ANTIGEN- AND RECEPTOR-DRIVEN REGULATORY MECHANISMS

VIII. Suppression of Idiotype-negative, *p*-Azobenzenearsonate-specific T Cells Results from the Interaction of an Anti-Idiotypic Second-Order T Suppressor Cell with a Cross-reactive-Idiotype-positive, *p*-Azobenzenearsonate-primed T Cell Target*

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Recent studies investigating the regulation of delayed-type hypersensitivity (DTH)[1] to *p*-azobenzenearsonate (ABA)-coupled cells have provided conclusive evidence for two distinct populations or subsets of interacting suppressor T cells (Ts) in the suppressor pathway (1, 2).

In the ABA system, the subcutaneous injection of ABA-conjugated spleen cells (ABA-SC) induces T cell-mediated (T_{DH}) delayed hypersensitivity. In contrast, intravenous administration of ABA-SC induces suppressor T cells (termed Ts_1) that bind to antigen-coupled plates and express cross-reactive idiotypic (CRI) determinants serologically similar to those present on antibodies specific for the same hapten (3, 4). Furthermore, second-order suppressor T cells (Ts_2) are induced by a CRI-bearing suppressor T cell factor (TsF) termed TsF_1 or by CRI-conjugated spleen cells (CRI-SC) and bind to idiotype-coupled polystyrene plates (5, 6). Recently, similar data on the specificity of Ts_1 and Ts_2 have also been obtained in a hapten-specific system using 4-hydroxy-3-nitrophenyl acetyl (7). Additional studies have shown that idiotype-bearing Ts_1 act only in an afferent mode, i.e., when administered at the induction phase of the immune response, whereas anti-idiotypic suppressor T cells (Ts_2) are

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[1] *Abbreviations used in this paper:* ABA, *p*-azobenzenearsonate; ABA-SC, ABA-coupled syngeneic spleen cells; CRI, cross-reactive idiotypic antibody from A/J mice; CRI-SC, CRI-coupled syngeneic spleen cells; DNFB, 2,4-dinitro-1-fluorobenzene; DTH, delayed type hypersensitivity; HBSS, Hanks' balanced salt solution; LN, lymph node; MHC, major histocompatibility complex; T_{aux}, auxiliary suppressor T cells; T_{DH}, T cells that mediate DTH; Ts, T suppressor cells; Ts_1, first-order Ts (induced by ABA-SC [idiotype positive]); Ts_2, second-order Ts (induced by CRI-SC [anti-idiotypic]); Ts_3, third-order T cell subset that serves as target of Ts_2; TsF, suppressor T cell factor; TsF_1, TsF produced by Ts_1; TsF_2, TsF produced by Ts_2.

efferent suppressors, active when transferred either at the time of immunization or challenge (8). In studies of the A/J humoral response, Ts with anti-idiotypic receptors (9) and TsF with either idiotypic or anti-idiotypic receptors (10) have been identified.

The experiments on DTH performed in the ABA system have revealed an interesting paradox. The efferent suppressors (Ts_2) are anti-idiotypic, and are able to suppress, upon adoptive transfer, an ABA-specific DTH reaction in syngeneic mice in the efferent phase. Yet, the DTH T effector cells, the ultimate targets of the suppression, do not bear serologically detectable idiotypic determinants; they are not lysed by anti-idiotypic antibody and complement (11). Moreover, we have recently determined that the failure of idiotype-coupled spleen cells, which stimulate anti-idiotypic Ts in A/J mice to induce T cell-mediated unresponsiveness in animals lacking the appropriate variable region of the Ig heavy chain (V_H) genes, appears to be a result of the lack of idiotype-matched targets (6). Thus, CRI-bearing antibodies from A/J ($H\text{-}2^a$, $Igh\text{-}1^e$) mice were conjugated to normal BALB/c ($H\text{-}2^d$, $Igh\text{-}1^a$) spleen cells in vitro. The CRI-bearing syngeneic cells, when injected intravenously into syngeneic BALB/c mice, failed to induce tolerance in these animals. Nevertheless, spleen cells taken from these CRI-SC-treated BALB/c animals transferred significant degrees of suppression to Igh-1-congenic C.AL-20 ($H\text{-}2^d$, $Igh\text{-}1^d$) but not to H-2 congenic, Igh-1 disparate B10.D2 ($H\text{-}2^d$, $Igh\text{-}1^b$) mice (C.AL-20 mice but not BALB/c or B10.D2 mice express the CRI in their humoral antibody). We concluded from these experiments that appropriate anti-idiotype and idiotype interactions are necessary for the expression of Ts_2 function, and that the target of these anti-idiotypic Ts_2 must be an idiotype-bearing cell. We speculated that these putative idiotype-bearing cells might be the precursors of the DTH effector cells or might represent helper T cells for the DTH response. Alternatively, we proposed that a population of idiotype-positive T cells—other than the T_{DH} cells or T helper cells— in the immune cell population may be the next, and perhaps last, T cell in the suppressor pathway. Such a cell has been shown previously to be required for in the suppression of the contact sensitivity to 2,4-dinitro-1-fluorobenzene (DNFB) (12).

This communication reports experiments that identify and characterize these idiotype-bearing target cells. Anti-idiotypic Ts_2 block the efferent step of DTH reactions by interacting with a population of idiotype-bearing Ts cells, which reside in the immune lymph node population, and which we have termed third-order suppressor cells, or Ts_3. Furthermore, incubation of Ts_2 with the appropriate Ts_3 results in suppression which is nonspecific for idiotype. Thus, these idiotype-bearing Ts_3 represent another set of suppressor cells in a coordinated series of cellular interactions determined by complementary receptor-anti-receptor binding.

Materials and Methods

Mice. Female BALB/c ($H\text{-}2^d$, $Igh\text{-}1^a$), and B10.D2 ($H\text{-}2^d$, $Igh\text{-}1^b$) mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. C.AL-20 ($H\text{-}2^d$, $Igh\text{-}1^d$) mice were obtained from the breeding colonies maintained at Brandeis University, Waltham, Mass. from stock originally provided by Dr. Michael Potter at the National Institutes of Health, Bethesda, Md.

Preparation of Antigen and Antigen-coupled Cells. These methods have been described in detail elsewhere (3). Briefly, a 40-mM solution of ABA diazonium salt was prepared from recrystallized *p*-arsanilic acid (Eastman Kodak Co., Rochester, N. Y.). The ABA solution was activated and conjugated to single-cell suspensions of erythrocyte-free splenocytes at a final concentration of 10 mM ABA. After washing in Hanks' balanced salt solution (HBSS), the ABA-SC were used to induce DTH.

Preparation of Idiotype-coupled Cells. The method used for coupling anti-ABA antibodies to spleen cells is a modification of the method of Wetzig et al. (13) and has been described in detail elsewhere (14). Briefly, a single-cell suspension of normal spleen cells was prepared in HBSS. Erythrocytes were lysed by treatment with isotonic Tris-buffered ammonium chloride (pH 7.6). The spleen cells were then washed three times in HBSS and once in 0.8% NaCl; 4×10^8–5×10^8 washed spleen cells were pelleted into a 17- × 100-mm Falcon plastic tube (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif.) and were resuspended in 1 ml of a 1 mg/ml solution in saline of CRI^+ ligand, affinity-purified (15), anti-ABA antibodies from A/J mice. The cells and the antibody solution were transferred to a small glass scintillation vial (15 × 60 mm) and 25 mg of crystalline 1-ethyl-3(3′-dimethylaminopropyl) carbodiimide (ECDI; Pierce Chemical Co., Rockford, Ill.) was dissolved in the coupling solution. The reaction was allowed to proceed for 90 min at 4°C with gentle stirring. (The coupling efficiency, as determined by using radiolabeled anti-ABA, was ~5–10%.) The CRI-coupled spleen cells (CRI-SC) were washed twice in HBSS, adjusted to a concentration of 1×10^8/ml, and 0.5 ml of the suspension was injected intravenously into appropriate recipients.

Induction and Elicitation of DTH to ABA. To induce DTH to ABA, a total of 3×10^7 ABA-coupled syngeneic cells were injected s.c. into separate sites on the dorsal flanks of the mice. Challenge was performed 5 d later by injecting 30 μl of 10 mM diazonium salt of *p*-arsanilic acid 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 the thickness of the challenged left footpad as compared with the untreated right footpad. Responses are given in units of 10^{-2} mm + SEM.

Transfer of Immunity with Immune Lymph Node Cells. Animals were killed 4 or 5 d after s.c. immunization with 3×10^7 ABA-coupled spleen cells and draining lymph nodes (bilateral, inguinal, and axillary) were obtained and made into single-cell suspensions as described earlier (3). Cells were washed twice with HBSS and resuspended in HBSS at a concentration of 1×10^8 cells/ml; 0.5 ml of the cell suspension, containing 5×10^5 cells, was injected i.v. into groups of syngeneic recipients. Within 2 h after cell transfer, recipient mice were challenged in the footpad with 30 μl of the diazonium salt as described (3).

Induction of Suppressor T Cells and the Blocking of Passive Transfer of Immunity with Suppressor Cells. Normal mice were injected 5×10^7 CRI-SC. 7 d afterwards, these mice were killed and served as donors of suppressor T cells. Spleens from such animals were removed, and a single-cell suspension was prepared in chilled HBSS. The cells were washed twice in HBSS and counted; 5×10^7 viable cells were injected i.v. into normal recipients, which were then primed s.c. with 3×10^7 ABA-SC and challenged 5 d later.

To measure the ability of these suppressor cells to block the passive transfer of immunity (efferent route), immune T cells were first mixed with suppressor T cells in vitro. They were then cotransferred to naive recipients. Footpad challenges with the diazonium salt were done within 1 h after cell transfer and increases in footpad swelling were measured 24 h later, as described. Controls were mice in which immune T cells were transferred without suppressor T cells.

Antiserum Treatment. Antisera to the CRI of A/J anti-ABA antibodies were prepared and quantitated as described (15). 1×10^8 immune lymph node cells were incubated for 45 min at 4°C anti-CRI antibodies (25 μg idiotype-binding capacity) in a 1-ml vol of HBSS. The cells were then washed twice, pelleted, and resuspended in 1 ml of a 1:10 dilution of Low-Tox rabbit complement (Cedarlane Laboratories, London, Ontario, Canada) for 30 min at 37°C. The cells were then washed twice in chilled HBSS, recounted, and resuspended for cell transfer. The number of cells transferred was determined by the viability counts of treated cells.

Anti-Thy-1.2 hybridoma antibodies were kindly provided by Dr. P. Lake, University College, London, England. Briefly, 1×10^8 cells were incubated with 1 ml of 1:20 dilution of anti-Thy-1.2 hybridoma antibodies for 45 min at 0°C, washed once in chilled HBSS, and incubated again with 1 ml of a 1:10 dilution of Low-Tox rabbit complement for 30 min at 37°C. The cells were then washed twice in HBSS then counted and adjusted to the appropriate concentration for transfer.

Results

Treatment of Immune Lymph Node (LN) Cells with Anti-CRI Antibodies and Complement Rendered them Nonsusceptible to Suppression by Anti-Idiotypic Ts_2. To investigate the target of anti-idiotypic Ts_2, we first determined whether we could transfer immunity into BALB/c mice that had been previously injected with CRI-SC to stimulate anti-idiotypic Ts_2 responses. To transfer immunity, ABA-immune LN cells from BALB/c or C.AL-20 mice were used. In addition, we investigated the effect of treating the immune LN cells with anti-CRI antibodies and complement just before the passive transfer of immunity.

The results of a representative experiment are depicted in Fig. 1. It was found that BALB/c ABA-immune LN cells transferred significant levels of immune reactivity into syngeneic BALB/c mice that had been injected with CRI-SC 7 d earlier. The effectiveness of the transfer was not influenced appreciably when the lymph node cells were treated with anti-CRI and complement. Immune LN cells taken from C.AL-20 mice, when treated with complement alone as control, also transferred significant immunity into normal nontreated BALB/c mice. However, such immune T cells failed to transfer immunity into CRI-SC-pretreated BALB/c mice. Of particular interest was the observation that the failure of C.AL-20-immune LN cells to transfer immunity into CRI-SC treated BALB/c mice could be effectively reversed by treating the immune LN cell population with anti-CRI antibodies and complement.

From this experiment, we can first conclude that the effector T_{DH} cell is itself insensitive to treatment with anti-CRI antibodies and complement. Second, as shown previously and documented again herein, injection of CRI-SC into BALB/c induced efferent suppression via the elicitation of anti-idiotypic Ts_2 cells. Third, and most important, there are idiotype-bearing cells that reside within the immune LN population that are apparently required for the expression of Ts_2 function. When these

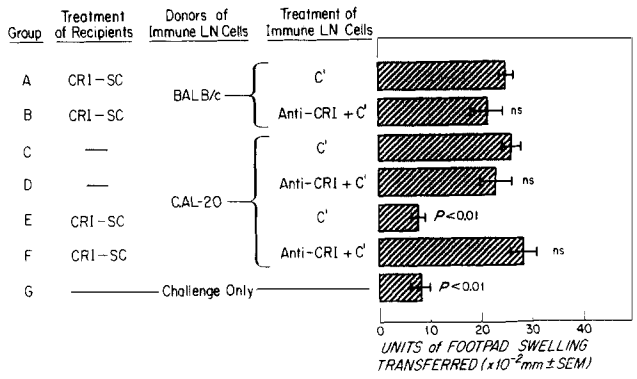

FIG. 1. Treatment of immune LN cells with anti-CRI antibodies and complement rendered them nonsusceptible to suppression by anti-idiotypic Ts_2. Groups of normal BALB/c mice were injected with 5×10^7 BALB/c CRI-SC to induce anti-idiotypic Ts_2. 7 d later, these animals were the recipients of immune LN cells. These immune LN cells were obtained from other groups of BALB/c or C.AL-20 mice that had been injected with 3×10^7–6×10^7 ABA-SC s.c. 5 d earlier. Before transfer into the appropriate recipients, these immune LN cells were first treated with either complement (C') alone as control, or with anti-CRI antibodies and complement. 5×10^7 immune LN cells in 0.5 ml were then injected intravenously into the appropriate recipients. Within 1 h after cell transfer, the animals were challenged with 30 μl of the diazonium salt and increases in footpad swelling were measured 24 h after challenge. Each bar represents the mean ± SEM of measurement of at least four mice.

idiotype-bearing cells were removed, the remaining cells were able to transfer DTH in the presence of Ts_2.

We designed a second experimental protocol to obtain additional evidence for the existence of an idiotype-bearing cell serving as target of Ts_2. Anti-CRI Ts_2 generated in BALB/c were mixed with C.Al-20 immune LN cells which had been treated earlier with anti-CRI antibodies and complement or complement alone as a control. These cells were then cotransferred to naive BALB/c recipients which were then challenged within 1 h after cell transfer. The result of such an experiment is shown in Fig. 2. As can be seen, normal BALB/c spleen cells, when cotransferred with immune LN cells taken from C.AL-20 mice, did not interfere with their ability to transfer immunity into normal BALB/c mice. Immunity was transferred irrespective of whether the immune LN cells had been treated with anti-CRI antibodies and complement or complement alone. However, spleen cells obtained from BALB/c mice treated 7 d earlier with CRI-SC, when cotransferred with ABA-immune LN cells taken from C.AL-20 mice, inhibited their ability to transfer immunity. This inhibition was eliminated if the immune LN cells were first treated with anti-CRI and complement. Therefore, using two different experimental approaches, we have provided substantial evidence that an idiotype-bearing cell is required for the expression of the suppressor activity of anti-idiotypic Ts_2 suppression.

Interaction of Anti-Idiotypic Ts_2 with Its Appropriate Target Results in an Idiotype-nonspecific Suppression. As discussed above, the effector T_{DH} cell in A/J and C.AL-20 mice appears not to bear detectable idiotype on its surface. Accordingly, we must conclude that the final suppression which limits T_{DH} activity, and which occurs after interactions between Ts_2 and their idiotypic target, is not idiotype specific.

To determine directly whether idiotype-nonspecific suppression indeed occurs, we mixed BALB/c anti-CRI Ts_2 with BALB/c immune LN cells in a cotransfer experiment. In addition we also added a defined number of C.AL-20-immune LN cells as potential targets of Ts_2 or B10.D2-immune LN cells as a control (C.AL-20 and B10.D2 are CRI^+ and CRI^- strains, respectively. Both strains as well as BALB/c are $H\text{-}2^d$).

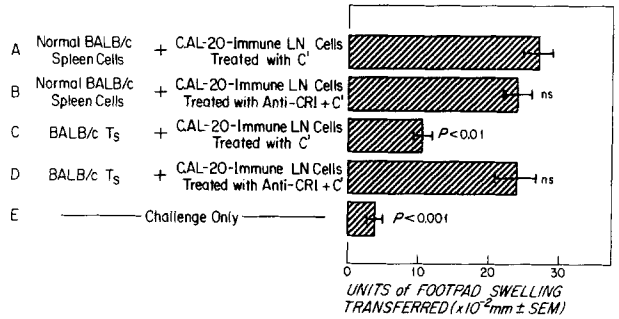

FIG. 2. Failure of Ts_2 to block the passive transfer of immunity if the immune LN cells had been treated with anti-CRI antibodies and complement. Normal BALB/c mice were injected with 5×10^7 BALB/c CRI-SC i.v.; 7 d later, they were the donors of Ts_2. C.AL-20 mice were injected with 3×10^7–6×10^7 C.AL-20 ABA-SC s.c. 4–5 d later, they were the donors of immune LN cells. 5×10^7 Ts_2 or normal BALB/c spleen cells were mixed with 5×10^7 immune LN cells either treated with complement (C') alone as control or with anti-CRI antibodies and complement. The cell mixture (in 0.5 ml) was then injected i.v. into normal BALB/c mice. Footpad challenges were done within one h after cell transfer, and increases in footpad swelling were measured 24 h after challenge. Each bar represents the mean ± SEM of measurement of at least four mice.

The results of such an experiment are shown in Fig. 3. BALB/c immune LN cells, when cotransferred with normal BALB/c spleen cells and C.AL-20 immune LN cells, transferred significant degrees of immunity into syngeneic BALB/c mice. BALB/c-immune LN cells, when mixed with BALB/c anti-CRI Ts_2 in the presence of B10.D2-immune LN cells, likewise still retained their ability to transfer immunity. However, BALB/c-immune LN cells, when mixed with BALB/c anti-idiotypic Ts in the presence of a defined number of C.AL-20-immune LN cells, failed to transfer immunity. Therefore, after anti-idiotypic Ts_2 interacts with its idiotype-bearing target, the final suppression appears to be idiotype nonspecific. Furthermore, using a different experimental protocol, in this case transferring BALB/c Ts_2 and C.AL-20-immune LN cells into normal BALB/c animals that were then immunized subcutaneously with ABA-SC (and footpad challenged 5 d later), similar results have been obtained (data not shown).

The Targets of Ts_2 Are T Cells. In an attempt to characterize further the cellular nature of the idiotype-bearing cells residing in the immune lymph node, we next investigated whether treatment with anti-Thy-1 and complement eliminates their activity.

The results of such an experiment are shown in Fig. 4. BALB/c immune LN cells, when cotransferred with BALB/c Ts_2 transfer significant degrees of immunity into syngeneic BALB/c mice. BALB/c-immune LN cells, when mixed with BALB/c Ts_2, in the presence of C.AL-20-immune LN cells that have been treated with complement alone as control, failed to transfer immunity. However, BALB/c-immune LN cells when mixed with BALB/c Ts in the presence of C.AL-20-immune LN cells that had been treated with anti-Thy-1 plus complement regain their ability to transfer immunity. Therefore, we can conclude that the tragets of Ts_2 are Thy-1-bearing, CRI^+ T cells.

Discussion

The experiments reported in this communication serve to extend our previous observations on the T cell-T cell interactions in the ABA-specific suppressor pathway

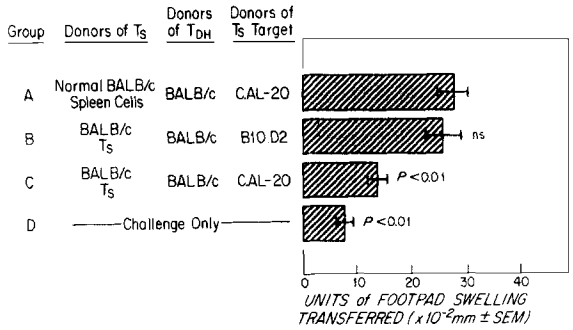

FIG. 3. By providing the appropriate CRI^+ target, the suppression observed in the cotransfer experiment becomes idiotype nonspecific. BALB/c mice injected 7 d earlier with 5×10^7 BALB/c CRI-SC (i.v.) were the donors of Ts_2. Another group of BALB/c, C.AL-20, and B10.D2 animals were immunized with 3×10^7 ABA-SC s.c. and 4–5 d later, they were the donors of immune T_{DH} cells. In a cotransfer experiment, 5×10^7 Ts_2 were mixed with 5×10^7 immune T_{DH} and 1×10^7 various Ts_2 targets (from C.AL-20 or B10.D2 mice) in 0.5 ml. The cell mixtures were then injected intravenously into groups of normal BALB/c recipients. Within 1 h after cell transfer, the animals were challenged in the footpad with 30 μl of the diazonium salt and increases in footpad swelling measured 24 h later. Each bar represents the mean ± SEM of measurement of at least four mice.

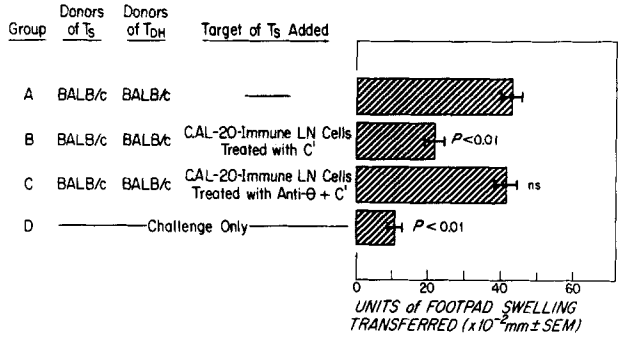

FIG. 4. The CRI$^+$ targets of Ts_2 are T cells. BALB/c mice injected with 5×10^7 BALB/c CRI-SC 7 d earlier were the donors of suppressor T cells. BALB/c mice immunized subcutaneously with 3×10^7 ABA-SC 4–5 d earlier were the donors of immunme T_{DH} cells. C.AL-20 mice immunized subcutaneously with 3×10^7 ABA-SC 7 d earlier were the donors of Ts_2 targets. The experimental protocol is exactly the same as the one described in Fig. 3, except that C.AL-20-immune LN cells were first treated with complement (C') alone as control or with anti-Thy-1 (θ) antibodies and complement. The cell mixtures were then injected intravenously into groups of normal BALB/c recipients. Within 1 h after cell transfer, the animals were challenged in the footpad with 30 μl of the diazonium salt and increases in footpad swelling measured 24 h later. Each bar represents the mean ± SEM of measurement of at least four mice.

(1, 2). In this suppressor pathway, idiotype and anti-idiotype interactions have been shown to play a decisive role in the propagation of suppressor signals.

We have herein provided evidence for the existence of an idiotype-bearing T cell subpopulation that resides in the immune LN cell population, and which is required for the function of anti-idiotypic suppressor T cells (Ts_2). These idiotype-bearing T cells have been termed the Ts_3 subset. These results are compatible with reports defining T cell-T cell interactions in the activation of many different T cell subsets (16–20).

These studies are consistent with an earlier finding on the suppression of contact sensitivity to DNFB (12). It was observed that Ts that block the efferent limb of sensitivity can inhibit the passive transfer of immunity mediated by DNFB-immune T_{DH} cells. However, these Ts failed to inhibit the passive transfer of immunity if the T_{DH} cells were obtained from animals pretreated with cyclophosphamide, a drug known to eliminate suppressor T cell precursors (21–23). The latter observations and the results described herein provide conclusive evidence that LN cells from sensitized mice contain not only T_{DH} cells, but also another T cell subpopulation which serves as target of efferent Ts. Furthermore, it was previously shown that the auxiliary suppressor T cells (T_{aux}) in the DNFB system are sensitive to adult thymectomy and bear I-J determinants. If indeed Ts_3, in the ABA system, are similar to, if not identical to, the T_{aux} subset, we expect the precursors of Ts_3 to be sensitive to cyclophosphamide or to adult thymectomy and possibly to bear I-J-subregion-encoded determinants.

We should also consider our current understanding of the generation of Ts_3 cells. Ts_3 were activated by subcutaneous immunization with ABA-SC but were not induced by intravenous injection of the same antigen. This latter route of administration favors the activation of first-order, idiotype-bearing suppressor T cells (Ts_1). Furthermore, only ABA-immune LN T cells—but not normal T cells—can provide the relevant idiotypic target for Ts_2 (M.-S. Sy, unpublished results). It might be postulated that the activation of Ts_3 might require two signals: one provided by the

antigen, possibly in the context of H-2 antigen, and the additional signal provided by anti-idiotypic Ts_2. The postulate that appropriate activation of T cells, similar to the activation of B cells, might require two different signals has received considerable support (24). Recently, we have obtained evidence indicating that Ts_1 activation requires two discrete signals (25), and it should also be noted that optimal activation of helper T cells and cytotoxic T cells also requires two types of signals (17, 26).

More important, the demonstration of such an idiotype-bearing T_3 cell serving as target of Ts_2 has resolved some of our earlier unexplained data. Specifically, we had observed and could not readily explain how Ts_2 cells were able to suppress apparently idiotype-negative effector T_{DH} cells (6, 11). It is now apparent that the relationship between Ts_2 and Ts_3 is governed by interactions that relate to anti-idiotypic and idiotypic structures either present on their cell surface or their soluble products. However, the final manifestation of suppression may be idiotype nonspecific. This latter notion was supported by our observation that by providing the appropriate idiotype-bearing Ts_3 (from C.AL-20 mice) relevant to the anti-diotypic Ts_2 (from BALB/c mice), suppression can indeed occur across an allotype barrier. This experiment provides strong evidence that Ts_3 are indeed the effector Ts. However, the exact mechanism that allows Ts_3 to act across an allotype barrier is not clear, and experiments are now in progress to determine whether the final suppression mediated by Ts_3 is antigen specific.

There are also several questions regarding Ts_3 that remain to be answered. We should consider the possibility that Ts_3 is a later stage of differentiation of the antigen-specific idiotypic Ts_1 after it has interacted with antigen and anti-idiotypic Ts_2. In this regard, we have recently found that whereas Ts_1 cells are Lyt-$1^+2,3^-$, Ts_3 cells are Lyt-$1^-2,3^+$ (M.-S. Sy, M. Takaoki, A. Nisonoff, M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene, manuscript in preparation).

We have recently demonstrated that anti-idiotypic Ts_2 produce a TsF that we termed TsF_2 (8). Similar to the dichotomy between Ts_1 and Ts_2, TsF_2 differs from TsF_1 with respect to receptor specificity, mode of action, and apparent genetic restriction. TsF_2 bears anti-idiotypic determinants and inhibits the development of ABA-specific DTH by suppressing the elicitation phase of the immune response. The observation that Ts_2 and Ts_3 interaction is necessary for the manifestation of suppressor function suggests that Ts_3 may also be required for the function of TsF_2. Experiments are now in progress to determine whether TsF_2 function also requires the presence of Ts_3.

Because our previous results indicated that Ts_2 require both H-2 and Igh-1 identity between donors of Ts_2 or TsF_2 and the recipients for successful transfer of suppression (8), we considered that Ts_2 were functionally H-2 restricted as well as Igh restricted. Our present results indicate that Ts_2 and Ts_3 may be functionally related by their idiotypic and anti-idiotypic structures. It is possible however that the observed H-2 restriction may, in fact, occur through events related to Ts_3 activity, for example, the interaction between Ts_3 and T_{DH} cells. Therefore, the possibility must be entertained that two different subsets of Ts (Ts_2 and Ts_3) may contribute independently to dictate H-2 and Igh restrictions, and that this dual restriction may not be the function of a single Ts_2.

The observation that the final suppression of ABA reactivity appears to be idiotype

nonspecific (because the T_{DH} and Ts_3 do not, of necessity, share the same idiotype) differs from the earlier reports of Owen et al. (9) in the regulation of the humoral response to ABA-KLH. In the antibody response, anti-idiotypic suppressor T cells only inhibit that portion of the anti-ABA antibodies that bear the major CRI with little effect on the total anti-ABA response. It is possible and indeed likely, based on experiments reported earlier (27), that in the antibody response, anti-idiotypic Ts_2 may be able to interact directly with the corresponding CRI-bearing B cells. This would be consistent with observations that suppression occurs only in the CRI-bearing portion of the anti-ABA antibodies. Ts_2 cells may be decisive in the regulation of antibody responses, whereas Ts_3 subsets may play a lesser or alternate role. Experiments are now in progress to determine whether by providing ABA-primed Ts_3 cells we can suppress both the CRI bearing and the non-CRI-bearing anti-ABA antibody response in vitro and in vivo.

In conclusion, it is clear that a subset of T cells which may be the last link in the suppressor T cell network appears concurrently with T effector cells. Analysis of the properties of this subset, termed Ts_3, may further our understanding of the genetic restriction and of the mechanism of suppression of T cell reactions.

Summary

The suppressor pathway that regulates the T cell response to *p*-azobenzenearsonate (ABA)-coupled cells has been studied. It has been found that the ability of anti-idiotypic second-order T suppressor cells (Ts_2) to inhibit T cell-dependent delayed-type hypersensitivity (DTH) responses depended upon the presence of cross-reactive-idiotype (CRI)-bearing T cells present in ABA-primed mice. This suppressor T cell subset, termed Ts_3, coexists with CRI-negative T cells that mediate DTH in vivo. It appears that antigen-activated CRI^+ Ts_3 require signals from the anti-CRI Ts_2 subset to suppress DTH reactions in an idiotype-nonspecific manner. The relevance of these observations to a comprehensive scheme of T and B cell regulation is discussed.

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