypic antisera were mixed with 15 μl of MOPC 104E and 15 μl of (B6 × DBA/2)F_1 ascitic fluids followed by 3–7 ng of ^{125}I-labeled specifically purified anti-NP antibodies or control ligands. Bound ^{225}I-labeled ligands were precipitated by excess rabbit anti-guinea pig immunoglobulin antiserum. Supernate and precipitate were separated by centrifugation and counted with a γ-scintillation counter. The percent idiotype binding was calculated with a Wang (Wang Laboratories, Inc., Lowell, Mass.) programmable calculator. Inhibition of idiotype binding assays were carried out with suboptimal quantities of anti-idiotypic antiserum that bind ~25–75% ^{125}I-labeled ligand. Various quantities of inhibitors were added before the addition of ^{125}I-labeled ligand. Results were expressed as micrograms of antibody needed to inhibit 50% idiotype binding. In some experiments the results were expressed as percent inhibition of idiotype binding.

Quantitative Determination of λ-bearing Antibodies. This was also carried out with a double antibody method. The reaction mixture contains 30 μl of normal guinea pig sera (Pel Freeze Biological Inc., Rogers, Ark.), 2 μg of MOPC 21 κ chains (Litton Bionetics Inc.), 5 μg of κ-bearing hybridoma IgM anti-poly($Glu^{60}Ala^{30}Tyr^{10}$) antibodies, 5 μg of MOPC 315 (α, λ_2) myeloma protein, and various quantities of rabbit anti-λ_1 chain antibodies (Litton Bionetics Inc.). After incubating at 37°C for 30 min, excess goat anti-rabbit immunoglobulin (Ig) antiserum was added to precipitate the bound ^{125}I-labeled ligand. To quantitate the amount of λ_2 chain-bearing molecules, we took advantage of the ability of unadsorbed anti-N-hybridoma antiserum to react with ^{125}I-MOPC 315 (α, λ_2) in a λ_2-specific fashion. The reaction mixture contains 30 μl of normal goat serum, 2 μg of MOPC 21 κ chain, 5 μg of κ-bearing hybridoma IgM anti-poly($Glu^{60}Ala^{30}Tyr^{10}$) antibodies, 5 μg of MOPC 104E myeloma protein, and various quantities of unadsorbed anti-N-hybridoma antisera. After incubating at 37°C for 30 min, excess rabbit anti-guinea pig Ig antiserum was added to precipitate the bound ^{125}I-labeled MOPC 315 myeloma protein. Concentrations of λ_2-bearing antibody molecules were determined by inhibition of ^{125}I-MOPC-315 binding using unlabeled MOPC 315 myeloma protein as a standard.

Adsorption Experiments. Adsorptions were carried out with 0.3-ml Sepharose 4B beads conjugated with appropriate antiserum γ-globulins. 30–50 μl of immune sera together with 50 μl of normal goat sera were added. In addition, 50 μg of MOPC 104E was added for anti-λ_2 adsorption experiments and 50 μg of MOPC 315 was added for anti-λ_1 adsorption experiments. After incubating at room temperature for 1 h with occasional shaking, the unbound fractions were separated and tested for NP-1 idiotype.

Results

Idiotype Binding. The activity of guinea pig anti-idiotypic antisera made against specifically purified anti-NP antibodies from primary B6 anti-NP antisera, SJL anti-NP antisera, and N-hybridoma antibody were tested by idiotype-binding analyses (Table I). Under maximum conditions, each antiserum reacted with a significant portion of its idiotypic antibodies but bound a nonsignificant level of two control ligands of ^{125}I-labeled MOPC 104E and MOPC 315 myeloma proteins. Interestingly, all antisera exhibited strong binding activity to ^{125}I-labeled N-hybridoma antibody. The cross-reactive binding of N-hybridoma is stronger than the binding of serum anti-NP antibodies to their respective anti-idiotypic antisera. This phenomenon is frequently observed with homogeneous IgM hybridoma antibody bearing defined idiotypes (16) and is attributable, at least in part, to the high density of local idiotypic determinants on IgM molecules.

Specificity of Idiotype Binding. Table II demonstrated the specificity of idiotype binding. Thus, the binding of ^{125}I-labeled B6 anti-NP antibodies and N-hybridoma antibody to suboptimum quantities of various anti-idiotypic antisera was not inhibited by B6 normal mouse serum, SJL normal mouse serum, MOPC 104E, and MOPC 315 myeloma proteins. In sharp contrast, strong inhibition was achieved with 1 μl B6 anti-NP sera. Furthermore, idiotype binding could be specifically inhibited (>90% inhibition) with 0.06 μmol NP-caproate (pH 7.6). Under identical conditions, 6 μmol of *p*-aminoarsanilate (pH 7.6) caused <10% inhibition of idiotype binding in all cases (data not shown). Thus, most of the idiotypic binding involved combining-site-related determinants.

Table II also provided suggestive evidence for the presence of low levels of shared NP^b-idiotypic specificities on N-hybridoma antibody, as shown by the weak inhibition of the binding of ^{125}I-labeled B6 anti-NP antibodies to its anti-idiotypic antibodies (see below). Increasing N-hybridoma antibody to 10 μg caused no more than 40%

TABLE I
Cross-Idiotype Binding

Anti-idiotypic antiserum to	^{125}I-labeled idiotype ligand‡	Percent binding of ^{125}I-labeled idiotypic antibodies with various quantities of antisera*		
		4 μl	0.4 μl	0.04 μl
C57BL anti-NP	C57BL anti-NP	60	32	6
	N-hybridoma	91	78	28
	MOPC 104E	4	2	0
	MOPC 315	2	0	0
SJL anti-NP	SJL anti-NP	30	15	4
	N-hybridoma	96	96	52
	MOPC 104E	2	1	0
	MOPC 315	2	0	2
N-hybridoma	N-hybridoma	96	94	84
	MOPC 104E	4	3	0
	MOPC 315	3	0	0

* Background binding (0–6%) with 4 μl of normal guinea pig serum was subtracted in each idiotypic system.

‡ Control bindings with guinea pig anti-mouse Ig antiserum were 80–100% with each ^{125}I-labeled ligand.

TABLE II
Specificity of Idiotype Binding

Anti-idiotypic antiserum to	^{125}I-labeled idiotype ligand	Percent control binding	Percent inhibition of idiotype binding in the presence of inhibitor*				
			B6 or SJL NMS	MOPC 104E	MOPC 315	B6 anti-NP	N-hybridoma
			30 µl	*20 µg*	*20 µg*	*1 µl*	*1 µg*
B6 anti-NP	B6 anti-NP	28	11	7	7	82	29
B6 anti-NP	N-hybridoma	70	9	4	6	86	96
SJL anti-NP	N-hybridoma	50	10	2	6	80	92
N-hybridoma	N-hybridoma	60	2	3	8	80	97

* Results are the average of two to five experiments with <5% variation.

inhibition, whereas 2 µg of purified serum anti-NP antibodies inhibited 80% of idiotype binding. This phenomenon is similar to those of λ_1-bearing, B6 hybridoma anti-NP antibodies of the NP^b idiotypic family, previously reported by Imanishi-Kari et al. (2). These results provide direct evidence that λ_2-bearing anti-NP antibody can express some NP^b idiotypic specificities.

Comparison of the Strain Distribution Patterns of N-Hybridoma Idiotype and NP^b Idiotype. The strong cross idiotype binding of N-hybridoma antibody by anti-idiotype against B6 and SJL anti-NP antibodies suggested the presence of shared idiotypic specificities among B6 and SJL anti-NP antisera. Inhibition of idiotype binding to some of these anti-idiotypic antisera with individual anti-NP and anti-NIP antisera of various mouse strains was carried out. Antibody activity was quantitated with a modified Farr assay, and the results were expressed as the number of micrograms of antibodies needed to inhibit 50% idiotype binding (Table III). The same anti-NP and anti-NIP antisera were used to determine the strain distribution pattern of NP^b idiotype, i.e., binding of ^{125}I-labeled purified B6 anti-NP antibodies to its anti-idiotypic antisera. Indeed, this idiotypic interaction defines the NP^b idiotype, as evidenced by its nearly identical strain distribution pattern to that described by Karjalainen and Makela (3). Results obtained from anti-NP antisera were similar to those obtained from anti-NIP antisera (data not shown). The anti-NIP antibodies of B6 antisera exhibited high concentrations of NP^b idiotypic antibodies. C.B-20 mice, which possess the *Igh*^{b} haplotype on a BALB/c genetic background, express moderate but highly variable levels of NP^b idiotypic antibodies. A somewhat lower concentration of NP^b idiotypic antibodies was consistently observed with CWB mice bearing *Igh*^{b} haplotype on a C3H genetic background. Low to undetectable levels of NP^b idiotypic antibodies were noted in A/St and in *Igh* congenic C.AL-20 mice. Although SJL mice possess the appropriate *Igh-Np*^{b} allele, they produced anti-NP antibodies with extremely low levels of NP^b idiotype. This is due to a genetic defect in SJL mice to produce normal levels of λ_1 chain, which is required for the construction of NP^b-positive anti-NP antibodies (3). Anti-NIP antisera of six other mouse strains (*Igh* haplotypes *a, c, d, g, j,* and a recombinant) failed to achieve 50% inhibition of NP^b idiotype binding. Under the assay conditions, 30 µl of these sera caused 18–45% inhibition, and such weak cross-reactivity was not explored further.

Quantitative comparison of the strain distribution pattern of NP-1 idiotype with

TABLE III
Strain Distribution Patterns of NP^b *and NP-1 Hybridoma Antibody Idiotypes*

Strains*	Igh-V/ Igh-C	Anti-NP Ab‡	Micrograms Ab needed to inhibit 50% binding		
			^{125}I-B6 anti-NP + anti-(B6 anti-NP)	^{125}I-N-hybridoma + anti-(SJL anti-NP)	^{125}I-N-hybridoma + anti-N-hybridoma
		mg/ml			
BALB/c (4)	a/a	0.5 ± 0.1	>16 ± 3	>16 ± 3	>16 ± 3
B.C-8 (4)	a/a	0.23 ± 0.08	>6 ± 2	>6 ± 2	>6 ± 2
C57L (3)	a/a	0.4 ± 0.03	>10 ± 1	>10 ± 1	>10 ± 1
BAB/14 (4)	a/b	0.4 ± 0.2	>10 ± 5	>10 ± 5	>10 ± 5
C57BL/6 (4)	b/b	0.8 ± 0.2	0.08 ± 0.02	0.08 ± 0.02	0.04 ± 0.01
C.B-20 (5)	b/b	0.3 ± 0.1	0.56 ± 0.34	0.2 ± 0.1	0.05 ± 0.01
CWB (4)	b/b	0.6 ± 0.2	2.2 ± 0.8	0.3 ± 0.1	0.1 ± 0.06
SJL (3)	b/b	0.2 ± 0.06	≥3.8 ± 1.8	0.06 ± 0.02	0.01 ± 0.003
DBA/1 (5)	c/c	0.2 ± 0.04	>5.5 ± 1	>5.5 ± 1	>5.5 ± 1
AKR (4)	d/d	0.44 ± 0.11	>7.5 ± 3.6	>7.5 ± 3.6	>7.5 ± 3.6
A/St (4)	e/e	0.23 ± 0.07	≥2.6 ± 0.6	0.9 ± 0.3	0.3 ± 0.2
DA (3)	g/g	0.1 ± 0.05	>2.8 ± 1.6	>2.8 ± 1.6	>2.8 ± 1.6
C3H (4)	j/j	0.15 ± 0.05	>4.1 ± 1.1	>4.1 ± 1.1	>4.1 ± 1.1
C.AL-20 (1)	e/d	0.1	1.5	0.6	0.1

* Number of individual mice tested are listed in parentheses.
‡ Determined by Farr assays with ^{125}I-labeled NIP-BSA using specifically purified B6 anti-NP antibodies as standard.

the same panel of anti-NIP antisera of various mouse strains indicated three major differences. First, there is a dramatic contrast between the expression of high levels of NP-1 idiotype and the very low levels of NP^b idiotype in the immune sera of individual SJL mice. Furthermore, SJL anti-NIP antisera exhibited the highest concentration of NP-1 idiotype among all tested mouse strains. Second, other strains bearing the Igh^b haplotype (B6, C.B-20, and CWB) but not an *Igh* recombinant strain BAB/14 expressed NP-1 idiotype in their anti-NIP antisera. These results indicated that expression of NP-1 idiotype is controlled by $Igh\text{-}V^b$ genes, and, unlike NP^b idiotype, it is not apparently subject to the influence of gene(s) regulating λ_1 chain-bearing antibody production. Third, it was demonstrated that immune sera from individual A/St, or an *Igh* congenic strain C.AL-20 exhibited significant levels of NP-1 idiotype; this contrasts with the great variability in the ability to identify NP^b idiotype in these anti-NIP antisera. Interestingly, the concentration of NP-1 idiotype in CWB, A/J, and C.AL-20 anti-NIP antisera, similar to that found for NP^b idiotype (Table III and reference 3), was also significantly lower than that of B6 mice. Anti-NIP antisera from other strains were negative for NP-1 idiotype production because 30 μl of these sera consistently cause <20% inhibition of NP-1 idiotype binding. An identical strain distribution of the idiotype, defined by the binding of ^{125}I-labeled N-hybridoma antibody to anti-idiotypic antiserum made against SJL serum anti-NP antibodies, was obtained with the same panel of immune sera (Table III). These data suggest that NP-1 idiotypic specificities are responsible for the cross-reactive idiotype binding.

Mapping of Gene(s) Controlling the Synthesis of NP-1 Idiotype. Table IV presents the compiled data on the expression of NP-1 idiotype in the anti-NP antisera from various recombinant inbred mouse strains. A total of 45 individual antisera from L × B,

TABLE IV
Genetic Mapping of NP-1 Idiotype

Strains‡	*Igh*-gene marker*								Anti-NP antibody§	Percent inhibition of NP-1 idiotype binding‖	
	Src	*Bgl*	*Gte*	*Np* *Nbp* *Npl*	*Sa4*	*Sa2*	*Igh-C*	*Pre-1*		30 µl	3 µl
									mg/ml		
B × D 1, 2, 5, 13 14, 21, 22, 23, 29	B	B	B	B	B	B	B	B	2 ± 0.5 (30)	ND¶	78 ± 3
B × D 6, 11, 15, 16, 24, 28, 31, 32	D	D	D	D	D	D	D	D	3 ± 0.5 (38)	4 ± 2	ND
B × D 8, 19	B	ND	B	B	B	B	B	× D	1 ± 0.4 (8)	ND	76 ± 5
B × D 12	D	ND	D	D	D	D	D	× B	2 (1)	6	ND
B × D 27	B	B	B	B	B	× D	D	D	10 (1)	ND	75
B × D 9	B	× D	D	D	D	D	D	D	5 ± 2.2 (5)	7 ± 2	ND

* The symbols designate *Igh* gene markers and have been reviewed in reference 17.
‡ Additional strains tested include L × B, 58N × L, BR × 58N, and B × H recombinant strains. 30 µl of all (18) *Igh-1*^{b}-negative strains with 2 ± 0.8 mg/ml anti-NP antibody caused 7 ± 3% inhibition of NP-1 binding. In contrast, 3 µl of all (27) *Igh-1*^{b}-positive strains with 3.9 ± 0.9 mg/ml anti-NP antibody caused 81 ± 3% inhibition of NP-1 idiotype binding.
§ Secondary sera obtained at day 52 were used in these tests. Numbers in parentheses indicate the total number of samples tested.
‖ In the absence of inhibition, 48% binding was obtained.
¶ Not done.

58N × L, BR × 58N, and B × H recombinant inbred strains were tested, and a complete correlation was observed between the presence of the *Igh-1*^{b} allotype and expression of the NP-1 idiotype (Table IV, footnote). The results indicated that the *Igh-V* gene encoding NP-1 idiotype is closely linked to *Igh-C* genes. Mapping of the *Igh-Np1* gene was carried out by testing anti-NP antisera obtained from 83 individual B × D recombinant inbred mice. The results are totally consistent with other experiments mapping the *Igh-V* genes controlling the synthesis of NP, Gte, and Bgl idiotypes (17) and therefore map the *Igh-Np1* gene within the same segment of chromosome that encodes the NP, Gte, and Bg1 idiotypes (Table IV). The results strongly suggest that the heavy chains required for the construction of NP^{b} and NP-1 idiotypic antibodies are derived from the same germ line *Igh-V* genes.

Expression of NP-1 Idiotype on λ_1 *and* λ_2*-bearing Anti-NP Antibodies.* Because N-hybridoma antibody bears a λ_2 (or λ_2-like) light chain, it is possible that expression of serum NP-1 idiotype is preferentially associated with λ_2-bearing anti-NP antibodies. Anti-NP and anti-NIP antibodies that were specifically purified from SJL, B6, A/J, and C3H immune sera obtained at various times after immunization were analyzed for the levels of λ_1-, λ_2-, and NP-1-bearing antibodies. Indeed, the results showed a correlation between the level of λ_2-bearing antibodies and the expression of NP-1 idiotype in NP-1 positive strains (B6, SJL, and A/J) (Table V). No apparent relationship was observed with respect to the level of λ_1 (or κ)-bearing antibodies and NP-1 idiotype expression. Interestingly, antibodies purified from late immune sera exhibited lower levels of λ_2-bearing antibodies and NP-1 idiotype.

Additional experiments demonstrated that, although expression of NP-1 idiotype is not dependent on λ_1 chain-bearing antibodies, such antibodies do exhibit weak cross-reactive NP-1 idiotypes. B6 and SJL anti-NP antisera were adsorbed with Sepharose 4B beads conjugated with the γ-globulin fractions of normal guinea pig serum, anti-λ_1, and anti-λ_2 antisera (Table VI). Adsorption of SJL anti-NP antiserum

TABLE V

Correlation of λ_2 Chain and NP-1 Idiotype Levels

Strain	Antigen*	Source of purified antibodies‡	Percent λ chain-bearing antibodies§		Micrograms needed for 50% inhibition of NP-1 idiotype binding
			λ_1	λ_2	
SJL	NP-Ficoll	N-hybridoma	0	100	0.008
SJL‖	NP-BGG/CFA	1° sera	<5	10	0.1
SJL‖	NP-BGG/CFA	2° sera	<5	0.3	7
B6‖	NIP-BGG/MP	1° sera	92	5	0.2
B6‖	NIP-BGG/MP	2° sera	70	3	1
A/J	NP-BGG/CFA	1° sera	26	0.8	2.5
C3H	NP-BGG/CFA	1° sera	10	0.8	>30

* MP, Maalox pertussis vaccine.

‡ Primary (1°) sera were collected between days 12 and 23 after the initial immunization. Secondary (2°) sera were obtained between days 100 and 112 after the initial immunization and a secondary immunization at day 93.

§ λ_1 and λ_2 chain-bearing antibodies were determined by indirect binding and inhibition of λ_2 chain binding, respectively.

‖ Primary and secondary anti-NP or anti-NIP antibodies were obtained from the same group of mice.

TABLE VI

*Relationship between λ Chain and NP-1 Idiotype Expression**

Source of inhibitor	Percent inhibition of NP-1 idiotype binding after adsorption with beads conjugated with			
	NGPS	anti-λ_1	anti-λ_2	anti-λ_1 + anti-λ_2
SJL anti-NP sera	85	71	6	0
B6 anti-NP sera	80	61	48	2

* Adsorptions were carried out with 0.3-ml Sepharose 4B beads conjugated with appropriate antiserum γ-globulins. For adsorption with anti-λ_1-beads, 50 μg of MOPC 315 myeloma protein was added to the mixture. For anti-λ_2-beads, 50 μg of MOPC 104E myeloma protein was added. 30–50 μl of immune sera was used in this experiment. 3 μl equivalents of the original sera were tested in all cases except the absorption with NGPS-bead in which 1-μl equivalents were used. NGPS are normal guinea pig serum γ-globulins.

with anti-λ_2-coupled beads removed essentially all NP-1 idiotypic antibodies, as evidenced by the inability of unbound materials to inhibit NP-1 idiotype binding. The bound material, eluted with acid followed by neutralization, exhibited NP-1 idiotype. Furthermore, the eluted fraction but not the adsorbed fraction contained λ_2-bearing molecules (data not shown). These results indicated that in SJL anti-NP antibodies, the NP-1 idiotype is expressed predominantly on λ_2-bearing antibodies. In contrast, adsorption of B6 anti-NP sera under similar conditions could only partially reduce the level of NP-1 idiotype. λ_2-bearing, NP-1 idiotypic antibodies could be recovered in the acid eluted fraction (data not shown). The results suggest that both λ_1 and λ_2-bearing anti-NP antibodies possess NP-1 idiotype. The depletion of some NP-1 idiotypic activity by adsorption of B6 anti-NP sera with anti-λ_1-coupled beads is in agreement with the interpretation that NP-1 idiotypic determinants are expressed on both λ_1 and λ_2 molecules in B6 mice. Furthermore, adsorption of B6 anti-NP

antisera with beads conjugated with anti-λ_1 and anti-λ_2 antisera removed all NP-1 idiotypic antibodies.

Finally, direct evidence demonstrating that λ_1-bearing anti-NP antibodies express cross-reactive NP-1 idiotype was obtained by taking advantage of hybridoma anti-NP antibodies bearing the λ_1 chain and testing them for NP-1 idiotypic specificities. Assay of NP^b idiotype was also carried out to determine the relationship between expression of NP^b and NP-1 idiotypes (Table VII). It was found that most λ_1-bearing hybridoma anti-NP antibodies that exhibit high levels of NP^b idiotype also express cross-reactive NP-1 idiotypic specificities. However, such specific NP-1 idiotypic cross-reactivity was extremely weak in all hybridoma antibodies studied. Furthermore, complete inhibition of NP-1 binding did not occur under the experimental conditions. The results demonstrated that the shared NP-1 idiotypic specificities detected on these μ, λ_1- and γ_1, λ_1-bearing hybridoma anti-NP antibodies are nonidentical to those present on μ, λ_2-bearing N-hybridoma anti-NP antibody.

Discussion

The present study focused on the idiotypic specificities of a monoclonal anti-NP antibody (N-hybridoma) secreted from a hybridoma cell line derived from a fusion between NP-Ficoll-immunized SJL splenic cells and the SP2/0 cell line. The idiotypic specificities of N-hybridoma anti-NP antibody are recognized by anti-idiotypic antisera made against B6, SJL, and N-hybridoma anti-NP antibodies, and these idiotypic specificities are extensively shared among individual anti-NP and anti-NIP antisera from B6, SJL, and A/J mice. This demonstration of shared idiotypes on anti-NP and anti-NIP antibodies among B6, SJL, and A/J mice contrast dramatically to the strain distribution pattern of the NP^b idiotype that exists in high concentration in B6 antisera but is present in low or undetectable levels in SJL and A/J antisera (3, 4).

TABLE VII

Presence of Cross-reactive NP-1 Idiotype on λ_1-bearing Monoclonal Antibodies

Hybridoma*	Binding activity‡ (ABC_{50}/ml) $\times 10^{-3}$	Percent inhibition of idiotype binding					
		NP^b			NP-1		
		30 μl	3 μl	0.03 μl	30 μl	3 μl	0.03 μl
N-hybridoma (μ, λ_2)	125	30	27	19	100	100	68
6100-23 (γ_1, λ_1)	625	97	86	48	57	37	8
6100-21 (γ_1, λ_1)	25	78	52	20	18	18	5
6100-15 (μ_1, λ_1)	625	79	46	14	35	18	7
6100-9 (γ_1, λ_1)	125	79	58	3	49	45	8
6100-6 (γ_1, λ_1)	25	23	15	12	27	12	1
6100-5 (γ_1, λ_1)	125	90	74	27	80	52	0
6100-2 (γ_1, λ_1)	5	53	13	7	17	0	3
Pooled λ_1-bearing hybridomas	ND§	ND	ND	ND	50	20	9
Control ascites	0	8	0	0	0	9	0

* Ascitic fluids were used in this experiment. Control ascites were hybridoma cell lines that do not make anti-NP antibody. 30 μl of normal sera from C57BL and SJL mice always inhibited <20% of NP-1 and NP^b idiotype binding.

‡ Affinity of each hybridoma antibody was not determined. ABC_{50} refers to dilution of ascitic fluid that is sufficient to bind 50% of 10 ng ^{125}I-NIP-BSA in the Farr binding assays.

§ Not done.

The marked difference in the strain distribution pattern between NP-1 and NP^b idiotypes may at least in part be due to the high sensitivity of the NP-1 assay system, which detects low concentrations (0.5 μg/ml) of NP-1 idiotype in a heterogeneous antibody population. More importantly, this contrast in the strain distribution patterns of NP-1 and NP^b idiotypes stemmed from the fact that NP-1 idiotype is preferentially associated with λ_2-bearing anti-NP antibodies (although λ_1-bearing anti-NP antibodies also exhibit weak cross-reactive NP-1 idiotypic specificities). Because B6, SJL, and A/J mice do not have a genetic defect in λ_2 chain production, anti-NP and anti-NIP antibodies from these strains were able to express NP-1 idiotypes. On the other hand, the expression of NP^b idiotypes is dependent upon the ability of the mouse strain to produce λ_1-bearing anti-NP antibodies. Thus, early B6 anti-NP antibodies possess predominantly λ_1 chain and exhibit high levels of NP^b idiotype. A/J mice, in spite of having normal levels of serum λ_1 chain, utilize κ light chains to produce the major portion of anti-NP antibodies and consequently exhibit variable or low levels of NP^b idiotype (3). SJL mice, despite having the *Igh-V* gene required for NP^b idiotype production, were unable to make significant levels of NP^b idiotype because of a genetic defect in producing normal levels of λ_1-bearing antibody molecules (5). In any event, the unambiguous demonstration of NP-1 idiotype in B6, SJL, and A strains indicates that the *Igh-V* gene(s) encoding NP-1 idiotypic antibodies in these strains are similar (or identical).

The molecular basis for the inability of SJL mice to produce normal levels of λ_1 chain remains unclear. It has been suggested that the defect resides in one of the recognition sites involved in the joining of the $V\lambda_1$ gene to the joining segment (Jλ) or the splicing event leading VJ to the $C\lambda_1$ segment, resulting in inefficient expression of λ_1-bearing antigen-sensitive bone marrow-derived lymphocytes (5). Recent experiments using mouse-hamster somatic cell hybrids have demonstrated that $C\lambda_1$ and $C\lambda_2$ genes are on the same chromosome, i.e., number 16 (7). Furthermore, accumulated biochemical and genetic evidence indicates that there is at least one $V\lambda_1$ gene and one $V\lambda_2$ gene present on this chromosome (7, 11). Based on sequence data, it appears that the expression of each Vλ gene is selectively associated with a particular Cλ gene (11). This is consistent with the observation that the NP-1 idiotype is mainly associated with λ_2-bearing anti-NP antibodies in SJL and B6 anti-NP antibodies. However, we also observed that B6 λ_1-bearing anti-NP antibodies and λ_1-bearing, NP^b-positive hybridoma anti-NP antibodies weakly express the cross-reactive NP-1 idiotypic specificities. These results suggest that N-hybridoma anti-NP antibody bear $V\lambda_2$ or $V\lambda_2$-like sequences. The weak NP-1 idiotypic cross-reactivity of λ_1-bearing NP^b-positive hybridoma anti-NP antibodies is in agreement with the sequence data that indicate that a higher degree of homology exists between $V\lambda_1$ and $V\lambda_2$ than between Vλ and Vκ chains. Conversely, the sequence homology between the $V\lambda_1$ and $V\lambda_2$ region can explain the weak expression of NP^b idiotypic specificities on N-hybridoma antibody. However, it should be cautioned that sequence differences between $V\lambda_1$ and $V\lambda_2$ must be present to account for the inefficiency of λ_2 chain-bearing antibodies to fully express the NP^b idiotype in SJL mice or in λ_2-bearing anti-NP hybridoma antibody (8).

Recently, Bothwell et al. (18) demonstrated that there are seven potential germline genes encoding the heavy chain of the NP^b idiotypic family. Furthermore, they observed that the cDNA heavy chain variable region (V_H) of one hybridoma (B1–8)

has an identical sequence to one germ-line V_H gene, V(186-2), and another cDNA V_H gene from a second hybridoma (S43) that was chosen to be as different as possible was a somatic mutational product of germ-line gene V(186-2). Thus, it is possible that many λ_1-bearing NP^b-positive anti-NP antibodies were derived from a single germ-line V_H gene and that λ_2-bearing NP-1-positive anti-NP antibodies may selectively utilize one of the six other germ-line genes. This hypothesis can account for the preferential expression of NP-1 idiotype on λ_2-bearing anti-NP antibodies and the inability of λ_2-bearing anti-NP antibodies to complement the production of NP^b idiotype in SJL mice that possess all the germ-line V_H genes.

It should be noted that the above interpretation is based on the essential assumption that the heavy chain of N-hybridoma antibody is similar to that of the NP^b idiotypic family. Indeed, the genetic mapping data using 128 individuals of 52 recombinant inbred mouse strains indicated that the gene controlling NP-1 idiotype mapped in the same *Igh-V* gene segment that controls NP^b idiotype synthesis. Furthermore, recent molecular genetic analysis of the *Igh-NP*^{b} germ-line gene indicated that B6 and SJL mice exhibit an identical Southern blot pattern (18). These results strongly suggest that the heavy chains of NP-1 and NP^b idiotypes are derived from the same family of *Igh-NP*^{b} genes. Sequences of the seven potential germ-line genes encoding the heavy chains of NP^b idiotypic antibodies indicated the existence of extensive homology (18). Consequently, these heavy chains have a high probability of sharing extensive structural similarity. Indeed, further serological analyses have demonstrated the presence of additional shared idiotypic structures that are different from the NP^b idiotype and NP-1 idiotype and are also extensively shared among λ_1-bearing NP^b-positive hybridoma antibodies, λ_2-bearing N-hybridoma antibody, and serum anti-NP antibodies from A/J, SJL, and B6 mice (manuscript in preparation).

Another motivation for analyzing the idiotypes in the anti-NP system stems from previous observations that showed that nearly all NP-specific suppressor T cells (19, 20) and affinity-purified NP-specific receptors (21) of C57BL and SJL mice were cross-reactive with anti-idiotypic antisera made against C57BL anti-NP antibodies. Because of the extremely low levels of NP^b idiotype in NP-immunized SJL mice, it was concluded that T cell receptors of C57BL and SJL mice possess NP^b idiotypic specificities and that SJL T cell receptors were not passively acquired from serum Ig molecules. In view of the current study, this interpretation warrants reconsideration. Although it is clear that C57BL and SJL T cell receptors bearing idiotypic determinants can cross-react with anti-idiotypic antisera made against C57BL anti-NP antibodies, the fine anti-idiotypic activity responsible for this cross-reactivity has not been defined. Consequently, it remains possible that NP-1 idiotypic determinants are expressed on these T cell receptors. In this regard, we have recently produced several functionally active suppressor T cell hybridomas that are sensitive to treatment with anti-idiotypic antisera made against either C57BL anti-NP antibodies or N hybridoma antibody (22). The latter anti-idiotypic antiserum exhibits high levels of activity to NP-1 idiotypic determinants but very weak activity against the NP^b idiotypic specificities. These data suggest that a restricted fraction of antibody idiotypes may be expressed on T cell receptor molecules.

Summary

Hybridoma cell lines secreting antibodies specific to (3-nitro-4-hydroxyphenyl) acetyl (NP) were generated by fusion of NP-immunized SJL spleen cells with the

SP2/0 cell line. One hybridoma (N-hybridoma) anti-NP antibody (μ, λ_2) was found to partially inhibit (35–40%) the binding of the predominant idiotype in primary C57BL/6 anti-NP antibodies (NP^b). Iodinated hybridoma antibody could be completely bound with anti-idiotypic antiserum made against either specifically purified C57BL/6 anti-NP antibodies, SJL anti-NP antibodies, or N-hybridoma antibody. The idiotypic specificities defined with anti-idiotypic antiserum made against N-hybridoma antibody were termed NP-1 idiotype. Strain distribution and genetic mapping studies indicate that the gene(s) controlling the production of NP-1 idiotype is closely associated with $Igh\text{-}1^b$ and $Igh\text{-}1^e$ alleles and mapped within the same chromosomal segment that controls the synthesis of NP^b idiotype. However, unlike NP^b idiotype, the expression of NP-1 idiotype is not influenced by the gene(s) that control λ_1 chain synthesis. Thus, SJL mice that produce low or undetectable levels of NP^b idiotype due to a defect in λ_1 chain production express high levels of NP-1 idiotype. Specifically purified C57BL/6 and SJL anti-NP antibodies fully express NP-1 idiotype, the level of which correlates with the level of λ_2 chain-bearing molecules. Nonetheless, further experiments indicate that λ_1-bearing anti-NP antibodies can express extremely weak NP-1 idiotypic cross-reactivity.

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