ANALYSIS OF T CELL HYBRIDOMAS
I. Characterization of H-2- and Igh-restricted
Monoclonal Suppressor Factors*

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The sequential interactions between T lymphocyte subsets have been well characterized in several systems of antigen-specific immune suppression (1–2). The immune response to the hapten 4-hydroxy-3-nitrophenyl acetyl (NP) has become one of the most extensively characterized of these model systems (3–6). Previous studies from our laboratory have revealed that in this suppressor network system, at least three distinct sets of T cells are required for suppression. The first set of suppressor T cells, termed Tsl, are induced by antigen, bear an idiotypic receptor, and function early in the immune response. These Tsl induce a second set of suppressor T cells, termed Ts2 (3, 7). The Ts2 bear anti-idiotypic receptors and function late in the immune response (3–5). Furthermore, the Ts2 population or a soluble factor derived from these cells activates an antigen-primed Ts3 population that is thought to mediate the additional suppressor signals (4). Most studies, including these in the NP system, appear to be fragmentary in that the entire sequence of cell interactions has not been fully defined. A serious limitation of these studies has been the lack of suppressor cell clones or hybridomas to further characterize the different suppressor cell populations and the molecules responsible for the communication between these cells.

For a better understanding of the precise nature and function of each participating suppressor T cell subset, we have recently prepared a series of monoclonal T cell hybridomas that are specific for NP (7–8). We previously (7, 8) characterized these hybrids and demonstrated that they correspond to the Tsl population. We now characterize another group of T cell hybrids that corresponds to the Ts2 population. Furthermore, we characterize the biologically active suppressor factor (TsF2) produced by these cells and compare it with the TsF1 factor obtained from the Tsl hybrids.

Materials and Methods

Mice. All mice were either purchased from The Jackson Laboratory, Bar Harbor, Maine or were bred in the animal facilities at Harvard Medical School, Boston, Mass. Mice were used at

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1 Abbreviations used in this paper: C, complement; CS, cutaneous sensitivity; DMSO, dimethyl sulfoxide; DNFB, 2,4-dinitro-1-fluorobenzene; HBSS, Hanks' balanced salt solution; NP, 4-hydroxy-3-nitrophenyl acetyl hapten; NP-O-Su, NP-O-succinimide ester; RAMG, rabbit anti-mouse immunoglobulin; Tsl, Ts2, Ts3, first, second, or third order suppressor T cells, respectively; TsF1, TsF2, suppressor factor derived from Tsl or Ts2 cells, respectively.
3–12 mo of age and were maintained on laboratory chow and acidified, chlorinated water ad lib.

Antigens. NP-O-Succinimide (NP-O-Su) was purchased from Biosearch Co., San Rafael, Calif. Dimethylsulfoxide (DMSO) was purchased from Fisher Scientific Co., Pittsburgh, Pa. 2,4-dinitro-1-fluorobenzene (DNFB) was obtained from the Sigma Chemical Co., St. Louis, Mo.

NP-specific T Cell Hybridoma and TsF. The methods for preparation of T cell hybridomas by polyethylene glycol-mediated fusion with the BW5147 tumor line were described previously (8). Our previous results (8) indicated that the CKB-Ts1-17 (IgH^b, H-2^b) and B6-Ts1-29 (IgH^b, H-2^k) hybridoma lines, which are also used in this report, possess I-J and NP^b idiotypic determinants.

In addition to these Ts^1 lines, some Ts^2 hybrids were also produced from the above fusions (7). This was not surprising because the Ts^1 and Ts^2 populations were shown to coexist in the spleen at the time these cells were hybridized (3, 4, 9). The Ts^2 lines isolated in this fashion include CKB-Ts2-59, CKB-Ts2-223, and B6-Ts2-28. The other Ts^2 lines were obtained by hybridization of BW5147 cells with Ts^2-containing spleen cells. The enrichment of NP-specific Ts^2 was described previously (5). Briefly, NP-primed C57BL/6 spleen cells were first fractionated on rabbit anti-mouse immunoglobulin (RAMG)-coated polystyrene petri plates for 1 h at room temperature. The unbound cells were then incubated on another set of polystyrene petri plates coated with purified C57BL/6 anti-NP antibodies for 1 h at room temperature. Nonadherent cells were removed, and adherent cells were incubated on ice for 30 min. The adherent cells were resuspended by vigorous pipetting. These enriched Ts^2 populations were fused by polyethylene glycol with the BW5147 tumor cell line, as described in detail elsewhere (8).

The hybridized Ts^2 candidates were screened using a cytotoxicity test (8) with anti-I-J alloantisera. All the Ts^2 hybrids (CKB-Ts2-59, CKB-Ts2-217, CKB-Ts2-223, B6-Ts2-28, and B6-Ts2-14) were periodically passed over C57BL/6 anti-NP antibody-coated plates, and the idiotype-adherent cells were used to maintain the cell lines. The CKB-Ts2-59 and B6-Ts2-28 hybridoma lines were cloned by limiting dilution, and each of the five clones tested had the same phenotype and suppressive activity as the parental line.

All of the hybridomas were cultured in RPMI 1640 containing 8% fetal calf serum and 0.01 M Hepes buffer. The suppressor factors used in the present experiment were collected from the cultured supernates of cells at a density of ~7 × 10^6 cells/ml in the above medium.

Adsorption and Elution of TsF. The methods of adsorption and elution of TsF using protein-conjugated Sepharose 4B columns were described in detail previously (8).

Adsorption of TsF with T Cells. T cells were enriched from immune lymph node cells using RAMG-coated plastic petri dishes as described above. 1 ml of TsF factor was incubated with 4 × 10^7 immune lymph node T cells or 2 × 10^6 hybridoma T cells for 1 h on ice with intermittent mixing. Then the supernates were centrifuged and tested for suppressor activity at a 1:5 dilution.

Assay for the Ability of TsF to Induce Suppressor Cells. To make TsF cells for in vivo studies, hybridoma TsF1 (0.3 ml) was injected i.v. into naive mice for 4 successive days. On day 6, spleens from these mice were removed, and a single cell suspension was prepared in Hanks’ balanced salt solution (HBSS). 4 × 10^7 spleen cells were washed three times with HBSS and injected into NP-primed mice (7). The recipient mice were then challenged with NP, and the swelling responses were measured 24 h later, as described elsewhere (4).

Assay for Suppressive Activity of TsF on NP-mediated Cutaneous Sensitivity (CS) Responses. The assay for NP-specific CS responses was described elsewhere (4). Briefly, each animal was primed subcutaneously with 7 mg of NP-O-Su in DMSO. Unless indicated otherwise, the hybridoma supernates were tested in the effector phase, 5 or 6 d after priming. 0.4 ml of each hybridoma supernate or BW5147 control supernate was injected intravenously on the day before and the day of antigen challenge. 6 d after immunization, 25 μg of NP-O-Su in 0.025 ml of phosphate-buffered saline, pH 7.2, was injected into the left footpad. Footpad swelling was measured 24 h after challenge. Swelling was determined as the difference, measured in units of 10^-3 cm, between the left and right footpad thickness.

DNFB Responses. Contact sensitivity was induced by painting the shaved abdomen twice
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daily with 25 µl of 0.5% DNFB solution (Sigma Chemical Co.) in acetone:olive oil (4:1) (7). 6
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.

Percent Suppression. The percent suppression in the present study was calculated by the
following formula:

percent suppression = $\frac{100 \times \text{(swelling of BW5147 tumor supernate-injected group - swelling
of TsF-injected group)}}{\text{(swelling of BW5147 tumor supernate-injected group - swelling
of unprimed group)}}$.

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

Results

Screening of Hybridoma Suppressor Cells. Suppressor cells were initially induced by
the intravenous injection of $4 \times 10^7$ NP-coupled syngeneic spleen cells. The suppressor
T cells were purified by successive passage over RAMG and NPb idiotype-coated
plates. The idiotype-adherent T cells were fused with the AKR BW1547 thymoma
tumor line with polyethylene glycol; selection of hybrids seeded at a concentration of
$10^3$ spleen cells per well was carried out with hypoxanthine-aminopterin-thymidine
selective media. Hybridoma colonies were noted in ~25% of the wells seeded. When
the colonies were large enough for passage, an aliquot of cells was tested for the
presence of I-J alloantigenic determinants by microcytotoxicity testing. 23% of the
colonies tested could be specifically lysed with allele-specific anti-I-J alloantisera.
Supernates from the I-J-positive colonies were then screened for in vivo suppression
of NP-specific CS responses. 0.4 ml of supernatant fluid was administered intrave-
nously on the day before and the day of antigen challenge. In addition to the above
supernates, several other I-J-positive cell lines from previous fusions were also screened
in this assay system. Five of the cell lines that manifested at least 50% suppression of
the CS immune response were saved for further analysis.

The hybrid cell lines that produced suppressor factors were also screened for other
cell surface markers by microcytotoxicity testing. All hybridoma cell lines were lysed
with anti-Thy-1.1 antisera and with one exception (CKB-Ts2-59) were also lysed with
anti-Thy-1.2 antisera, which detected the Thy-1.2 gene products introduced by the
parental suppressor cell donors. Preliminary analysis with the anti-Lyt-2 monoclonal
antibody suggests that several of the Ts2 hybrids may also carry this cell surface
marker (data not shown). However, none of the cell lines could by lysed with anti-
NPb idiotype antiserum plus complement (C) (data not shown), which contrasts with
the results noted with a previous series of Ts1 hybrids that could by lysed by the latter
reagent (8).

Finally, it should be noted that the ability to lyse these cell hybrids with anti-I-J
alloantibody plus C has proven to be a variable feature of these cell lines. With time,
there appeared to be a continual decrease in the level of surface I-J determinants
expressed on these cells. We have recently noted similar decreases in the level of I-J
determinants expressed on the surfaces of Ts1 hybridoma cell lines.

Specificity of Suppressor Factors. To test the biological activity of these presumptive
suppressor hybrids, we screened for the ability of culture supernates obtained from
cells growing at a density of ~7 × 10^5 cells/ml to suppress in vivo CS responses. As
shown in Table I, intravenous injection of 0.4 ml of supernate from the B6-Ts2-28,
B6-Ts2-14, and CKB-Ts2-225 cell lines specifically suppressed NP-induced CS responses. The supernates demonstrated maximum or plateau levels of activity when given either undiluted or diluted 1:10. In these experiments the percent of suppression ranged from 50–75%. After further dilution, these materials no longer demonstrated suppressive activity. The failure of these factors to induce complete suppression of the CS response, coupled with the plateau level of suppression, suggested that they might be maximally effective if the different monoclonal factors derived from each strain were mixed. In three separate experiments, we observed that the percent of suppression was consistently greater when mice were given a mixture of suppressor factors, but the differences were small and not statistically significant.

The antigen specificity of these suppressor factors was demonstrated by their inability to suppress DNFB-induced contact sensitivity responses (Table I). The NP specificity of the CKB-Ts2-59 and CKB-Ts2-217 suppressor factors was similarly established (data not shown).

Comparison of Ts1 and Ts2-derived Factors. To compare this series of NP-specific suppressor factors with the previously characterized set of Ts1 hybridoma factors, we determined the phase of the immune response in which the various factors were active. 0.4 ml of factor was injected intravenously either on the day of and the day after antigen priming (induction phase) or the day before and the day of antigen challenge (effector phase). In confirmation of our previous data, the B6-Ts2-28 and CKB-Ts2-17 factors only demonstrated suppressive activity when administered during the induction phase of the immune response (Table II). In contrast, the B6-Ts2-28, B6-Ts2-14, CKB-Ts2-59, and CKB-Ts2-225 factors only suppress the NP response when administered during the effector phase (Table II).

We have previously demonstrated (7) that Ts1- and Ts1-derived factors induce a

| Table I | Specificity of Hybridoma Suppressor Factors* |
|---------|-------------------------------------------|
| Source of TsF | Strain tested | TsF dilution | Swelling response (x10^-6 cm) ± SE |
| BW5147 | C57BL/6 | Undiluted | 22.0 ± 3.4 | 12.6 ± 2.1 |
| B6-Ts2-28 | C57BL/6 | Undiluted | 8.5 ± 1.3† | 19.3 ± 4.6 |
|           |         | 1/10 | 8.0 ± 1.0† |  |
|           |         | 1/100 | 18.2 ± 2.4 |  |
| B6-Ts2-14 | C57BL/6 | Undiluted | 11.2 ± 1.3‡ | 19.6 ± 5.5 |
|           |         | 1/10 | 12.0 ± 1.0‡ |  |
|           |         | 1/100 | 19.0 ± 2.2 |  |
| BW5147 | B10.BR | Undiluted | 23.5 ± 1.3 | 17.6 ± 1.8 |
| CKB-Ts2-225 | B10.BR | Undiluted | 14.4 ± 1.7‡ | 17.3 ± 2.8 |
|           |         | 1/10 | 14.8 ± 1.4‡ |  |
|           |         | 1/100 | 23.5 ± 1.8 |  |

* Groups of four to five mice were immunized with either NP-O-Su or DNFB. On the day before and the day of antigen challenge, mice were given an injection of 0.4 ml i.v. of either control BW5147 or suppressor factors. The background responses of nonimmunized C57BL/6 mice were 3.5 ± 0.9 and 0.5 ± 0.6 for NP-O-Su and DNFB, respectively. The background responses of B10.BR mice were 6.4 ± 1.1 and 2.4 ± 0.9 for NP-O-Su and DNFB, respectively.
† Significant suppression, P < 0.05.
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Table II

| Source of TsF | Strain tested | NP-O-Su priming | Footpad swelling (× 10^{-6} cm) ± SE |
|--------------|---------------|----------------|-----------------------------------|
|              |               |                | Induction phase | Effector phase |
| None         | C57BL/6       | +              | 19.0 ± 0.9      | 19.0 ± 0.9     |
| B6-Ts1-29    | C57BL/6       | +              | 7.4 ± 0.7       | 18.6 ± 1.7     |
| B6-Ts2-28    | C57BL/6       | +              | 18.2 ± 1.3      | 12.2 ± 1.5‡    |
| B6-Ts2-14    | C57BL/6       | +              | 17.8 ± 1.1      | 12.2 ± 2.1‡    |
| None         | C57BL/6       | −              | 3.4 ± 0.6       | 5.4 ± 0.6      |

None         | B10.A         | +              | 25.0 ± 2.5      | 25.0 ± 2.5     |
| CKB-Ts1-17   | B10.A         | +              | 8.6 ± 2.6‡      | 31.0 ± 4.5     |
| CKB-Ts2-59   | B10.A         | +              | 26.8 ± 4.3      | 12.4 ± 2.4‡    |
| CKB-Ts2-225  | B10.A         | +              | 23.4 ± 4.5      | 11.4 ± 2.7‡    |
| None         | B10.A         | −              | 9.6 ± 1.4       | 9.6 ± 1.4      |

* Groups of four to five mice were immunized with NP-O-Su and were given TsF either at the time of antigen priming (induction phase) or antigen challenge (effector phase).
‡ Significant suppression, P < 0.01.

Table III

| Source of TsF | Strain for Ts generation | Transfer recipients | NP-O-Su priming | Footpad swelling ± SE |
|---------------|--------------------------|---------------------|----------------|-----------------------|
| BW5147        | B10.BR                   | +                   | 31.2 ± 1.9     |
| CKB-Ts1-17    | B10.BR                   | +                   | 12.4 ± 2.2‡    |
| CKB-Ts2-59    | B10.BR                   | +                   | 33.8 ± 1.9     |
| CKB-Ts2-225   | B10.BR                   | +                   | 33.8 ± 3.1     |
| None          | None                     | −                   | 8.2 ± 2.0      |

* Groups of four to five NP-O-Su-primed B10.BR mice received 4 × 10^7 splenic cells from donors that were treated with TsF. The mice were challenged 1–2 h after cell transfer. Footpad swelling was measured 24 h after challenge in units of 10^{-6} cm.
‡ Significant suppression, P < 0.01.

second population of suppressor T cells. To determine whether this new series of factors could also induce suppressor cells, B10.BR mice were given four (day 0 through day 3) intravenous injections of 0.4 ml of TsF. 6 d later the animals were killed, and 4 × 10^7 splenocytes were adoptively transferred into NP-O-Su-primed syngeneic recipients that were challenged 1–2 h after the cell transfers. The data shown in Table III confirm that hybridoma CKB-Ts1-17-derived factor can induce second-order suppressor cells. In contrast, the CKB-Ts2-59 and CKB-Ts2-225 factors fail to generate another population of suppressor cells. Thus, we can conclude that the factors characterized in the present report are different from the Ts1-derived factors previously characterized, and this new group of hybridoma cell lines represents Ts2 cells that produce a biologically active suppressor factor termed TsF2.

Immunological Characterization of TsF2. To further distinguish the CKB-Ts2-225,
CKB-Ts2-59, and B6-Ts2-28 factors from the previously described Ts2 factors, these materials were passed over a series of immunoadsorbent columns. The suppressive activity of the CKB (H-2k)-derived factors could be depleted by passage over anti-I-Jk columns but not anti-I-Jb columns. In contrast, the C57BL/6 (H-2b)-derived factor was specifically adsorbed by anti-I-Jb columns. The suppressive activity could be recovered by acid elution (Table IV). The suppressive activity of the B6-Ts2-28, CKB-Ts2-59 and CKB-Ts2-225-derived suppressor factors were not adsorbed by polyvalent guinea pig anti-immunoglobulin columns, guinea pig anti-idiotype columns, or NP-KLH columns. This contrasts with the results obtained with B6-Ts1-29-derived factor that could be specifically adsorbed by either anti-idiotype or NP-KLH immunoadsorbent columns (Table IV). However, the Ts2 derived but not Ts1-derived factors could be depleted on NPb idiotype columns. Furthermore, the TsF2 suppressive activity was recovered by acid elution from the NPb idiotype columns. The specificity of the adsorption was controlled by the demonstration that the factors could not be adsorbed on columns containing nonimmune mouse immunoglobulin (Table IV). Thus, we conclude that these TsF2 bear I-J determinants and have a specific receptor for NPb idiotype.

Cellular Absorption of TsF2. The binding of TsF2 suppressive activity to NPb idiotype-bearing immunoadsorbent columns indicated that these hybridoma factors bound to B cell-derived immunoglobulin products. In the next series of experiments, the ability of TsF2 to bind to T cells was established. 1.0 ml of CKB-Ts2-59-derived factor was absorbed with 4 × 10⁷ B10.BR RAMG-purified lymph node T cells from mice that were primed 1 wk previously with either NP-O-Su or DNFB. Only the NP-O-Su-primed cells could absorb the TsF2 suppressive activity (Table V). In addition, B6-Ts2-14-derived factor was also specifically absorbed with NP-O-Su immune C57BL/6 T cells (Table V). To assess the influence of H-2 genotype on the ability of NP-O-Su immune T cells to absorb TsF2 activity, CKB-Ts2-59 factor (I-Jk) was absorbed with C57BL/6 (H-2b) NP-O-Su-primed T cells. The data in Table V demonstrate that C57BL/6/6 T cells can absorb CKB-derived TsF2, indicating that H-2 homology is not required between the strain producing the TsF2 and the T cells.

### Table IV

| Immunoadsorbent columns | B6-Ts1-29 | B6-Ts2-28 | CKB-Ts2-59 | CKB-Ts2-225 |
|-------------------------|-----------|-----------|------------|------------|
| None                    | 83        | 75        | 65         | 68         |
| Anti-IJk                | 89 -17    | 63 19     | 5 75       | 23 77      |
| Anti-IJb                | 0 72      | 10 77     | 72 0       | 76 5       |
| Anti-Ig                 | 89 11     | 75 17     | 77 -1      | 50 1       |
| Anti-NPb                | 22 83     | 67 12     | 70 28      | 37 -1      |
| NPb                     | 71 -1     | 9 86      | 4 68       | 0 104      |
| Ig                      | 69 5      | 48 -6     |            |            |
| NP-KLH                  | 17 69     | 61 10     | 92 -11     |            |

*Hybridoma-derived TsF containing supernatant fluids were adsorbed onto various columns and the unbound and acid-eluted fractions were tested for suppressive activity in either C57BL/6 or B10.BR recipients. Some of the results using the control B6-Ts1-29-derived TsF were previously published (7, 8). The data are expressed as the percent suppression of the NP-O-Su CS response compared with BW5147 supernate controls. Less than 30% suppression is not considered significant.
TABLE V

Absorption of TsF2 Factors with T Cells*

| Source of TsF | T cells used for absorption | Percent suppression ± SE |
|--------------|-----------------------------|--------------------------|
|              | Strain or hybridoma | Priming antigen |               |
| BW5147       | None | None | 0 ± 8          |
| CKB-Ts2-59   | None | None | 71 ± 5§        |
|              | B10.BR | NP-O-Su | -2 ± 9        |
|              | B10.BR | DNFB | 61 ± 10‡      |
|              | C57BL/6 | NP-O-Su | 16 ± 5        |
|              | CKB | NP-O-Su | 4 ± 8         |
|              | C3H | NP-O-Su | 53 ± 8‡       |
|              | B6-Ts1-3 |       | -9 ± 12       |
|              | B6-Ts1-29 |       | 1 ± 17        |
|              | CKB-Ts1-17 |       | 14 ± 20       |
|              | CKB-Ts1-19 |       | 14 ± 24       |
|              | CKB-Ts1-39 |       | 8 ± 19        |
|              | AKR (BW5147 tumor) |       | 76 ± 13‡      |
| B6-Ts2-14    | None | None | 76 ± 7‡       |
|              | C57BL/6 | NP-O-Su | 15 ± 12       |
|              | C57BL/6 | DNFB | 71 ± 13‡      |

*1 ml of hybridoma-derived TsF2 was absorbed with either 4 × 10^7 NP-O-Su- or DNFB-primed lymph node T cells from mice of the strains listed or with 5 × 10^6 Ts1 hybridoma T cells. The factor was then diluted 1:5 and 0.4 ml was injected i.v. on the day before and the day of antigen challenge. The data represent the pooled results from three separate experiments (not all groups were included in each experiment). The data were normalized and the percent suppression ± SE was calculated.

§ Significant suppression, P < 0.01.

used for absorption. To determine whether a requirement for Igh homology exists, CKB-Ts2-59 factor was absorbed with CKB (Igh^b) or C3H (Igh^c) NP-primed lymph node T cells. The C3H cells failed to absorb significant levels of TsF2 suppressive activity, whereas T cells from the Igh congenic CKB strain depleted suppressive activity (Table V). Finally, we attempted to absorb TsF2 activity with 5 × 10^6 NP^b idiotype-bearing Ts1 hybridoma cell lines derived from either C57BL/6 (H-2^b) or CKB (H-2^c) donors. As also shown in Table V, each of these Ts1 hybrids absorbed TsF2 activity, whereas two nonfunctional B6 T cell hybridoma lines (data not shown) and the BW5147 tumor line that was used to prepare the hybrids failed to absorb the suppressive activity. Thus, Tss-derived suppressor factor can specifically bind antigen-primed T cells or hybridoma cells that bear idiotypic determinants. Furthermore, the binding of TsF2 to idiotypic T cells does not require H-2 homology.

Genetic Restriction of TsF2. We have previously characterized (4, 7, 9) the genetic restrictions of the splenic Ts2 cell population. The results of these past experiments indicated that this population had a dual restriction for both H-2 and Igh-V complex genes. Because these experiments always used a heterogeneous population of cells, it was never clear whether one group of Ts2 cells was Igh restricted and another subset was H-2 restricted. The production of monoclonal Ts2 hybridomas now permits definitive analysis of this issue. The data in Table VI demonstrate that monoclonal TsF2 factors are restricted by both H-2 and Igh genes. Thus, CKB-Ts2-59, CKB-Ts2-225, and CKB-Ts2-217 factors that are derived from cells that carry the H-2^b and
Igh<sup>b</sup> haplotypes only suppress NP-induced CS responses of CKB (H-2<sup>k</sup>, Igh<sup>b</sup>) or B10.BR (H-2<sup>k</sup>, Igh<sup>b</sup>) mice. These factors fail to suppress NP-induced CS responses in Igh congenic C3H (H-2<sup>k</sup>, Igh<sup>j</sup>) mice or H-2 congenic CWB (H-2<sup>b</sup>, Igh<sup>b</sup>) mice. Similarly, the C57BL/6 (H-2<sup>b</sup>, Igh<sup>a</sup>)-derived factors suppress the CS responses of C57BL/6 mice but fail to suppress NP-specific responses induced in Igh congenic B6.Igh<sup>a</sup> or H-2 congenic B10.BR recipients (Table VI). These experiments were repeated using monoclonal TsF<sub>2</sub> derived from the cloned B6-Ts2-28 and CKB-Ts2-59 cell lines; comparable data were obtained (data not shown).

**Intra-H-2 Mapping of TsF<sub>2</sub> Restrictions.** To determine which subregions of the H-2 complex restricted TsF<sub>2</sub> activity, the B6-Ts2-28, CKB-Ts2-59, and CKB-Ts2-225 factors were tested in B10.MBR (K<sup>b</sup>, I<sup>k</sup>, S<sup>k</sup>, D<sup>b</sup>), B10.A(3R) (K<sup>b</sup>, IA<sup>b</sup>, IB<sup>b</sup>, IJ<sup>b</sup>, IE<sup>b</sup>, IC<sup>d</sup>, SI<sup>d</sup>, D<sup>o</sup>), B10.A(4R) (K<sup>k</sup>, IA<sup>a</sup>, IB<sup>b</sup>, IJ<sup>b</sup>, IE<sup>b</sup>, IC<sup>b</sup>, S<sup>d</sup>, D<sup>o</sup>), and B10.A(5R) (K<sup>b</sup>, IA<sup>b</sup>, IB<sup>b</sup>, IJ<sup>k</sup>, IE<sup>b</sup>, IC<sup>d</sup>, SI<sup>d</sup>, D<sup>o</sup>) NP-O-Su-primed recipients. The results of three independent experiments were pooled (Table VII). The C57BL (H-2<sup>b</sup>)-derived B6-Ts2-28 factor suppressed NP responses in H-2K-, I-A-, I-B-, and I-J-compatible B10.A(3R) and I-B-, I-J-, I-E-, I-C-, S-, and H-2D-compatible B10.A(4R) mice but failed to suppress H-2K-compatible B10.MBR or H-2K-, I-A-, and I-B-compatible B10.A(5R) recipients. In contrast, the CKB (H-2<sup>k</sup>)-derived CKB-Ts2-59 and CKB-Ts2-225 factors suppressed I-A-, I-B-, I-J-, I-E-, I-C-, and S-compatible B10.MBR and I-J- and I-E-compatible B10.A(5R) recipients but failed to suppress I-E-compatible B10.A(3R) or H-2K- and I-A-compatible B10.A(4R) recipients. Thus, it appears that I-J homology is required between the TsF<sub>2</sub> donor and recipient strains for factor activity.

**Discussion**

We characterize five T cell hybridomas that correspond to the Ts<sup>a</sup> or Ts<sup>b</sup> cells previously identified in the NP system. These hybrids differ from three other NP-specific suppressor T cell hybridomas previously characterized that represented Ts<sub>1</sub> cells. The differences between the hybridoma Ts<sub>1</sub> and Ts<sub>2</sub> surface phenotypes correlate
with those noted between the splenic T{s} and T{s} populations in the NP and other systems (1, 2, 4). Thus, T{s} cells bear I-J, Thy-1, and idiotypic determinants, whereas T{s} cells bear the I-J and Thy-1 markers but lack NPb idiotypic determinants. The T{s} hybridoma cells generally display the same surface phenotype but occasionally some hybrids lose the parental Thy-1.2 marker, e.g., CKB-T{s}-59, presumably due to loss of the parental chromosome coding for this marker (8).

The antigen-specific suppressor activity of the T{s} hybridoma culture supernates also has several features that distinguish it from the previously characterized T{s} derived factors. First, T{s} functions during the effector phase of the immune response, whereas T{s} only induces suppression when given during the induction phase. These differences in the kinetics of T{s} activity reflect differences in the mechanism by which these factors function. Thus, T{s} induces a second population of suppressor cells (Table III and reference 8), whereas T{s} presumably activates an antigen-primed T{s} target population. Because T{s} does not function when given during the induction phase of the immune response, we assume that this material, in the absence of the appropriate target cells, has a very short biological half-life.

The second method used to distinguish these two I-J-bearing factors is to characterize the binding properties of each factor. As shown in Table IV, T{s} bears NPb idiotypic determinants and binds to hapten-coupled immunoabsorbant columns. In contrast, T{s} lacks NPb idiotypic determinants and hapten-binding specificity, but can specifically bind to NPb idioype or to T cells that bear NPb idiotypic determinants, i.e., the T{s} derived factor is anti-idiotypic. The specificity of these factors correlates with the specificity of the T{s} and T{s} cell surface receptors. Thus, T{s} cells bear NPb idiotypic determinants and can bind hapten, whereas the T{s} population bears anti-idiotypic receptors (3-5). These correlations between Ts and TsF specificity support the notion that TsF may represent a solubilized membrane receptor.

It is important to note the specificity of cellular absorption of TsF2 (Table V). Thus, TsF2 can be absorbed by H-2-compatible or H-2-incompatible NP immune T cells but not by T cells primed with another antigen. These findings contrast with those of
Taniguchi et al. (10), who demonstrated that in their system, suppressor-factor binding was H-2 restricted and their suppressor factor could be absorbed by normal T cells. These authors postulated (10) that TsF binding was controlled by an I-J acceptor site on a subpopulation of suppressor T cells. Because of these major differences between the two systems studied, we conclude that the factors and cellular interactions described in the latter report may relate to other suppressor products (perhaps Ts3) rather than those described in this report.

The third method for distinguishing between the TsF1 and TsF2 factors is by the genetic restrictions required for factor activity. Thus, TsF1 factor will suppress antigen-primed mice that are H-2 incompatible with the strain producing the factor (7). In contrast, TsF2 can only suppress antigen-primed mice that share H-2 genes with the strain producing the TsF2 factor. The nature of the TsF2 H-2 restriction is not clear because NPb idiotype columns that lack H-2 determinants and NP-immune T cells from H-2 incompatible mice can absorb the factor (Tables IV and V). To account for these paradoxical observations we propose the following possibilities: (a) the H-2 restriction reflects a pseudogenetic restriction in which TsF2 activates an H-2-restricted Ts3 cell population. Pseudogenetic restrictions involving Tsa cells and factors have been well documented in the NP and other systems (7, 11, 12). Alternatively, (b) the Ts3 cells (which are considered to represent the cellular targets of TsF2) might have a receptor that recognizes H-2 determinants on TsF2. Activation of Ts3 cells might therefore require the simultaneous stimulation of both idiotypic and H-2 receptors, resulting in an apparent dual genetic restriction. This could account for the ability of H-2-incompatible cells to absorb the H-2-restricted TsF2. Further experiments are required to understand the nature of the dual H-2 and Igh restriction of the TsF2 factor. However, the present results demonstrate that these dual restrictions are properties of monoclonal factors from a series of hybridoma T cell lines.

The genetic restrictions of antigen- or TsF1-induced Ts2 cells have been previously characterized. We and others (3, 4, 6) have demonstrated that this cell population is both Igh and H-2I restricted. One series of experiments attempted to dissect the H-2 restriction of Ts9 cells and demonstrated that genes in the I-A subregion control this restriction. However, those previous experiments (9) involved the in vivo transfer of immunocompetent splenic Ts2 cell populations into allogeneic recipients. We assumed that the potential allogeneic effects had no influence on suppressive activity. However, Bromberg et al. (13) recently demonstrated the activation of Ts suppressor cells by an allogeneic effect. Thus, the conclusions from our previous experiment must be reinterpreted in view of this complicating factor. In the present experiment, three different monoclonal TsF2 factors were used to investigate genetic restrictions. Because soluble factors were used in the present experiments instead of immunocompetent cells, we believe that the potential complications introduced by allogeneic effects have been eliminated. Therefore, the I-J and Igh restrictions observed in the current experiments probably reflect the restrictions on the Ts2 cell population. Tada et al. (14) had previously demonstrated the involvement of I-J restrictions in a different suppressor cell system, but the restrictions in other systems have mapped to different regions of the H-2 complex (15-17). The reasons for these apparent discrepancies are not clear; they might reflect interactions between different suppressor cell subsets or alternative receptors on some Ts populations.

The present results can be combined with previous data on the NP suppressor cell
CHARACTERIZATION OF TsF₂ SUPPRESSOR FACTOR

FIG. 1. Immunoregulation of NP-induced immune responses. A schematic representation of the cellular populations in the suppressor pathway. See text for details.

The Ts₂ cell population bears Lyt-2 and I-J determinants and has an anti-idiotypic receptor (3, 4). In contrast to inducer Ts₁ cells, Ts₂ cells suppress during the effector phase of the immune response. The hybridoma Ts₂ cells described in this report produce a suppressor factor termed TsF₂, which bears I-J determinants and has binding specificity for NPβ idiotypic determinants; this factor probably corresponds to the factor characterized by Dietz et al. (19) in another suppressor cell system. Monoclonal TsF₂ factor is restricted by both H-2 and Igh genes. There are suggestions that the Ts₂ population might be heterogeneous (20). The presumed target of TsF₂ is an antigen-primed, I-J⁺, Lyt-2⁺ cell population that bears idiotypic determinants (4). This third

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cell population is termed TsS. NP-specific TsS cells are poorly characterized but have been identified in cell preparations from antigen-primed lymph nodes (4). Hybridoma TsS lines have not yet been described. Thus, some of the properties of TsS cells may be attributable to distinct T cell subsets that are present in the mixed TsS population. The TsS population or a factor (TsF3) produced by this population may interact directly or indirectly (via other cellular intermediates) with the T cells that mediate CS or helper activity for NPb-specific B cells. The precise nature and mechanism of the final phase of immune suppression remains unresolved. Experiments are underway to prepare TsS hybridomas to help clarify some of the outstanding questions concerning the final phases of the suppressor T cell pathway.

Summary

Five hybridoma T cell lines were prepared by fusion of second order suppressor T cells (Ts2) with the BW5147 thymoma. The culture supernates from these T cell hybrids contained a factor, TsF2, which specifically suppressed 4-hydroxy-3-nitrophenyl acetyl hapten (NP)-induced cutaneous sensitivity responses. TsF2 activity was observed when the factor was administered during the effector phases of the immune response. TsF2 bears I-J determinants and has binding specificity for NPb idiotypic determinants. TsF2 suppressor activity could be absorbed on antigen-primed H-2-incompatible T cells but cannot suppress H-2-incompatible mice. In addition to this H-2 restriction, which maps to the I-J subregion, monoclonal TsF2 also has an Ig genetic restriction. The present results are combined with previous data to describe the cellular interactions leading to immune suppression.

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