ANTIGEN- AND RECEPTOR-DRIVEN
REGULATORY MECHANISMS

VII. H-2-restricted Anti-Idiotypic Suppressor Factor from Efferent Suppressor T Cells*

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Interactions between T lymphocyte subsets characterize virtually all well-studied models of antigen-specific immune suppression (1-11). In addition, soluble factors possessing antigen-binding sites and I region-coded determinants have been found to play major roles in these cell-cell interactions (reviewed in 12, 13). However, no clear consensus has been reached as to the precise phenotype of each participating T cell, the total number of cells involved in the overall pathway from initial antigen stimulation to actual suppression, or the importance of H-2 and/or variable region of immunoglobulin heavy chain (V\textsubscript{H})\textsuperscript{1}-linked genetic restrictions at each step. At least some of the difficulty in resolving these issues arises because most studies to date appear to involve only a portion of a much larger sequence of cell interactions.

Over the past several years these laboratories have attempted to explore these issues by analyzing the sequence of T-T interactions in several T suppressor (Ts) models. Previous studies have defined in detail the production of antigen-specific T cell-derived suppressor factors (TsF) from antigen-induced first-order Ts (Ts\textsubscript{i}) (we have termed this TsF "TsF\textsubscript{i}") (14-17). In four different models, such TsF have been shown to bear I-J-subregion-coded determinants and to function by inducing a second set of Ts, termed Ts\textsubscript{2}, from resting T cell populations (2, 4, 17-21). This induction occurs across H-2 and background genetic differences (19, 22, 23). More-recent studies have shown that these TsF share serologically detectable idiotypic determinants with antibody of the same specificity (24, 25), and, in a model system involving suppression of delayed-type hypersensitivity (DTH) responses to the hapten azobenzenearsonate (ABA), that the Ts\textsubscript{2} induced by idiotypic ABA-TsF\textsubscript{i} are, in fact, anti-idiotypic (10). The present study continues this work by investigating the suppressive activity of such anti-idiotypic Ts\textsubscript{2}. The data demonstrate that in contrast to Ts\textsubscript{i}, Ts\textsubscript{2} are able to

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Abbreviations used in this paper: ABA, azobenzenearsonate; ABA-SC, ABA-coupled spleen cells; CRI, cross-reactive major idiotype of A/J anti-ABA antibodies; CRI-Ig, CRI-containing Ig; DNBS, dinitrobenzenesulfonate; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salts solution; NP, 4-hydroxy-3-nitrophenyl acetyl; PBS, phosphate-buffered saline; TNP, trinitrophenyl; Ts, suppressor T cell(s); Ts\textsubscript{i}, antigen-induced first-order Ts; Ts\textsubscript{2}, TsF\textsubscript{i} induced second-order Ts; TsF\textsubscript{2}, antigen-specific T cell-derived suppressor factors; TsF\textsubscript{2}, soluble suppressor factor from Ts\textsubscript{i}; TsF\textsubscript{2}, soluble suppressor factor from Ts\textsubscript{2}.
suppress already immune animals. Furthermore, the Ts2 produce a second soluble factor, TsF2, which lacks Ig-constant-region determinants, bears H-2-coded determinants, is anti-idiotypic, and fails to act across H-2 genetic differences. These results provide the basis for a hypothesis integrating a number of suppressor models into a single overall scheme.

Materials and Methods

**Mice.** A/J (H-2b, Igh-1~), A.By (H-2b, Igh-1~), B10.A (H-2a, Igh-1b) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All animals in these experiments were 8–10 wk of age at the time of the experiment. All experimental groups consisted of at least four animals per group.

**Preparation of Antigen and Antigen-coupled Cells.** These procedures have been described in detail elsewhere (26). Briefly, a 40-mM solution of diazonium salt was prepared from p-arsanilic acid (Eastman Kodak Co., Rochester, N.Y.). The ABA solution was activated as previously described and conjugated to single-cell suspensions of erythrocyte-free spleen cells at a final concentration of 10 mM ABA. After washing in Hank’s balanced salts solution (HBSS), the ABA-coupled spleen cells (ABA-SC) were used to induce DTH or Ts. Trinitrophenyl (TNP)-coupled spleen cells were prepared as described earlier (27).

**Induction and Elicitation of DTH to ABA- to TNP-coupled Cells.** To induce DTH to ABA or TNP, a total of 3 × 10^7 ABA-coupled syngeneic cells or TNP-coupled syngeneic spleen cells were injected subcutaneously into two separate sites on the dorsal flanks of mice. Challenge was performed four d later by injecting 30 #! of 10 mM diazonium salt of p-arsanilic acid or 30 #! of 10 mM 2,4,6-trinitrobenzene sulfonic acid, pH 7.4, (Eastman Kodak Co.) into the left footpad. 24 h after the footpad challenge, DTH reactivity was assessed by measuring the swelling of the footpad using a Fowler Micrometer (Schlesinger’s for Tools, Brooklyn, N.Y.). The magnitude of the DTH was expressed as the increment of thickness of the challenged left footpad as compared with the untreated right footpad. Responses are given in units of 10^-2 mm ± SEM.

**Induction of Suppressor Cells and Preparation of Suppressor Factors.** Normal A/J mice were injected intravenously with 5 × 10^7 ABA-SC. 7 d later, these mice served as donors of Ts1 (10). Spleens from such animals were removed, and a single-cell suspension was prepared in HBSS. The cells were washed twice in HBSS and counted. To assay for the ability of such cells to inhibit ABA-specific DTH, 5 × 10^7 viable cells were injected intravenously into normal recipients, which were then primed subcutaneously with ABA-SC and challenged five d later. Production of TsF1 was done precisely as described earlier (17). Briefly, 1 × 10^8–5 × 10^8 washed spleen cells in 1 ml of HBSS were subjected to alternate snap freezing at -78°C and thawing at 37°C. This was repeated four times and followed by centrifugation at 10,000 g for 90 min. The supernates were adjusted to 5 × 10^7 cell-equivalents/ml and frozen until use at -40°C. To test the ability of TsF1 to inhibit ABA-specific DTH, the extract was injected intravenously into normal mice beginning at the time of immunization with 3 × 10^7 ABA-SC. 2 × 10^7 cell-equivalents of factor were administered each day for five successive d, at which time the animals were immunized and tested for DTH activity by adoptive transfer to normal recipients, which were immunized and challenged as described above. TsF2 was prepared from the spleens of TsF1-treated animals in the same manner as TsF1. TsF2 was usually assayed by administering 2 × 10^7 cell-equivalents of extract/d per mouse on the day before and the day of footpad challenge of subcutaneously primed mice. As indicated in Results, however, Ts1, TsF1, TsF2, and TsF2 were also tested according to other schedules to assess afferent versus efferent suppressive activity.

**Affinity Chromatography of TsF2.** Solid-phase immunoabsorbent columns were prepared and characterized as described earlier (17, 25). Soluble TsF2 was fractionated on immunoabsorbents in the following manner. 5-ml plastic columns containing Sepharose 4B conjugated with the relevant protein were prepared and extensively washed with phosphate-buffered saline (PBS), pH 7.2, immediately before loading of the suppressor factors. The adsorption of the factor was performed at 4°C by allowing 2.4 × 10^8 cell-equivalents of suppressor factor in 1 ml to enter
the gel matrix; the suppressor factor was then allowed to remain in the column for at least 60 min at 4°C. The column was then washed with at least five times its own void volume of PBS, pH 7.2. Such effluents were termed filtrates. Materials that remained adsorbed to the column were rapidly eluted with five bed-volumes of a glycine-HCl buffer, pH 2.8. The collected eluate was immediately neutralized to pH 7.0 with 1 N NaOH as the material emerged from the column. Both the filtrate and the eluate were concentrated to the original volume by negative-pressure dialysis at 4°C and were thereafter frozen at -40°C. Such materials were thawed and adjusted to the appropriate concentration immediately before use.

Purification of Suppressor Cells on Antigen-coated Plates. Specific suppressor cells were separated according to a modification of the method of Taniguchi and Miller (28), as reported earlier (29).

Antiserum Treatment. Anti-Thy 1.2 hybridoma antiserum was kindly provided by Dr. P. Lake, University College, London, England. 1 × 10^6 spleen cells from animals treated with TsF1 were incubated with 1 ml of 1:20 dilution of anti-Thy 1.2 hybridoma antibody for 45 min at 0°C, washed once in HBSS, and then incubated again with 1 ml of a 1:10 dilution of Low-Tox rabbit complement (Cedarlane Laboratories Ltd., London, Ontario, Canada) for 30 min at 37°C. The cells were then washed twice in HBSS and passed over a Ficoll-Hypaque density gradient to remove the dead cells. The cells at the interface were recovered, extensively washed, counted (>90% viability as determined by trypan blue exclusion), and adjusted to the appropriate concentration for the preparation of TsF2.

Statistical Analysis. Analysis of the significance of the differences in the data obtained was carried out using a Wang programmable computer. The means and SEM were expressed as well as the relevant P value obtained with the two-tailed Student's t test.

Results

Ts1 and Ts2 Differ in Their Ability to Suppress an Immunized Animal. Ts able to regulate the response of an immunized animal are frequently termed "efferent" suppressors and have been reported in several systems (30–32). This is in contrast to those Ts that act only when given before or at the time of initial antigen priming, the so-called "afferent" suppressors (31). Because Ts1 arise before and stimulate Ts2, the ability of these two cell types to suppress when transferred at different times during the ABA-DTH response was investigated. Ts1 and Ts2 cells were prepared as in Materials and Methods and transferred to sets of mice that were primed either on various days before or on the day of Ts transfer. All groups were challenged in the footpad 4 d after priming, and the DTH response assayed on day five. As shown in Fig. 1, both Ts1 and Ts2 suppress when given on the day of priming (afferent suppression). Ts1 fail to decrease responses when transferred 2 or more d after priming. Ts2, in contrast, could suppress the DTH responses of mice when transferred 2 or 3 d after priming, indicating that they could function in an efferent mode. These experiments also clearly indicate that in addition to differential receptor specificity, as reported earlier (10), Ts1 and Ts2 are functionally distinct suppressor T cell subsets.

The Ts2 Capable of Suppressing When Transferred Late in an Evolving Immune Response are Anti-Idiotype. In an earlier report (10) the Ts2 induced by TsF1 were shown to be anti-idiotypic by the demonstration that such cells adhered to plates coated with cross-reactive major idiotype of A/J anti-ABA antibodies (CRI)-containing mouse immunoglobulin (CRI-Ig). In that report, the anti-idiotypic cells were transferred to mice at the time of immunization. To insure that the Ts2 that were able to act late in an ongoing immune response were the same anti-idiotypic Ts2 described earlier, plate-separated T cells were transferred to immune mice 1 d before challenge (Fig. 2).

This experiment demonstrates that efferent suppressor cells were depleted from the whole T cell population by incubation on CRI-Ig-coated plates, but not on control
Fig. 1. $T_s$ and $T_{ss}$ differ in their ability to suppress an immunized animal. A/J mice were immunized with $3 \times 10^7$ ABA-SC subcutaneously on day 0. $5 \times 10^7 T_s$ (○), induced by intravenous injection of ABA-SC, or $T_{ss}$ (△), induced by TsF, were transferred intravenously into different groups of mice either on the day of immunization (day 0) or day 2, or day 3, or day 4. All mice were challenged 4 d after immunization with 30 μl of the diazonium salt of p-arsanilic acid as described in Materials and Methods. 24 h later, the degree of footpad swelling was determined. The percentage of suppression transferred was calculated according to the following formula:

$$\text{percent tolerance} = \left( \frac{\text{positive control} - \text{experimental value}}{\text{positive control} - \text{negative control}} \right) \times 100.$$ * Not significantly different from control.

Fig. 2. The $T_s$ capable of suppressing when transferred late in an evolving immune response are anti-idiotypic. $T_s$ were obtained from A/J mice treated with $2 \times 10^7$ cell-equivalents of TsF (intravenously) for 5 successive d. They were then incubated first on rabbit anti-mouse Ig plates to remove the immunoglobulin-bearing B cells. Part of the enriched T cell population was assayed for suppressor activity (group C) or subjected to further purification on plates coated either with normal A/J immunoglobulin (groups D and E) or anti-ABA antibodies containing CRI determinants (groups F and G). The various populations were transferred to different groups of A/J mice which had been immunized with $3 \times 10^7$ ABA-SC 3 d earlier to assay for efferent suppression. All the mice and the appropriate control animals were challenged with the diazonium salt 24 h after cell transfer and increase in footpad swelling was determined 24 h after challenge. The bars represent the mean footpad swelling of at least four mice/group.
Ig-coated plates. In addition, such cells were recovered only from CRI-Ig-coated plates. Therefore, the Ts2 capable of inhibiting an ongoing immune response have anti-idiotypic receptors.

Ts2 and a Soluble Suppressor Factor (TsF2) Derived from Ts2 Suppress Fully Immune Mice. The preceding experiments, although demonstrating the ability of Ts2 to act late after priming, did not prove that such Ts could inhibit the elicitation of DTH in a fully immune animal already able to give a response at the time of Ts transfer. The inability of Ts2 to suppress when given on the day of challenge (Fig. 1) might indicate either that these cells cannot inhibit fully differentiated primed T cells or, alternatively, that additional time is necessary for optimal Ts2 activity after transfer, either alone or in concert with additional cells of the recipient. To explore these issues, and to determine if Ts2 acted via a soluble factor in analogy to Ts1 and TsF1, a series of experiments was carried out in which challenge was delayed until day 5, permitting Ts2 or TsF2 transfer beginning on day 4, a time at which DTH can be readily elicited. As shown in Fig. 3, either Ts2, administered on day 4, or a soluble extract of Ts2, termed TsF2, given on days 4 and 5, markedly inhibited ABA DTH responses, indicating their ability to suppress the activity of already immune cells. Further, the source of TsF2 is shown to be a T cell by the loss of TsF2 activity from an anti-Thy 1.2 plus C-treated cell population.

Suppression by TsF2 is Antigen Specific. In an earlier report, it was shown that TsF1 acted in an antigen-specific fashion (17). Because antigen-specific suppressor pathways may terminate with a nonspecific effector molecule (33-34), the antigen specificity of the TsF2 was tested. The results in Fig. 4 show that TsF2 was not able to suppress DTH to the hapten TNP. Hence, TsF2 functions in an antigen-specific fashion.

Functional Characterization of TsF2. To further analyze the activity of TsF2, a series of functional experiments comparing it to TsF1 was carried out. In the experiment

![Graph Image](image-url)

Fig. 3. Ts2 and a soluble suppressor factor TsF2 derived from Ts2 suppress fully immune mice. Ts2 were obtained from A/J mice as described in Materials and Methods. Some of the Ts2 were assayed for suppressor activity by adoptive transfer (group B), whereas others were either not treated (group C) or treated with complement alone as control (group D) or with anti-Thy 1.2 antibody and complement (group E). After the antiserum treatment, viable cells were recovered by density gradient separation using Ficoll-Hypaque. These cells (groups C, D, and E) were then subjected to several freeze-thaws to produce TsF2 as described in Materials and Methods. To assay for TsF2 activity, A/J mice were immunized with 3 × 10^7 ABA-SC on day 0. On day 4 or 5, they were treated with 3 × 10^7 cell equivalents of TsF2 (intravenously) for two successive d. All the animals and the appropriate controls were challenged on day 5 and the increased footpad swelling measured 24 h later. The bars represent the mean footpad swelling of at least four mice/group.
TsF₂ is antigen specific. A/J mice were immunized with either $3 \times 10^7$ ABA-SC or $3 \times 10^7$ TNP-SC subcutaneously on day 0. On days 3 and 4, they received $3 \times 10^7$ cell equivalents of TsF₂ obtained from animals injected with ABA-specific TsF₁ (groups B and E). All the animals and the appropriate controls were challenged on day 4 with the diazonium salt of p-arsanillic acid or TNBS in the footpad. 24 h later, the degree of footpad swelling was determined. The bars represent the mean footpad swelling of at least four mice/group.

![Suppressor Factor Administration Table](image)

Depicted in Fig. 5, TsF₁ and TsF₂ were each administered to different sets of immunized A/J mice. They were either given over 5 d, beginning with the day of immunization and ending with the day of challenge, or they were administered only on the day before and the day of challenge. TsF₁ was able to suppress only when administered beginning with the day of immunization. TsF₂ was able to suppress when administered in either fashion. This pattern paralleled that seen with the cells, i.e., TsF₁ acts only in the afferent mode, whereas TsF₂ can act in the efferent mode.

A characteristic feature of TsF₁ studied in these laboratories is activity across H-2 differences. This contrasts markedly with data on antigen-specific TsF active in secondary humoral responses as reported by Tada et al. (35), and recent work showing that 4-hydroxy-3-nitrophenyl acetyl (NP)-specific efferent Ts failed to suppress across I-region differences (37). TsF₂ was thus tested for its ability to act across various genetic differences. Fig. 6 shows that TsF₁ from A/J mice was able to suppress DTH in the H-2-congenic strain A.BY (H-2²). On the other hand, TsF₂, produced from Ts₂ induced by the same lot of TsF₁, was unable to suppress the A.BY strain when
Fig. 6. H-2 restriction of ABA-TsF2 activity. A total of 10^6 cell-equivalents of A/J-derived TsF1 or 6 x 10^7 cell-equivalents of TsF2 was administered on days 0-4 or 4 and 5 to A/J and A.BY mice, respectively. These mice were all immunized with ABA-syngeneic spleen cells subcutaneously on day 0 and challenged in the footpad with diazonium salt on day 5. Footpad swelling was determined 24 h later.

Additional experiments were then carried out to assess whether or not gene products of non-H-2 loci also played a role in restricting TsF2 activity. Furthermore, it was important to insure that the inability of the A.By mice to be suppressed in the efferent mode by A/J TsF2 was not because of some inherent defect in TsF2 activity in A.By mice. Therefore, in the experiment depicted in Fig. 7, TsF2 was produced not only in
A/J, but also in the H-2-congenic strain A.By and in B10.A, which, like A/J, is H-2\(^a\) but which differs in its background. The TsF\(_2\) was produced in each of the three strains by administration of TsF\(_1\) derived from the same strain. A/J-derived TsF\(_2\) was only able to suppress the immune response in A/J mice. A.By and B10.A mice were nonetheless suppressed by syngeneic TsF\(_2\). Thus, both H-2 and non-H-2 gene products play roles in genetically restricting the function of TsF\(_2\).

**Immunochemical Characterization of TsF\(_2\).** A/J TsF\(_1\) has been shown to bear H-2-coded determinants, but no Ig constant region determinants (17, 25). In addition, TsF\(_1\) binds to ABA-conjugated Sepharose 4B columns and is retained by an anti-CRI column. TsF\(_2\) was similarly characterized immunochemically in a series of experiments, the results of which are shown in Table I. In these studies, the filtrates and acid eluates of TsF\(_2\) passed over a variety of immunoadsorbents were tested in the efferent mode. TsF\(_2\) from A/J (H-2\(^a\)) mice was retained by an anti-H-2\(^a\) column, but not an anti-H-2\(^b\) or by an anti-mouse Ig column. The activity retained on the anti-H-2\(^a\) absorbent could be recovered in the acid eluate. Furthermore, TsF\(_2\) was retained by and eluted from a column to which CRI-Ig was coupled, but not to columns to which nonspecific immune A/J Ig was coupled nor to columns to which ABA was.

### Table I

**Immunochemical Characterization of ABA-TsF\(_2\)**

| Experiment | Immunoadsorbent fraction* | Footpad size‡ | Percent suppression§ |
|------------|---------------------------|---------------|----------------------|
| 1          | None (positive control)   | 25.2 ± 2.0    | —                    |
|            | RAMIg, filtrate           | 11.3 ± 1.1    | 79                   |
|            | RAMIg, eluate             | 29.8 ± 1.8    | 0                    |
|            | B10 anti-B10.A, filtrate | 21.8 ± 2.1    | 20                   |
|            | B10 anti-B10.A, eluate   | 14.0 ± 1.5    | 64                   |
| 2          | None (positive control)   | 23.2 ± 2.0    | —                    |
|            | Unfractionated ABA-TsF\(_2\)                             | 5.0 ± 1.1    | 100                  |
|            | B10 anti-B10.A, filtrate | 18.7 ± 0.9    | 21                   |
|            | B10 anti-B10.A, eluate  | 7.5 ± 0.2     | 80                   |
|            | (B10.D2 × B10.BR) anti-B10.6R, filtrate | 5.5 ± 0.8 | 86                   |
|            | (B10.D2 × B10.BR) anti-B10.6R, eluate | 23.0 ± 2.4 | 0                    |
| 3          | None (positive control)   | 29.3 ± 3.0    | —                    |
|            | Unfractionated ABA-TsF\(_2\)                             | 7.8 ± 1.2    | 100                  |
|            | A/J control Ig (CRI\(^1\)), filtrate          | 9.0 ± 0.9    | 100                  |
|            | A/J control Ig (CRI\(^1\)), eluate            | 30.5 ± 3.4   | 0                    |
|            | A/J anti-ABA Ig (CRI\(^1\)), filtrate         | 25.0 ± 1.4   | 22                   |
|            | A/J anti-ABA Ig (CRI\(^1\)), eluate           | 16.5 ± 0.9   | 65                   |
|            | ABA-FyG, filtrate                | 13.8 ± 2.3   | 79                   |
|            | ABA-FyG, eluate                  | 28.3 ± 1.7   | 0                    |

RAMIg, rabbit anti-mouse Ig; FyG, fowl gamma globulin.

* A/J ABA-TsF\(_2\) was passed over indicated immunoadsorbent and filtrate, then acid eluate collected, and tested in the efferent mode. B10 anti-B10.A = anti-H-2\(^b\), (B10.D2 × B10.BR); anti-B10.6R = anti-H-2K\(^b\).15.

‡ P value is in comparison to positive control.

§ Percent supression = \(\frac{\text{positive control DTH} - \text{experimental DTH}}{\text{positive control DTH} - \text{negative control DTH}}\) × 100%.
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coupled in the form of ABA-fowl gamma globulin. These experiments characterize
TsF2 as having H-2 determinants and anti-idiotypic specificity. It does not have anti-
antigen binding activity.

Discussion

The studies reported above provide significant new information about the T cells
and T cell-derived products involved in antigen-specific immune suppression of ABA
DTH (10, 17, 25, 26, 29). The data indicate that antigen-specific Ts1 and TsF1 act in
the afferent mode, before immunization, whereas Ts2 and the soluble product of these
anti-idiotypic cells, TsF2, act in the efferent mode in fully immune animals. TsF2
from the A/J strain has been characterized functionally and immunochemically. In
contrast to TsF1, TsF2 fails to act across either H-2 or non-H-2 genetic differences, as
shown by the observations that TsF2 from A/J mice was not suppressive in A.By or
B10.A mice. The active material lacks Ig constant region determinants and ABA-
binding activity, but possesses H-2-coded determinants and has anti-idiotypic (anti-
CRI) binding specificity. Similar idiotypic and anti-idiotypic TsF that suppress the
idiotypic component of the humoral anti-ABA response have been reported previously
(36).

Two major issues are raised by these results. The first involves the difference in
mode of action between TsF1 and TsF2. Several earlier studies have demonstrated
that some Ts act only in nonimmune animals to prevent priming, whereas other Ts
can inhibit responses of immune animals (31, 37). The present report confirms these
observations, in a model in which the direct relationship between the two cell types
has been established. It is not clear if Ts1 have any actual suppressive activity, or if
they function only via Ts2 induction. The time (24-48 h) needed for this inductive
step would account for the inability of Ts1 to function late in the immune response.
Ts2 clearly act in primed animals, but whether or not Ts2 are actually effector Ts is
not apparent. Their failure to suppress when given on the day of challenge may be
simply a quantitative or homing problem, or may, in fact, reflect the necessity for Ts2
to act on a third cell type which might be the actual effector suppressor cell. Whether
or not the target of Ts2 activity is another cell in the suppressor pathway or is the
immune cell being suppressed, the specificity of action shown by the anti-idiotypic
Ts2 and the previous demonstration of the failure of such cells to suppress mice lacking
the appropriate allotype linkage group of genes necessary for CRI expression indicate
that the target cell bears idiotypic (CRI) determinants. Recent experiments have in
fact identified a CRI+ T cell distinct from the DTH effector cell that is required for
Ts2-mediated suppression (M.-S. Sy, unpublished observations).

The second major issue raised by this study involves the genetic restrictions evident
in TsF2, but not TsF1 activity. Examining TsF obtained from Ts in the l-glutamic
acid60-l-alanine60-l-tyrosine60, l-glutamic acid60-l-tyrosine60, or ABA (M.-S. Sy,
manuscript in preparation) models (19, 20) has not revealed the I-J restrictions of TsF
function reported by Tada et al. (35). In each of these cases, TsF1 activity across H-2
differences is quantitatively and qualitatively undistinguishable from that observed
in H-2-identical animals. The finding that ABA-TsF2 fails to act in H-2-different mice
suggests strongly that one explanation for the apparent discrepancy in the earlier
studies is that the TsF studied by Tada et al. (35) is a TsF2 and not TsF1, and that
only such TsF2 show H-2-restricted activity. This suggestion is consistent with the
prolonged (4 wk) time period used to generate the keyhole limpet hemocyanin-specific Ts (38) and the ability of these cells to suppress primed T cell populations (39). It may be relevant that the suppressor cells identified by Owen et al. (40) after hyperimmunization and a prolonged rest period were anti-idiotypic in their specificity. We have recently proposed (41) an overall pathway of T cell-TsF activity based on this analysis that integrates the major features of several reported Ts models into a single scheme. In this model, antigen-specific, H-2-unrestricted TsF induces Ts2, which produces TsF2. This material in turn acts in an H-2-restricted manner on antigen-primed idiotypic auxiliary Ts (Ts3; Taux) that mediate effector suppression.

Other groups have also demonstrated a role for H-2 gene products in Ts activity. Thus, Miller et al. (42) have shown that suppression requires identity between Ts donor and recipient at the H-2D locus in the dinitrophenyl-coupled cell model. Weinberger et al. (37) showed that NP-specific Ts failed to act across I-region differences. Epstein and Cohn (43), exploring alloinduced Ts, and Jandinski et al. (44), studying TNP-modified, self-induced Ts active in humoral immune responses, observed H-2-restricted suppressor function. Rich and Rich (45) showed that TsF active in inhibiting mixed lymphocyte responses required I-C subregion identity between producer and acceptor (45). Moorehead (46, 47) found that dinitrobenzenesulfonate (DNBS)-induced TsF was also H-2K- or H-2-D-restricted in its interaction with its target cell. Recently, Yamuchi et al.2 have found a sheep erythrocyte-specific TsF in the feedback suppression model which shows H-2 restriction. Thus, such H-2 genetic restrictions are common in suppressor systems, although the subregion involved appears to differ in the various examples, and the site in the pathway at which the genetic influence exists has not always been evaluated. In all of these studies, it is not yet clear if the restriction reflects the receptor specificity of the TsF itself, as is true of H-2-restricted cytotoxic lymphocytes, the receptor specificity of the target cell, or some other interaction process controlled by H-2-linked genes.

A final point concerns the influence of background genes on TsF2 activity. Several groups have demonstrated a role for non-H-2-, non-Vn-linked genes in Ts activity (48, 49). More recently, Weinberger et al. (37) and Eardley et al. (50) have reported on the critical role of Vn-linked genes in T-T interaction in Ts pathways. Based on the known anti-idiotypic specificity of TsF2, and the finding that Ts2 fail to act across allotype differences in congenic strains of mice (50), it is most likely that the influence of non-H-2 genes on TsF2 activity in the ABA model reflects a requirement for idiotype expression in the recipient to serve as the TsF2 target. Thus, mapping studies currently in progress might be expected to reveal that the relevant non-H-2 genes controlling TsF2 function are allotype-linked (VnH) genes. It should be pointed out that the requirement for idiotype expression to permit detection of Ts2 activity can lead to detection of pseudorestrictions of TsF1 activity. Sy et al. (51) have recently demonstrated that the apparent failure of ABA-TsF1 (M.-S. Sy, manuscript in preparation) or CRI-coupled spleen cells to suppress CRI+ mice is not a result of a failure of Ts2 induction by these materials because of VnH restrictions, but rather, of a failure of Ts activity because of the absence of an idiotypic target in these animals. Thus, the apparent VnH restriction of TsF1 activity under such circumstances does not truly reflect the activity of the TsF itself but rather reflects genetic constraints on later

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parts of the suppressor pathway. These findings must be kept in mind in evaluating any apparent restrictions of Ts interactions.

These experiments have extended our knowledge about the cells and factors involved in a single Ts model and pointed out the similarities and differences involved in T-T interactions in two successive stages of the ABA suppressor pathway. These observations have also been of help in understanding the relationship among the various suppressor systems in the literature. Future studies may reveal the basis for initiation of the suppressor pathway and the actual means by which Ts decrease immune responses.

Summary

Azobenzenearsonate (ABA)-specific T cell-derived suppressor factor (TsF1) from A/J mice was used to induce second-order suppressor T cells (Ts2). Comparison of suppressor T cells induced by antigen (Ts1) with Ts2 induced by TsF1 revealed that Ts1 were afferent suppressors active only when given at the time of antigen priming, and not thereafter, whereas Ts2 could act when transferred at any time up to 1 d before antigen challenge for a delayed-type hypersensitivity response. This was true even when the recipient could be shown to be fully immune before transfer of Ts2, thus defining these cells as efferent suppressors. The anti-idiotypic specificity of the Ts2 was demonstrated by the ability of Ts2 to bind to idiotypic (cross-reactive idiotypic [CRI])-coated Petri dishes. A soluble extract from Ts2 (TsF2) was also capable of mediating efferent suppression that was functionally antigen- (ABA) specific. Comparison of TsF1 with this new factor, TsF2, revealed that both lack Ig-constant-region determinants, possess H-2-coded determinants, and show specific binding (to ABA and to CRI′-Ig, respectively). TsF1 acts in strains that differ with respect to H-2 and background genes, whereas TsF2 shows H-2- and non-H-2-linked genetic restrictions. This existence of H-2 restriction of TsF2 activity suggests that the apparent discrepancies in studies on H-2 restriction of TsF may be a result of the analysis of two separate classes of TsF, only one of which shows genetically restricted activity, thus unifying several models of suppressor cell activity.

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