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Immunology

Functional association of idiotypic and I-J determinants on the antigen receptor of suppressor T cells

(T-cell receptor/hybridoma T cells/suppressor factor)

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ABSTRACT **The serological characteristics of the antigen receptor on 4-hydroxy-3-nitrophenylacetyl (NP) specific suppressor T cell hybridomas were analyzed. Three T-cell hybrids could be lysed with anti-idiotype and complement. The reactivity pattern observed from a panel of anti-idiotypic reagents indicated that NP^b determinants were detected on all three hybrid lines. NP conjugates with bovine serum albumin or caproic acid specifically inhibited the complement-mediated lysis of these cells by both anti-NP^b idiotype and anti-I-J antisera. These hapten conjugates failed to block lysis by anti-Thy 1 or anti-H-2K antisera on the same target cell populations. The data indicate that both I-J and Igh variable region gene products are intimately involved in the recognition of antigen by suppressor T cells. Finally, the suppressor cell hybrids produce soluble factors that mediate antigen-specific suppression. The characteristics of the cells and their factors indicate that the hybrids correspond to the Ts^i or first-order suppressor cells in the suppressor cell pathway.**

Immune responses to specific antigens are controlled by the activity of suppressor T lymphocytes. The sequence of cellular interactions resulting in suppression of the cellular and humoral immune responses to antigen has been well characterized in the 4-hydroxy-3-nitrophenylacetyl (NP) system. At least three distinct cell populations have been implicated in this suppressor cell pathway (1–5), thereby making the NP system one of the most thoroughly characterized of the suppressor cell models. Furthermore, the conclusions derived from the NP system are consistent with most other observations concerning the cellular interactions involved in immune suppression (6–10). However, in spite of considerable data relating to the cell surface phenotype of the T cells involved in immune suppression, little is actually known about the interrelationships of such membrane markers and the nature of the antigen receptors on these cells.

To investigate the nature of the T cell antigen receptor and to characterize the molecules responsible for the communication between cells, in the suppressor pathway, large numbers of functional suppressor T cells must be obtained. To achieve this goal we have utilized the techniques of somatic cell hybridization (11). These techniques have been used previously to prepare monoclonal cell lines that maintain the biological activity of the original suppressor cell population, including the ability to secrete biologically active suppressor factors (12–14).

We now describe a series of suppressor cell hybrids that produce soluble products capable of specifically suppressing NP immune responses. The relationship of these hybrids to the overall suppressor cell pathway is analyzed. These hybridoma cell lines also provide a source of monoclonal cells that are used to probe the T cell antigen receptor. The data demonstrate that both I-J and idiotypic determinants are intimately associated with the antigen receptor on suppressor T lymphocytes.

MATERIALS AND METHODS

Mice. C57BL/6J(B6), C3H/HeJ, and B10.BR mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All other inbred strains were bred in the animal facilities of Harvard Medical School. Mice were used at 2–12 months of age.

Antigens. The preparation of hapten-conjugated proteins has been described (15). The molar conjugation ratio of haptenic groups used in this work was: dinitrophenyl (DNP)/bovine serum albumin, 12:1; NP/albumin, 12:1; and 4-hydroxy-5-iodo-3-nitrophenyl (NIP)/albumin, 7:1. The caproic acid (Cap) derivatives of DNP and NP were purchased from Biosearch (San Raphael, CA). DNP-Cap and NP-Cap were dissolved in phosphate-buffered saline at 10 mM and the pH was adjusted to 7.3 with 1 M NaOH. NP-$(CH_2)_6$-NP (0.1 mM) was dissolved in 0.25 M phosphate buffer (pH 7.8) containing 20% dimethyl sulfoxide.

Alloantisera. B10.A(3R) anti-B10.A(5R) ($I\text{-}J^k$), B10.A(5R) anti-B10.A(3R) ($anti\text{-}I\text{-}J^b$), (B10 × LP.RIII)F_1 anti-B10.A(4R) ($anti\text{-}K^k$ and $I\text{-}A^k$), and (B10.A(4R) × B10.GD)F_1 anti-B10 ($anti\ K^b + I\text{-}A^b$) were produced by immunization with spleen and lymph node cells as described (4, 16). Anti-brain-associated T-cell antigen (anti-BAT) antibody was made by the technique described by Golub (17). Monoclonal anti-Thy 1.1 and Thy 1.2 antibodies were purchased from New England Nuclear.

Anti-Idiotypic Antisera. C57BL/6, A/J, SJL, and C3H mice were immunized intraperitoneally with 100 μg of NP-BGG and pertussis vaccine (2). Primary anti-NP antibodies were specifically purified by addition of pooled sera to Sepharose 4B beads (Pharmacia) conjugated with NP-albumin and elution with 0.2 M glycine·HCl buffer (pH 2.35) into tubes containing 2 M Tris·HCl at pH 8.0. Guinea pigs were immunized with 100 μg of anti-NP antibodies emulsified with complete Freund's adjuvant, followed by repeated intramuscular and subcutaneous injections with the same immunogen. Sera were rendered idiotype specific by adsorption with Sepharose 4B beads coupled with a gamma globulin fraction of MOPC 104E ascites and normal serum from the appropriate mouse strains. The binding of ^{125}I-labeled B6 anti-NP antibodies to its anti-idiotypic antiserum could be strongly inhibited by 1 μl of B6 anti-NP antiserum but not by 30 μl of B6 normal mouse serum or MOPC 104E ascites. The idiotypic specificities detected were closely associated with NP binding sites because 0.06 μmol of NP-Cap or NIP-Cap but not 6 μmol of *p*-aminoarsanilate completely inhibited the binding. Furthermore, strain distribution studies indicated that the anti-idiotypic antiserum defines NP^b-idiotypic specificities similar to those previously reported (18).

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Abbreviations: NP, 4-hydroxy-3-nitrophenylacetyl; Cap, caproic acid; DNP, dinitrophenyl; NIP, 4-hydroxy-5-iodo-3-nitrophenyl; NBrP, 4-hydroxy-5-bromo-3-nitrophenyl.

Anti-idiotypic antisera to B6 anti-4-hydroxyl-5-bromo-3-nitrophenyl (NBrP) and B6 anti-NIP, characterized in a similar fashion, also detected NP^b idiotypic specificities.

Anti-NP-1 idiotypic antiserum was made in guinea pigs against NP-1 hybridoma anti-NP antibody (μ, λ) secreted by a hybridoma cell line generated by fusion of SJL spleen cells immunized to NP-Ficoll with the SP2/0 cell line. The anti-idiotypic antiserum was adsorbed as described above. The adsorbed antiserum reacted strongly with NP-1 hybridoma anti-NP antibody but not with MOPC 104E or MOPC 315 myeloma protein or SJL normal mouse serum. The specific idiotype binding, termed "NP-1 idiotype," was inhibited completely in the presence of 0.06 μmol of NP-Cap or NIP-Cap but not 6 μmol of *p*-aminoarsanilate. NP-1 idiotypic specificities were shared by B6, SJL, and A/J anti-NP antisera and were not detectable in seven other mouse strains not bearing Igh^b or Igh^e alleles. Additional serological analyses indicated that anti-idiotypic antisera against B6 anti-NP, B6 anti-NBrP, B6 anti-NIP, and SJL anti-NP crossreactively bound NP-1 hybridoma anti-NP antibody. However, the binding strength of these anti-idiotypic antisera were $\frac{1}{10}$ to $\frac{1}{3}$ that of anti-idiotypic antiserum to NP-1 hybridoma antibody.

Anti-idiotypic antisera against CGAT and hybridoma F17.148.3 of B6 origin have been described (19).

Preparation of Antigen Specific Suppressor T Cells. The methods for the preparation and enrichment of NP-specific suppressor T cells have been described (1, 3).

Hybridization. Suppressor T-enriched C57BL/6J or CKB splenic lymphocytes were hybridized with BW5147 T lymphoma cells. The hybridizing method was as described by Taniguchi *et al.* (12).

To provide an additional specificity control, in some experiments another T cell hybrid, prepared by fusion of BW5147 and T cells from DBA/1 GAT nonresponder mice pretreated with GAT to induce suppressor T cells, was studied. This hybrid line, F65,121 (termed "D1-121" in this report) was the gift of Ronald N. Germain (Harvard Medical School).

Screening for $I\text{-}J^+$ and Id^+ Hybrids. Soon after the hybrid cells began to grow, both $I\text{-}J^+$ and Id^+ hybrids were selected by cytotoxic tests (20). Briefly, 2 μl of cell suspension (1×10^6 cells per ml) was treated with 2 μl of diluted antisera in L-15 medium (Microbiological Bioproducts, Walkersville, MD) containing 0.5% fetal calf serum. The reaction was carried out at room temperature in Terasaki microplates (no. 3034, Falcon). After 20 min, the cells were washed with one drop of serum-free L-15 medium. Then 2 μl of a 1:5 dilution of selected rabbit complement in serum-free L-15 medium was allowed to react with these washed cells for 40 min at 37°C. Then these cells were stained with 0.2% nigrosine and the percentage of dead cells was determined.

To inhibit the cytotoxicity test, 1 μl of DNP-albumin, albumin, NP-albumin, or NIP-albumin solution (2 mg/ml) in phosphate-buffered saline or 0.01 M DNP-Cap, 0.01 M NP-Cap, or 0.1 mM NP-$(CH_2)_6$-NP were mixed with 1 μl of cell suspension (2×10^6 cells per ml) prior to the addition of antiserum.

Adsorption and Elution of Suppressor Factor. NP-albumin, specifically purified antibodies, and gamma globulin of various antisera were conjugated to CNBr-activated Sepharose 4B beads according to the manufacturer's protocol. Two to 4 mg of protein was coupled to 1 ml of beads and the product was packed into a 3-ml syringe. Adsorptions were carried out at room temperature by repeatedly passing 5 ml of culture supernatants through the columns. Unbound material was saved. After each column was washed with 10 ml of phosphate-buffered saline, bound materials were eluted with 4.2 ml of 0.2 M glycine·HCl (pH3.2) into tubes containing 0.5 ml of fetal calf serum and 0.3 ml of 2 M Tris·HCl (pH 8.0). The pH of the eluted fractions was adjusted to 7.4–7.6 when necessary. Fractions were stored at −20°C until used.

Assay for Suppressive Activity of Culture Supernatant or Fractionated Samples on Cutaneous Sensitivity Responses. The assay has been described in detail (21). Footpad swelling was determined as the difference, in units of 10^{-3} cm, between left and right footpad thicknesses.

Data Analysis. Statistical analysis of the experimental data with respect to controls utilized the two-tailed Student's *t* test. Data are expressed as the mean (±SEM) incremental footpad swelling.

RESULTS

Serological Analysis of T-Cell Hybridoma Cell Lines. Two series of fusions using either B6 ($H\text{-}2^b$, Igh^b) or CKB ($H\text{-}2^k$, Igh^b) antigen-enriched suppressor cells and the BW5147 AKR thymoma were analyzed. The cell fusions were performed with polyethylene glycol, and the hybrids were selected by growth in hypoxanthine/aminopterin/thymidine medium. Of 1050 wells plated, 30% developed hybrids. All hybrids were screened with anti-I-J alloantisera and subsequently with guinea pig anti-NP^b idiotype antisera; 16% of the hybrids were specifically lysed with allele-specific anti-I-J alloantisera and 29% of the I-J-bearing hybrids were specifically lysed with anti-NP^b idiotype antisera. For the present study one B6- and two CKB-derived hybrids that were susceptible to lysis with both anti-I-J and anti-NP^b antisera were selected. In addition one CKB hybrid that had I-J determinants but lacked idiotypic determinants was selected as a control.

The specificity of lysis with these reagents is shown in Table 1. The anti-$I\text{-}J^b$ antiserum only lysed hybrids of B6 ($H\text{-}2^b$) origin; the anti-$I\text{-}J^k$ reagent only lysed cells of CKB origin. Several batches of anti-I-J antisera were used and all gave concordant results.

The results from adsorption experiments indicated that the lytic activity of the anti-$I\text{-}J^k$ antisera could be specifically absorbed with spleen cells from $H\text{-}2^k$, but not $H\text{-}2^b$-bearing mice. The specificity of the anti-idiotypic serum was demonstrated by its ability to lyse the B6-29, CKB-39, and CKB-17 hybrids but not T-cell hybrids produced by fusion with GAT-induced suppressor T lymphocytes. As a reciprocal control, guinea pig anti-CGAT idiotype failed to react with the NP hybrids but lysed the DBA/1-derived GAT suppressor cell hybrid. To verify the T-cell nature of these hybrids, each cell line was tested with

Table 1. Serological analysis of T-cell hybridomas

Antisera	% specific cytotoxicity*					
	B6-29	CKB-17	CKB-39	CKB-70	D1-121	BW5147
Anti-$I\text{-}J^b$	35	0	0	0	0	5
Anti-$I\text{-}J^k$	0	70	81	90	0	5
Anti-NP^b	30	26	60	0	0	0
Anti-CGAT	0	5	12	0	45	0
Anti-Thy 1.2	100	94	0	70	95	11
Anti-Thy 1.1	89	84	90	88	95	86

The results are from a single experiment, but each cell line has been tested on at least three separate occasions with comparable results. Each reagent was diluted 1:10 for use in cytotoxicity assays.

* The data were calculated as 100 × (% experimental lysis − % control lysis) ÷ (% total lysis − % control lysis). Control lysis was 5–20%. In a large series of experiments with control sera, we observed that specific lysis <15% is within control limits and specific lysis >20% is considered significant.

Table 2. Immunochemical characterization of B6-29 hybridoma suppressor factor

Source of factor	NP-O-Su priming	Immuno-adsorbent column	Column fraction	
			Unbound	Eluate
BW5147	+	—	26.8 ± 4.6 (5)	
B6-29	+	—	11.8 ± 2.3 (5)*	
B6-29	+	Anti-NP^b	22.8 ± 2.4 (5)	12.0 ± 1.6 (6)*
B6-29	+	Anti-NIP^b	25.2 ± 5.0 (5)	12.8 ± 2.1 (4)*
B6-29	+	Anti-Ig	10.5 ± 2.0 (4)*	25.0 ± 9.6 (4)
B6-29	+	Anti-I-J^b	27.4 ± 3.9 (5)	13.8 ± 0.7 (5)*
B6-29	+	Anti-I-J^k	10.8 ± 1.8 (5)*	30.0 ± 6.4 (5)
B6-29	+	NP-albumin	24.3 ± 3.5 (4)	14.5 ± 1.5 (4)*
BW5147	−	—	8.8 ± 1.8 (5)	

C57BL/6 mice were primed with NP-O-Su followed by daily intravenous injections of the column fractions until the day prior to antigen challenge. The results are expressed as the mean (±SEM) increment of footpad swelling. The number of mice per group is indicated in parentheses. The data are from one experiment; a second experiment yielded comparable results.
* Significant suppression, $P < 0.05$.

monoclonal anti-Thy 1 reagents. With the exception of the CKB-39 cell line, all were lysed with the anti-Thy 1.2 reagent which fails to lyse the parental BW5147 line, indicating that most of these hybrids contained the Thy 1.2 allele derived from the suppressor T lymphocyte donor.

The percentage of cells lysed with the anti-I-J and -NP^b reagents varied considerably in different experiments, from 20% to 90%. Similar results were noted with cloned cell lines. Preliminary experiments suggest that this variation is correlated with the stage of the cell cycle (22). The percentage of cells susceptible to lysis with anti-I-J and anti-idiotypic antisera appeared to decrease after prolonged maintenance in cell culture.

Functional Analysis of T-Cell Hybrids. Supernatants from the four hybridoma cells were assayed for their ability to inhibit NP-specific cutaneous sensitivity responses. The B6-29, CKB-39, CKB-17, and CKB-70 hybrid supernatants inhibited the NP-O-Su response 90%, 69%, 59%, and 12%, respectively, if given daily from the time of antigen priming until the day prior to antigen challenge. The inhibition by the first three hybrids was antigen specific in that DNFB contact sensitivity responses were not inhibited by the same supernatants (the inhibition was 9%, 11%, and 11%, respectively). Controls for these experiments included testing supernatants from the BW5147 tumor line, which failed to inhibit cutaneous sensitivity responses.

To characterize the biologically active factors present in these supernatants, we selected supernatants from the B6-29 hybrid for analysis. Culture supernatants from these cells were passed over immunoadsorbent columns and the adsorbed fractions were eluted with glycine·HCl buffer (pH 3.2). The suppressive activity was adsorbed by anti-idiotype, anti-I-J^b, and NP-albumin columns (Table 2). Furthermore, suppressive activity could be recovered specifically by elution from these columns. The suppressor factor lacked conventional immunoglobulin determinants because polyvalent guinea pig anti-immunoglobulin antisera failed to absorb the activity. Furthermore, a control anti-I-J^k immunoadsorbent column failed to remove the activity of the H-2^b-derived suppressor factor.

Idiotype Analysis of Hybridoma T-Cell Lines. In order to characterize which idiotypic determinants were present on the hybridoma cell lines, the hybrids were screened against a series of guinea pig anti-idiotypic reagents prepared against purified antibodies to NP or to NP derivatives such as NIP or NBrP (Table 3). The anti-idiotypic reagents prepared against B6 anti-NP, anti-NIP, or anti-NBrP were specifically lytic for the B6-29, CKB-39, and CKB-17 hybrids. In contrast, none of the other anti-idiotypic reagents prepared against purified NP antibodies derived from other strains of mice could lyse these cell lines. Furthermore, anti-idiotypic antibody prepared against the NP^b idiotype bearing SJL B cell hybridoma were specifically lytic for the three functional suppressor T cell hybrids (see below). As a control, guinea pig anti-idiotypic antibodies specific for the GAT-specific B-cell hybridoma F17. 148.3 were found to be nonlytic.

Antigen-Specific Inhibition of Anti-Idiotype- and Anti-I-J-Mediated Lysis. In order to verify the specificity of the complement-mediated lysis with anti-idiotype antisera, we attempted to inhibit this reaction with antigen. Either NP-albumin or NIP-albumin was used to inhibit cytolysis, and DNP-albumin and albumin were used as controls. Each of these inhibitors was used at a final concentration of 0.5 mg/ml, and the antisera were titrated to give 30–80% lysis. Addition of either NP-albumin or NIP-albumin specifically inhibited lysis by anti-idiotype (Table 4). Furthermore, addition of NP- or NIP-albumin also blocked lysis by anti-I-J alloantisera on the B6-29, CKB-39, and CKB-17 targets. Neither of these hapten conjugates blocked anti-I-J cytolysis of the idiotype-negative I-J-bearing CKB-70 cells. In addition, NP-albumin and NIP-albumin could not inhibit complement-mediated cytolysis by anti-H-2K, anti-Thy 1.2, or anti-BAT antiserum.

To determine if the NP-albumin or NIP-albumin inhibition of anti-idiotype and anti-I-J was due to steric hindrance, we attempted to block cytolysis with the low molecular weight mono- and bivalent Cap derivatives NP-Cap and NP-$(CH_2)_6$-NP. NP-Cap at 10 mM and NP-$(CH_2)_6$-NP at 0.1 mM could

Table 3. Idiotype analysis of T-cell hybridoma cell lines

Anti-idiotype code	Idiotype		% specific cytotoxicity				
	Strain	Immunogen	B6-29	CKB-17	CKB-39	CKB-70	BW5147
Anti-NP^b	B6	NP	30	36	63	0	0
Anti-NIP^b	B6	NIP	39	42	53	0	0
Anti-NBrP^b	B6	NBrP	28	26	63	0	0
Anti-NP-1	SJL	Monoclonal anti-NP	38	26	63	0	5
Anti-SJL	SJL	NP	0	0	0		0
Anti-A/J	A	NP	0	5	0		0
Anti-C3H	C3H	NP	11	10	0		0
Anti-CGAT	D1.LP	GAT	6	5	12		0
Anti-148.3	B6	Monoclonal anti-GAT	0	10	0		0

See legend to Table 1.

Table 4. Antigen-specific inhibition of anti-idiotype and anti-I-J complement-mediated lysis

Cell line	Antiserum	% specific cytotoxicity: Albumin	DNP-albumin	NP-albumin	NIP-albumin
B6-29	Anti-NP^b	29	29	5	0
	Anti-I-J^b	35	29	0	5
	Anti-K^b	70	65	79	70
	Anti-Thy 1.2	94	100	100	94
CKB-17	Anti-NP^b	38	36	0	5
	Anti-NIP^b	39	36	0	5
	Anti-I-J^k	63	68	12	5
	Anti-K^k	63	79	78	75
	Anti-Thy 1.2	75	79	68	63
	Anti-BAT	63	79	79	63
CKB-39	Anti-NP^b	47	57	0	6
	Anti-NIP^b	58	57	0	0
	Anti-I-J^k	89	93	5	5
	Anti-K^k	79	79	89	95
	Anti-Thy 1.2	0	0	0	0
	Anti-BAT	79	71	79	75
CKB-70	Anti-NP^b	0	0	0	0
	Anti-NIP^b	0	0	0	0
	Anti-I-J^k	47	53	47	37
	Anti-K^k	63	59	48	56
	Anti-Thy 1.2	63	59	59	75

Antisera were titrated in order to obtain 30–80% specific lysis. All inhibitors were used at a final concentration of 0.5 mg/ml. See legend to Table 1.

Table 5. Hapten inhibition of anti-I-J and anti-NP^b lysis

Cell line	Antiserum	% specific cytotoxicity: No inhibitor	NP-$(CH_2)_6$-NP (0.1 mM)	NP-Cap (10 mM)	DNP-Cap (10 mM)
B6-29	Anti-NP^b	37	3	6	30
	Anti-NIP^b	48	6	3	33
	Anti-I-J^b	37	13	12	33
	Anti-K^b	74	77	82	76
	Anti-Thy 1.1	76	63	63	58
CKB-17	Anti-NP^b	25	0	0	25
	Anti-NIP^b	28	2	13	25
	Anti-I-J^k	52	5	25	63
	Anti-K^k	63	55	81	63
	Anti-Thy 1.1	57	72	75	69
CKB-39	Anti-NP^b	41	0	10	35
	Anti-NIP^b	36	4	15	31
	Anti-I-J^k	55	9	19	56
	Anti-K^k	60	61	69	69
	Anti-Thy 1.1	63	63	70	72
CKB-70	Anti-I-J^k	47	39	33	44
	Anti-K^k	50	67	—	—
	Anti-Thy 1.1	50	33	—	—
CKB-59	Anti-NP^b	0	0	0	0
	Anti-NIP^b	3	3	6	0
	Anti-I-J^k	47	42	65	65
	Anti-K^k	58	63	53	53
	Anti-Thy 1.1	58	63	53	65

See legend to Tables 1 and 4.

block the lysis mediated by either anti-NP^b or anti-NIP^b anti-idiotypic antiserum (Table 5). Furthermore, these compounds specifically blocked anti-I-J lysis in the B6-29, CKB-17, and CKB-39 cell lines but failed to inhibit anti-I-J lysis of the non-functional CKB-70 hybrid or the CKB-59 cell line. Preliminary analyses suggest that the CKB-59 hybrid represents an effector-phase second-order suppressor T cell and presumably bears an anti-idiotypic receptor. The specificity of the inhibition was demonstrated by the inability of NP-Cap or NP-$(CH_2)_6$-NP to block anti-H-2K or anti-Thy 1 lysis and the inability of DNP-Cap to block anti-idiotype- or anti-I-J-mediated lysis.

Finally, the data suggest that NP-$(CH_2)_6$-NP at 0.1 mM is a better inhibitor of anti-idiotype and anti-I-J lysis than is a 100-fold excess of monovalent NP-Cap. Although this trend has been observed in nearly all experiments, more data are required to evaluate adequately the role of valency in suppressor T-cell–antigen interactions.

DISCUSSION

The suppressor cell pathway resulting in the modulation of cellular and humoral NP responses has been well characterized. The first population of suppressor cells (Ts_1 or Ts^i) bear I-J and idiotypic determinants and function during the induction phase of the immune response (1, 3). The Ts_1 population induces a second complementary population of suppressor cells (Ts_2 or Ts^e) which bear I-J determinants and anti-idiotypic receptors (3). The latter population functions during the effector phase of the immune response in previously primed animals (2, 3). However, this second population does not contain the final effector suppressor T cells. The available data suggest that the Ts_2 cells activate a third population of I-J- and idiotype-bearing suppressor T cells (Ts_3) in the immune recipient (4, 9). The B6-29, CKB-17, and CKB-39 T-cell hybrids described here appear to correspond with the Ts_1 population. In support of this, we note that (*i*) the cells were induced and selected by the same procedures that have been shown previously to generate NP-specific Ts_1 (1), (*ii*) these hybrid cells have the same surface phenotype as the Ts_1 population (4), and (*iii*) preliminary data indicate that the supernatants from these hybrid cells can generate a Ts_2 population (data not shown).

The relationship of the B cell idiotypic determinants with those on hybridoma suppressor T cell lines was compared by using a panel of anti-idiotypic antisera. The anti-NP^b, anti-NIP^b, and anti-NBrP^b antisera have similar specificity patterns—i.e., each of these guinea pig antisera bind purified anti-NP antibodies from Igh^b-bearing C57BL/6 mice and the predominant idiotypic determinants detected by these reagents have a strain distribution similar to that described for the allotype-linked NP^b idiotype (18). Another reagent was also capable of specifically lysing the Ts hybrids. Anti-idiotype against a SJL monoclonal anti-NP hybridoma antibody which possessed some NP^b idiotypic determinants and demonstrated weak binding of NP^b ligand could also lyse the three functional T cell hybrids. Because this anti-idiotypic reagent was prepared against purified monoclonal hybridoma antibodies, it excludes the possibility that this reagent contained contaminating antibodies against T cell-derived products that may have copurified with the anti-NP antibodies obtained from conventional immune serum.

Anti-idiotype reagents prepared against purified anti-NP or anti-NIP antibodies from C3H or A/J mice (which lack the Igh^b allotype) failed to lyse the NP-specific T cell hybrids. Finally, anti-idiotype prepared against purified SJL anti-NP antibodies,

which carry the Igh^b allotype but lack the NP^b idiotype due to a defect in the production of λ light chain (18), also failed to lyse these hybrids. Anti-idiotype prepared against these SJL antibodies can bind the NP-1 hybridoma protein which possesses some of the NP^b idiotypic determinants, but this reagent failed to lyse the T cell hybridoma lines. These findings suggest that only selected idiotypic determinants on NP-1 hybridoma anti-NP antibody are expressed on individual hybrid T cell lines.

The availability of a homogeneous population of antigen-specific suppressor T cells permitted serological analysis of the T cell's antigen receptor. The ability of NP-albumin or NIP-albumin to inhibit lysis by anti-idiotype specifically verifies the specificity of this lysis. The finding that NP-albumin or NIP-albumin could also block lysis by anti-I-J alloantisera on hybrids that possessed both the NP^b and I-J antigenic determinants indicated that the I-J determinant is closely associated with the antigen receptor of the T cell. Furthermore, monovalent NP-Cap and bivalent NP-$(CH_2)_6$-NP also were able to block anti-idiotype- and anti-I-J-mediated lysis. Because these compounds are smaller than 500 daltons, the results indicate that (*i*) there is a functional association of *H-2* and *Igh* gene products on the Ts cell surface, (*ii*) the predominant idiotypic determinants detected on Ts are associated with the combing site, not framework variable region, determinants, and (*iii*) I-J determinants are intimately associated with the combining site. It is important to note that these hapten conjugates failed to inhibit the lysis of anti-I-J antisera on the I-J-bearing but NP^b-idiotype-negative CKB-70 hybridoma. This hybridoma lacks NP-specific suppressor function and presumably lacks an anti-NP receptor. Furthermore, these NP conjugates could not block anti-H-2K- or anti-Thy 1-mediated lysis.

Finally, among the small number of hybrids tested, there is an apparent correlation between the expression of cell surface I-J and NP^b determinants and the ability of factors from these hybrids to mediate NP-specific suppression. Only one of the hybridoma factors was characterized. The antigen-binding B6-29 factor had both I-J and NP^b determinants which correlates with the phenotype of the antigen receptor on the cell from which it was derived. The properties of this factor indicate that it corresponds to a series of suppressor factors described in other systems (10).

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