The immune system normally appears to exist in a stable, steady state. As proposed by Jerne (1), the stability of the immune system is in part the result of a network of interactions involving idiotypes (Id) and anti-idiotypes (anti-Id). Several systems have described a critical requirement of Id recognition in the induction of antibody synthesis (2-6). Evidence has also accumulated in recent years (7, 8) indicating that Id-related mechanisms play a role in pathways among interacting T cells that eventually suppress hapten-specific delayed-type hypersensitivity (DTH). The down-regulation of the immune response by suppressor T cells (Ts) is complex and not fully understood. Recent reports describing the heretofore unrecognized immunoregulatory functions of T cells such as "contrasuppressors" (9) and "abrosuppressors" (10) add still more to the complexity involved in the biological function, immunoregulation.

In an effort to understand the role of the idiotypic network in the regulation of the immune response to a small synthetic antigen L-tyrosine-p-azophenyltrimethylammonium [tyr(TMA)], we have been studying the regulation of antibody and DTH responses directed to the TMA hapten. In the course of the study, we found that a single injection of tyr(TMA) in Freund’s complete adjuvant (FCA) induced Ts that shut down only the cross-reactive Id component of the anti-TMA antibody response (11). Recently (12), we have characterized the tyr(TMA)-induced Ts and directly demonstrated the idiotypic specificity of these cells by absorption procedures. Further, we demonstrated that these anti-idiotypic second-order Ts (Ts2) cells (8) can also shut down DTH reactions (12) in addition to Id+ antibody formation (11). The regulatory function of these cells on TMA-specific DTH responses was shown by adoptive transfer into naïve recipients. However, when the anti-idiotypic Ts-bearing mice were themselves immunized and tested for TMA specific DTH, they unexpectedly exhibited normal responses. Thus, although the anti-idiotypic Ts could readily suppress DTH upon transfer to normal recipients, these same cells could not function intrinsically.
This anomaly led us to attempt to analyze a probable lesion in the suppressor pathway. The results reported here demonstrate the apparent loss of function of an idiotypic T8 essential for the anti-idiotypic T5-mediated DTH suppression. This apparent loss of function of the idiotypic T8 in anti-idiotypic T5-bearing mice is discussed as a modulatory cell type critical for fine-tuning in immunoregulation.

Materials and Methods

**Mice.** Male A/J (H-2*, Ig-1*) mice were obtained from The Jackson Laboratory, Bar Harbor, ME and were 6–8 wk of age when used in these experiments.

**Antigen Preparations.** A 110-mM solution of trimethylaminoaniline (TMA) (Bachem Inc., Torrance, CA) diazonium salt was prepared as described earlier (13). The procedure for obtaining syngeneic spleen cells free of erythrocytes, and coupling with diazonium salt of TMA has already been described (14). In brief, syngeneic spleen cells devoid of erythrocytes were coupled with the diazonium salt of TMA at a final concentration of 10 mM at pH 8.2.

**Immunization and Challenge.** For the induction of DTH reactivity to TMA, routinely 3 × 10^7 freshly coupled syngeneic spleen cells (TMA-SC) were injected subcutaneously into separate sites on the dorsal flanks of the animal. In some experiments, anti-idiotypic antisera raised in rabbits were used for the induction of TMA-specific DTH. The induction, isolation, and purification of anti-Id and the determination of idiotype-binding capacity (IBC) of anti-Id have already been described (11, 13). 1.0 µg IBC equivalent of anti-idiotypic antisera in 0.2 ml phosphate-buffered saline was injected subcutaneously to induce DTH (14). 5 d after immunization by either method, mice were challenged with 25 µl of 10 mM diazonium salt in H2O into the right footpad. The contralateral footpad was left uninjected and served as the control. The footpad thickness was measured with a Mitutoyo micrometer (Schlesiingers for Tools Ltd., Brooklyn, NY) 24 h after the challenge. The magnitude of DTH reaction was expressed as the increment of footpad swelling between the challenged and unchallenged control footpads. Nonimmunized mice challenged similarly served as negative controls. In some experiments, for suppression of DTH, 20 µg IBC equivalent of anti-Id was injected intravenously through a lateral tail vein 1 h before challenge as described previously (14).

**Cyclophosphamide (CY) Treatment.** Mice were treated intraperitoneally with (CY) (cytoxan; Mead Johnson & Co., Evansville, IN) reconstituted in sterile distilled H2O and diluted in physiological saline. CY was administered 2 d before immunization if 200 mg CY/kg body weight was used, or 1 d after immunization if 20 mg CY/kg was used.

**Induction of Suppressor Cells.** Naïve A/J mice were inoculated intraperitoneally with 0.1 ml containing 100 µg of tyr(TMA) and FCA (containing H37Ra, Difco Laboratories, Detroit, MI). Spleens were obtained from tyr(TMA)-inoculated mice 6–7 wk later, and a single cell suspension was prepared in sterile balanced salt solution (BSS). Viability was determined using trypan blue dye exclusion method. To adoptively transfer suppression, 6 × 10^7 viable splenic cells were inoculated intravenously through a lateral tail vein. To study the intrinsic unresponsiveness, tyr(TMA)-immunized mice were injected subcutaneously with TMA-SC or anti-Id, usually 6–7 wk after tyr(TMA) inoculation, except when specifically mentioned, and challenged in the footpad 5 d later.

**Adoptive Transfer and Treatment of Lymph Node Cells.** Mice were immunized with TMA-SC, and 5 d later the animals were killed. The superficial cervical, axillary, brachial, superficial inguinal, and mesenteric lymph nodes were removed, and a single-cell suspension was prepared using a fine mesh screen. After washing, viability was determined and desired number of cells were resuspended in BSS. Before adoptive transfer, the lymph node cells were treated with several reagents, washed extensively in BSS, and resuspended in BSS at a desired density.

The cells were suspended at 1 × 10^7 cells/ml concentration in BSS for treatment with several reagents. Anti-Thy 1 monoclonal antibody secreted by the hybridoma T24/40.7 (kindly provided by Dr. J. Kappler and Dr. P. Marrack, The National Jewish Hospital, Denver) was used at 1:30 dilution. Anti-Lyt.2 monoclonal antibody secreted by the hybridoma 243.1 and anti-Lyt.1 monoclonal antibody secreted by the hybridoma 78.10.1 were used at 1:50 dilution. Both hybridomas were obtained from Dr. Barbara Araneo (The Jewish Hospital, St. Louis) and were originally produced in Dr. Frank Fitch's laboratory, University of Chicago. Cells
were incubated with antisera for 1 h on ice, spun, and resuspended at \(3.3 \times 10^7/ml\) concentration and incubated at \(37^\circ C\) for 30 min with low toxic rabbit complement (C) at 1:30 dilution. After incubation cells were extensively washed and used for transfer. Treatment of lymph node cells with anti-Id antisera and C was performed according to the method described by Sy et al. (15). Briefly, lymph node cells were suspended at \(10^7\) cells/ml in BSS and incubated on ice for 1 h with 25 \(\mu\)g IBC equivalent of anti-Id per every \(10^8\) cells. After incubation with anti-Id sera, cells were spun and resuspended in rabbit C (1:30) and incubated at \(37^\circ C\) for 30 min. All the lymph node cell preparations were injected intravenously through the lateral tail vein into appropriate recipients. The mice were challenged in the footpad within 1 h of cell transfer.

**Statistical Analysis.** All data were analyzed for significance by using a two-tailed Student’s \(t\) test. Percent suppression was calculated using the formula: percent suppression = \(((\text{positive control} - \text{experimental group})/\text{positive control} - \text{negative control})) \times 100\). More than 50% suppression was always highly significant.

**Results**

**Transferable Suppression and Intrinsic Unresponsiveness.** Mice were injected intraperitoneally with tyr(TMA) in FCA. These will be referred to as tyr(TMA)-immune mice. Spleen cells obtained from these mice 6 wk after tyr(TMA) injection when transferred intravenously into normal mice suppressed the DTH responses induced with TMA-SC (12). In conformity with this, we observed that tyr(TMA)-immune spleen cells suppressed the DTH responses of normal mice immunized either with TMA-coupled spleen cells (TMA-SC) or anti-Id antibodies (Table I) at the effector phase. However, when tyr(TMA)-immune mice were immunized with TMA-SC or anti-Id antibodies, no suppression was observed. This is referred to as the lack of intrinsic unresponsiveness. Thus, the tyr(TMA) inoculation of A/J mice led to the development of anti-

| Group | Treatment | Immunization | Challenge | Response mm ± 1 SEM | Percent suppression |
|-------|-----------|--------------|-----------|---------------------|---------------------|
| 1     | —         | TMA-SC       | +         | 1.12 ± 0.10 (5)     | —                   |
| 2     | tyr(TMA)  | TMA-SC       | +         | 1.43 ± 0.13 (4)     | -46                 |
| 3     | —         | Anti-Id      | +         | 0.90 ± 0.10 (7)     | —                   |
| 4     | tyr(TMA)  | Anti-Id      | +         | 0.84 ± 0.06 (7)     | 12                  |
| 5     | tyr(TMA)  | —            | +         | 0.53 ± 0.13 (4)     | —                   |
| 6     | —         | —            | +         | 0.40 ± 0.04 (4)     | —                   |
| 7     | —         | TMA-SC       | +         | 0.78 ± 0.05 (4)     | —                   |
| 8     | splenic suppressor cells | TMA-SC | + | 0.43 ± 0.10 (4) | 79 |
| 9     | —         | Anti-Id      | +         | 0.83 ± 0.08 (11)    | —                   |
| 10    | splenic suppressor cells | Anti-Id | + | 0.56 ± 0.03 (8) | 63 |
| 11    | —         | —            | +         | 0.40 ± 0.03 (5)     | —                   |

* A/J mice were immunized with either \(3 \times 10^7\) TMA-SC or 1.0 \(\mu\)g IBC equivalent of anti-Id and 3 d later challenged in the footpad with activated diazonium salt of TMA. Footpad swelling was measured 24 h later. Number of mice tested is given in parentheses. Percent suppression was calculated as described in Materials and Methods. In the experiment to study the intrinsic unresponsiveness, mice were inoculated intraperitoneally with \(100 \mu\)g of tyr(TMA) + FCA 6 wk before immunization. The ability of suppressor cells generated by tyr(TMA) + FCA injection 6 wk before, was tested by transferring \(6 \times 10^7\) splenic suppressor cells intravenously through a lateral tail vein into normal mice immunized with appropriate immunogens 24 h before challenge.
TABLE II

Kinetics of Intrinsic Unresponsiveness*

| Period after tyr(TMA) inoculation | Response | Percent suppression |
|-----------------------------------|----------|--------------------|
|                                   | mm ± 1 SEM |                    |
| **Experiment A**                  |          |                    |
| Positive controls                 | 0.90 ± 0.04 (4) | —                   |
| 1 wk                              | 0.04 ± 0.08 (5) | 10                  |
| 2 wk                              | 0.50 ± 0.06 (3) | 64§                 |
| 3 wk                              | 0.78 ± 0.05 (4) | 19                  |
| 4 wk                              | 0.73 ± 0.06 (4) | 13                  |
| 6 wk                              | 0.98 ± 0.12 (4) | —                   |
| Negative controls                 | 0.27 ± 0.03 (3) | —                   |
| **Experiment B**                  |          |                    |
| Positive controls                 | 1.40 ± 0.10 (5) | —                   |
| 2 wk                              | 0.72 ± 0.10 (5) | 68§                 |
| 8 wk                              | 1.10 ± 0.10 (3) | 30                  |
| 9 wk                              | 1.13 ± 0.06 (4) | 27                  |
| 10 wk                             | 1.30 ± 0.10 (4) | 10                  |
| Negative controls                 | 0.40 ± 0.05 (5) | —                   |

* A/J mice were immunized with 3 × 10⁷ TMA-SC at different time intervals after 100 μg of tyr(TMA) + FCA inoculation and challenged in the footpad 5 d later. Footpad swelling was measured 24 h after challenge. Number of mice tested is given in parentheses. Positive controls indicate the response of normal A/J mice that received no tyr(TMA) + FCA. Naive mice not immunized with TMA-SC but challenged in the footpad served as the negative controls.

§ Significantly different from positive controls; P < 0.01.

idiotypic Tₐ₁ (12), which could transfer suppression to normal mice without obviously impairing the ability of tyr(TMA)-immune mice to mount a normal DTH response when immunized with TMA-SC or anti-Id antibodies. Since no intrinsic suppression was noted at the 6-wk period, we next determined if tyr(TMA) inoculation led to intrinsic unresponsiveness at some earlier or later time point. Accordingly, mice were inoculated with tyr(TMA) in FCA and each week thereafter immunized with TMA-SC and assayed for DTH reactivity 5 d later. As seen in Table II, no significant suppression was seen at 3 wk and thereafter up to 10 wk after tyr(TMA) inoculation. The lack of suppression at the 1-wk period was not surprising, as we have previously shown (16) that this population does not contain suppressor activity, but in fact contains T cell mediated helper activity for anti-TMA antibody production. Clear-cut suppression was noted only at the 2-wk period, but this suppression is clearly mediated by idiotypic Tₐ₁ with different functional and phenotypic characteristics from that of anti-idiotypic Tₐ₂ induced 6 wk after tyr(TMA) injection (manuscript in preparation).

In summary, the data presented indicate that tyr(TMA) inoculation first leads to the appearance of idiotypic Tₐ₁ which can function intrinsically. At later time points (6 wk) anti-idiotypic Tₐ₂ emerge (12), which can only be detected by adoptive transfer experiments (see Table I). The failure of these Tₐ₂ to function intrinsically led us into a series of experiments to understand this apparent anomaly.

**Lack of Intrinsic Unresponsiveness Is Not Caused by Compensatory Increases of Id⁻ T cells that Mediate DTH (T_DTH).** Previous (14) as well as data presented herein indicate that
the T_{DTH} population bear surface Id^- receptors. Given these facts, it was possible that the anti-idiotypic T_{62} were suppressing Id^- T_{DTH}, which then resulted in the expression of a compensating Id^- T_{DTH} population after TMA-SC inoculation. If this were the case, then inoculation of the anti-Id just before challenge should not block the DTH response in the tyr(TMA)-primed group. As shown in Table III, the DTH responses in the normal as well as the tyr(TMA)-immune mice were blocked equally well, suggesting an intact Id^- T_{DTH} component. In addition, the blocking of DTH by anti-Id occurred even when mice were pretreated with high or low doses of CY. This suggests that the anti-Id-mediated suppression is not the result of the activation of CY-sensitive suppressor cells by the anti-Id, but rather a blockade or elimination of Id^+ T_{DTH}. Thus, it appears that the Id^- component of the T_{DTH} is not remarkably influenced by the coexistence of anti-idiotypic T_{62} in tyr(TMA)-immune mice.

Requirement of a CY-sensitive Cell for Anti-Idiotypic T_{62}-mediated Suppression. Because anti-idiotypic T_{62} function could only be demonstrated by transfer into naive recipients, we reasoned that the recipients were supplying some needed cell type for suppressor function. Reports have recently emerged (17, 18) describing a CY-sensitive T cell that is necessary in some systems for effector-phase T_s function and is activated upon antigen priming. To test whether this described cell was operating in our system, we treated naive mice with high or low doses of CY either before or after immunization with TMA-SC. 24 h before antigen challenge, spleen cells from 6-wk tyr(TMA)-immune mice were transferred into both the CY-treated and control mice. As seen in

| Immunization          | CY treatment | Anti-Id inoculation | Response | Percent suppression |
|-----------------------|--------------|---------------------|----------|---------------------|
| Normal mice           |              |                     |          |                     |
| TMA-SC                |              |                     | 1.23 ± 0.05 (8) |          |
| TMA-SC                | 200          |                     | 1.03 ± 0.08 (4) | 22       |
| TMA-SC                | 20           |                     | 1.10 ± 0.13 (4) | 14       |
| TMA-SC                |              | +                   | 0.64 ± 0.06 (9) | 66       |
| TMA-SC                | 200          | +                   | 0.50 ± 0.05 (5) | 81       |
| TMA-SC                | 20           | +                   | 0.48 ± 0.13 (4) | 83       |
| tyr(TMA) immune mice  |              |                     |          |                     |
| TMA-SC                |              |                     | 1.36 ± 0.07 (10) |        |
| TMA-SC                | 200          |                     | 1.03 ± 0.03 (3) | 32       |
| TMA-SC                | 20           |                     | 1.42 ± 0.12 (6) | -6       |
| TMA-SC                |              | +                   | 0.74 ± 0.12 (8) | 60       |
| TMA-SC                | 200          | +                   | 0.52 ± 0.06 (5) | 82       |
| TMA-SC                | 20           | +                   | 0.72 ± 0.06 (5) | 62       |
| Negative Controls     |              |                     | 0.33 ± 0.04 (11) |        |

* Both normal A/J mice and mice inoculated intraperitoneally with tyr(TMA) + FCA 6 wk before were immunized with 3 × 10^7 TMA-SC and challenged in the footpad with the activated diazonium salt of TMA 5 d later. Response was measured 24 h after challenge, and the number of mice tested is given in parentheses. 200 mg CY was injected intraperitoneally 2 d before immunization with TMA-SC, and 20 mg CY was injected 1 d after immunization with TMA-SC. Mice were injected intravenously with 20 μg IBC of anti-Id just before challenge. Negative controls indicate naive mice challenged only.
Table IV, pretreatment of the recipients with either low or high doses of CY did not alter the DTH reaction induced by TMA-SC inoculation. However, these doses of CY markedly altered the ability of the transferred suppressor population to function when compared with the non-CY-treated control group (Table IV). Thus, the data suggest that the anti-idiotypic Ts require a CY-sensitive cell, which is activated by antigen priming, to eventually suppress TMA-specific DTH.

**tyr(TMA)-immune Mice Lack a Critical Cell Type Involved in Ts-mediated Suppression.** As previously pointed out, the CY-sensitive cell necessary for Ts-mediated DTH suppression can be activated by priming normal mice with TMA-SC. Based on this fact, we tested whether this procedure could activate the necessary cell type in tyr(TMA)-immune mice. To assay for this activity, both control and tyr(TMA)-immune mice were primed with TMA-SC, and 5 d later their lymph nodes removed and the immune lymph node cells (ILNC) were transferred into naïve recipients with or without splenic Ts from 6-wk tyr(TMA)-immune mice. As seen in Table V, ILNC from normal mice (N-ILNC) transfer DTH but are suppressed by the addition of the splenic Ts population. No such suppression of DTH was observed when ILNC originated from the tyr(TMA)-immune mice. To test whether ILNC from tyr(TMA)-immune mice can be suppressed, ILNC from both sources were mixed and transferred along with the splenic suppressor cells into primed recipients. As seen in Table V, suppression of DTH was evident, indicating that the ILNC from the tyr(TMA) mice are indeed subject to suppression when mixed with N-ILNC.

**Phenotypic Characteristics of the Modulatory T Cell.** In this next series of experiments, we asked if the 6-wk tyr(TMA)-immune mice could be intrinsically suppressed if we

| Immunization | CY Treatment | Suppressor Cells | Response | Percent Suppression |
|--------------|--------------|------------------|----------|---------------------|
|              | mg | mm ± 1 SEM |          |                     |
| Experiment A |     |             |          |                     |
| TMA-SC      | 200 | +           | 0.73 ± 0.09 (4) | 55‡ |
| TMA-SC      | 200 | +           | 1.08 ± 0.06 (5) | 7   |
| Negative Controls | — | —          | 0.40 ± 0.12 (3) | —   |
| Experiment B |     |             |          |                     |
| TMA-SC      | 20  | +           | 0.75 ± 0.03 (4) | 11  |
| TMA-SC      | 20  | +           | 0.40 ± 0.04 (4) | 85  |
| Negative Controls | — | —          | 0.30 ± 0.03 (3) | —   |

* Normal A/J mice were immunized and challenged as described before. 200 mg CY was injected 2 d before immunization, and 20 mg CY was injected 1 d after immunization. 6 × 10⁷ splenic suppressor cells were inoculated intravenously through a lateral tail vein 24 h before challenge. Suppressor cells were obtained from mice inoculated intraperitoneally with 100 μg of tyr(TMA) + FCA 6 wk before. Number of mice tested is given in parentheses. Negative controls indicate naïve mice challenged only.

‡ Significantly different from positive controls; P < 0.02.
Table V

| Cells transferred | Suppressor cells | Response | Percent suppression |
|-------------------|------------------|----------|--------------------|
|                   |                  | mm ± 1 SEM |                     |
| N-ILNC -          | -                | 0.76 ± 0.07 (5) | -                  |
| N-ILNC +          | +                | 0.40 ± 0.04 (4) | 72                 |
| tyr-ILNC -        | -                | 0.90 ± 0.09 (5) | -                  |
| tyr-ILNC +        | +                | 0.79 ± 0.06 (7) | 17                 |
| N-ILNC + tyr-ILNC-| -                | 0.88 ± 0.04 (6) | -                  |
| N-ILNC + tyr-ILNC+| +                | 0.46 ± 0.07 (9) | 68                 |
| Negative controls | -                | 0.26 ± 0.02 (7) | -                  |

*Both normal (N) and tyr(TMA) + FCA-injected (6 wk before) A/J mice were immunized with 3 × 10⁷ TMA-SC, and 3 d later their immune lymph nodes were removed. 3 × 10⁷ viable lymph nodes were transferred intravenously into naive recipients with or without 6 × 10⁷ splenic suppressor cells derived from A/J mice injected with tyr(TMA) + FCA 6 wk before. In some experiments, 1 × 10⁷ N-ILNC and 2 × 10⁷ tyr-ILNC were mixed immediately before transfer with or without additional splenic suppressor cells. All mice were challenged in the footpads within 1 h of cell transfer. Negative controls indicate the naive mice challenged only. Number of mice tested is given in parentheses.

introduced ILNC from normal mice immunized with TMA-SC (N-ILNC) before challenge. Normal A/J mice were immunized with TMA-SC, and 5 d later ILNC were harvested and transferred into tyr(TMA)-immune mice, which had been previously primed with TMA-SC. 1 h after cell transfer, the recipients were challenged with the diazonium salt of TMA, and the footpad swelling was recorded 24 h later. As seen in Table VI, 6-wk tyr(TMA)-immune mice, when immunized with TMA-SC, give a normal DTH response. If N-ILNC are transferred just before challenge, the DTH (group 2) response is suppressed by 70%. To characterize the cells that convert the tyr(TMA) mice to intrinsic suppressors, the N-ILNC were pretreated with various antibody reagents plus C before transfer. These reagents were directed to the Thy-1, Lyt-2, and Lyt-1 antigens as well as the cross-reactive idiotypes associated with anti-TMA antibodies. As seen in Table VI, all of the antibody reagents, with the exception of the anti Lyt-1 (group 6), abolished the ability of the cells to mediate suppression. Furthermore, we show that transfer of tyr(TMA) (group 7) ILNC into the tyr(TMA) mice does not convert these mice, confirming our similar previous finding that tyr(TMA)-ILNC do not have modulatory cell activity when transferred into naive animals (see Table V). To control for any specific or nonspecific suppressor element in the transfer system, either N-ILNC or ILNC from 6-wk tyr(TMA)-immune mice were transferred into normal mice immunized 5 d previously with TMA-SC. Transfer of these populations into normal mice (groups 17, 18) had no effect on the DTH reaction. Therefore, it would appear that the modulatory cell necessary for the function of T₄ is itself a T cell, bearing the Lyt-2 antigen as well as surface idiotypes.

Because the ILNC transfer DTH as well as modulatory cell activity, we wanted to determine if the cells mediating DTH could be phenotypically distinguished from the
**Table VI**

Phenotype of the Modulatory Cells and $T_{DTH}^*$

| Group | Recipients | Cells transferred | Response $\text{mm} \pm 1 \text{SEM}$ | Percent suppression |
|-------|------------|-------------------|--------------------------------------|-------------------|
| 1     | tyr(TMA)-immune mice | None | 1.02 ± 0.03 (37) | — |
| 2     | tyr(TMA)-immune mice | N-ILNC + C | 0.55 ± 0.03 (18) | 70 |
| 3     | tyr(TMA)-immune mice | N-ILNC + anti-Thy-1 + C | 0.88 ± 0.09 (6) | 21 |
| 4     | tyr(TMA)-immune mice | N-ILNC + anti-Id + C | 0.83 ± 0.03 (10) | 25 |
| 5     | tyr(TMA)-immune mice | N-ILNC + anti-Lyt-2 + C | 0.82 ± 0.03 (6) | 30 |
| 6     | tyr(TMA)-immune mice | N-ILNC + anti-Lyt-1 + C | 0.64 ± 0.02 (5) | 57 |
| 7     | tyr(TMA)-immune mice | tyr-ILNC + C | 0.96 ± 0.09 (5) | 9 |
| 8     | Naive mice | None | 0.35 ± 0.01 (30) | — |
| 9     | Naive mice | N-ILNC + C | 0.72 ± 0.04 (20) | — |
| 10    | Naive mice | N-ILNC + anti-Thy-1 + C | 0.26 ± 0.03 (7) | 124 |
| 11    | Naive mice | N-ILNC + anti-Id + C | 0.43 ± 0.07 (8) | 78 |
| 12    | Naive mice | N-ILNC + anti-Lyt-2 + C | 0.62 ± 0.03 (6) | 27 |
| 13    | Naive mice | N-ILNC + anti-Lyt-1 + C | 0.28 ± 0.03 (6) | 119 |
| 14    | Naive mice | tyr-ILNC + C | 0.91 ± 0.06 (9) | — |
| 15    | Naive mice | tyr-ILNC + anti-Id + C | 0.54 ± 0.05 (8) | 66 |
| 16    | Normal mice | None | 0.87 ± 0.07 (6) | — |
| 17    | Normal mice | N-ILNC + C | 0.95 ± 0.03 (4) | 15 |
| 18    | Normal mice | tyr-ILNC + C | 0.78 ± 0.03 (4) | 17 |

* tyr(TMA) + FCA injected (6 wk before) and untreated normal A/J mice were immunized with $3 \times 10^7$ TMA-SC, and 5 d later lymph nodes were removed from some of them. The remaining mice served as the recipients of lymph node cells. In addition, nonimmune, naive A/J mice were used as recipients to monitor for the ability of treated lymph node cells to transfer DTH (line 9-15). The lymph node cells were treated with several reagents as indicated, washed extensively, and transferred intravenously into appropriate recipients. Nonimmune, naive mice received no cells served as negative controls (line 8). All mice were challenged in the footpad within 1 h of cell transfer, and the footpad swelling was recorded 24 h later. Data from eight experiments were pooled. Number of animals tested is given in parentheses.

modulatory cell population. Accordingly, we treated the ILNC with the same reagents used in the previous experiments and then tested the ability of these treated cells to transfer TMA specific DTH. The transfer of DTH is abolished by (group 10, 11) anti-Thy-1 and the anti-Id reagent, the latter confirming our previous contention that $T_{DTH}$ bear idiotypic determinants. However, the anti-Lyt-2 + C treatment did not affect the ability of ILNC to transfer DTH, whereas the anti-Lyt-1 + C completely abolished the transfer of DTH. In addition, the ability of ILNC from tyr(TMA) (group 15) mice to transfer DTH was greatly affected by treatment with anti-Id antibodies, confirming that the $T_{DTH}$ present in tyr(TMA) mice are similar to the $T_{DTH}$ in normal mice. However, the ability of ILNC from 2,4-dinitrofluorobenzene (DNFB)-painted A/J mice to transfer contact sensitivity was not abolished after treatment with anti-Id and C, confirming the specificity of the reagent (data not shown). Thus, the $T_{DTH}$ are Id*, Lyt-1−,2−; the modulatory T cells are Id*, Lyt-1−,2+. In summary, it would appear that the inability of anti-idiotypic Ts2-bearing mice to exhibit intrinsic unresponsiveness is caused by the lack of function in an antigen-primed, Thy-1*, Id*, Lyt-1−,2+ modulatory cell population.
Discussion

We have reported earlier that a single intraperitoneal injection of tyr(TMA) in FCA in A/J mice induced a suppressor cell population that was able to suppress the TMA-specific DTH responses when adoptively transferred into naïve mice (12). In addition, it was observed that these Ts (termed Ts2 [8]) bear anti-idiotypic receptors, Lyt-2 alloantigen, and I-J subregion-encoded products and were both H-2- and Igh-V-restricted for their action (12). The data presented in this report demonstrate that Ts2 can suppress not only the TMA-SC-induced DTH but also the anti-Id-induced DTH when adoptively transferred into normal recipients at the effector phase (Table I). However, when these Ts-bearing tyr(TMA)-immune mice were intentionally immunized with either TMA-SC or anti-Id, they failed to exhibit intrinsic unresponsiveness. This paradoxical observation, wherein the presence of Ts2 can only be demonstrated by adoptive transfer into normal recipients but failed to function intrinsically, is not caused by the inability of A/J mice in general to exhibit intrinsic unresponsiveness. This conclusion is based on the finding that 2 wk after tyr(TMA) + FCA injection, A/J mice exhibited intrinsic unresponsiveness (Table II). However, the Ts induced at this time were found to bear idiotype, act only at the induction phase of DTH when adoptively transferred into normal recipients, and the factor extracted from these Ts found to act across MHC barriers (manuscript in preparation). Thus, it is clear that the reason for the failure to observe suppressor activity intrinsically in mice bearing anti-idiotypic Ts2 is not caused by the general inability of A/J mice to exhibit intrinsic unresponsiveness. The fact that a normal DTH response can be evoked in Ts2-bearing mice with anti-Id (Table I) suggests that the DTH response in these mice is not qualitatively different from normal mice. This contention is further supported by two lines of evidence. Previously (14) we have shown that the intravenous administration of a large dose of anti-Id just before challenge blocked the DTH responses specific to TMA. In addition, it was observed that this mode of blocking of DTH occurred only in strains of mice possessing the same allotype as that of A/J strain, suggesting the presence of Id on the surface of TdTH. To rule out the possibility that the anti-Id-induced blocking of DTH was not caused by the activation of suppressor cells, the mice were treated with doses of CY known to deplete suppressor T cell precursors (19–24). Irrespective of the treatment to mice with CY, DTH induced in normal as well as in tyr(TMA)-immune mice was effectively blocked with anti-Id (Table III), suggesting the presence of Id on TdTH as observed in other systems (25, 26). Direct evidence for the function of the idiotypic component of TdTH in tyr(TMA)-immune mice comes from experiments involving treatment of TdTH with anti-Id + C before adoptive transfer into naïve recipients. Such a treatment abolished the ability of TdTH derived from normal as well as tyr(TMA)-immune mice to transfer immunity into naïve recipients (Table VI, groups 9, 11, 14, and 15) equally well. Taken together, these results strongly indicate that the idiotypic component of TdTH was not functionally deleted as a result of the coexistence of anti-idiotypic Ts2 in tyr(TMA)-immune mice.

The notion that the auxiliary cells may help the manifestation of suppressor function has been previously suggested (8, 17, 18, 27–31). Such auxiliary (17) or Ts3 (18, 30, 31) cells have been shown to be essential for the function of anti-idiotypic Ts2 in nitrophenylacetyl-specific cutaneous hypersensitivity (18) and azobenzenearsonate-specific DTH (30) systems. Based on the fact that anti-idiotypic Ts2 cannot intrinsi-
ally suppress the DTH, it was reasoned that this apparent inability of $T_{\lambda 2}$ to function could be caused by the loss of the function of a cell type similar to an auxiliary or $T_{\lambda 3}$ cell. Data in Table IV suggest that the ability of $T_{\lambda 2}$ to suppress the DTH response when adoptively transferred into normal mice at the effector phase depends on an antigen-activated, CY-sensitive suppressor cell type in the recipients. This is in line with the findings that the CY-sensitive auxiliary (17) or $T_{\lambda 3}$ cells (18) are needed for the manifestation of effector-phase suppressors. It is likely that an absence of this CY-sensitive suppressor cell activity, induced after TMA-SC immunization, is a major factor in the lack of expression of suppressor activity intrinsically in $T_{\lambda 2}$-bearing mice.

Further experiments in fact indicated that the ILNC from tyr(TMA)-immune mice were not suppressed when adoptively transferred together with $T_{\lambda 2}$ into naive recipients (Table V). However, the addition of ILNC from normal mice to ILNC derived from tyr(TMA)-immune mice conferred the ability to be suppressed by $T_{\lambda 2}$. It is thus evident that the anti-idiotypic, Lyt-2+, I-J$^+$ $T_{\lambda 2}$ is not the effector suppressor, but the CY-sensitive cell type present in the antigen-activated immune lymphoid population could bring about suppression of TMA-specific DTH. Because the presence or absence of the function of the latter cell type determines whether suppression should occur, this cell type will be referred to as "modulatory" as suggested (32).

Because the transfer protocols do not really mimic the physiological situation, it was decided to see if we could convert the $T_{\lambda 2}$-bearing mice into intrinsically suppressed mice by providing activated modulatory cell populations from normal mice. The data show that it is possible to make the $T_{\lambda 2}$ functional in situ by providing extraneous modulatory cells (Table VI). In addition, it was shown that the cells responsible for the function of $T_{\lambda 2}$ are T cells that bear Lyt-2 alloantigen as previously shown for the $T_{\lambda 3}$ (18) and amplifier of suppressor cells (28). Like the target cell of $T_{\lambda 2}$ in another system (30), the modulatory cells involved in TMA-specific $T_{\lambda 2}$-mediated DTH suppression also bear the Id on their surface. It should be noted that the $T_{\lambda 3}$ cells have been shown to bear the I-J subregion-encoded products and are restricted by H-2 and Igh-complex products (18). Thus, it appears that the modulatory cells, which are nonfunctional in anti-idiotypic $T_{\lambda 2}$-bearing mice, share many of the properties attributed to auxiliary (17), $T_{\lambda 3}$ (18, 30), and amplifier of suppressor cells (28). The consensus from all these studies is that the modulatory cells are very critical for the expression of suppressor activity and may be the final cell type in the suppressor pathway (8, 30, 31). Because both $T_{DTH}$ and modulatory cells appear to bear Id on their surface (Table VI), and the stimulation of $T_{\lambda 2}$ or modulatory cell alone do not bring about any suppression, these data support the hypothesis that the $T_{\lambda 2}$ and modulatory cells interact with each other by Id-anti-Id recognition, which releases a factor that directly or indirectly suppress the function of $T_{DTH}$ (8, 30, 31).

We have previously shown (11) that if 6-wk tyr(TMA)-immune mice were inoculated with TMA coupled to a protein, the resulting anti-TMA antibody lacked the Id$^+$ component of the response, which normally constitutes on an average 50% of the response. Because this suppressor activity is $T_{\lambda 2}$ mediated, an apparent difference in the ability of these mice to intrinsically modulate antibody vs. DTH responses exists. The discrepancy between the results would indicate that $T_{\lambda 2}$ may act directly on Id$^+$ B cells through direct receptor interaction (33, 34) or through an antigen bridge. Alternatively, the immunization protocol used to generate antibody production could have activated the modulatory population. At present we cannot rule out any one of
these possibilities. Nevertheless, under a certain set of conditions the T\textsubscript{s2} population is unable to shut down DTH but can mediate suppression at the humoral level. Taken together, the results indicate that the modulatory population can potentially play a strategic role in immune regulation. In this particular situation the modulatory population can split control-mediated by T\textsubscript{s2} cells, allowing the suppression of antibody on one hand and intact DTH responses on the other. Rather than the necessity to regulate a variety of responses at the T\textsubscript{s2} level, which would remove suppressor potential at both the antibody and DTH levels, the system via the modulatory cell is now capable of a more sophisticated form of control or “fine tuning.” Thus, the induction of T\textsubscript{s} using tyr(TMA) in FCA appears to offer the potential to study the fine regulation of antibody and DTH responses in a naturally controlled fashion.

Summary

A single intraperitoneal injection of the monovalent synthetic antigen, tyrosinated trimethylaminoaniline [tyr(TMA)] in Freund’s complete adjuvant induces an anti-idotypic second-order T suppressor (T\textsubscript{s2}) cell population 6 wk later. This population was able to suppress TMA-specific delayed-type hypersensitivity (DTH) responses when adoptively transferred into normal syngeneic recipients. However, they failed to function intrinsically. The inability of the T\textsubscript{s2} to function intrinsically was not caused by compensating idiotype-negative T cells that mediate DTH. Rather, this paradoxical observation was found to be caused by the absence or loss of function of a critical modulatory T cell population in the suppressor cell-bearing mice. This cell is functionally active in normal mice immunized for DTH responses and is sensitive to cyclophosphamide treatment. In addition, this cell type bears idiotype on its surface and is Thy-1\textsuperscript{−} and Lyt-1\textsuperscript{−},2\textsuperscript{+}. It was demonstrated that by adoptively transferring the activated modulatory T cells from normal mice into tyr(TMA)-immune recipients, it was possible to observe suppressor cell function intrinsically. The potential importance of modulatory T cell function in the regulation of antibody and DTH responses is discussed.

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