I-J RESTRICTIONS ON THE ACTIVATION
AND INTERACTION OF PARENTAL AND
F1-DERIVED T3 SUPPRESSOR CELLS*

BY MUTSUHIKO MINAMI, SHUICHI FURUSAWA, AND MARTIN E. DORF

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

Data from a variety of systems indicate that several distinct populations of T lymphocytes are involved in the process of immune suppression (1–3). These suppressor T cells (T3) function in a defined sequence. The nature of these cells and the T3-derived factors (T3F) involved in the suppressor pathway have not been fully resolved, but in at least two independent systems three separate T3 populations have been identified (4–6). These T3 populations have been termed T31, T32, and T33. Many of the T3 described in the literature have properties similar to one of these three populations. Although it is difficult to classify all T3 reported in this simplified suppressor cell cascade, many of the discrepancies might reflect differences in the various assay conditions used rather than implying the existence of several totally distinct suppressor cell pathways.

One of the most frequently defined T3 cell types appears to correspond to the T3a population identified in the 4-hydroxy-3-nitrophenyl acetyl (NP) suppressor system. This suppressor cell population is derived from antigen-primed mice, may represent the final or effector cell in the T3 pathway, has the Lyt 1−, Lyt 2+, I-J+ phenotype, and produces a soluble T3F that may under selected conditions be nonspecific (4, 7). T3 cells that fit most of these criteria have also been identified in the azobenezearsonate (6, 8), dinitrophenyl (9), trinitrophenyl (10), keyhole limpet hemocyanin (11), and sheep erythrocyte (12) systems.

This report focuses on the mechanism of T3a cell activation and the specificity of T3a cells, especially those obtained from F1 hybrid mice. The NP suppressor system was chosen to study these parameters because the methods for assaying T3a activity independent of T31 or T32 activity had been established (4, 5). Furthermore, we previously characterized (13) suppressor factors (T3F2) derived from a series of monoclonal T3a hybridomas that could be used to activate T3a cells. The present data demonstrate that the suppressive activity of the T3a population is not manifest unless these cells are specifically activated by T3F2. Furthermore, the data suggest that

* Supported in part by grant SO 7 RR 05381-20 awarded by the Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health, and by grant CA-14723 from the National Cancer Institute, and a grant from the Cancer Research Institute.

1 Abbreviations used in this paper: CS, cutaneous sensitivity; CY, cyclophosphamide; DMSO, dimethyl sulfoxide; DNFB, 2,4-dinitrofluorobenzene; HBSS, Hanks' balanced salt solution; NP, 4-hydroxy-3-nitrophenyl acetyl hapten; NPb, common idiotype on C57BL anti-NP antibodies; NP-O-Su, NP-O-succinimide ester; PBS, phosphate-buffered saline; T31, T32, T33, first, second, or third order suppressor T cells, respectively; T3, suppressor T cells; T3F1, T3F2, T3F3, suppressor factor derived from T31, T32, or T33, respectively.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/82/08/0465/15 $1.00
Volume 156 August 1982 465–479
distinct clones of F1-derived suppressor cells are restricted to each parental H-2 haplotype. Thus, Ts cells, like helper T cells, appear to be restricted in their ability to recognize antigen in the context of major histocompatibility complex gene products, but in the Ts pathway, antigen may be associated with I-J products instead of products of the I-A or I-E loci.

Materials and Methods

Mice. All mice were either purchased from The Jackson Laboratory, Bar Harbor, ME, or were bred in the animal facilities at Harvard Medical School, Boston, MA. Mice were used at 3-12 mo of age and were maintained on laboratory chow and acidified, chlorinated water ad lib.

Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication (NIH) 78-23, revised 1978).

Antigens. NP-O-Succinimide (NP-O-Su) was purchased from Biosearch Co., San Rafael, CA. Dimethylsulfoxide (DMSO) was purchased from Fisher Scientific Co., Pittsburgh, PA. 2,4-dinitro-1-fluorobenzene (DNFB) was obtained from Eastman Kodak Co., Rochester, NY.

Antisera. Both B10.A(3R) anti-B10.A(5R) (anti-I-Jk) and B10.A(5R) anti-B10.A(3R) (anti-I-Jb) were produced by immunization with spleen and lymph node cells as described elsewhere (14).

Treatment of Lymph Node Cells with Anti-I-J Antisera and Complement. 7.5 × 10^7 NP-immune lymph node cells were pelleted and incubated in 1.0 ml of a 1:5 dilution of B10.A(3R) anti-B10.A(5R) (anti-I-Jk) or B10.A(5R) anti-B10.A(3R) (anti-I-Jb) antisera. After 30 rain at room temperature, cells were spun and resuspended in 1.0 ml of rabbit complement diluted 1:5 or 1:8 in Hanks’ balanced salt solution. After an additional 30-min incubation at 37°C, the cells were washed three times and then activated with TsF2, as detailed below.

In Vitro Activation of NP-primed Lymph Node Ts3 Cells with TsF2. Regional lymph node cells from mice that had been immunized subcutaneously with 2 mg NP-O-Su were used as the source of Ts3 cells. B6-Ts2-28 and CKB-Ts2-59-derived TsF2, which have been characterized and described (13), were used for activation of lymph node Ts3 cells in vitro. 5 × 10^7 NP-primed lymph node cells were cultured for 2 or 48 h in 10 ml RPMI 1640 with 10% fetal calf serum and 0.1 mM Hepes plus 50 μl TsF2 ascites fluid derived from B6-Ts2-28, CKB-Ts2-59, or BW5147 cells that were grown in (AKR × B6)F1, (AKR × CKB)F1, or AKR mice, respectively. After culture, these activated lymph node cells were washed three times with Hanks’ balanced salt solution and resuspended.

Functional Analysis of the Activated NP-primed Lymph Node Ts3 Cells in Cyclophosphamide-treated Antigen-primed Mice. Mice were primed subcutaneously with 2 mg of NP-O-Su in DMSO on day 0, as described elsewhere (15). 24 h later, they were treated with an intraperitoneal injection of 20 mg/kg cyclophosphamide (CY) in saline. On day 6, each mouse received intravenously 1 × 10^7 NP-primed lymph node cells activated with TsF2 or control BW5147 factors, as described above, or received 0.5 ml of TsF2 or control BW5147 factors. Immediately after transfer, mice were challenged in the left footpad with 0.025 ml PBS solution containing 30 μg of NP-O-Su (prepared by mixing 25 μl of a 2% NP-O-Su/DMSO solution in 0.4 ml PBS). Footpad swelling was measured 24 h later. Swelling was determined as the difference, in units of 10^-3 cm, between the left and right footpad thickness. It should be noted that 1 × 10^7 immune lymph node cells are not sufficient to transfer immunity under these experimental conditions.

DNFB Contact Sensitivity Responses. Contact sensitivity was induced by two daily paintings on the shaved abdomen with 25 μl of 0.5% DNFB solution in acetone: olive oil (4:1) (16). 5 d after the last painting, 20 μl of 0.2% DNFB in the same vehicle was applied to the left ear, and the ear swelling was measured as the difference between the left and right ear thicknesses.

Double Antigen Ear Challenge. Individual mice were immunized with either DNFB alone or DNFB + NP-O-Su, as described above. Mice were challenged in the left ear by painting with 0.2% DNFB, injecting 0.015 ml containing 6 μg NP-O-Su (prepared by mixing 0.025 ml of
0.7% NP-O-Su in DMSO with 0.4 ml PBS, pH 7.7), or with both antigens. The incremental ear swelling was measured 24 h thereafter. The concentration and volume of NP-O-Su used to challenge was predetermined to elicit high specific ear swelling and low nonspecific backgrounds.

**Percent Suppression.** The percent suppression in the present study was calculated by the following formula: percent suppression = 100 × [(swelling of group receiving Tss cells activated with BW5147 tumor ascites - swelling of group receiving Tss cells activated with TsF2)/ (swelling of group receiving Tss cells activated with BW5147 tumor ascites - swelling of unprimed group)].

**Data Analysis.** Statistical analysis of the experimental data with respect to controls was calculated using the two-tailed Student’s t test.

**Results**

*In Vitro Activation of Tss Cells.* To demonstrate that Ts2-derived factor could activate Tss cells, we took advantage of past observations on the biological properties of the Tss cell population. Thus, it was previously shown that the Tss population was sensitive to (CY) treatment and, furthermore, that lymph node cells from antigen-primed mice could be used in adoptive transfer experiments to restore Tss activity to the CY-treated recipients (4). To directly activate Tss cells, we incubated 0.05 ml of BW5147 control or Ts2 hybridoma-derived ascites with 5 × 10⁷ NP-O-Su-primed lymph node cells in 10 ml of RPMI 1640 media containing 10% fetal calf serum. The cells were cultured for 48 h at 37° in 10% CO₂. After 48 h of in vitro culture, the cells were washed extensively, and 1 × 10⁷ viable lymph node cells were injected intravenously into syngeneic recipients that had been previously primed with NP-O-Su and treated 24 h later with 20 mg/kg CY. In confirmation of previous findings (5), CY-treated recipients were not sensitive to suppression by monoclonal B6-Ts2-28 or CKB-Ts2-59 suppressor factor (Table 1). However, significant suppression of the cutaneous sensitivity (CS) response was observed when CY-treated recipients were given lymph node cells derived from NP-O-Su-primed C57BL/6 mice that were activated in vitro with B6-Ts2-28-derived TsF2. As specificity controls, factors from the BW5147 tumor line or from the CKB-Ts2-59 line failed to activate suppressive activity in these cells. The failure of CKB-Ts2-59-derived TsF2 to activate C57BL/6 antigen-primed lymph node cells is presumably due to the H-2-linked (I-J) genetic restriction of TsF2 (13). Thus, the B6-Ts2-28 factor that is derived from C57BL/6 (H-2b, Ighb) cells is only active in recipients that are matched at the I-J and Igh regions (13). The CKB-Ts2-59 factor is of CKB (H-2k, Ighk) origin and is also genetically restricted by I-J and Igh genes. To verify that the CKB-Ts2-59 factor was capable of activating antigen-primed lymph node cells of the appropriate strain, a reciprocal experiment was performed. As shown in Table I, the CKB-Ts2-59 factor activated Tss suppressive activity when incubated with H-2 and Igh-matched B10.BR lymph node cells, whereas the C57BL/6-derived Ts2 factor failed to induce suppression under the same experimental conditions.

**Kinetics of Tss Activation.** Lymph node cells from C57BL/6 mice were cultured with B6-Ts2-28 or control BW5147-derived factors for various intervals ranging from 5 min to 48 h. The cells were then washed and assayed for suppressive activity in NP-O-Su-primed CY-treated C57BL/6 recipients. As shown in Fig. 1, maximum suppressive activity was noted after 1-2 h of in vitro activation with TsF2. Activation of Tss cells with TsF2 for up to 48 h did not result in an increased level of immune suppression.

**Specificity of In Vitro Activated Tss Cells.** The specificity of in vitro activated Tss cells
ACTIVATION AND INTERACTION OF T₈ SUPPRESSOR CELLS

### Table I

| T₈F source | Donor of NP-primed lymph node cells | NP-primed, CY-treated recipients | Footpad swelling ± SE (10⁻³ cm) |
|------------|-------------------------------------|---------------------------------|-------------------------------|
| BW5147     | None                                | C57BL/6                         | 38.8 ± 1.4                    |
| B6-T₈-28   | None                                | C57BL/6                         | 38.0 ± 2.0                    |
| BW5147     | C57BL/6                             | C57BL/6                         | 37.5 ± 1.0                    |
| B6-T₈-28   | C57BL/6                             | C57BL/6                         | 15.8 ± 1.8‡                   |
| CKB-T₈-59  | C57BL/6                             | C57BL/6                         | 35.0 ± 3.8                    |
| BW5147     | None                                | B10.BR                          | 27.0 ± 1.8                    |
| CKB-T₈-59  | None                                | B10.BR                          | 28.0 ± 2.6                    |
| BW5147     | B10.BR                              | B10.BR                          | 26.3 ± 1.3                    |
| CKB-T₈-59  | B10.BR                              | B10.BR                          | 14.3 ± 1.2‡                   |
| B6-T₈-28   | B10.BR                              | B10.BR                          | 27.3 ± 1.7                    |

* Regional lymph node cells from mice that had been immunized subcutaneously with 2 mg NP-O-Su were cultured for 48 h with T₈F₂ or control BW5147 ascites for activation, then washed and transferred to designated recipients. Groups of recipient mice were immunized with 2 mg NP-O-Su. 24 h later, they were treated with intraperitoneal injections of 20 mg/kg CY. On day 6, each mouse received 1 X 10⁷ activated NP-primed lymph node T₈ cells, and the recipients were challenged after cell transfer. The data were expressed as the increment of footpad swelling ± SE in units of 10⁻³ cm. The background response of nonimmunized C57BL/6 mice was 12.5 ± 1.3 and that of B10.BR was 7.3 ± 1.1.

‡ Significant suppression, P < 0.001.

---

**Fig. 1.** Kinetics of in vitro activation of lymph node cells from NP-O-Su-primed mice with T₈F₂. C57BL/6 mice were immunized with 2 mg NP-O-Su. After 6 d, the regional lymph nodes were removed, teased, and the cells were cultured for 5 min to 48 h with T₈F₂ or control BW5147 ascites for activation. The T₈ cells were then washed and used for transfer. Groups of recipient mice were immunized with 2 mg NP-O-Su. 24 h later, the recipients were treated with an intraperitoneal injection of 20 mg/kg CY. On day 6, each mouse received 1 X 10⁷ activated NP-primed lymph node T₈ cells intravenously. The mice were then challenged. The data represent pooled results from two separate experiments. The data were normalized and the percent suppression ± SE was calculated.

● T₈F₂, ○, BW.
was evaluated in two ways. First, NP-O-Su or DNFB antigen-primed C57BL/6 lymph node cells were used as the source of Ts3 cells for activation with TsF2. Second, these activated cells were tested for suppressive activity in syngeneic C57BL/6 recipients primed with either DNFB or DNFB + NP-O-Su. In these experiments the mice were challenged by injection of NP-O-Su into the left ear pinna or by painting the left ear with DNFB or both. The control right ear was untreated. As shown in Table II, the only condition in which significant levels of suppression were observed was when hybridoma-derived TsF2 was used to activate Ts3 cells from NP-O-Su-primed mice and when these activated Ts3 cells were tested in animals primed and challenged with NP-O-Su. The suppression was not due to the carry over of B6-Ts2-28 factor because intravenous injection of TsF2 did not suppress CY-treated recipients (Table II). NP-specific TsF2 would not activate lymph node cells from DNFB-primed mice, even when these cells were tested in DNFB-primed and challenged recipients. Furthermore, there is no apparent suppression of a bystander DNFB response when activated Ts3 cells are transferred to recipients that had been either doubly primed or challenged with DNFB + NP-O-Su (Table II).

**Genetic Restrictions on Ts3 Cell Activation and Function.** One of the advantages of activating Ts3 cells in vitro is that it permits independent analysis of the genetic restrictions for Ts3 activation and Ts3-target cell interactions. Control BW5147, C57BL/6 (H-2b, Ighb), and CKB (H-2k, Ighb)-derived TsF2 were incubated with C57BL/6, B10.BR (H-2k, Ighb), CKB, or C3H (H-2k, Ighb) NP-O-Su-primed lymph node cells. Ts3 activation was assessed by adoptively transferring the in vitro activated

| Table II | Specificity of in Vitro Ts3 Cell Activation* |
|----------|---------------------------------------------|
|          | 48-h Ts3 activation                         |
|          | Tsf2 source | Antigen for Ts3 priming | Priming of CY-treated recipient | Antigen for ear challenge |
|          |             |                           | NP-O-Su | DNFB | NP-O-Su + DNFB |
| BW5147 (i.v.) | — | NP + DNFB | 16.3 ± 0.3 | 10.8 ± 0.3 |
| B6-Ts2-28 (i.v.) | — | NP + DNFB | 17.3 ± 0.5 | NT‡ |
| BW5147 | NP-O-Su | NP + DNFB | 16.5 ± 0.6 | 10.5 ± 0.3 |
| B6-Ts2-28 | NP-O-Su | NP + DNFB | 8.3 ± 1.0§ | 10.3 ± 0.5 |
| BW5147 | DNFB | NP + DNFB | 14.3 ± 3.4 | 10.0 ± 1.1 |
| B6-Ts2-28 | DNFB | NP + DNFB | 18.0 ± 0.4 | 11.8 ± 1.8 |
| BW5147 | — | None | 4.0 ± 0.4 | 1.0 ± 0.6 |
| BW5147 (i.v.) | — | DNFB | 18.0 ± 0.8 |
| B6-Ts2-28 (i.v.) | — | DNFB | 18.8 ± 0.5 |
| BW5147 | NP-O-Su | DNFB | 17.3 ± 0.5 |
| BW5147 | DNFB | DNFB | 16.8 ± 0.5 |
| B6-Ts2-28 | NP-O-Su | DNFB | 18.8 ± 1.2 |
| B6-Ts2-28 | DNFB | DNFB | 17.0 ± 1.2 |
| BW5147 | — | None | 5.0 ± 0.6 |

* Regional lymph node cells from mice that had been immunized with DNFB or NP-O-Su were cultured with B6-Ts2-28 or control BW5147 ascites for activation. Groups of recipient mice were primed with DNFB alone or DNFB and NP-O-Su. 24 h later, all groups were given 20 mg/kg CY. 6 d later, mice were given the in vitro activated Ts3 cells and challenged with DNFB or NP-O-Su alone or with DNFB and NP-O-Su.

‡ Not tested.
§ Significant suppression, P < 0.001.
ACTIVATION AND INTERACTION OF Ts3 SUPPRESSOR CELLS

Ts3 cells to antigen-primed CY-treated C57BL/6, B10.BR, CKB, or C3H recipients. Activated Ts3 cells were transferred during the effector phase of the CS response, i.e., on the day of antigen challenge. Such effector phase transfers minimize potential allogeneic effects because the Ts3 cells are only present in the allogeneic environment for 24 h before termination of the assay. Furthermore, the BW5147-activated Ts3 lymph node population serves as a control for nonspecific suppression. The data shown in Table III were derived from seven independent experiments that were normalized and pooled. Activation of the Ts3 population was generally assayed after 2 h of incubation with TsF2. After activation of the Ts3-containing lymph node population, suppressive activity was only noted in combinations of TsF2, Ts3, and recipients that were matched at the H-2 and IgH gene complexes. Thus, after a 2 h in vitro activation, C57BL/6 (H-2b)-derived TsF2 activated C57BL/6 but not B10.BR

| Table III |
| Genetic Restrictions on Ts3 Cell Activation and Function*‡ |
| TsF2 source | Ts3 donor | CY-treated recipients | Normalized percent suppression ± SE |
| BW5147 | C57BL/6 | C57BL/6 | 0 ± 3 (7) |
| B6-Ts2-28 | C57BL/6 | C57BL/6 | 51 ± 5 (8)‡ |
| CKB-Ts3-59 | C57BL/6 | C57BL/6 | 5 ± 7 (4) |
| BW5147 | B10.BR | C57BL/6 | 0 ± 4 (4) |
| B6-Ts2-28 | B10.BR | C57BL/6 | -5 ± 4 (4) |
| CKB-Ts3-59 | B10.BR | C57BL/6 | 2 ± 6 (4) |
| BW5147 | C57BL/6 | B10.BR | 0 ± 3 (4) |
| B6-Ts2-28 | C57BL/6 | B10.BR | 7 ± 8 (4) |
| CKB-Ts3-59 | C57BL/6 | B10.BR | 6 ± 19 (4) |
| BW5147 | B10.BR | B10.BR | 0 ± 7 (4) |
| B6-Ts2-28 | B10.BR | B10.BR | 3 ± 9 (4) |
| CKB-Ts3-59 | B10.BR | B10.BR | 55 ± 10 (4)‡ |
| BW5147 | CKB | B10.BR | 0 ± 9 (4) |
| B6-Ts2-28 | CKB | B10.BR | -1 ± 11 (4) |
| CKB-Ts3-59 | CKB | B10.BR | 80 ± 8 (4)‡ |
| BW5147 | C3H | B10.BR | 0 ± 6 (8) |
| B6-Ts2-28 | C3H | B10.BR | -4 ± 7 (7) |
| CKB-Ts3-59 | C3H | B10.BR | 2 ± 7 (8) |
| BW5147 | CKB | CKB | 0 ± 6 (9) |
| CKB-Ts3-59 | CKB | CKB | 59 ± 4 (9)‡ |
| BW5147 | CKB | C3H | 0 ± 7 (8) |
| CKB-Ts3-59 | CKB | C3H | 3 ± 5 (8) |
| BW5147 | None | C57BL/6 | 0 ± 7 (5) |
| B6-Ts2-28 | None | C57BL/6 | -3 ± 5 (4) |
| BW5147 | None | B10.BR | 0 ± 8 (5) |
| CKB-Ts3-59 | None | B10.BR | -3 ± 19 (4) |

* In vitro activation of regional lymph node Ts3 cells from NP-O-Su-primed mice with TsF2 was done as described in Materials and Methods. Activation was continued for 2 h except for one experiment in which a 48-h activation was used. Recipient mice were primed with NP-O-Su; 24 h later all mice were given 20 mg/kg cyclophosphamide, and 6 d later received 1 × 10⁷ activated Ts3 before antigen challenge. The data represent the pooled results from seven separate experiments (not all groups were included in each experiment). The data were normalized and the percent suppression ± SE was calculated. The number of mice is indicated in parentheses.

‡ Significant suppression, P < 0.01.
(H-2k) T3 cells that only functioned when adoptively transferred to syngeneic C57BL/6 recipients. Similarly, after 2 h of activation with CKB (H-2k)-derived TsF2, only B10.BR or CKB T3-containing lymph node cells were activated. Furthermore, the activated B10.BR or CKB T3 population would suppress CS responses in H-2 and Igh-matched B10.BR and CKB recipients but not in Igh-disparate C3H mice (Table III). To prove that the suppression was mediated by activated T3 cells instead of TsF2 that might have been passively transferred along with the T3 cells, we injected TsF2 intravenously into NP-O-Su-primed CY-treated recipients. As shown in Table III, administration of TsF2 without added T3 cells was unable to suppress CS responses in antigen-primed CY-treated recipients.

**I-J Restriction of Activated T3.** Because after a 2-h activation period the TsF2-T3-target cell interactions are H-2 restricted, we next asked which subregion within the H-2 complex was responsible for this genetic restriction. Based on several previous studies that indicated that suppressor cell restrictions were generally mediated through the I-J subregion (5, 13, 17), we tested two congenic strains of mice, 3R(I-Jb) and 5R(I-Jk), that only differ with respect to their I-J subregions. The data in Table IV demonstrate that using 2-h activation conditions, suppression is only observed when the TsF2-T3 and the recipient strain are matched at the I-J subregion. The controls for these experiments were similar to those used in the previous experiments and demonstrate that the results are not due to carry over of TsF2 (Table IV).

**Activation and Function of T3 Cells Derived from F1 Mice.** To further analyze the

| TsF2 source | T3 donor | NP-primed, CY-treated recipients | Normalized percent suppression ± SE |
|-------------|----------|---------------------------------|-------------------------------------|
| BW5147      | 5R       | 5R                              | 0 ± 6 (8)                           |
| B6-Tsz-28   | 5R       | 5R                              | 7 ± 5 (7)                           |
| CKB-Tsz-59  | 5R       | 5R                              | 50 ± 6 (8)‡                         |
| BW5147      | 3R       | 5R                              | 0 ± 4 (8)                           |
| B6-Tsz-28   | 3R       | 5R                              | 3 ± 5 (9)                           |
| CKB-Tsz-59  | 3R       | 5R                              | 15 ± 3 (8)                          |
| BW5147      | None     | 5R                              | 0 ± 4 (8)                           |
| CKB-Tsz-59  | None     | 5R                              | 8 ± 13 (8)                          |
| BW5147      | 5R       | 3R                              | 0 ± 5 (8)                           |
| B6-Tsz-28   | 5R       | 3R                              | 6 ± 7 (7)                           |
| CKB-Tsz-59  | 5R       | 3R                              | −1 ± 10 (8)                         |
| BW5147      | 3R       | 3R                              | 0 ± 11 (8)                          |
| B6-Tsz-28   | 3R       | 3R                              | 59 ± 8 (8)‡                         |
| CKB-Tsz-59  | 3R       | 3R                              | −11 ± 11 (8)                        |
| BW5147      | None     | 3R                              | 0 ± 6 (10)                          |
| B6-Tsz-28   | None     | 3R                              | 7 ± 5 (8)                           |

* Refer to legend for Table III for protocol. Regional lymph node cells from NP-primed mice were cultured with TsF2 for 2 h. The data represent the pooled results from three separate experiments. The data were normalized and the percent suppression ± SE was calculated.

‡ Significant suppression, *P* < 0.001.
restrictions on Ts3 cell interactions and to evaluate whether allogeneic effects could influence the results, we activated NP-O-Su-primed (B10 × B10.BR)F1 lymph node cells with C57BL/6, CKB, or control BW5147-derived TsF2 for 2 h in vitro. These activated F1 cells were transferred to NP-O-Su-primed CY-treated C57BL/6 (H-2b) or B10.BR (H-2k) recipients. The data in Table V again clearly demonstrate an absolute requirement for H-2 homology between the TsF2 and the recipient strain to obtain immune suppression. Thus, C57BL/6 (H-2b)-derived TsF2 activates (B10 × B10.BR)F1 Ts3 cells, but these cells only function in C57BL/6 (H-2b) not B10.BR (H-2k) mice. In a reciprocal experiment, CKB (H-2k)-derived TsF2 activated (B10 × B10.BR)F1 Ts3 cells, but again these F1 cells only produce suppression when transferred into H-2k-bearing B10.BR recipients.

The simplest hypothesis that would account for the above observation is that two distinct Ts3 populations exist in lymph node cells derived from F1 animals; one population is restricted by I-Jb gene products and the other by I-Jk gene products. This hypothesis parallels the situation observed with helper T cells derived from F1 mice in which two functionally distinct populations exist and each is restricted by different I region genes (18, 19). Another possibility to account for these observations is that the I-J gene products are allelically expressed on the F1 cells. To test the latter possibility, B10.BR, C57BL/6, or (B10 × B10.BR)F1 NP-O-Su-primed lymph node cells were treated with anti-I-Jb or anti-I-Jk alloantisera plus complement before a 2-h activation with TsF2. As shown in Table VI, treatment of B10.BR Ts3 cells with anti-I-Jb specifically depleted the ability to generate suppressive activity. In reciprocal groups, treatment of C57BL/6 Ts3 cells with anti-I-Jk but not anti-I-Jb alloantisera completely eliminated Ts3 cell activity. When the same anti-I-J alloantisera were used to lyse (B10 × B10.BR)F1 Ts3 cells, both anti-I-Jb and anti-I-Jk alloantisera eliminated the ability to generate functional Ts3 cells. Thus, it appears that Ts3 cells derived from (B10 × B10.BR)F1 donors carry both the I-Jb and I-Jk antigenic determinants in a codominant fashion.

**Suppression of H-2 Heterozygous F1 Recipients by Activated Ts3 Cells.** Finally, to evaluate

| TsF2 source | Ts3 donor | CY-treated recipients | Normalized percent CS suppression ± SE |
|-------------|-----------|-----------------------|----------------------------------------|
| BW5147     | (B10 × B10.BR)F1 | C57BL/6               | 0 ± 4 (8)                              |
| B6-Ts3-28  | (B10 × B10.BR)F1 | C57BL/6               | 43 ± 3 (8)i                            |
| CKB-Ts3-59 | (B10 × B10.BR)F1 | C57BL/6               | 1 ± 5 (8)                              |
| BW5147     | (B10 × B10.BR)F1 | B10.BR                | 0 ± 6 (8)                              |
| B6-Ts3-28  | (B10 × B10.BR)F1 | B10.BR                | −2 ± 6 (8)                             |
| CKB-Ts3-59 | (B10 × B10.BR)F1 | B10.BR                | 54 ± 6 (7)i                            |
| BW5147     | None       | C57BL/6               | 0 ± 5 (10)                             |
| B6-Ts3-28  | None       | C57BL/6               | −2 ± 3 (6)                             |
| BW5147     | None       | B10.BR                | 0 ± 6 (10)                             |
| CKB-Ts3-59 | None       | B10.BR                | −4 ± 9 (6)                             |

*Refer to legend for Table III for protocol. The 2-h-activation data represent the pooled results from two separate experiments. The data were normalized and the percent suppression ± SE was calculated.

‡ Significant suppression, \( P < 0.01 \).
**Table VI**

*F₂-derived T₃₅ Cells Bear Both Parental I-J Determinants*

| TsF₂ source | Ts donor | Ts treatment | NP-primed | Normalized percent suppression ± SE |
|-------------|----------|--------------|-----------|-------------------------------------|
|             |          |              | CY-treated |                                    |
|             |          |              | recipient |                                    |

- BW5147 B10.BR — B10.BR 0 ± 4 (12)
- B6-T₃₅-28 B10.BR — B10.BR -1 ± 11 (4)
- CKB-T₃₅-59 B10.BR — B10.BR 78 ± 4 (12)‡
- CKB-T₃₅-59 B10.BR Anti-I-J⁺ + C B10.BR 7 ± 8 (12)
- CKB-T₃₅-59 B10.BR Anti-I-J⁺ + C B10.BR 80 ± 6 (8)‡
- BW5147 C57BL/6 — C57BL/6 0 ± 4 (8)
- B6-T₃₅-28 C57BL/6 — C57BL/6 64 ± 5 (6)‡
- B6-T₃₅-28 C57BL/6 Anti-I-J⁺ + C C57BL/6 43 ± 9 (7)‡
- B6-T₃₅-28 C57BL/6 Anti-I-J⁺ + C C57BL/6 -1 ± 4 (8)
- BW5147 (B10 × B10.BR)F₁ — B10.BR 0 ± 3 (12)
- B6-T₃₅-28 (B10 × B10.BR)F₁ — B10.BR 2 ± 5 (12)
- CKB-T₃₅-59 (B10 × B10.BR)F₁ — B10.BR 61 ± 5 (11)‡
- CKB-T₃₅-59 (B10 × B10.BR)F₁ Anti-I-J⁺ + C B10.BR 3 ± 6 (12)
- CKB-T₃₅-59 (B10 × B10.BR)F₁ Anti-I-J⁺ + C B10.BR 11 ± 7 (8)
- BW5147 (B10 × B10.BR)F₁ — C57BL/6 0 ± 3 (12)
- CKB-T₃₅-59 (B10 × B10.BR)F₁ — C57BL/6 4 ± 4 (12)
- B6-T₃₅-28 (B10 × B10.BR)F₁ — C57BL/6 55 ± 4 (12)‡
- B6-T₃₅-28 (B10 × B10.BR)F₁ Anti-I-J⁺ + C C57BL/6 5 ± 5 (11)
- B6-T₃₅-28 (B10 × B10.BR)F₁ Anti-I-J⁺ + C C57BL/6 4 ± 3 (8)

* Before activation of regional lymph node cells from NP-O-Su-primed mice, the lymph node cells were treated with anti I-J antisera and C, as described in Materials and Methods. Activation of the lymph node cells was done as in Table III. The data represent the pooled results from three separate experiments. The data were normalized and the percent suppression ± SE was calculated.

‡ Significant suppression, P < 0.001.

The potential role of the recipient strains in directing the genetic restrictions, H-2 heterozygous F₁ recipients were given in vitro activated T₃₅ cells. In the first experiment, C57BL/6 (H-2ᵇ) or B10.BR (H-2ᵏ) NP-O-Su-primed lymph node cells were used as the source of the T₃₅ population. The T₃₅ cells were activated for 2 h in vitro with monoclonal B6-T₃₅-28 (H-2ᵏ origin) or CKB-T₃₅-59 (H-2ᵏ origin) TsF₂ and then adoptively transferred to (B10 × B10.BR)F₁ (H-2ᵇ × H-2ᵏ) NP-O-Su-primed CY-treated recipients. As shown in Table VII, significant suppression was only noted when the TsF₂ and T₃₅ cells were derived from strains that shared H-2 haplotypes. It should be noted that after a 2-h activation, CKB (H-2ᵏ)-derived TsF₂ failed to activate C57BL/6 (H-2ᵇ) T₃₅ cells, even when the potential suppressive activity of these cells was assayed in H-2ᵇ × H-2ᵏ recipients. These data again indicate that under these experimental conditions a definite requirement for H-2 homolgy exists among the TsF₂, T₃₅ cells and the recipient strain.

In a second experiment, the role of genes linked to the Igh complex was also evaluated. Thus, C57BL/6 (H-2ᵇ, Igh¹), CKB (H-2ᵏ, Igh³), and C3H (H-2ᵏ, Igh³) T₃₅ cells were activated for 2 h with either C57BL/6- or CKB-derived TsF₂. The activated cells were then adoptively transferred to (C57BL/6 × CBA)F₁ (H-2ᵇ/H-2ᵏ; Igh¹/Igh³) recipients during the effector phase of the CS response. Only in those combinations
TABLE VII

Suppression of F1 Recipients by Activated Parental Ts2 Cells*

| TsF2 source | Ts2 donor | CY-treated recipients | Footpad swelling ± SE |
|-------------|-----------|------------------------|-----------------------|
| BW5147     | C57BL/6   | (B10 × B10.BR)F1       | 48.5 ± 2.4            |
| B6-Ts2-28  | C57BL/6   | (B10 × B10.BR)F1       | 30.5 ± 1.9            |
| CKB-Ts2-59 | C57BL/6   | (B10 × B10.BR)F1       | 47.3 ± 2.8            |
| BW5147     | B10.BR    | (B10 × B10.BR)F1       | 50.0 ± 1.9            |
| B6-Ts2-28  | B10.BR    | (B10 × B10.BR)F1       | 47.7 ± 2.2            |
| CKB-Ts2-59 | B10.BR    | (B10 × B10.BR)F1       | 35.4 ± 2.9            |
| BW5147     | C57BL/6   | (B6 × CBA)F1           | 56.6 ± 2.3            |
| B6-Ts2-28  | C57BL/6   | (B6 × CBA)F1           | 31.3 ± 2.3            |
| CKB-Ts2-59 | C57BL/6   | (B6 × CBA)F1           | 54.0 ± 3.5            |
| BW5147     | CKB       | (B6 × CBA)F1           | 56.8 ± 2.7            |
| B6-Ts2-28  | CKB       | (B6 × CBA)F1           | 56.0 ± 2.8            |
| CKB-Ts2-59 | CKB       | (B6 × CBA)F1           | 35.5 ± 2.2            |
| BW5147     | C3H       | (B6 × CBA)F1           | 50.0 ± 2.2            |
| B6-Ts2-28  | C3H       | (B6 × CBA)F1           | 56.8 ± 2.2            |
| CKB-Ts2-59 | C3H       | (B6 × CBA)F1           | 59.5 ± 2.5            |

* Refer to legend for Table III for protocol. The data were expressed as the increment of footpad swelling ± SE in units of 10⁻³ cm. The background response of nonimmunized (B10 × B10.BR)F1 mice was 12.0 ± 1.2 and that of (B6 × CBA)F1 mice was 10.0 ± 1.1.

‡ Significant suppression, P < 0.01.

in which the donor of the TsF2, the Ts2, and the recipients shared genes in both the H-2 and Igh complexes were significant levels of suppression noted (Table VII).

Discussion

The past several years have witnessed numerous advances in our knowledge of the mechanisms of immunoregulation. In some systems, three distinct T lymphocyte subpopulations act in a defined sequence to mediate immune suppression (20, 21). For example, suppression of both cellular (5) and humoral (7) immune responses to the NP require a similar cellular cascade involving Ts1, Ts2, and Ts3 cells as well as factors derived from each of these cell types. Previous reports (5, 13, 16) from our laboratory characterized a series of hybridoma T cell lines representing each of these functional populations. Furthermore, we compared the suppressor factors (TsF) released by each of these Ts cells. The TsF2 and TsF3 factors, which both function during the effector phase of the immune response, have similar genetic restrictions. Thus, TsF2 and TsF3 only suppress strains of mice that are homologous with the factor-producing strain at both the H-2 complex (I-J subregion) and the Igh complex (5, 13). Because the basis for these dual restrictions had not been clarified, it was postulated that at least some of the restrictions might represent "pseudogenetic restrictions," as were initially described for TsF1 factors and cells (16, 22, 23). These pseudogenetic restrictions reflect requirements for homology between H-2 or Igh determinants that are present at different ends of the suppressor cell cascade (16). The hypothesis that the dual genetic restrictions of TsF2 reflected a pseudo-restriction was based on the observation that TsF2 activity could be absorbed by Ts2 cells derived...
The present protocol was designed to determine whether the allogeneic cells that could absorb Tsf could become activated. Thus, we developed an experimental system in which the genetics of activation of Ts cells by Tsf could be analyzed in vitro, independent of the ability of activated Ts cells to interact with their targets. The transfer of Ts cells was performed during the effector phase, i.e., along with the NP-O-Su challenge and within 24-26 h of the termination of the CS response, to minimize potential allogeneic effects. Additional controls to exclude potential allogeneic affects included the transfer of nonactivated Ts cells that were cultured with control BW5147-derived factor. Furthermore, F1-derived Ts cells and F1 recipients were used in combinations in which the direction of the allogeneic effect could be controlled (Tables V and VII).

The data demonstrated that NP-specific Ts cells are generated in NP-O-Su-immune animals concomitant with the CS effector cell population. In contrast to CS effector cells, Ts are very sensitive to low dose CY treatment. The Ts cells must be specifically activated by Tsf to manifest suppression (Table I). Normally, in a primary NP-O-Su immune response, the Ts cells are not activated. However, later in the response the Ts cells may play an important immunoregulatory role in modulating both the cellular and humoral immune response (4, 7). The present data directly demonstrate the role of Tsf in suppressor cell activation. The triggering of Ts cells with Tsf is rapid. Thus, after 1-2 h of in vitro exposure to Tsf, the activation of Ts cells appears irreversible and results in optimum levels of suppression (Fig. 1). This rapid activation process presumably reflects the fact that the antigen-primed Ts cells have already expanded and differentiated. These cells apparently await a terminal signal for activation and/or release of biologically active mediators, such as Tsf.

The specificity of Ts cell-mediated suppression was demonstrated in two ways. First, NP-specific Ts cells are generated after NP-O-Su priming, whereas immunization with another antigen (e.g., DNFB) does not generate NP-reactive Ts cells. Furthermore, once NP-O-Su-induced Ts cells are activated with Tsf, they suppress only NP-O-Su-induced CS responses even in animals that have been doubly primed or challenged with NP-O-Su plus DNFB (Table II). Although under the experimental conditions described in this report immune suppression is antigen specific, nonspecific suppression of immune response has been noted in other systems in which different experimental conditions are used (10-12). This disparity might reflect the requirement for the suppressor cell and the potential targets to be in very close proximity to mediate suppression.

Genetic analyses of the Tsf-Ts-target cell interaction indicated the requirement for Igk homology was absolute. Thus, CKB (Ighb)-derived Tsf would only activate an Igk-compatible Ts population, which in turn only suppressed Igk homologous recipients (Table III). These results, along with previous data (24) demonstrating anti-idiotypic receptors on Ts cells and factors as well as previous data demonstrating the presence of NP-β-related idiotypic determinants on Ts1 and Ts2 cells, suggests that suppressor T cell interactions proceed via a series of idiotypic-anti-idiotypic interactions in accord with Jerne's network hypothesis. In addition to the absolute requirement for Igk homology with respect to the cells involved in suppression of the effector phase of the contact sensitivity response, there is also an H-2 restriction that controls the interaction of these cells. Thus, after activation, the series of interactions between Tsf, Ts, and the recipient strain appears to be completely H-2 restricted. These
H-2 restrictions can be more precisely mapped to the I-J subregion of the H-2 complex (Table IV), which has also been shown to regulate suppressor cell interactions in other systems (17, 25, 26). The physiological meaning of this I-J restriction is unknown. We have not yet determined the directionality of the restriction; i.e., do Ts3 cells have a receptor for I-J determinants on a target population or are the I-J determinants present on Ts3 cells and factors recognized by the target population?

To further evaluate the genetic restrictions on activated Ts3 cells, (B10 × B10.BR)F1 hybrid-derived Ts3 were cultured with either H-2b- or H-2k-derived TsF2, and the activated Ts3 were tested for suppressive activity in either C57BL/6 (H-2b) or B10.BR (H-2k) recipients. The data again demonstrate that the critical requirements for H-2 homology were between the H-2 type of the TsF2 donor and the H-2 type of the recipients of activated Ts3 cells. Thus, a C57BL/6-derived TsF2 activated (B10 × B10.BR)F1-derived Ts3 cells, as evidenced by their ability to suppress NP-induced CS responses in C57BL/6 mice. It should be noted that the same population of activated Ts3 cells failed to suppress NP-O-Su CS responses in B10.BR recipients (Table V). Reciprocal data were obtained when CKB-derived TsF2 was used to activate F1-derived Ts3 cells (Table V). The simplest explanation for these observations is that two distinct populations of Ts3 cells exist in heterozygous F1 donors, each restricted to a parental I-J determinant. This hypothesis is analogous to the findings noted with F1-derived helper T cells (18, 19). By extending this analogy with helper T cells further, one can postulate that the induction of I-J restrictions might reflect the requirement for the initial presentation of antigen in the context of I-J determinants. Preliminary experiments support the latter postulate.

The next series of experiments was aimed at determining whether I-J determinants were allelically excluded in the H-2 heterozygous Ts3 population. If only one I-J determinant was expressed on each subset of F1-derived Ts3 cells, it could help to explain the directionality of the genetic restriction. The data in Table VI clearly demonstrate that these I-J determinants are not allelically excluded in confirmation of the results reported by Okuda et al. (27), who arrived at similar conclusions in a different type of experimental system. However, because both I-J determinants are expressed on Ts3 cells of F1 origin, it will be important to analyze TsF3 of F1 origin to determine whether both I-J determinants are also present on these factors. Separate experiments are planned to address these questions.

Finally, we evaluated the role of the recipient strain in these genetic restrictions. By using F1 recipients, we again confirmed the requirements for homology at both H-2 and Igh complexes. The recipient strain must contain the cells that are the target of the activated Ts3 population. However, the present data do not permit us to determine the nature of these target cells. The target cells could be the CS effector cells, a Ts4 population, or even an antigen-presenting cell. Whatever the nature of the target, we expect that it will either bear I-J determinants or receptors for I-J, and it may also bear anti-idiotypic receptors. Furthermore, the data obtained after a 2-h activation argue against the notion that the dual genetic restrictions of TsF2 and TsF3 are pseudogenetic restrictions, as were defined for Ts4-derived factors (16, 22, 23). In addition, some experimental data indicate that TsF2 may have a two-chain structure, one polypeptide containing I-J determinants and the other idiotypic determinants (28) (Furusawa, et al., unpublished data). The dual genetic restriction of Ts3 cells and factors might therefore reflect the requirement of target cells to interact with both
portions of the TsF2 molecule. The significance of these dual restrictions (I-J and Igh) might lie in the fact that two recognition signals are required for the activation of effector-suppressor cells. Such a two-signal model could account for the specificity of suppression as well as the molecular structure of the factor.

Summary

An experimental system was developed to independently analyze the H-2 and Igh genetic restrictions at two steps of the 4-hydroxy-3-nitrophenylacetyl hapten (NP) suppressor cell pathway. This experimental system allowed genetic analysis of the activation of Ts3 cells by hybridoma-derived TsF2 and independent analysis of the genetic restrictions that controlled the interaction of the Ts8 cells with their target population. Thus, Ts3 cells were activated in vitro with monoclonal H-2b or H-2k-derived TsF2. The activated Ts8 cells were then adoptively transferred to Ts3-depleted (cyclophosphamide-treated) recipients of various genotypes. When the Ts3-containing lymph node population was activated in vitro for 2 h, suppressive activity was only noted in combinations of TsF2, Ts8, and recipients that were matched at both the I-J and Igh gene complexes. The data indicate that TsF2 can activate Ts3 cells and that both the activation and the interaction of Ts8 cells are I-J and Igh restricted. Using (B10 × B10.BR)F1 mice as Ts3 donors, we noted that H-2b-derived TsF2 activated these F1 Ts3 cells to suppress NP-specific cutaneous sensitivity responses in H-2b but not in H-2k recipients. Reciprocal experiments using H-2k-derived TsF2 demonstrated that only an H-2k-restricted population was activated in the F1-derived Ts3 cells. The simplest explanation to account for these observations is that two distinct populations, each of which is restricted to a parental I-J determinants, exists in the heterozygous F1 Ts3 population. Furthermore, we demonstrated that both I-Jb and I-Jk determinants are expressed on F1-derived Ts3 cells. These observations are discussed in terms of the mechanisms involved in immunoregulation.

We would like to express our appreciation to Mrs. Nancy Axelrod and Mrs. Mary Jane Tawa for their excellent assistance in the preparation of this manuscript. In addition, we thank Ms. Ann Marie Fay for her outstanding technical help.

Received for publication 19 April, 1982.

References

1. Waltenbaugh, C., J. Thèze, J. A. Kapp, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic acid50-L-tyrosine60 (GT). III. Generation of suppressor T cells by a suppressive extract derived from GT-primed lymphoid cells. J. Exp. Med. 146:970.
2. Taniguchi, M., and T. Tokuhisa. 1980. Cellular consequences in the suppression of antibody response by the antigen-specific T cell factor. J. Exp. Med. 151:517.
3. Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F. W. Shen, R. K. Gershon, and H. Cantor. 1978. Immunoregulatory circuits among T-cell sets. I. T-helper cells induce other T-cell sets to exert feedback inhibition. J. Exp. Med. 147:1106.
4. Sunday, M. E., B. Benacerraf, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. VIII. Suppressor cell pathways in cutaneous sensitivity responses. J. Exp. Med. 153:811.
5. Okuda, K., M. Minami, S. Furusawa, and M. E. Dorf. 1981. Analysis of T cell hybridomas.
II. Comparisons among three distinct types of monoclonal suppressor factors. J. Exp. Med. 154:1838.
6. Sy, M.-S., A. Nisonoff, R. N. Germain, B. Benacerraf, and M. I. Greene. 1981. Antigen- and receptor-driven regulatory mechanisms. VIII. Suppression of idiotype-negative, \( \beta \)-azobenzene arsonate-specific T cells results from the interaction of an anti-idiotypic second-order T suppressor cell with a cross-reactive-idiotype-positive, \( \beta \)-azobenzene arsonate-primed T cell target. J. Exp. Med. 153:1415.
7. Sherr, D. H., and M. E. Dorf. 1982. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XIII. Characterization of a third order T cell (Ts3) involved in suppression of in vitro PFC responses. J. Immunol. 128:1261.
8. Thomas, W. R., F. I. Smith, I. D. Walker, and J. F. A. P. Miller. 1981. Contact sensitivity to azobenzene arsonate and its inhibition after interaction of sensitized cells with antigen-conjugated cells. J. Exp. Med. 153:1124.
9. Sy, M.-S., S. D. Miller, J. W. Moorhead, and H. N. Claman. 1979. Active suppression of 1-fluoro-2,4-dinitrobenzene-immune T cells. Requirement of an auxiliary T cell induced by antigen. J. Exp. Med. 149:1197.
10. Asherson, G. L., and M. Zembala. 1982. The role of the T acceptor cell in suppressor systems: antigen-specific T suppressor factor acts via a T acceptor cell: this releases a non-specific inhibitor of the transfer of contact sensitivity when exposed to antigen in the context of I-J. N. Y. Acad. Sci. In press.
11. Tada, T., and K. Okumura. 1980. The role of antigen-specific T cell factors in the immune response. Adv. Immunol. 28:1.
12. Fresno, M., L. McVay-Boudreau, G. Nabel, and H. Cantor. 1981. Antigen-specific T lymphocyte clones. II. Purification and biological characterization of an antigen-specific suppressive protein synthesized by cloned T cells. J. Exp. Med. 153:1260.
13. Minami, M., K. Okuda, S. Furusawa, B. Benacerraf, and M. E. Dorf. 1981. Analysis of T cell hybridomas. I. Characterization of H-2- and Igh-restricted monoclonal suppressor factors. J. Exp. Med. 154:1390.
14. Okuda, K., M. Minami, S.-T., Ju, and M. E. Dorf. 1981. Functional association of idiotypic and I-J determinants on the antigen receptor of suppressor T cells. Proc. Natl. Acad. Sci. U. S. A. 78:4557.
15. Sunday, M. E., J. Z. Weinberger, B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. IV. Specificity of cutaneous sensitivity responses. J. Immunol. 125:1601.
16. Okuda, K., M. Minami, D. H. Sherr, and M. E. Dorf. 1981. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. XI. Pseudogenetic restrictions of hybridoma suppressor factors. J. Exp. Med. 154:468.
17. Taniguchi, M., T. Tada, and T. Tokuhisa. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of anti-body response of the mouse. III. Dual gene control of the T-cell mediated suppression of the antibody response. J. Exp. Med. 144:20.
18. Paul, W. E., E. M. Shevach, S. Pickeral, D. W. Thomas, and A. S. Rosenthal. 1977. Independent populations of primed F1 guinea pig T lymphocytes respond to antigen-pulsed parental peritoneal exudate cells. J. Exp. Med. 145:618.
19. Sredni, B., and R. H. Schwartz. 1981. Antigen-specific, proliferating T lymphocyte clones. Methodology, specificity, MHC restriction and alloreactivity. Immunol. Rev. 54:187.
20. Germain, R. N., and B. Benacerraf. 1981. A single major pathway of T-lymphocyte interactions in antigen-specific immune suppression. Scand. J. Immunol. 13:1.
21. Benacerraf, B., M. I. Greene, M.-S. Sy, and M. E. Dorf. 1982. Suppressor T cell circuits. Conference on Immunological Tolerance to Self and Non-Self. Ann. N. Y. Acad. Sci. In press.
22. Sunday, M. E., J. Z. Weinberger, S. Wolff, and M. E. Dorf. 1981. Anti-receptor antibody-
induced suppression of murine H-Y-specific delayed-type hypersensitivity responses. *Eur. J. Immunol.* 11:626.

23. Sy, M.-S., M. H. Dietz, A. Nisonoff, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. V. The failure of idiotype-coupled spleen cells to induce unresponsiveness in animals lacking the appropriate V<sub>H</sub> genes is caused by the lack of idiotype-matched targets. *J. Exp. Med.* 152:1226.

24. Jerne, N.-K. 1974. Towards a network of the immune system. *Ann. Immunol. (Paris).* 125C:373.

25. Meruelo, D., B. Deak, and H. O. McDevitt. 1977. Genetic control of cell-mediated responsiveness to an AKR tumor-associated antigen. Mapping of the locus involved to the I region of the H-2 complex. *J. Exp. Med.* 146:1367.

26. Kapp, J. A., and B. A. Araneo. 1982. Antigen-specific suppressor T cell interactions. I. Induction of an MHC-restricted suppressor factor specific for L-glutamic acid<sup>20</sup>-L-tyrosine<sup>30</sup>. *J. Immunol.* 128:2447.

27. Okuda, K., C. S. David, and D. C. Shreffler. 1977. The role of gene products of the I<sub>J</sub> subregion in mixed lymphocyte reactions. *J. Exp. Med.* 146:1561.

28. Taniguchi, M., T. Saito, I. Takei, and T. Toluhi. 1981. Presence of interchain disulfide bonds between two gene products that compose the secreted form of an antigen-specific suppressor factor. *J. Exp. Med.* 153:1672.