ANTIGEN- AND RECEPTOR-DRIVEN REGULATORY MECHANISMS*

V. The Failure of Idiotype-coupled Spleen Cells to Induce Unresponsiveness in Animals Lacking the Appropriate V_H Genes is Caused by the Lack of Idiotype-matched Targets

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The level of an immunological response to a T-dependent antigen is determined by a series of interactions among different subpopulations of effector and regulatory T lymphocytes (1, 2). The nature of the signals that mediate these communications is still being investigated. Previous studies have revealed that genes in the major histocompatibility complex play an essential role in some of these interactions (3, 4). Recent experiments have provided evidence indicating that genes linked to the Igh-1 allotype locus may also govern the interactions among certain T cell subsets (5, 6), in accordance with the operation of a T cell network of idiotype and anti-idiotype interactions as proposed by Jerne (7). According to this hypothesis, both antigens and their immune receptors (idiotype) were considered capable of modulating immune responses.

To investigate further the secondary aspect of the immune network activated by receptor- or idiotype-stimulated events, we have injected anti-*p*-azobenzenearsonate (ABA)[1] antibodies bearing cross-reactive idiotypic (CRI) determinants, coupled to normal spleen cells (SC) in an attempt to modulate ABA-specific delayed-type hypersensitivity (DTH) (8). The administration of idiotypic determinants presented to a naive animal on spleen cells was meant to mimic the rapid appearance of antigen binding cells in vivo. It was found that the injection of anti-ABA antibodies, coupled covalently to normal syngeneic spleen cells, into normal animals, suppresses ABA-specific DTH and also suppresses the major idiotypic component of a humoral anti-ABA response (9) upon subsequent immunization. Moreover, adoptive transfer experiments provided evidence that this unresponsiveness was mediated, at least in part,

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[1] *Abbreviations used in this paper*: ABA, *p*-azobenzenearsonate; ABA-SC, *p*-azobenzenearsonate-coupled syngeneic spleen cells; CRI, cross-reactive idiotype common to anti-ABA antibodies of A/J mice; CRI-SC, spleen cells conjugated CRI+ antibodies; DNFB, 2,4-dinitrofluorobenzene; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salt solution; NMG, normal A/J immunoglobulin; NP, 4-hydroxy-3-nitrophenyl acetyl; PBS-5, phosphate-buffered saline with 5% fetal calf serum; RAMIg, rabbit anti-mouse immunoglobulin; Ts, suppressor T cells.

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by suppressor T cells. Furthermore, genetic analysis revealed that the ability of CRI-coupled spleen cells (CRI-SC) to inhibit ABA-specific DTH was linked to Igh-1 heavy chain allotype. BALB/c (H-2^d, Igh-1^a) animals that do not produce CRI+ anti-ABA antibodies were insensitive to suppression induced by CRI-SC. In contrast, CRI-SC suppressed ABA-DTH in CAL-20 (H-2^d, Igh-1^d) mice, which possess the heavy chain allotype of the AL/N strain on a BALB/c background and produce anti-ABA antibodies bearing the CRI.

This restriction in the ability to respond to CRI-SC was postulated to reflect one of two possibilities. (*a*) The receptor repertoire of cells operative in the idiotypic network may be genetically restricted to self idiotypes so that only those mice which express CRI+ receptors at either the T or B cell level can recognize these elements. Accordingly, the receptor for these idiotypic structures (i.e. anti-idiotype) would also appear to be genetically linked to the Igh-1 locus and BALB/c mice which do not possess CRI would be unable to generate a discernible anti-idiotypic (anti-CRI) reaction at the T cell level; or (*b*) Restriction with respect to anti-idiotypic receptors does not exist. In this case BALB/c mice would be expected to respond to CRI-SC because the CRI+ element is antigenic. The suppressor T cell (Ts) so generated, however, would only be able to suppress immune T cells which use this CRI element as a component for their receptor. Because CRI is not expressed in BALB/c animals, suppression will never be observed in the absence of a suitable target.

In this paper, we will provide evidence indicating that the second interpretation is correct. Intravenous injection of CRI-SC into mice unable to express the appropriate V_H genes which code for the CRI indeed induces anti-idiotypic Ts in these animals. These Ts can only be functionally demonstrated upon transfer to animals with the capacity to express CRI. The significance of these findings will be discussed in terms of a modified network theory of regulation of the immune response.

Materials and Methods

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

Preparation of Antigen and Antigen-coupled Cells. These methods have been described in detail elsewhere (10). Briefly, a 40 mM solution of ABA diazonium salt was prepared from 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 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.

Induction and Elicitation of DTH to ABA-coupled Cells. To induce DTH to ABA, a total of 3×10^7 ABA-coupled syngeneic cells were injected subcutaneously into separate sites on the dorsal flanks of 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 after the footpad challenge, DTH reactivity was assessed by measuring the swelling of the footpad with a Fowler micrometer (Schlesingers for Tools Ltd., Brooklyn, N. Y.). The magnitude of 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 and Elicitation of Contact Sensitivity to DNFB. Contact sensitivity was induced by two daily paintings on the clipped abdomen with 25 ul of 0.5% 2,4-dinitro-fluorobenzene (DNFB) solution (Sigma Chemical Co., St. Louis, Mo.) in acetone:olive oil (4:1). 5 d after the last painting, 20 μl of 0.2% DNFB in the same vehicle was applied to the dorsal surface of each ear,

and ear swelling was measured 24 h later with a Mitutoyo engineer's micrometer (Mitutoyo/MTI Corp., New York).

Preparation of Idiotype-coupled Cells. The method used for coupling anti-ABA antibodies to spleen cells is a modification of the method of Miller et al. (11). 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. 4–5 × 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 of CRI+ affinity-purified (12) 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 (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 radiolabeled CRI was ~5–10%.) The CRI-SC were washed twice in HBSS and adjusted to a concentration of 10^8/ml, and then 0.5 ml of the suspension was injected intravenously into appropriate recipients.

Transfer of Ts Induced with CRI-SC. 7 d after tolerization with CRI-SC, the animals were sacrificed, their spleens were removed, and single cell suspensions were then prepared as described (8). These cells were washed twice in HBSS and adjusted to a concentration of 10^8 cells/ml, and 0.5 ml as injected intravenously into naive recipients.

Antiserum Treatment. Anti-Thy 1.2 hybridoma antibodies were kindly provided by Dr. P. Lake, University College, London. 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 HBSS, and then incubated again with 1 ml of a 1:10 dilution of Low Tox rabbit complement (Cedarlane, London, Ontario) for 30 min at 37°C. The cells were then washed twice in HBSS and resuspended and adjusted to 10^8 viable cells/ml, and then 0.5 ml of the suspension was injected intravenously into appropriate recipients.

Purification of Ts on Idiotype-coated Plates. B cells were removed from a single cell suspension of spleen cells by a modification of the method described by Mage et al. (13). 4 ml of rabbit anti-mouse immunoglobulin (RAMIg) prepared as described (14) was added at a concentration of 0.7–1.0 mg/ml in phosphate-buffered saline (PBS), and was added to 100 × 15-mm polystyrene petri dishes (Falcon Labware) for 1 h at room temperature. Plates were washed extensively with PBS, pH 7.2, and then incubated for 20 min with 4 ml PBS with 5% fetal calf serum (PBS-5) before addition of spleen cells. After removal of PBS-5, 60–75 × 10^6 spleen cells depleted of erythrocytes by incubation with 0.83% (Tris) NH_4Cl were added to each plate in a 5-ml volume of PBS-5. After 30 min at 4°C, the plates were gently rocked, and after a 1-h incubation, nonadherent cells were removed by gently swirling and washing the plates with PBS-5. These nonadherent populations contained ~5–8% surface immunoglobulin-bearing cells as judged by immunofluorescence using fluorescenated-RAMIg. A/J anti-ABA antibodies, >30% of which were CRI, were prepared and purified as described (9, 12). CRI- or normal A/J immunoglobulin (NMG)-coated dishes were prepared according to a modification of the method of Abbas et al. (15). Briefly, 4 ml of purified CRI+ anti-ABA antibodies or NMG (0.5–1.0 mg/ml in PBS) as incubated on 100 × 15-mm polystyrene petri dishes for 2–3 h at room temperature. After removal of the immunoglobulin solutions, the plates were washed with PBS, incubated 20–30 min with PBS-5, and washed extensively with PBS before addition of T cells. 60–65 × 10^6 plate-purified T cells were added to NMG- or idiotype-coated plates in a 5-ml total volume PBS-5. After incubating for 1 h at 20°C, nonadherent cells were removed from NMG- or CRI-coated plates with two cycles of swirling and washing with warm (20°C) PBS-5. Then, 5 ml of chilled PBS-5 was added to all plates, which were then placed at 4°C for an additional 30 min. The adherent cells were suspended by vigorous pipetting. After two additional washes with chilled PBS-5 with vigorous pipetting, the adherent cells were pooled, spun at 200 *g* for 10 min, resuspended in PBS-5, and then counted. The cells were aliquoted into groups, washed twice with chilled HBSS, and brought to the appropriate volume before transfer. These separation procedures routinely yielded a number of T cells equal to 35–50% of the original spleen cell population added to the RAMIg plates; cells adherent to CRI- or NMG-

coated dishes comprised 5–15% of the applied T cell population or ~2–7% of the initial spleen cell population.

Statistical Analysis. Analysis of the significance of differences between the experimental and control groups was performed with the Wang programmable computer. The means and standard error of the mean are given, as well as the relevant P value obtained with the two-tailed Student's t test.

Results

CRI-SC Induces Suppressor Cells in BALB/c Mice Whose Activity Can be Demonstrated in CRI+ C.AL-20 Mice but Not in CRI⁻ B10.D2 Recipients. Our previous studies had indicated that CRI-SC, when injected intravenously into normal BALB/c mice, did not interfere with the development of ABA-specific DTH in this strain (8). To investigate whether undetected suppressor cells had nevertheless been induced in these mice, we explored the question of whether spleen cells taken from CRI-SC-treated BALB/c mice could transfer suppression for ABA-specific DTH to H-2 syngeneic but allotype-distinct C.AL-20 and B10.D2 mice.

The results of one such experiment are shown in Fig. 1. Spleen cells from BALB/c mice, which had received CRI-SC 7 d earlier, failed to transfer any significant suppression to syngeneic BALB/c (H-2^d, Igh-1^a) animals or to H-2 identical but allotype-distinct B10.D2 (H-2^d, Igh-1^b) animals. In contrast, the same number of spleen cells from CRI-SC-treated BALB/c mice transferred significant suppression to CRI+ C.AL-20 (H-2^d, Igh-1^d) mice. Because CRI-SC-primed BALB/c spleen cells transferred to B10.D2 mice failed to suppress ABA-specific immunity, and because normal BALB/c spleen cells transferred into C.AL-20 mice also did not suppress ABA-specific DTH (data not shown), the inhibition of the ABA response in C.AL-20 mice is not caused simply by the effect of transferring cells into an allotype-different strain.

These experiments reveal that intravenous injection of CRI-SC does induce ABA-

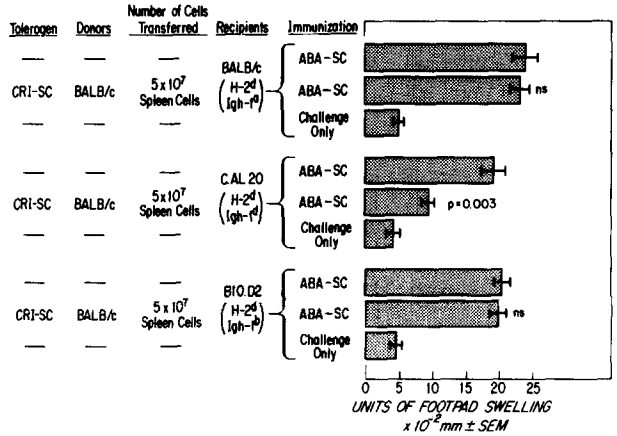

FIG. 1. Induction of suppressor cells in BALB/c mice with CRI-SC. Normal BALB/c animals were injected intravenously with 5×10^7 CRI-SC. 7 d later they were the donor of suppressor cells, and 5×10^7 spleen cells from these were transferred into either syngeneic BALB/c or H-2 congeneic C.AL-20 and B10.D2 animals. Within 2 h after transfer, these animals and their appropriate controls were immunized with 3×10^7 ABA-SC subcutaneously. 5 d later they were challenged in the footpad with 30 μl of 10 mM ABA diazonium salt and increases in footpad swelling were measured 24 h later. Bars represent mean footpad swelling for groups of four to five mice ± SEM.

specific suppressor cells in BALB/c mice. Because BALB/c mice are unable to express CRI+ receptors, these putative Ts are immunologically silent in their own environment caused by the lack of suitable targets, in this case, CRI+ structures on ABA-specific cells. However, upon transfer to animals capable of expressing CRI (C.AL-20), their activities can be fully revealed.

To further investigate that the ability to express CRI+ receptors is a prerequisite for the demonstration of CRI-SC-induced suppressor cells, we determined whether suppressor T cells induced in C.AL-20 mice can transfer ABA-specific suppression to H-2 congenic BALB/c mice.

The results of a representative experiment are depicted in Fig. 2. Spleen cells from C.AL-20 mice inoculated with CRI-SC inhibited the development of ABA-specific DTH in syngeneic C.AL-20 mice. However, the same number of spleen cells failed to transfer any significant degree of suppression to BALB/c mice. Thus, the ability to express CRI on ABA-specific antibody and also on T cells with anti-ABA receptors is a prerequisite for the expression of these suppressor T cells.

Suppressor Cells Induced with CRI-SC in BALB/c Mice Are Sensitive to In Vitro Treatment with Anti-Thy Serum and Complement. Previously we reported that CRI-SC could induce suppressor cells in A/J animals (8). In addition, treatment with anti-Thy 1.2 serum plus complement completely abrogated the ability of these cells to transfer suppression. Thus, this active suppression induced in A/J mice by CRI-SC is a T cell-dependent phenomenon. To determine whether the suppressor cells induced in BALB/c mice are also T cells, CRI-SC-primed spleen cells were treated in vitro with either normal mouse serum or anti-Thy 1.2 antibodies before transfer to C.AL-20 mice. The results of these experiment are shown in Fig. 3. Suppressor cells treated in vitro with normal serum and complement transfer significant suppression to C.AL-20 mice. In contrast, cells treated in vitro with monoclonal anti-Thy 1.2 antibodies and

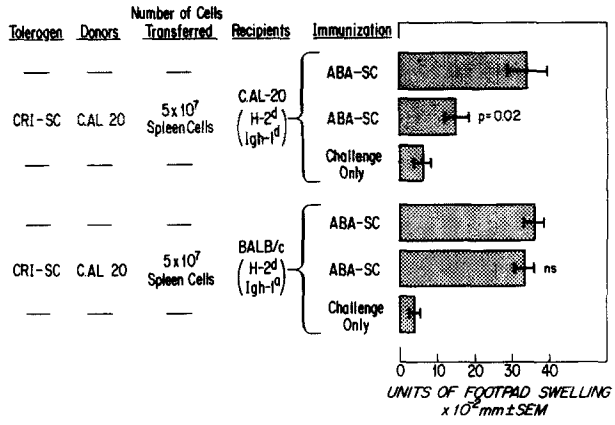

FIG. 2. Allotype restriction of suppressor cells induced in C.AL-20 with CRI-SC. Normal C.AL-20 mice were injected intravenously with 5×10^7 CRI-coupled syngeneic spleen cells. 7 d after tolerization, 5×10^7 spleen cells from these mice were transferred to naive syngeneic C.AL-20 mice or BALB/c mice which were concomitantly immunized subcutaneously with 3×10^7 ABA-SC. 5 d later, the recipients and the controls were challenged with ABA in the footpad. Increases in footpad swelling were measured 24 h after challenge. Bars represent the mean footpad swelling for groups of four to five mice ± SEM.

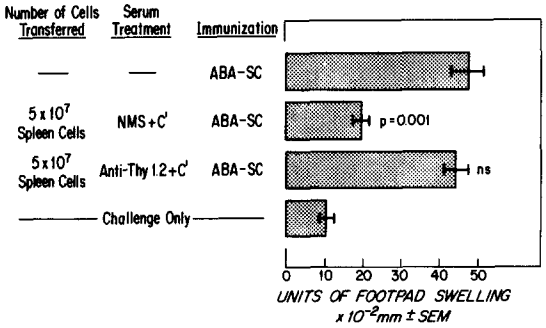

FIG. 3. Sensitivity of suppressor cells induced with CRI-SC in BALB/c mice to anti-Thy 1.2 antibodies and complement treatment. Spleen cells from BALB/c animals which received CRI-SC intravenously 7 d earlier were treated with anti-Thy 1.2 antiserum or normal mouse serum and complement before transfer to naive C.AL-20 animals. All recipients and appropriate controls were immunized with 3×10^7 ABA-SC subcutaneously within 2 h after cell transfer. 5 d later, they were challenged in the footpad with ABA diazonium salt and increases in footpad swelling were measured 24 h after challenge. Bars represent the mean footpad swelling of groups of four to five mice.

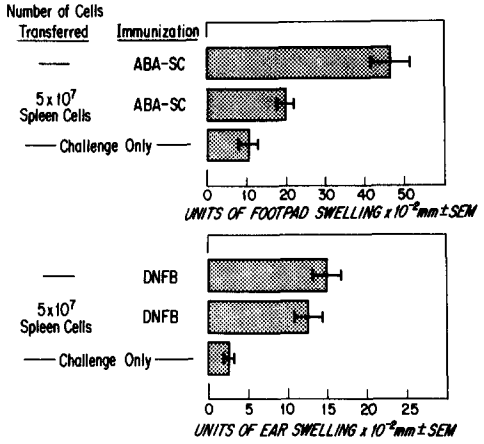

FIG. 4. Antigen specificity of suppressor T cells induced in BALB/c mice with CRI-SC. Suppressor cells were induced in BALB/c mice by the intravenous administration of CRI-SC, 5×10^7 spleen cells were transferred into two different groups of C.AL-20 recipients. One group was immunized with 3×10^7 ABA-SC subcutaneously, while the other groups were skin painted with DNFB. 5 d after immunization, these animals and their appropriate controls were challenged with the appropriate agent and DTH was measured 24 h after challenge. Bars represent either the mean footpad or mean ear swelling of at least four to five mice ± SEM.

complement were unable to transfer suppression. Thus, the ability to transfer suppression is a T-cell-dependent phenomenon.

Antigen Specificity of Suppressor T Cells Induced with CRI-SC. Because these suppressor T cells could conceivably recognize some allotypic markers associated with the A/J anti-ABA antibodies rather than idiotypic elements, the specificity of the suppression observed had to be established. Suppressor T cells were induced in BALB/c mice with CRI-SC; 7 d later, their spleen cells were transferred to different groups of C.AL-20 mice, one sensitized with ABA-SC and the other with DNFB. The results of such an experiment are depicted in Fig. 4. Spleen cells from CRI-SC-treated animals suppress ABA-specific DTH; in contrast, the same number of spleen cells failed to

suppress the development of contact sensitivity to DNFB. Therefore, suppressor T cells induced with CRI-SC are operationally antigen specific.

Suppressor Cells Induced in BALB/c Mice Are Anti-Idiotypic and Bind to Idiotype-coated Plates. Both idiotypic and anti-idiotypic Ts have been found in the regulation of ABA-specific DTH (16). Therefore, the specificity of the suppressor T cells induced by CRI-SC in BALB/c mice for CRI determinants was investigated. BALB/c mice were tolerized with CRI-BALB/c cells and their spleens removed 7 d later. Single cell suspension of spleen cells were allowed to adhere to dishes to which a polyvalent rabbit anti-mouse immunoglobulin was attached. The nonadherent cells comprising predominantly of T cells were then tested directly for suppression or alternatively subjected to further tests of adherence to CRI or to NMG-coated plates. The adherent or nonadherent cells from such plates were then evaluated for their ability to suppress the development of ABA-specific DTH in C.AL-20 mice. As can be seen in Table I the Ts cells from BALB/c mice responsible for the observed suppression adhered selectively to CRI-coated plates. Thus, Ts induced by CRI-SC are anti-idiotypic.

Discussion

The studies documented herein extend our earlier observation that idiotypic determinants coupled to cell surfaces are useful probes for the study of immunoregulatory events (8, 9, 17). In this report we presented evidence that A/J CRI bearing anti-ABA antibodies, coupled to BALB/c cells, failed to induce ABA-specific immune unresponsiveness in normal BALB/c animals, but nevertheless induced anti-CRI suppressor T cells, whose activity could be demonstrated by transfer to appropriate CRI+ recipients.

The suppressor T cells generated in this manner in BALB/c mice were found to be functionally antigen specific, but perhaps most importantly, anti-idiotypic, because they were bound directly to CRI determinants used in the plate separation studies. Anti-idiotypic suppressor T cells have been reported in many different experimental systems. Owen et al. (18) observed that suppressor T cells specific for the CRI+ components of the anti-ABA antibody response bear anti-CRI receptors, and Bona et al. (19) reported the presence of anti-idotypic suppressor T cells specific for the MOPC 460 myeloma protein in normal animals. More recently, we have found that second-order suppressor T cells (Ts-2) induced by intravenous administration of ABA-specific suppressor T cell factors (TsF-1) similarly bear anti-idiotypic receptors (16). In addition, anti-idiotypic Ts has been reported in the regulation of DTH to 4-hydroxy-3-nitrophenyl acetyl (NP) by Weinberger and colleagues (20). Anti-idiotypic, as well

TABLE I

Ts Induced in BALB/c Mice by CRI-SC Are Anti-idiotypic and Bind to CRI-coated Plates

Cell enrichment	Number of cells transferred	Immunization	Units of footpad swelling $\times 10^{-2}$ mm ±SEM	*P* value
—	—	ABA-SC	25 ± 0.9	<0.001
T cells	19×10^6	ABA-SC	14.0 ± 0.9	<0.001
NMG, nonadherent	19×10^6	ABA-SC	9.0 ± 1.0	<0.001
NMG, adherent	1.4×10^6	ABA-SC	22.0 ± 2.5	=0.2
CRI, nonadherent	19×10^6	ABA-SC	21.7 ± 3.1	=0.3
CRI, adherent	1.4×10^6	ABA-SC	6.5 ± 1.7	<0.001

as idiotypic receptors have also been identified on T cell factors that can suppress the major idiotypic component of a humoral anti-ABA response (21).

Although anti-idiotypic Ts are readily induced by intravenous injection of CRI-SC in BALB/c mice, these Ts cannot exhibit their suppressive effect unless they are placed in an environment in which CRI is expressed, such as in C.AL-20 mice. Thus, idiotype:anti-idiotype interactions are required for the manifestation of ABA-specific suppression. This was further supported by the observation that Ts induced in C.AL-20 mice failed to suppress the development of ABA-specific DTH when transferred to BALB/c mice.

Furthermore, these studies demonstrate that the generation of anti-idiotypic Ts by CRI-SC is not in itself restricted by Igh-1 phenotype of the responding cells. The restriction we observed in an intact animal is actually a reflection of the role of Igh-1 gene products at a later stage in T cell-mediated suppression. Although some investigators have demonstrated that the activity of anti-idiotypic Ts may be modulated by anti-idiotypic antibody, the results presented in this study do not appear to reflect such antibody-mediated effects (19).

It should be noted that other systems have similarly described suppressor T cell interactions that are limited by Igh linked genes. Eardley et al. (5) have reported that Ts generation with sheep erythrocytes in vitro requires a V_H-restricted interaction between an Ly 1+Qa 1+ inducer and Lyt 1+2+3+ Qa 1+ acceptor cells. However, it is impossible to distinguish whether the restriction observed in their experiments actually reflects the interaction between the Ly 1+ inducer and its Ly 1+2+3+ acceptor or the reaction of the effector suppressor with its target(s). The studies documented herein reveal that V_H restriction which in some systems had been interpreted to reflect requirement of V_H identity between two interacting sets may rather be the consequence of idiotypic anti-idiotypic restrictions imposed on the system by the inductive signal. More recently, interactions between T cell subsets limited by the Igh-V locus has also recently been reported in the regulation of DTH specific for NP by Weinberger and colleagues (6, 20). Moreover, we have also demonstrated, that ABA-coupled spleen cells given intravenously induce CRI idiotype-bearing Ts in mice of the appropriate Igh-1 allotype.[2] Likewise, anti-idiotypic antisera when administered to naive mice by different routes could only induce immunity mediated by idiotype bearing T cells or induce suppression mediated by CRI+ Ts, in strains of the appropriate heavy chain allotype (22; and Unpublished results.).

The regulation of antibody specificities produced in response to an external stimulus could proceed from two basic mechanisms. In the first case, one could envisage that the expression of idiotype (CRI) and anti-idiotype (anti-CRI) would be genetically linked and totally interdependent. If this were the case, then we should have never observed the generation of anti-idiotypic Ts in BALB/c, because A/J major CRI components are not expressed in BALB/c mice. Secondly, it has been suggested that the immune system comprises an internal image set of specificities present on different receptors that cross-react with antigens (7). Thus, "determinants" on receptors against antigen "y" would fortuitously cross-react with antigen "x", and as a result, all antigenic specificities would be represented within the immune system. For example,

[2] Dietz, M. H., M.-S. Sy, M. I. Greene, A. Nisonoff, B. Benacerraf, and R. N. Germain. 1980. Antigen and receptor driven regulatory mechanisms. VI. Demonstration of cross-reactive idiotypic determinants of azobenzenearsonate specific antigen-binding suppressor cells producing soluble suppressor factor(s). *J. Immunol.* In press.

in the BALB/c (or in B10.D2) mouse, CRI idiotypic determinants which cross-react with antigen "x" would simply expand some already existing anti-idiotypic set of cells, and induce what we have herein observed as anti-idiotypic Ts. The data in this study illustrate that the absence of certain V_H genes poses no restriction on the ability of the immune system to develop anti-idiotype receptors against other V_H sequences.

We conclude that the immunogenicity of CRI molecules itself expands the population of anti-idiotypic Ts, and can do so in any recipient strain because of the large repertoire of T cell specificities within the host.

Summary

A/J anti-*p*-azobenzenearsonate (ABA) antibodies bearing cross-reactive idiotypic (CRI) determinants, when coupled to spleen cells and then injected intravenously into naive animals, stimulate suppressor T cell (Ts) responses. Moreover, previous studies have demonstrated that the ability of such idiotype-coupled spleen cells to induce immune unresponsiveness to subsequent immunization with ABA-coupled spleen cells is linked to Igh-1 genes. Thus, CRI bearing antibodies from A/J mice, when conjugated to normal BALB/c spleen cells in vitro and then injected intravenously to syngeneic BALB/c mice, failed to induce tolerance in these animals. However, spleen cells taken from these animals transferred significant degrees of suppression to Igh-1 congenic C.AL-20 but not to H-2 congenic, Igh-1 distinct B10.D2 mice. Therefore, the failure of CRI-coupled spleen cells to induce suppressor cell-mediated unresponsiveness in animals unable to express the appropriate V_H genes (i.e. BALB/c and B10.D2) appears to be caused by the lack of idiotype-matched targets. The notion that the ability to express certain V_H genes in the recipient animal is a prerequisite for suppressor cell function was further supported by the observation that suppressor cells induced in C.AL-20 mice failed to transfer any degree of suppression to BALB/c mice. The ability to transfer suppression from BALB/c mice to C.AL-20 mice is a T cell-dependent phenomenon, since in vitro treatment with anti-Thy 1.2 antiserum and complement completely abrogated suppressor cell function. Furthermore, these suppressor T cells are antigen specific and can be enriched on idiotype-coated petri dishes, indicating they possess anti-idiotypic receptors. Therefore, appropriate anti-idiotype and idiotype interaction is essential for the manifestation of suppressor T cell function in ABA-specific suppressor pathways.

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References

1. Benacerraf, B., and R. N. Germain. 1979. Specific suppressor responses to antigen under I region control. *Fed. Proc.* **38:**2053.
2. Cantor, H., and R. K. Gershon. 1979. Immunological circuits: cellular composition. *Fed. Proc.* **38:**2058.
3. Katz, D. H., and B. Benacerraf. 1975. The function and interrelationships of T cell receptors, Ir genes and other histocompatibility gene products. *Transplant. Rev.* **22:**175.
4. Doherty, P. C., R. V. Blanden, and R. M. Zinkernagel. 1976. Specificity of virus-immune effector T cells for H-2K or H-2D compatible interactions: Implications for H-antigen diversity. *Transplant. Rev.* **29:**89.

5. Eardley, D. D., F. W. Shen, H. Cantor, and R. K. Gershon. 1979. Genetic control of immunoregulatory circuits. Genes linked to the Ig locus govern communication between regulatory T-cell sets. *J. Exp. Med.* **150:**44.
6. Weinberger, J. Z., B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. III. Interaction of effector suppressor T cells is restricted by H-2 and Igh-V genes. *J. Exp. Med.* **151:**1413.
7. Jerne, N-K. 1974. Towards a network theory of the immune system. *Ann. Inst. Pasteur (Paris).* 373.
8. Sy, M.-S., Bach, B. A., A. Brown, A. Nisonoff, B. Benacerraf, and M. I. Greene. 1979. Antigen- and receptor-driven regulatory mechanisms. II. Induction of suppressor T cells with idiotype-coupled syngeneic spleen cells. *J. Exp. Med.* **150:**1229.
9. Dohi, Y., and A. Nisonoff. 1979. Suppression of idiotype and generation of suppressor T cells with idiotype-conjugated thymocytes. *J. Exp. Med.* **150:**909.
10. Bach, B. A., L. Sherman, B. Benacerraf, and M. I. Greene. 1978. Mechanisms of regulation of cell-mediated immunity. II. Induction and suppression of delayed-type hypersensitivity to azobenzenearsonate coupled syngeneic cells. *J. Immunol.* **121:**460.
11. Miller, S. D., R. P. Wetzig, and H. N. Claman. 1979. The induction of cell-mediated immunity and tolerance with protein antigens coupled to syngeneic lymphoid cells. *J. Exp. Med.* **149:**758.
12. Tung, A. S., S.-T Ju, S. Sato, and A. Nisonoff. 1976. Production of large amounts of antibodies in individual mice. *J. Immunol.* **116:**676.
13. Mage, M. G., L. L. McHugh, and T. L. Rothstein. 1977. Mouse lymphocyts with and without surface immunoglobulin: preparative scale preparation in polystyrene tissue culture dishes coated with specifically purified anti-immunoglobulin. *J. Immunol. Methods.* **15:**47.
14. Weinberger, J. Z., R. N. Germain, S-T. Ju, M. I. Greene, B. Benacerraf, and M.E. Dorf. 1979. Hapten specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. *J. Exp. Med.* **150:**761.
15. Abbas, A. K., L. L. Perry, B. A. Bach, and M. I. Greene. 1980. Idiotype specific T-cell immunity. I. Generation of effector and suppressor T lymphocytes reactive with myeloma idiotypic determinants. *J. Immunol.* **124:**1160.
16. Sy, M.-S., M. H. Dietz, R. N. Germain, B. Benacerraf, and M. I. Greene. 1980. Antigen- and receptor-driven regulatory mechanisms. IV. Idiotype-bearing I-J^+ suppressor T cell factors induce second-order suppressor cells which express anti-idiotype receptors. *J. Exp. Med.* **151:**1183.
17. Greene, M. I., B. A. Bach, M.-S. Sy, A. R. Brown, A. Nisonoff, and B. Benacerraf. 1979. Antigen and receptor stimulated regulation. The relationship of idiotype and MHc products to regulatory networks. *In* T and B Lymphocytes: Recognition and Function. F. Bach, B. Bonavida, and E. S. Vitetta, Editors. Academic Press, Inc., New York. 361.
18. Owen, F. L., S-T. Ju, and A. Nisonoff. 1977. Binding to idiotypic determinants of large proportions of thymus-derived lymphocytes in idiotypically suppressed mice. *Proc. Natl. Acad. Sci. U. S. A.* **74:**204.
19. Bona, C., R. Hooghe, P. A. Cazenave, C. Leguérn, and W. E. Paul. 1979. Cellular basis of regulation of expression idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. *J. Exp. Med.* **149:** 815.
20. Weinberger, J. Z., R. N. Germain, B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. V. Role of idiotypes in the suppressor pathway. *J. Exp. Med.* **152:**161.
21. Hirai, Y., and A. Nisonoff. 1980. Selective suppression of the major idiotypic component of an antihapten response by soluble T cell-derived factors with idiotypic or anti-idiotypic receptors. *J. Exp. Med.* **151:**1213.
22. Sy, M.-S., B. A. Bach, Y. Dohi, A. Nisonoff, B. Benacerraf, and M. I. Greene. 1979. Antigen- and receptor-driven regulatory mechanisms. I. Induction of suppressor T cells with anti-idiotypic antibodies *J. Exp. Med.* **150:**1216.