# HAPTEN-SPECIFIC T-CELL RESPONSES TO 4-HYDROXY-3-NITROPHENYL ACETYL

## II. Demonstration of Idiotypic Determinants on Suppressor T Cells*

By JUDAH Z. WEINBERGER, RONALD N. GERMAIN, SHYR-TE JU, MARK I. GREENE, BARUJ BENACERRAF, AND MARTIN E. DORF

*From the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115*

The interaction of the immune system with its antigenic environment is governed in part by available recognition structures on its cellular elements, the T and B cells. The nature of the T-cell antigen receptor has recently been the subject of intense study. Evidence for V-gene products being involved in the cell surface T-cell receptor has primarily involved production of various anti-idiotypic antisera and investigation of their biological effects (1–4). Recently, we have utilized a different approach based on the genetic analysis of functional fine-specificity markers to provide evidence of $V_H$ control of delayed-type hypersensitivity (DTH)[1]-reactive T-cell specificity in common with serum antibody against the same antigenic moiety (5). The purpose of the present study is to extend these observations to another functional class of T cells, namely the suppressor T-cell population. The present experiments use three approaches to establish the idiotypic nature of the suppressor T-cell antigen receptor.

Indirect evidence concerning the idiotypic nature of the suppressor T-cell antigen receptor has been previously reported in several systems (6, 7). Eichmann was able to induce A5A idiotype-specific suppressor T cells in A/J mice by treating these mice with the $IgG_2$ fraction of guinea pig anti-idiotype to A5A (7). The other major line of evidence concerning this issue comes from the study of antigen-specific T-cell-derived suppressor factors. Germain et al. (8) have shown that T-cell-derived suppressor factor specific for the random terpolymer poly-(L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$) (GAT) was adsorbed onto and fully recoverable from a guinea pig anti-GAT idiotype-Sepharose immunoadsorbent. These data were interpreted as suggesting a sharing of V-region structures between B-cell antibody and T-cell suppressor factor. Similarly, Bach et al. (9) demonstrated the presence of the A/J anti-azobenzenearsonate (ARS) idiotype on the A/J ARS-specific T-cell-derived suppressor factor.

In the work presented below, we have utilized (*a*) a fine specificity marker, (*b*) an

* Supported by grants AI-14732, AI-00152, and GM-02220 from the National Institutes of Health and by grant PCM 75-22422 from the National Science Foundation.

[1] *Abbreviations used in this paper:* ARS, azobenzenearsonate; BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund's adjuvant; DTH, delayed-type hypersensitivity; GAT, poly-(L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$); GLØ, poly-(L-glu, L-lys, L-phe); MEM, Eagle's minimum essential medium; MHC, major histocompatibility complex; NIP, (4-hydroxy-5-iodo-3-nitrophenyl)acetyl hapten; NP, (4-hydroxyl-3-nitrophenyl)acetyl hapten; NP-$T_s$ NP suppressor T cells; PBS, phosphate-buffered saline; RAMIg, rabbit anti-mouse immunoglobulin.

idiotypic marker on serum antibody against (4-hydroxy-3-nitrophenyl)acetyl (NP), and (*b*) genetic analysis to characterize an NP-suppressor T-cell population. The basis of this approach comes from observations concerning the nature of the serological primary anti-NP response. It was found that the primary anti-NP antibody response in most mice of the Ig-$1^b$ allotype has a heteroclitic fine specificity (10). These antibodies bind analogues of the immunizing hapten such as (4-hydroxy-5-iodo-3-nitrophenyl)acetyl (NIP) with greater affinity than the immunizing hapten, NP, itself. The antibodies in these strains share common, strain-specific clonotypes as shown by isoelectric focusing (11). Furthermore, two laboratories have prepared anti-idiotypic antibodies against purified C57BL/6 anti-NP antibodies (12, 13). These anti-idiotypic reagents have been used to identify at least one germ-line gene which maps to the $V_H$ region of Ig-$1^b$ heavy-chain linkage group. This gene has been termed $V_HNP^b$ (12, 14). The idiotype-positive antibody comprises the predominant antibody population of the primary anti-NP response of C57BL/6 mice (>85%) and also in $F_1$ hybrid mice between C57BL/6 and an idiotype-negative strain (13). Finally, the idiotype-positive antibody expresses the λ-light chain as its predominant light chain species. SJL mice, despite producing Ig-$1^b$ allotypic antibody, have been shown to produce a nonheteroclitic, idiotype negative, primary anti-NP response. The $V_HNP^b$ gene, however, is thought to be present in SJL, as is shown by the demonstration of idiotype production in primary anti-NP responses of (CBA × SJL)$F_1$ or (BALB/c × SJL)$F_1$ hybrid mice (12, 13). The explanation advanced for this phenomenon is that SJL mice, which have a genetic defect resulting in the production of very low levels of immunoglobulins bearing the λ-light chain (15), cannot express the $V_HNP^b$-gene product in the absence of a gene which permits expression of the λ-light chain. Thus, the primary anti-NP antibody response is associated with a characteristic fine-specificity marker, clonotype, λ-light chain bearing immunoglobulins, and idiotypic markers.

These characteristics of the V-region markers of anti-NP antibodies have also been used in characterization of NP-binding receptor material isolated from enriched populations of NP-primed splenic B and T lymphocytes. Antigen-binding material was isolated by adsorption of lymphocytes to hapten-conjugated nylon wool, followed by a temperature shift to release the cells, and elution of the receptor material with acidic buffer or free hapten. Amounts of receptor material isolated from C57BL/6 splenic lymphocytes lacking immunoglobulin C-region determinants of either heavy or light chains correlated with the relative number of T cells. It was concluded that this material was of T-cell origin, although the functional properties of these T cells were not determined. It was concluded that the T-cell receptor for antigen bears similar $V_H$-region markers as those found on immunoglobulin of the same specificity (16, 17).

We have recently reported (5) that after cyclophosphamide pretreatment and immunization with (4-hydroxy-3-nitrophenyl)acetyl-bovine gamma globulin (NP-BGG), several strains of mice which did not bear the Ig-$1^b$ heavy-chain linkage group made a NP-specific DTH response when challenged with NP-bovine serum albumin (BSA) and failed to respond to challenge with NIP-BSA. Strains of NP-BGG-primed mice bearing the Ig-$1^b$ allotype, including SJL, responded to challenge with either NP-BSA or NIP-BSA. Genetic mapping of the NIP cross-reactive DTH response localized the gene(s) controlling the trait to the $V_H$ region of the Ig-$1^b$ heavy-chain linkage group. Thus, the fine-specificity pattern of the DTH effector T-cell anti-NP

response, and the genetic mapping of this trait, were analogous to the reported fine specificity and mapping data of the humoral heteroclitic anti-NP response. In this report we utilize serological markers, fine specificity, and genetic analyses to demonstrate that another functional subpopulation of T cells, i.e., NP-specific suppressor T cells also utilize receptors sharing idiotypic structures with anti-NP antibodies.

## Materials and Methods

*Mice.* C57BL/6J, B10.BR, CBA/J, SJL/J, and (BALB/c × C57BL/6)$F_1$ strain mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. C.B-20 mice were bred in the animal facilities of Harvard Medical School. Mice were used at 2–10 mo of age, and were maintained on laboratory chow and acidified, chlorinated water ad libitum.

*Antigens.* BSA, and BGG were purchased from Sigma Chemical Co., St. Louis, Mo. NP-O-succinimide and NIP-O-succinimide were purchased from Biosearch, San Rafael, Calif. The preparation of NP- and NIP-conjugated proteins has been previously described (5). The molar conjugation ratio of haptenic groups used in this work was $NP_{17}$-BGG, $NIP_{18}$-BGG, $NP_{11}$-BSA, and $NIP_9$-BSA. The random synthetic terpolymer GAT with an average mol wt of 40,000 daltons was custom synthesized by Vega-Fox Biochemicals, Div., Newberry Energy Corp., Tucson, Ariz.

*Haptenated Cell Preparation.* Single cell suspensions of spleen cells were prepared in Eagle's minimum essential medium (MEM) containing 0.5% heparin. The suspension was pelleted and treated with Tris-$NH_4Cl$ to lyse erythrocytes. After two washes, the spleen cells were resuspended in pH 7.6 phosphate-buffered saline (PBS) at $10^8$ cells/ml. 25 μl of a 24 mg/ml solution of NP-*o*-succinimide or 32 mg/ml solution of NIP-O-succinimide in dimethylsulfoxide was added per milliliter of cells. After allowing the reaction to proceed for 3 min at room temperature, 10–30 ml cold pH 7.4 PBS containing 1.2 mg/ml glycyl-glycine was added to stop the reaction. The cells were extensively washed in MEM containing 0.5% heparin before use.

*Immunization.* Experimental animals were primed with NP-BGG, NIP-BGG, BSA, or GAT-emulsified in complete Freund's adjuvant (CFA) (Difco Laboratories, Detroit, Mich.) containing 2 mg/ml *Mycobacterium butyricum*, so that the final antigen concentration was 500 μg/ml in the CFA emulsion. 3 d before antigen priming, animals were pretreated with 20 mg/kg cyclophosphamide (Mead Johnson & Co., Evansville, Ind.) in 0.2 ml PBS injected intraperitoneally. A total of 0.2 ml antigen emulsion was injected subcutaneously divided between two sites on the dorsal flanks.

*DTH Response.* 7 d after immunization, mice were challenged for the DTH response by injecting 25 μl of 1 mg/ml of NP-BSA, NIP-BSA, BSA, or GAT in PBS into the left footpad using a 27-gauge needle. Footpad swelling was measured 24 h after challenge using an engineer's micrometer (Schlesingers For Tools Ltd., Brooklyn, N. Y.). Swelling was determined as the difference, in units of $10^{-4}$ in, between the left footpad thickness and the right footpad thickness. All animals were measured by two independent observers, whose measurements were then averaged. The responses thus elicited have been previously demonstrated to be a classical T-cell-mediated DTH reaction by the following criteria: (*a*) kinetically the reaction reached a peak at 20–36 h after challenge; (*b*) T cells were required to transfer this reactivity to naive recipients; and (*c*) major histocompatibility complex (MHC) identity at the I-A subregion was necessary for transfer of the reactivity (5).

*Antisera.* Rabbit anti-mouse immunoglobulin (RAMIg) was prepared as follows. After several immunizations of a rabbit with purified normal mouse immunoglobulin, the rabbit antisera was passed over an immunoadsorbent prepared by coupling a 40% saturated ammonium sulfate precipitate of normal mouse serum to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). The rabbit anti-mouse immunoglobulin was eluted from the column with 0.1 M glycine-HCl solution at pH 2.8, neutralized in 2 M Tris buffer at pH 7.9, dialyzed against PBS, and adjusted to a final protein concentration of 1 mg/ml.

Guinea pig anti-$NP^b$ anti-idiotypic antisera were prepared using affinity purified B6 anti-NP antibodies obtained from primary sera of a pool of C57BL/6 mice immunized with 100 μg NP-BGG in alum with 0.1 ml Pertussis vaccine (U. S. Department of Public Health, Ann Arbor, Mich.). The antibodies were purified by precipitation with 45% saturated ammonium

sulfate followed by affinity purification on a NP-BSA-coupled Sepharose 4B immunoadsorbent. Specific antibody was eluted from the column by washing the column with a 0.03 M NIP-caproate (Biosearch) in PBS solution. The eluate was extensively dialyzed in PBS. The eluate from this column contained only $\lambda_1$-light chain and $\mu$, $\gamma_1$, and small amounts of $\gamma_{2a}$-heavy chains as detected by radioimmunodiffusion (18). Furthermore, isoelectric focusing of a sample of the radioiodinated purified immunoglobulin in a pH 3–10 range polyacrylamide gel revealed a remarkably restricted spectrotype. Guinea pigs were immunized biweekly with 200 $\mu$g of purified C57BL/6 anti-NP antibodies in CFA. 2 wk after the third injection, the animals were bled. The antisera were adsorbed on NP-GL, MOPC 104E ($\mu$, $\lambda_1$), and normal mouse serum adsorbents prepared by glutaraldehyde cross-linking. These gels were prepared according to the procedure of Averameas and Ternynck (19). After exhaustive adsorptions on these gels, the specificity of this reagent was verified using an inhibition of idiotype binding assay, according to the method of Ju et al. (20). 30 $\mu$l of normal mouse sera, MOPC 104E ascites, 5 $\mu$l A/J anti-NP sera, and $1.25 \times 10^{-4}$ M DNP-caproate did not inhibit idiotype binding. However, 1 $\mu$l of primary B6 anti-NP sera, $4.25 \times 10^{-6}$ M NP-caproate or $2.00 \times 10^{-6}$ M NIP-caproate could significantly inhibit the idiotype binding. Thus, the anti-idiotypic antiserum used detects combining site determinants as previously described by other groups (12, 13). The control anti-idiotypic antiserum used in these experiments was prepared in guinea pigs against purified anti-poly-(L-glu, L-lys, L-phe) (GLØ) antibodies (anti-BGL) as detailed elsewhere (21).

*Cell Fractionation.* T cells were purified according to Mage et al. (22). 5 ml of a 1 mg/ml solution of purified RAMIg were added to 100 × 15 mm polystyrene Petri plates (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif.) and incubated for 1 h at room temperature. Unbound antibody was removed and the plates were then washed extensively in PBS containing 5% fetal calf serum. Spleen cells, previously treated for 3 min at 37°C with Tris-$NH_4Cl$ to lyse erythrocytes, were resuspended at a concentration of $1.2 \times 10^7$ cells/ml. 6 ml of cells were added to the antibody-coated plates and incubated for 1 h at 4°C. The nonadherent T-cell population was recovered by gentle swirling.

For experiments in which T cells were treated with anti-idiotypic antiserum plus complement, spleen cells were fractionated using a nylon wool column according to the method of Julius et al. (23). Briefly, nylon wool (Fenwal Inc., Walter Kidde & Co., Inc., Ashland, Mass.) was boiled three times in twice distilled water, and 2 g in dry weight was packed into a 35-cc syringe. Before application of the cells, nylon wool was rinsed and saturated with MEM containing 10% fetal calf serum. Up to $5 \times 10^8$ spleen cells in 5 ml of this medium were washed into the column and incubated at 37°C for 1 h. After incubation, nonadherent cells were eluted during a 45-min period using ≅40 ml of media.

NP-binding T cells were prepared by a modification of the procedure described by Taniguchi and Miller (24). Briefly, 5 ml of a 1 mg/ml solution of NP-BSA were added to 100 × 15 mm polystyrene Petri plates and incubated overnight at 4°C. Unbound NP-BSA was decanted and the plates extensively washed with 5% fetal calf serum in PBS. T cells, previously recovered from RAMIg-coated plates, were washed and suspended to $10^7$ cells/ml. 5 ml of the cell suspension was added to each plate, and the cells were allowed to adhere for 1 h at room temperature. Nonadherent cells were removed by gentle swirling, and the plates were then incubated at 4°C for 20 min. The latter procedure permitted the removal of adherent cells from the plate by gentle pipetting. The sum of the NP-binding T cells and nonadherent T cells recovered was >95% of the original cell number applied to the antigen-coated plates.

*Antisera Treatments.* Whole spleen lymphocyte populations of SJL strain mice or nylon wool-purified T cells of other mouse strains were treated with anti-idiotypic antiserum and complement. A maximum of $3 \times 10^8$ cells were pelleted in a 17 × 100 mm plastic tube (Falcon Labware, Div. of Becton, Dickinson, & Co.), and resuspended in 0.5 ml of unabsorbed anti-idiotypic antiserum. The suspension was incubated at room temperature for 30 min. The antisera was then washed out and, the cells resuspended in 1.5 ml of prescreened low toxicity rabbit complement. This suspension was incubated at 37°C for 45 min, the cells then washed three times, counted, and transferred to the appropriate recipients.

## Results

*Nonresponsiveness Induced with NP-coupled Spleen Cells.* Erythrocyte-free spleen cells from C57BL/6 mice were derivatized with NP-*O*-succinimide. $2 \times 10^7$ NP-derivatized

TABLE I

*Tolerance of NP-induced DTH Responses with NP-coupled Spleen Cells in C57BL/6 Mice**

| Cells transferred | Immunogen | DTH challenge‡ | |
|---|---|---|---|
| | | NP-BSA | NIP-BSA |
| $2 \times 10^7$ control | NP-BGG | 47.6 ± 5.7 | 60.7 ± 4.8 |
| $2 \times 10^7$ NP-spleen | NP-BGG | 27.1 ± 0.4§ | 15.3 ± 5.6§ |
| none | CFA | 11.0 ± 1.7 | 29.5 ± 3.7 |

* See text for experimental protocols.

‡ Responses of a minimum of five mice per group, expressed as mean ± 1 SE in units of $10^{-4}$ in.

§ $P < 0.005$ compared to mice receiving control spleen cells.

or normal spleen cells were injected intravenously into C57BL/6 mice. After 7 d, mice were treated with 20 mg/kg cyclophosphamide. 3 d later, these mice were immunized subcutaneously with 100 μg NP-BGG in CFA. Another group of mice, serving as DTH-negative controls, were similarly cyclophosphamide pretreated then immunized subcutaneously with CFA alone. 7 d after priming, animals were challenged with 25 μg NP-BSA or NIP-BSA in 25 μl PBS injected into the left footpad. The data presented in Table I indicate that i.v. administration of NP-derived syngeneic spleen cells 10 d before antigen priming induced a significant state of NP nonresponsiveness. The data in Table I also confirm previous results that the NP-induced DTH response to C57BL/6 mice is highly cross-reactive with the related compound NIP. The intravenous administration of NP coupled syngeneic cells also tolerized recipients with respect to this cross-reactive NIP response.

*Cellular Requirement for Transfer of NP Nonresponsiveness.* To determine if this tolerance was mediated in part by suppressor T cells, several experiments were performed. In the first experiment, animals were given $2 \times 10^7$ NP-derivatized or normal spleen cells intravenously. 7 d later, these animals were sacrificed and $3 \times 10^7$ spleen cells were transferred to cyclophosphamide-pretreated mice. 4–6 six h after transfer, the mice were primed with 100 μg NP-BGG in CFA. 6 d later, the mice were challenged with 25 μg NP-BSA or NIP-BSA. The results of this experiment, indicated in Table II, show that virtually complete suppression of both the NP and the cross-reactive NIP responses, could be effected by transfer of spleen cells from animals which had received NP-derivatized syngeneic spleen cells 7 d earlier. Thus, spleen cells from mice treated 7 d earlier with $2 \times 10^7$ NP-derivatized syngeneic spleen cells will be referred to as NP-suppressor cells.

To determine if T cells alone were sufficient to transfer the suppression of these responses, the following experiment was performed. T cells were purified from the NP-suppressor cell population produced as described above. This was accomplished by negative selection of T cells using RAMIg-coated Petri plates. Recovery was generally 20–30% of the Tris-$NH_4Cl$-treated spleen cell population. In earlier experiments this method was shown to yield T cells with <5% contaminating Ig-positive cells as determined by immunofluorescence. Graded numbers of these cells were transferred into cyclophosphamide-pretreated C57BL/6 mice, which were then primed with NP-BGG. The results of a typical experiment of this sort are shown in Table II, exp. 2. When $2 \times 10^7$ splenic NP suppressor cells were transferred to C57BL/6 mice on the day of priming, the DTH response was suppressed to the same

TABLE II

*T-Cell-mediated Suppression of NP DTH Responses in C57BL/6 Mice**

| Experiment No. | Cells transferred | Immunogen | DTH challenge‡ | |
|---|---|---|---|---|
| | | | NP-BSA | NIP-BSA |
| 1 | $3 \times 10^7$ control | NP-BGG | 57.3 ± 4.2 | 59.3 ± 4.2 |
| | $3 \times 10^7$ NP-tolerized spleen cells | NP-BGG | 12.5 ± 2.5§ | 11.3 ± 2.5§ |
| | None | CFA | 11.0 ± 1.7 | 29.5 ± 3.7 |
| 2 | $2 \times 10^7$ control | NP-BGG | 55.1 ± 6.4 | NT |
| | $2 \times 10^7$ NP-tolerized spleen cells | NP-BGG | 28.1 ± 3.3§ | — |
| | $5 \times 10^5$ NP-tolerized T cells | NP-BGG | 40.3 ± 4.1‖ | — |
| | $2 \times 10^6$ NP-tolerized T cells | NP-BGG | 29.1 ± 2.2§ | — |
| | $7 \times 10^6$ NP-tolerized T cells | NP-BGG | 14.3 ± 3.3§ | 10.9 ± 2.6§ |
| | $7 \times 10^6$ control T cells | NP-BGG | 60.0 ± 5.7 | 54.6 ± 6.4 |
| | None | CFA | 12.1 ± 2.5 | 7.2 ± 2.6 |

NT, not tested.

* See legend to Table I.

‡ Responses of a minimum of four mice per group, expressed as mean ± SE in units of $10^{-4}$ in.

§ $P < 0.005$ compared to mice receiving control cells.

‖ $P < 0.03$ compared to mice receiving control T cells.

degree as achieved by transferring $\cong 2 \times 10^6$ purified T cells isolated from this starting population. Furthermore, the purified T cells could also suppress the cross-reactive NIP response. The data strongly suggest that suppressor T cells demonstrable 7 d after induction were at least in part, if not wholly, responsible for nonresponsiveness observed in the intact NP-spleen treated animals.

*Specificity of NP-induced Suppressor Cells in C57BL/6 Mice.* As controls for the above experiments a different population of suppressor cells was induced by the intravenous administration of syngeneic NIP-coupled spleen cells. The NIP-suppressor cells were also obtained from the spleens of mice 7 d after induction. To determine the specificity of the NP-suppressor cells in C57BL/6 mice, mice of this strain was pretreated with cyclophosphamide. Three days later these mice received $2.5 \times 10^7$ NP- or NIP-induced suppressor cells, or $2.5 \times 10^7$ control spleen cells. These mice were then immunized with either NP-BGG, NIP-BGG, or GAT. 7 d later, the animals were challenged in the left footpad with NP-BSA, NIP-BSA, or GAT for DTH responses. Results of two experiments are summarized in Table III.

Several points were noted. First, the NP-induced suppressor cells could suppress the NP-induced DTH responses elicited by NP and NIP. Furthermore, the NP-induced suppressor cells not only suppressed the NP-BGG-primed responses, but also suppressed responses primed by NIP-BGG. Thus, the NP-induced suppressor cell cross-reactively suppresses NIP-primed responses in C57BL/6 mice as well as NP-primed responses. However, the NP-induced suppressor cells could not suppress the GAT DTH response, demonstrating the specificity of this cell population.

NIP-induced suppressor cells were able to specifically suppress NIP-BGG-primed NIP responses, whereas not suppressing GAT primed, GAT responses. Interestingly, the NIP-induced suppressor cells given at the day of priming with NP-BGG were able to selectively suppress the cross-reactive DTH response elicited with NIP. However, the same population of NIP-induced suppressor cells did not affect the NP-BGG-induced NP DTH response.

TABLE III

*Specificity of NP- and NIP-induced Suppressor Cells in C57BL/6 Mice*

| Cells transferred | NP-BGG primed | | NIP-BGG primed | | GAT primed |
|---|---|---|---|---|---|
| | NP challenge* | NIP challenge* | NP challenge | NIP challenge | GAT challenge* |
| Control | 34.4 ± 3.0 (14) | 37.0 ± 3.7 (15) | 15.2 ± 2.5‡ (15) | 47.1 ± 4.7 (11) | 52.8 ± 3.3 (5) |
| NP-S.C. | 7.4 ± 4.0§ (9) | 9.6 ± 2.2§ (10) | NT | 17.0 ± 6.4§ (7) | 46.7 ± 5.1‖ (5) |
| NIP-S.C. | 38.8 ± 3.3‖ (9) | 8.1 ± 2.2§ (10) | NT | 11.1 ± 2.5§ (9) | 48.4 ± 4.8‖ (5) |

* Background responses of cyclophosphamide pretreated, CFA-primed animals: NP-10.0 ± 1.8, NIP-11.5 ± 2.6, and GAT-0.6 ± 1.1. Responses in units of $10^{-4}$ in. The number of mice per group is indicated in parentheses. NT indicates not tested.

‡ $P > 0.10$ as compared with animals receiving control cells.

§ $P < 0.001$ as compared with group receiving control cells.

‖ $P > 0.30$ as compared with animals receiving control cells.

*Purification of NP-specific Suppressor T Cells.* Spleen cells from C57BL/6 strain mice which, 7 d earlier, had been injected intravenously with $2 \times 10^7$ NP-derivatized syngeneic spleen cells were first depleted of B cells by passage over a RAMIg-coated plate. As previously noted, recovery at this step was 20–30% of the original cell population. The purified T cells were resuspended at $10^7$ cells/ml, and incubated 1 h at room temperature on NP-BSA coated plates. The nonadherent cells were removed (NP-nonbinding T cells). The plates were then incubated at 4°C for 20 min and the cells were removed by gentle pipetting with a Pasteur pipette; these cells are referred to as NP-binding suppressor T cells. The recovery at this step was ≅3–5% of the original T-cell population.

Graded doses of purified unfractionated suppressor T cells, NP-binding suppressor T cells, NP-nonbinding T cells, and control normal T cells were given to cyclophosphamide-pretreated C57BL/6 mice. The control normal T cells were incubated on NP-BSA coated plates for 1 h at 20°C and then incubated for 20 min at 4°C before recovery from the plates. These cells were used to control for the possibility that the NP-binding suppressor T-cell population were mediating suppression via carryover of small amounts of NP-BSA from the Petri dishes. Within 4–6 h after transfer of these cells into the experimental animals, the mice were primed with NP-BGG, NIP-BGG, or BSA, the latter serving as a specificity control. 6 d after priming, the animals were challenged with NP-BSA, NIP-BSA, or BSA for DTH responses. The results of such an experiment are shown on Table IV.

By interpolation from the data, $1.2 \times 10^6$ unfractionated NP-suppressor T cells could suppress 50% of the NP primed, NP response. The suppressive activity of the adherent suppressor T cells recovered from the NP-coated plates were highly enriched compared to the original T-cell population. Thus, $5 \times 10^4$ adherent NP suppressor T cells could suppress 50% of the NP DTH response. Furthermore, the NP-BSA nonbinding cells did not have any detectable suppressive activity, indicating that the specific suppression was mediated by cells of the NP-binding population rather than by passive carryover of small amounts of NP-BSA. On a quantitative basis, virtually all of the original suppressive activity in the T-cell population could be found in the NP binding T-cell population. Furthermore, $5 \times 10^4$-NP binding suppressor T cells could inhibit the NIP response of NIP-BGG primed mice completely. This implies that the NP-derivatized spleen cells induce a population of cells which are roughly 10-fold more efficient, in terms of cell number, at suppressing NIP-primed responses as they are at suppressing NP-primed responses. Specificity controls demonstrated

TABLE IV

*Purification of NP-specific Suppressor T Cells**

| Cells transferred | Immunogen | DTH challenge‡ | | |
|---|---|---|---|---|
| | | NP-BSA | NIP-BSA | BSA |
| None | NP-BGG | 49.2 ± 4.5 | | |
| $2.3 \times 10^5$ NP-$T_s$ cells | NP-BGG | 59.1 ± 9.6 | | |
| $7 \times 10^5$ NP-$T_s$ cells | NP-BGG | 31.5 ± 4.3§ | | |
| $2 \times 10^6$ NP-$T_s$ cells | NP-BGG | 26.1 ± 4.9‖ | | |
| $6 \times 10^6$ NP-$T_s$ cells | NP-BGG | 7.9 ± 5.4¶ | | |
| $5 \times 10^3$ NP-binding $T_s$ cells | NP-BGG | 47.2 ± 4.8 | | |
| $5 \times 10^3$ NP-binding $T_s$ cells | NIP-BGG | | 65.8 ± 5.8§ | |
| $5 \times 10^4$ NP-binding $T_s$ cells | NP-BGG | 29.0 ± 1.7¶ | | |
| $5 \times 10^4$ NP-binding $T_s$ cells | NIP-BGG | | 16.2 ± 4.7¶ | |
| $5 \times 10^5$ NP-binding $T_s$ cells | NP-BGG | 10.3 ± 4.2¶ | | |
| $7 \times 10^6$ NP non-adherent T cells | NP-BGG | 47.2 ± 6.9 | | |
| $5 \times 10^4$ control T cells | NP-BGG | 48.2 ± 4.6 | | |
| $5 \times 10^5$ control T cells | NP-BGG | 44.3 ± 1.9 | | |
| None | CFA | 10.3 ± 4.6 | 18.2 ± 4.5 | 11.3 ± 5.2 |
| None | NIP-BGG | | 63.2 ± 4.7 | |
| None | BSA | | | 46.6 ± 10.0 |
| $5 \times 10^5$ NP-binding $T_s$ cells | BSA | | | 56.4 ± 5.1§ |

* See legend to Table I.

‡ Responses of a minimum of four mice per group, expressed as mean ± SE in units of $10^{-4}$ in.

§ $P > 0.05$ as compared with group of mice receiving control cells.

‖ $P < 0.05$ as compared with group of mice receiving control cells.

¶ $P < 0.005$ as compared with group of mice receiving control cells.

that these adherent NP-suppressor T cells did not influence the BSA response (Table IV).

*Ability of Anti-idiotype to Remove NP-suppressor Cell Activity.* To further elucidate the nature of the receptor on NP suppressor cells able to bind to NP-BSA Petri dishes, experiments were carried out to investigate the relationship between idiotypic determinants on anti-NP antibodies and determinants on NP-induced suppressor T cells. An anti-idiotype was raised in a guinea pig against a pool of C57BL/6 primary anti-NP antibodies (Ig-$1^b$ allotype). The characterization of this antiserum has been described under Materials and Methods.

Suppressor cells were induced in various strains of mice by injection of $2 \times 10^7$ derivatized syngeneic spleen cells. 7 d later, the animals were sacrificed and T cells were obtained from B10.BR and CBA mice by passage of the spleen cells over a nylon wool column. Because SJL mice lack B cells bearing the $NP^b$ idiotype, whole spleen cells were used rather than purified T cells. The purified T cells or SJL strain spleen cells were treated with either the anti-$NP^b$ anti-idiotypic antiserum plus complement, or with a control anti-idiotypic antiserum (anti-BGL) plus complement. This control antiserum is also a guinea pig anti-idiotypic antiserum raised against anti-GLØ antibodies of the Ig-$1^b$ allotype (21). Furthermore, the genes controlling the $NP^b$- and BGL-idiotypic markers map to the same region of the $V_H{}^b$ haplotype (T. J. Kipps, B. Taylor, and M. E. Dorf, manuscript in preparation). After the anti-idiotype treatment the cells were transferred into syngeneic, cyclophosphamide-pretreated mice. 4–6 h after transfer, the mice were primed with 100 μg NP-BGG in CFA, subcutaneously. Responses were assayed 6 d after priming by injection of 25 μg of NP-BSA into the

TABLE V

*Ability of Anti-NP$^b$ Anti-idiotypic Antisera to Specifically Remove NP-induced Suppressor T Cells**

| Strain | Heavy-chain linkage group | Cells transferred | Treatment of cells | Immunogen | NP-BSA‡ challenge |
|---|---|---|---|---|---|
| B10.BR | b | Control | None | NP-BGG | 53.5 ± 3.4 |
| | | NP-$T_s$ | Anti-NP$^b$ + C | NP-BGG | 41.7 ± 3.9§ |
| | | NP-$T_s$ | Anti-BGL + C | NP-BGG | 11.8 ± 3.3‖ |
| | | None | — | CFA | 5.5 ± 3.7 |
| SJL | b | Control | None | NP-BGG | 65.9 ± 8.4 |
| | | NP-S.C. | Anti-NP$^b$ + C | NP-BGG | 61.0 ± 10.2§ |
| | | NP-S.C. | Anti-BGL + C | NP-BGG | 16.2 ± 3.7‖ |
| | | None | — | CFA | 8.9 ± 6.7 |
| CBA | j | Control | None | NP-BGG | 40.9 ± 0.9 |
| | | NP-$T_s$ | Anti-NP$^b$ + C | NP-BGG | 7.1 ± 3.4‖ |
| | | NP-$T_s$ | Anti-BGL + C | NP-BGG | 7.9 ± 1.6‖ |
| | | None | — | CFA | 3.9 ± 2.5 |

* See legend to Table I.

‡ Responses of a minimum of four mice per group, expressed as mean ± SE in $10^{-4}$ in.

§ $P > 0.05$ as compared with group given control cells.

‖ $P < 0.001$ as compared with group given control cells.

left footpad. 24 h after challenge, relative footpad swelling was determined. Results of these experiments are shown in Table V.

In two strains, B10.BR and SJL, having identical immunoglobulin heavy-chain linkage groups (Ig-1$^b$, $V_H{}^b$), treatment of NP-suppressor T cells (NP-$T_s$) with control anti-BGL anti-idiotypic antiserum plus complement did not interfere with the ability of these cells to significantly suppress the NP response. Treatment of NP-$T_s$ from the B10.BR strain (Ig-1$^b$ allotype) with anti-NP$^b$ anti-idiotypic antiserum essentially abrogated ($P > 0.05$) the ability of these cells to transfer significant suppression. When the NP-$T_s$ of SJL origin were examined with respect to the ability of anti-NP$^b$ anti-idiotypic antiserum plus complement to affect transfer of NP-specific suppression, it was found that this treatment also abrogated the suppressive activity of this cell population. As specificity controls NP-$T_s$ from CBA/J mice were treated with anti-NP$^b$ anti-idiotype plus complement. The latter strain carries the Ig-1$^j$ heavy-chain linkage group, does not make heteroclitic antibody, and lacks the NP$^b$ idiotype. There was no decrease in the ability of NP-$T_s$ of CBA strain origin to transfer specific suppression to NP after treatment with anti-NP anti-idiotype plus complement. Thus, treatment with anti-NP$^b$ anti-idiotype plus complement was able to distinguish the NP-suppressor cells originating from Ig-1$^b$ allotype strains and NP-suppressor cells from a strain not bearing this allotype.

*Genetic Mapping of NP Suppressor T-Cell Fine Specificity.* Because a strain distribution was observed for the ability of anti-NP$^b$ anti-idiotype plus complement to remove NP-suppressor T-cell activity, experiments were performed to determine whether, in analogy to the situation at the DTH effector cell level (5) the gene(s) controlling the fine specificity of the suppressor T cell would be allotype linked. Using the same strains as were used in the study with anti-idiotypic antisera, namely B10.BR and CBA, $2 \times 10^7$ NP-derivatized syngeneic spleen cells were injected into mice of each strain. 7 d later, $2.5 \times 10^7$ spleen cells from these suppressed mice were transferred intravenously to cyclophosphamide-pretreated syngeneic recipients. The recipients

TABLE VI

*Genetic Analysis of the Fine Specificity of NP-induced Suppressor T Cells**

| Strain | Heavy-chain allotype | Cells transferred | NP-BGG primed | | NIP-BGG primed | BSA primed |
|---|---|---|---|---|---|---|
| | | | NP-BSA challenge | NIP-BSA challenge | NIP-BSA challenge | BSA challenge |
| CBA‡ | j | Control | 40.9 ± 0.9 | NT | 38.6 ± 2.6 | NT |
| | | NP-S.C. | 6.9 ± 2.5§ | NT | 38.4 ± 5.5 | |
| | | NIP-S.C. | NT | NT | 2.4 ± 1.6 | |
| B10.BR‡ | b | Control | 53.5 ± 3.4 | 54.3 ± 7.3 | 56.7 ± 7.8 | NT |
| | | NP-S.C. | 5.9 ± 9.2§ | 7.9 ± 4.3§ | 15.7 ± 4.5§ | |
| | | NIP-S.C. | NT | | 10.2 ± 2.0§ | |
| C.B-20‡ | b | Control | 55.3 ± 10.1 | 39.9 ± 6.5 | 49.4 ± 3.8 | NT |
| | | NP-S.C. | 22.6 ± 6.1‖ | 9.1 ± 3.1 | 8.7 ± 1.4§ | |
| | | NIP-S.C. | NT | NT | 13.2 ± 2.2 | |
| BALB/c‡ | a | Control | 43.1 ± 3.0 | NT | 48.8 ± 4.4 | NT |
| | | NP-S.C. | 14.1 ± 2.2‖ | NT | 52.4 ± 4.5 | |
| | | NIP-S.C. | 43.0 ± 6.2 | NT | 10.8 ± 7.2 | |
| (BALB/c × C57BL/6)$F_1$‡ | a/b | Control | 49.2 ± 2.5 | 54.4 ± 7.4 | 63.3 ± 5.0 | NT |
| | | NP-S.C. | 25.2 ± 2.0§ | 25.6 ± 3.5§ | 41.8 ± 5.2‖ | |
| | | NIP-S.C. | NT | NT | 16.2 ± 2.9 | |
| SJL‡ | b | Control | 65.9 ± 8.4 | 66.9 ± 12.4 | 47.2 ± 3.6 | 71.3 ± 6.0 |
| | | NP-S.C. | 14.6 ± 3.1§ | 13.8 ± 8.7§ | 11.8 ± 4.1§ | 83.1 ± 14.9 |
| | | NIP-S.C. | NT | NT | 18.7 ± 2.6 | NT |

* See legend to Table I.

‡ CFA-primed negative controls challenged with NP-BSA, NIP-BSA, or BSA. The response for each strain was $<10 \times 10^{-4}$ in for all antigens with the exception of the NIP response in CFA primed (BALB/c × C57BL/6)$F_1$ hybrid mice which were $19.3 \pm 6.3 \times 10^{-4}$ in.

§ $P < 0.005$ as compared with mice given control cells.

‖ $P < 0.05$ as compared with mice given control cells.

were then primed with 100 μg NP-BGG, NIP-BGG, or BSA in CFA. 6–7 d after priming, animals were challenged with NP-BSA, NIP-BSA, or BSA. DTH responses were determined 24 h after challenge by measurement of footpad swelling.

As seen on Table VI, NP-suppressor cells from B10.BR donors suppress responses to NP-BSA or NIP-BSA, after NP-BGG priming. Furthermore, the response to NIP-BSA after NIP-BGG priming is also suppressed. This is the same fine specificity pattern as previously noted with C57BL/6 mice (Table III). However, CBA-strain NP suppressor cells are unable to suppress NIP-BGG primed responses, despite their ability to significantly suppress NP-BGG primed responses (Table VI). This correlates with the inability of anti-$NP^b$ anti-idiotypic antiserum to remove the suppressive activity of CBA NP-suppressor cells.

Additional experiments were carried out to determine if the fine specificity of NP-suppressor cells mapped to the heavy-chain linkage group. C.B-20 and BALB/c mice are genetically identical except that C.B-20 strain mice bear the heavy-chain linkage group of C57BL mice, $Ig\text{-}1^b$. As seen in Table VI, C.B-20 NP suppressor cells suppress NIP-BGG-primed responses, whereas BALB/c NP-suppressor cells do not. Thus, the gene(s) controlling the ability of NP-suppressor cells to suppress the NIP-primed DTH response are linked to the heavy-chain linkage group of the $Ig\text{-}1^b$ allotype.

The data in Table VI also indicate that (BALB/c × C57BL/6)$F_1$ hybrids (produced between a strain with NIP-cross-reactive NP suppressors, C57BL/6, and a noncross-reactive strain, BALB/c) are phenotypically cross-reactive. It is important to note that

only partial suppression was obtained with $F_1$ NP suppressor cells on the NIP-induced DTH response. Suppression of NIP-BGG primed responses with NP suppressor cells is therefore considered a codominant phenotype in the $F_1$ strain combination tested.

Finally, mice of the SJL strain were studied. As a result of a defect in λ-chain production, SJL mice do not make idiotype bearing, heteroclitic anti-NP antibody responses, despite possessing the Ig-$1^b$ heavy-chain allotype. To compare the fine specificity of the B cell and suppressor T-cell responses of this strain, NP-suppressor cells were transferred to mice which were then challenged with NP-BGG, NIP-BGG, or BSA. These NP-induced suppressor cells were able to cross-reactively suppress the NIP-BGG-primed DTH response. The specificity of the SJL NP suppressor cells was tested by attempting to suppress a BSA DTH response. As is indicated in Table VI, the NP-suppressor cells were unable to suppress the BSA response.

## Discussion

The experiments described demonstrate the ability of intravenously administered NP-derivatized syngeneic spleen cells to induce suppressor T cells (Tables I and II). The administration of antigen-conjugated syngeneic cells has been shown to induce various states of nonresponsiveness (25–32). Several studies have implicated suppressor T cells in such states of nonresponsiveness (29–32). The data in Table II indicate that in the NP system, suppressor T cells may be isolated from the spleens of animals given syngeneic NP-derivatized cells intravenously.

It has previously been shown that in certain strains of mice, in particular those bearing the Ig-$1^b$ heavy-chain linkage group, such as C57BL/6 or SJL, immunization of cyclophosphamide-pretreated mice with NP-BGG sensitizes for elicitation of a DTH response with NP-BSA or NIP-BSA (5). Administration of syngeneic NP-induced suppressor cells to mice of these strains on the day of priming with NP-BGG caused specific suppression of responses elicited with either NP-BSA or NIP-BSA (Tables III, VI). The mode of action of these suppressor cells has not been addressed in this work. However, an inference may be made from the effect of NIP-induced suppressor cells on the NP-BGG response. The NIP-induced suppressor cells, if given to C57BL/6 mice on the day of priming with NP-BGG, caused selective suppression of the heteroclitic DTH response elicited with NIP-BSA, but did not affect the DTH response that was elicited with NP-BSA. The NIP-suppressor cells may divert the entire NP-BGG response in such a way as to eliminate clones of T cells with cross-reactivity to NIP. Alternatively, the normal NP-BGG response may lead to the maturation of separate clones of T cells with reactivity for NP alone or the NIP alone. The NIP-suppressor cell would then block inhibition or function of only the NIP-reactive clones. In preliminary experiments, NIP-suppressor cells transferred intravenously on the day of challenge to C57BL/6 mice inhibited responses with either NP-BSA or NIP-BSA (J. Z. Weinberger, B. Benacerraf, and M. E. Dorf, unpublished observations). Thus, it seems more likely that NIP suppressors, given at the time of antigen priming, effectively divert the NP-BGG response and allow maturation of a population of distinct NP-reactive T cells with no cross-reactivity for NIP. However, when NIP-suppressor cells are given after the maturation of T-cell clones, the majority of which are reactive with both NP and NIP, the inhibition of NIP-reactive clones is phenotypically expressed as an inhibition of NP and NIP reactivity.

Examination of the specificity of the NP-suppressor cell (Table III) indicates that

the suppressor cell has a fine specificity comparable to the effector cell; NP-induced C57BL/6 suppressor cells not only influenced responses after NP-BGG priming, but also effectively suppressed responses induced after NIP-BGG priming. This observation suggested that, possibly, similar genes control the specificity of the anti-NP antibodies, NP-specific DTH effector T cells, and NP-suppressor T cells or their products.

As we approached further studies on the fine specificity of the NP-suppressor T cell, experiments were also carried out to examine the question of whether the NP-suppressor cell could be enriched or purified, in a manner analogous to that reported in other systems (24, 33). Table IV indicates that a fraction of spleen cells from animals immunized 7 d previously with NP-derivatized spleen cells isolated on NP-BSA-coated Petri dishes could efficiently induce suppression of an NP response in an adoptive syngeneic host. The adherent NP-suppressor T cells were ≅50-fold enriched in NP-suppressive activity as compared with the whole suppressor T-cell population. The nonadherent fraction of cells was depleted of detectable suppressive activity. Quantitatively, all the suppressive activity of the original T-cell population was accounted for in the adherent cell population. Thus, the suppression-generating cell population was effectively purified by this two-step method. Although this population was termed the NP-binding suppressor T cells other possibilities cannot be excluded, e.g., these cells may be suppressor precursors, feedback helper cells, or some other functional T-cell subset. Additional experiments are required to elucidate the nature of this cell population.

Several further points of interest concerning the purification of NP-suppressor cell populations were noted. NP-suppressor cells were found to adhere to NP-BSA-coated dishes in the absence of H-2 antigens. This suggests that these cells do not have an obligate requirement for binding NP in association with an MHC antigen. Another interesting point was noted when dose-response experiments were performed with the NP-binding T-cell population titrated for suppressive activity in NP-BGG-primed mice and in NIP-BGG-primed mice. The data in Table IV indicate that the NP-suppressor cell population was roughly 10-fold more efficient in suppressing responses after NIP-BGG priming than those after NP-BGG priming. This is similar to the relative affinities of C57BL/6 primary anti-NP antibodies for NP as compared with NIP (10). In analogy to the antibody situation, we term NP-suppressor cells which significantly suppress responses after NIP-BGG priming heteroclitic.

Having noted the heteroclitic fine specificity of C57BL/6 NP suppressor cells, we proceeded to investigate whether there was detectable $NP^b$ idiotype on suppressor cells of various strains (Table V). B10.BR and SJL strain mice bear the Ig-$1^b$ heavy-chain linkage group; the suppressor cells from these strains were susceptible to inactivation by treatment with guinea pig anti-$NP^b$ plus complement. NP-suppressor cells from the CBA strain of a different heavy-chain immunoglobulin allotype, Ig-$1^j$, were not susceptible to inactivation by anti-$NP^b$ plus complement.

Although the most likely interpretation of these experiments is that NP-suppressor cells bear idiotypic determinants in common with anti-NP antibodies, several caveats are necessary. Although the immunogen for the preparation of anti-idiotype consisted of ammonium sulfate precipitated, NP-affinity column purified, primary C57BL/6 anti-NP antibodies, it is possible that a T-cell product copurified with the antibodies, and thus the immunogen might contain T-cell factor or T-cell receptor. In this regard

it is noteworthy that the anti-idiotype used in these experiments was effective against NP-suppressor cells from mice of differing H-2 haplotypes, namely SJL ($H\text{-}2^s$) and B10.BR ($H\text{-}2^k$), but did not eliminate NP-suppressor cells obtained from another $H\text{-}2^k$ bearing strain, CBA. Thus, if anti-T-cell product antibodies exist in the anti-$NP^b$ antiserum, these antibodies do not recognize H-2 determinants on these molecules.

Because of some of the aforementioned inherent difficulties in the serological approach to characterization of NP-suppressor T cells, an independent set of experiments was carried out to elucidate the genetic control of the fine specificity of these cells (Table VI). In the case of the antibody response, presence of $NP^b$ idiotype in the serum correlates with demonstrable heteroclitic antibody (12, 13). It was first noted that B10.BR ($Ig\text{-}1^b$ heavy-chain allotype) strain NP-suppressor cells were cross-reactive, or heteroclitic, in possessing the ability to suppress NIP priming; CBA ($Ig\text{-}1^j$ heavy-chain allotype) NP-suppressor cells were not, however, similarly cross-reactive. Furthermore, when NP-suppressor cells originating from allotype congenic BALB/c ($Ig\text{-}1^a$) and C.B-20 ($Ig\text{-}1^b$) strain mice were examined, only the C.B-20 NP-suppressor cells were heteroclitic. Thus, genes in the heavy-chain linkage group control the expression of the heteroclitic fine specificity for NP-suppressor cells, as well as DTH-effector cells (5), and primary anti-NP antibody (12). Thus, the genetic data on fine specificity of NP-induced suppression (Table VI) taken together with the serological data demonstraing that anti-$NP^b$ anti-idiotypic serum plus complement can specifically eliminate the ability of splenic T cells of NP-suppressed mice carrying the $Ig\text{-}1^b$ allotype genes to transfer NP-specific suppression (Table V), imply that $V_H$-region gene(s) control the fine specificity of the NP suppressor T-cell population. Together with our previous report (5) the data demonstrate two populations of NP-specific idiotype-positive T cells; the first is an idiotype positive, MHC-restricted T cell in the DTH effector pathway, and the second, is the suppressor cell dealt with here, which also bears idiotypic determinants.

A further point noted in the strain distribution study on the fine specificity of NP suppressors is that an $F_1$ hybrid between nonheteroclitic and heteroclitic mouse strains, (BALB/c × C57BL/6)$F_1$, when given NP-derivatized $F_1$ spleen cells intravenously, responds with the development of cross-reactive NP suppressors. Again, this is analogous to the situation observed for antibody (10), and effector DTH function (5).

SJL strain mice, when immunized with NP-protein conjugates, do not make idiotype positive, heteroclitic anti-NP antibodies (12, 13). This is in spite of their possession of the requisite $Ig\text{-}1^b$ allotype-linked $V_H$ genes coding for the $NP^b$ idiotype. It has been theorized that SJL mice, defective in adequate production of λ-light immunoglobulin chains, cannot make idiotypic antibody because of this defect. Tables V and VI demonstrate that at the T-cell level, NP-induced suppressor T cells of SJL origin possess idiotypic determinants and have a heteroclitic fine specificity in common with other mouse strains of the $Ig\text{-}1^b$ allotype which do produce heteroclitic, idiotypic anti-NP antibody responses. This observation has also been made for the DTH effector T-cell population of the SJL strain (5). This implies one of two possibilities: (*a*) the T cells subsets examined do not use conventional λ-light chains in the make up of their receptors, or (*b*) these T cells may have a method of circumventing the inability of the B cell to produce adequate amounts of λ-light chain. Finally, the

data from SJL strain suppressor cells also implies that the T-cell receptor described functionally in this paper must be produced by the T cell itself rather than passively absorbed by the T cell from serum immunoglobulin.

The mechanism of NP-induced suppression remains to be elucidated. Several possible mechanisms can be considered at this time. NP suppressor cells can interact via an antigen bridge or an antigen-containing factor bridge to select appropriate effector clones to inactivate. This may occur at the induction phase of sensitization or the efferent response phase of the response. Another possibility is that the NP suppressors induce another cell population with specificity for the idiotypic determinants on the effector cell. The effects of such suppressor cells have been established in the idiotype-regulated system described by Eichmann and associates (34, 35). These investigators reported that idiotype-specific suppression induced early in the maturation of the immune response leads to maturation of clones of T or B cells, specific for the original antigen, yet not bearing the original idiotype (34, 35). However, this possibility is less likely in the NP system because administration of the NP-suppressor cell at the day of priming interferes with the complete NP-BGG induced response; there seems to be no diversion of the response to one of a nonheteroclitic nature. That such a diversion could lead to expression of clones of T cells with nonheteroclitic fine specificity can be seen by the effect of NIP suppressors given to C57BL/6 mice on the day of priming with NP-BGG (Table III).

In summary, we have demonstrated by idiotypic, genetic, and fine specificity analyses that NP-induced suppressor cells bear an idiotypic receptor. The direct demonstration of idiotypic structures on such T-cell subpopulations supports the network theory of immunoregulation (36).

## Summary

The ability of NP-coupled syngeneic spleen cells to induce antigen-specific T-suppressor cells capable of binding to NP-BSA-coated Petri dishes and mediating transfer of specific suppressive activity to NP was demonstrated. Furthermore, in strains of mice bearing the Ig-$1^b$ allotype, including SJL, and in (non-Ig-$1^b$ × Ig-$1^b$)$F_1$ hybrids, the NP-specific suppressor cell also interferes with expression of immunity after priming with NIP-BGG. Anti-$NP^b$ anti-idiotype antiserum plus complement treatment effectively abrogated the ability to transfer suppression. Formal genetic mapping of the fine specificity of cross-reactivity with Ig-1 allotypic congenic mice implies that expression of this trait is linked to the Ig-$1^b$ heavy chain linkage group. The sensitivity of NP-suppressor cells of appropriate strains to anti-idiotype treatment was also consistent with the formal mapping data. These experiments suggest that there are shared V-region structures on antibody and T cells that are crucial in the suppression pathway for the same antigen.

We wish to thank Ms. Ofra Weinberger for helpful discussions and Ms. Terri Greenberg for expert secretarial assistance.

*Received for publication 17 May 1979.*

## References

1. Binz, H., and H. Wigzell. 1977. Antigen-binding, idiotypic T-lymphocyte receptors. *Contemp. Top. Immunobiol.* **7**:113.

2. Rajewsky, K., and K. Eichmann. 1977. Antigen receptors of T helper cells. *Contemp. Top. Immunobiol.* **7**:69.
3. Cosenza, H., M. H. Julius, and A. A. Augustin. 1977. Idiotypes as variable region markers: analogies between receptors on phosphorylcholine-specific T and B lymphocytes. *Immunol. Rev.* **34**:3.
4. Cazenave, P.-A., J. M. Cavillon, and C. Bona. 1977. Idiotypic determinants on rabbit B- and T-derived lymphocytes. *Immunol. Rev.* **34**:34.
5. Weinberger, J. Z., M. I. Greene, B. Benacerraf, and M. E. Dorf. 1979. Hapten specific T cell responses to 4-hydroxy-3-nitrophenylacetyl I. Genetic control of delayed-type hypersensitivity by $V_H$ and I-A region genes. *J. Exp. Med.* **149**:1336.
6. Nisonoff, A., S.-T. Ju, and F. L. Owen. 1977. Studies of structure and immunosuppression of a cross-reactive idiotype in Strain A mice. *Immunol. Rev.* **34**:89.
7. Eichmann, K. 1974. Idiotype suppression. I. Influence of the dose and of the effector functions of anti-idiotypic antibody on the production of an idiotype. *Eur. J. Immunol.* **4**: 296.
8. Germain, R. N., S.-T. Ju, T. J. Kipps, B. Benacerraf, and M. E. Dorf. 1979. Shared idiotypic determinants on antibodies and T-cell-derived suppressor factor specific for the random terpolymer L-glutamic acid$^{60}$-L-alanine$^{30}$-L-tyrosine$^{10}$. *J. Exp. Med.* **149**:613.
9. Bach, B. A., M. I. Green, B. Benacerraf, and A. Nisonoff. 1979. Mechanism of regulation of cell mediated immunity. IV. Azobenzene-arsonate specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy chain allotype linkage group of genes. *J. Exp. Med.* **149**:1084.
10. Imanishi, T., and O. Mäkelä. 1974. Inheritance of antibody specificity. I. Anti-(4-hydroxy-3-nitrophenyl)acetyl of the mouse primary response. *J. Exp. Med.* **140**:1498.
11. McMichael, A. J., J. M. Phillips, A. R. Williamson, T. Imanishi, and O. Mäkelä. 1975. Inheritance of an isoelectric focused spectrotype linked to the Ig-1$^b$ allotype. *Immunogenetics.* **2**:161.
12. Karjalainen, K., and O. Mäkelä. 1978. A mendelian idiotype is demonstrable in the heteroclitic anti-NP antibodies of the mouse. *Eur. J. Immunol.* **8**:105.
13. Jack, R. S., T. Imanishi-Kari, and K. Rajewsky. 1977. Idiotypic analysis of the response of C57BL/6 mice to the (4-hydroxy-3-nitrophenyl)acetyl group. *Eur. J. Immunol.* **7**:559.
14. Mäkelä, O., and K. Karjalainen. 1977. Inherited immunoglobulin idiotype of the mouse. *Immunol. Rev.* **34**:119.
15. Gechler, W., J. Faversham, and M. Cohn. 1978. On a regulatory gene controlling the expression of murine λ, light chain. *J. Exp. Med.* **148**:1122.
16. Krawinkel, U., M. Cramer, T. Imanishi-Kari, R. S. Jack, K. Rajewsky, and O. Mäkelä. 1977. Isolated hapten-binding receptors of sensitized lymphocytes. I. Receptors from nylon wool-enriched mouse T lymphocytes lack serological markers of immunoglobulin constant domains but express heavy chain variable portions. *Eur. J. Immunol.* **7**:566.
17. Reth, M., T. Imanishi-Kari, R. S. Jack, M. Cramer, U. Krawinkel, G. J. Hämmerling, and K. Rajewsky. 1977. The Immune System: Genes and the Cells in Which They Function. *ICN-UCLA Symp. Mol. Cell. Biol.* **8**:139.
18. Williams, C. A. 1971. *In* Methods of Immunology and Immunochemistry. C. A. Williams and M. W. Chase, editors. Academic Press, Inc., New York. 463.
19. Averameas, S., and Ternynck, T. 1969. The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry.* **6**:53.
20. Ju, S.-T., T. J. Kipps, J. Theze, B. Benacerraf, and M. E. Dorf. 1978. Idiotype analysis of anti-GAT antibodies. I. Presence of common idiotypic specificities in both responder and nonresponder mice. *J. Immunol.* **121**:1034.
21. Kipps, T. J., B. Benacerraf, and M. E. Dorf. 1977. Presence of common idiotypes on antibodies induced by glutamic acid-lysine-containing terpolymers in responder and non-responder mice with the Ig-1$^b$ heavy chain allotype. *Eur. J. Immunol.* **7**:865.

22. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocytes with and without surface immunoglobulin: Preparative scale separation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* **15:**47.
23. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1974. Purification of T cells in high yield using nylon wool columns. *Eur. J. Immunol.* **3:**645.
24. Taniguchi, M., and J. F. A. P. Miller. 1977. Enrichment of specific suppressor T cells and characterization of their surface markers. *J. Exp. Med.* **146:**1450.
25. Battisto, J. R., and B. R. Bloom. 1966. Dual immunological unresponsiveness induced by cell membrane coupled hapten or antigen. *Nature (Lond.).* **212:**156.
26. Asherson, G. L., and M. Zembala. 1974. Suppression of contact sensitivity by T cells in the mouse. I. Demonstration that suppressor cells act on the effector phase of contact sensitivity; and their induction following in vitro exposure to antigen. *Proc. R. Soc. Lond. B Biol. Sci.* **187:**329.
27. Miller, S. D., and H. N. Claman. 1976. The induction of hapten-specific T cell tolerance by using hapten-modified lymphoid cells. I. Characteristics of tolerance induction. *J. Immunol.* **117:**1519.
28. Long, C. A. R., and D. W. Scott. 1977. Role of self-carriers in the immune response and tolerance. II. Parameters of tolerance induced by haptenated lymphoid cells. *Eur. J. Immunol.* **7:**1.
29. Moody, C. E., J. B. Innes, G. W. Siskind, and M. E. Weksler. 1978. Tolerance induced by TNP-derivatized syngeneic erythrocytes: evidence for cooperation between hapten-specific T and hapten-specific B lymphocytes in the immune response. *J. Immunol.* **120:**844.
30. Miller, S. D., M. Sy, and H. N. Claman. 1977. H-2 restriction of suppressor T-cell induction by hapten-modified lymphoid cells in tolerance to 1-fluoro-2,4-dinitrobenzene contact sensitization. *J. Exp. Med.* **145:**1071.
31. Scott, D. W. 1978. Role of self carriers in the immune response and tolerance. III. B cell tolerance induced by hapten-modified self involves both active T cell mediated suppression and direct blockade. *Cell Immunol.* **37:**327.
32. Cheung, N.-K., D. H. Sherr, K. M. Heghinian, B. Benacerraf, and M. E. Dorf. 1978. Immune suppression in vivo with antigen-modified syngeneic cells. I. T-cell-mediated suppression to the terpolymer poly-(Glu, Lys, Phe)$_n$. *J. Exp. Med.* **148:**1539.
33. Lewis, G. K., and J. W. Goodman. 1978. Purification of functional, determinant-specific, idiotype-bearing murine T cells. *J. Exp. Med.* **148:**915.
34. Hetzelberger, D., and K. Eichmann. 1978. Recognition of idiotypes in lymphocyte interactions. I. Idiotypic selectivity in the cooperation between T and B lymphocytes. *Eur. J. Immunol.* **8:**846.
35. Eichmann, K., I. Falk, and K. Rajewsky. 1978. Recognition of idiotypes in lymphocyte interactions. II. Antigen-independent cooperation between T and B lymphocytes that possess similar and complementary idiotypes. *Eur. J. Immunol.* **8:**853.
36. Jerne, N. K. 1974. Towards a network theory of the immune system. *Ann. Immunol. (Paris).* **125C:**373.