ANTIGEN- AND RECEPTOR-DRIVEN
REGULATORY MECHANISMS

II. Induction of Suppressor T Cells with
Idiotype-coupled Syngeneic Spleen Cells*

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Jerne proposed that after antigenic stimulation and clonal proliferation, a particular
idiotype might stimulate an anti-idiotypic antibody response and presumably there-
after an anti-anti-idiotype antibody response. Thus, these linked humoral responses
were envisioned to be active in a balanced network of immune reactions. According
to this hypothesis, both antigen and their immune receptors (idiotype) were considered
capable of modulating an immunological response (1). These cascade regulatory
mechanisms have been later shown to occur both at the thymus-derived (T) cell and
bone marrow (B) cell level (2-6).

Evidence supporting this postulate at the T-cell level was obtained from the studies
on T-cell subpopulations, in particular, suppressor T cells (Ts). It has been observed
that suppressor T cells induced by antigens could be enriched on antigen-coated
plates (7, 8) or columns (9) and are sensitive to lysis with anti-idiotype anti-serum
plus complement (8, and M.-S. Sy, B. A. Bach, N. Nisonoff, B. Benacerraf, and M. I.
Green. Manuscript in preparation.). These findings provide direct evidence that T,
bear idiotypic determinants similar to those of B cells and their antibody products. In
contrast to these results, there is also evidence indicating that suppressor T cells in
some experimental systems bear anti-idiotypic determinants, and as such, these
suppressor T cells are idiotype specific and can be identified and enriched with
idiotype-coupled erythrocytes (10, 11) or idiotype-coupled plates (12). Anti-idiotype
induced Ts can suppress p-azobenzene arsonate (ABA)-specific responses in strains
expressing the cross-reactive idiotype (CRI). One interpretation of these sets of data
is that suppressor T cells bearing the ABA CRI-induced with antigen represent an
antigen-driven first order regulatory pathway; whereas, anti-idiotype-bearing sup-
pressor T cells represent a receptor- or idiotype-driven second order suppressor
pathway, a notion discussed elsewhere (13).

* Supported in part by grant PO1-CA-14723 from the Department of Health, Education and Welfare
and grants AI-12907 and AI-12908 from the National Institutes of Health.
† Recipient of a postdoctoral fellowship from the Arthritis Foundation.

Abbreviations used in this paper: ABA, p-azobenzene arsonate; ABA-SC, p-azobenzene arsonate-coupled
syngeneic spleen cells; CRI, cross-reactive idiotype common to anti-ABA antibodies of A/J mice; DNFB,
2,4-dinitro-fluorobenzene; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salt solution;
HIS, hyperimmune suppressed; NMG, normal A/J immunoglobulin; Ts, suppressor T cells.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/79/11/1229/12 $1.00
Volume 150 November 1979 1229-1240
In this paper, we will provide evidence indicating that suppressor T cells that are able to suppress ABA DTH can be induced by the ABA CRI when they are coupled to normal spleen cells. Intravenous injection of idiotype-coupled cells 7 d earlier rendered animals unresponsive to subsequent immunization to ABA-coupled cells. In addition, this suppression can be passively transferred to naive recipients. Thus, idiotype, when coupled to normal syngeneic spleen cells, can preferentially induce suppressor T cells. The significance of this finding in terms of antigen- and idiotype-driven regulatory pathways will be discussed.

Materials and Methods

Mice. Female BALB/c (H-2d, Igh-1a) and A/J (H-2a, Igh-1e) mice were obtained from The Jackson Laboratories, Bar Harbor, Maine. Mice were between 8 and 10 wk of age when used in these experiments. C.AL-20 (H-2b, Igh-1e) mice were from breeding colonies maintained at Brandeis University, from stock originally produced by Dr. Michael Potter at the National Institutes of Health.

Preparation of ABA-coupled Cells and the Induction and Elicitation of ABA-specific DTH. All the procedures on the preparation of ABA-coupled spleen cells and the protocols for immunization and elicitation are exactly those described in the accompanying paper (14).

Induction and Elicitation of Contact Sensitivity to DNFB. Contact sensitivity was induced by two daily paintings on the clipped abdomen with 25 μl of 0.5% 2,4-dinitro-1-fluorobenzene (DNFB) solution (Sigma Chemical Co., St. Louis, Mo.). 4 d after the last painting, 20 μl of 0.2% DNFB was applied to the dorsal surface of each ear, and increased ear swelling was measured 24 h later with an engineer's micrometer.

Preparation of Anti-ABA Antibodies in Normal and Hyperimmune-suppressed (HIS) Animals. Preparation and characterization of anti-ABA antibodies in normal and HIS animals has been described earlier (15, 16).

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. (17). Briefly, a single cell suspension of normal spleen cells was prepared in Hanks' balanced salt solution (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-positive anti-ABA antibodies specifically purified from A/J mice as described (15). The cells in the antigen solution were transferred to small glass scintillation vial (15 × 60 mm) and 25 mg of crystalline ECDI [1-ethyl-3(3'-dimethylaminopropyl)carbodiimide] (Pierce Chemical Co., Rockford, Ill.) was dissolved into the coupling solution. The reaction was allowed to proceed for 90 min at 4°C, while gently stirred. After coupling, the cells were washed twice in HBSS and were adjusted to a vol of 10^8/ml and 0.5 ml was injected intravenously into appropriate recipients.

Transfer of Ts Induced with Idiotype-coupled Cells. 7 d after tolerization with either normal mouse immunoglobulin-coupled cells of CRI-coupled cells. The animals were sacrificed, their spleens were removed, and single cell suspensions were prepared as described (14). These cells were washed twice in HBSS and adjusted to a vol of 10^9/ml and 0.5 ml were injected intravenously into naive recipients.

Anti-Thy 1.2 Serum Plus Complement Treatment. Treatment of suppressor cells with anti-Thy 1.2 or normal mouse serum is identical to the protocol described in the accompanying paper (14).

Results

Inhibition of Delayed-type Hypersensitivity (DTH) to ABA by Idiotype-coupled Cells. We directly coupled CRI-positive anti-ABA antibodies to normal A/J spleen cells and investigated whether they behave similarly to antigen-coupled cells as tolerogens for
ABA-specific DTH. In these experiments, A/J mice were given $5 \times 10^7$ idiotype (CRI)-coupled spleen cells or the same number of spleen cells coupled with normal A/J immunoglobulin (NMG) intravenously. 7 d later, the recipients were immunized subcutaneously with ABA-coupled spleen cells and ABA-specific DTH was assessed 5 d later. The results are shown in Fig. 1. Mice injected with NMG-coupled cells developed comparable immunity to control animals. However, mice injected with CRI-coupled cells did not manifest significant immunity. Thus, idiotype-coupled cells are excellent tolerogens for ABA-specific T-cell immunity.

**Anti-ABA Antibodies from HIS Animals When Coupled to Spleen Cells Failed to Inhibit ABA-specific DTH.** A/J mice that are treated with rabbit anti-idiotypic antibodies before immunization with the ABA hapten produce anti-ABA antibodies in high titer that lack the CRI (22). Idiotypes present on the anti-ABA antibodies of such mice are generally difficult to identify in other immunized A/J mice, either suppressed or nonsuppressed (15).

To examine whether the ability of idiotype-coupled cells to function as tolerogen is mainly a result of the CRI-positive components of the antibodies, or the CRI-negative components, we coupled anti-ABA antibodies obtained from HIS animals and tested the ability of these molecules once covalently linked to cell surfaces to inhibit ABA-specific DTH.

The results of such an experiment are depicted in Fig. 2. Anti-ABA antibodies possessing CRI components when coupled to spleen cells were potent tolerogens. However, the same total amount of anti-ABA antibodies from HIS animals coupled to syngeneic cells failed to induce discernible inhibition. From these observations, we concluded that the ability of anti-ABA antibody coupled cells to function as tolerogen relies solely on the presence of CRI antibodies.

**Suppression Induced with Idiotype-coupled Cells Is Linked to Heavy Chain Igh-1 Allotype.** The ability of an animal to manifest an anti-ABA antibody response which bears CRI components is linked to heavy chain Igh-1 allotype (18). We therefore
investigated whether the ability of CRI-coupled cells to induce tolerance to ABA is also linked to the same genetic locus. CRI or NMG from A/J mice were coupled to either A/J (H-2^a, Igh-1^a) or allotype congenic C.AL-20 (H-2^d, Igh-1^b) or BALB/c (H-2^d, Igh-1^b) spleen cells which bear a different Igh-1 allotype. 50 million of such CRI-coupled cells were then injected intravenously into their appropriate syngeneic recipients to assess their ability to induce ABA-specific tolerance as described above.

The results of a representative experiment are shown in Fig. 3. A/J or C.AL-20 mice injected with CRI-coupled cells 7 d earlier, showed significant reduction in ABA-specific DTH (panels A and C), whereas BALB/c mice similarly treated were unaffected (panel B). These experiments suggested that the ability of CRI-coupled cells to induce tolerance is also linked to the Igh-1 allotype locus.

To rule out the possibility that this allotype-restricted phenomenon was a result of the carrier (in this case the spleen cells) rather than the CRI^+ antibodies, we asked whether CRI-coupled BALB/c cells, which were incapable of inducing tolerance in BALB/c, could induce suppression in A/J mice. The results also shown in Fig. 3 (panel D) and clearly demonstrated that CRI-coupled BALB/c spleen cells induced a significant degree of suppression in A/J mice. Thus, the cell surface carrier does not seem to play any significant role in the induction of ABA-specific tolerance.

**Idiotype-coupled Cells Administered Intravenously Induce Suppression Which is Transferable by Cells.** In light of our previous observations that intravenous administration of ABA-coupled cells was a potent means of inducing antigen-specific suppressor T cells (19), experiments were initiated to examine whether animals injected with CRI-coupled cells can serve as donors of suppressor cells.

Results presented in Fig. 4 clearly demonstrated that tolerance to ABA DTH can be transferred to naive recipients using either thymocytes or spleen cells from animals injected with CRI-coupled cells 7 d previously. On the other hand, cells from animals which received NMG-coupled spleen cells were unable to manifest detectable suppression.

**Suppressor Cells Induced with CRI-coupled Cells Are Sensitive to Lysis with Anti-Thy 1.2**
Fig. 3. Inhibition of ABA-specific DTH by idiotype-coupled cells is linked to heavy chain Ig-1 allotype. Normal A/J, BALB/c, C.AL-20 mice were injected (i.v.) with $5 \times 10^7$ idiotype-coupled syngeneic spleen cells or normal immunoglobulin-coupled syngeneic cells (intravenously). In addition, a group of A/J mice were injected with idiotype-coupled BALB/c spleen cells. 7 d later, all these animals were immunized with $3 \times 10^7$ ABA-coupled syngeneic spleen cells (ABA-SC) subcutaneously. Challenge in the footpad was done 5 d after immunization, and increases in footpad swelling measured 24 h later. Bars represent the mean footpad swelling for groups of five mice ± SEM.

**Serum Plus Complement.** The demonstration that tolerance to ABA-specific DTH could be induced by intravenous injection of CRI-coupled spleen cells and that this suppression can be transferred to naive recipients, raised the issue of the type of cells responsible for the transfer of suppression. To examine this question, suppressor cells generated by the administration of CRI-coupled cells were treated with anti-Thy 1.2 serum or normal mouse serum and complement in vitro, before transfer to naive recipients.

The results are presented in Fig. 5. Both spleen cells and thymocytes from animals received CRI-spleen cells (SC) 7 d earlier, when treated with NMS and complement were capable of transferring suppression. However, the ability to transfer suppression was completely abrogated when either the SC or thymocytes were treated with anti-Thy 1.2 serum plus complement. Thus, suppression induced with CRI-coupled cells is a T-cell-dependent phenomenon.
Fig. 4. Induction of suppressor cells with idiotype coupled spleen cells. A/J mice were injected with idiotype-coupled spleen cells or normal mouse globulin coupled spleen cells (intravenously). 7 d after tolerization, \(5 \times 10^7\) spleen cells or thymocytes from these mice were transferred to naive recipient mice which were concomitantly immunized subcutaneously with \(3 \times 10^7\) ABA-coupled spleen cells. 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 five mice ± SEM.

Fig. 5. Suppressor cells induced with idiotype-coupled cells are sensitive to anti-Thy 1.2 and complement. Thymocytes and spleen cells from animals which received idiotype-coupled cells (intravenously) were treated with either anti-Thy 1.2 antiserum or normal mouse serum and complement before transfer to naive recipients. All recipients and appropriate controls were immunized with \(3 \times 10^7\) ABA-SC subcutaneously within 2 h after cell transfer. 5 d later, they were challenged in the footpad with ABA and increases in footpad swelling measured 24 h after challenge. Bars represent the mean footpad swelling of groups of five mice ± SEM. The number in the parentheses indicates the percentage of tolerance transferred. It was calculated according to the following formula:

\[
\text{Percent tolerance} = \left( \frac{\text{positive control} - \text{experimental}}{\text{positive control} - \text{negative control}} \right) \times 100.
\]
Antigen Specificity of Suppressor Cells Induced with Idiotype-coupled Cells.

To investigate whether suppressor T cells induced with CRI-coupled cells exhibit functional antigen specificity, we examined the ability of the suppressor T cells to inhibit the development of DTH to an unrelated hapten DNFB. A/J mice were injected with \(5 \times 10^7\) CRI-coupled cells intravenously. 7 d later, they were the donors of suppressor cells, and \(5 \times 10^7\) spleen cells were injected into two different recipient groups which were then sensitized with either ABA-coupled cells or DNFB as described in Materials and Methods.

The results shown in Fig. 6 clearly demonstrated that suppressor T cells were able to inhibit ABA-specific DTH. However, the same number of suppressor T cells were unable to inhibit DTH to an unrelated hapten DNFB.

Discussion

We have been interested in the possibility of using either antigen or the receptors for antigen to modulate an immunological response (13). We have chosen DTH to ABA as a model system (13, 19–21) because ABA is a simple hapten and in addition, a great deal is known regarding the humoral aspect of the immune responses to ABA in A/J mice (22).
In previous studies, we have found that suppressor T cells specific for ABA can be readily induced with passively administered antigen-coupled cells (19). Furthermore, these suppressor T cells (M.-S. Sy, B. A. Bach, A. Nisonoff, B. Benacerraf, and M. I. Greene. Manuscript in preparation.) and suppressor factors derived from suppressed A/J mice bear idiotypic determinants detected with anti-idiotypic antibodies prepared in rabbit against the ABA CRI (13, 20, 21). Because both suppressor T cells and suppressor factors were induced as a consequence of injection of tolerogenic forms of antigen, this represents an antigen-initiated regulatory event. In the accompanying paper, we have provided additional evidence that in this DTH system, suppressor T cells can also be induced with anti-idiotypic antibodies (14). Thus, anti-idiotypic antibodies can functionally duplicate the action of antigens.

Jerne proposed the network theory of immune response (1) which can be extended to suggest that antigen might initiate regulatory pathways, in which receptors (idiotypes) influence and modulate the outcome of an immune response, either in a positive (help) or negative (suppression) fashion. To further investigate the secondary aspect of the immune network which represents the receptor- or idiotype-stimulated events, we utilized a probe in which idiotypes were coupled to normal syngeneic spleen cells in an attempt to suppress ABA-specific T-cell dependent cell-mediated immunity (CRI). The rationale was to use idiotype, in this case CRI\textsuperscript{+} anti-ABA antibodies, presented to the recipient on cell surface to mimic physiologically the rapid appearance of antigen binding cells in vivo.

In this paper, we have provided evidence that idiotype-coupled cells, when injected intravenously into normal A/J mice 7 d before immunization, are potent tolerogens, and stimulate regulatory T cells capable of limiting T-cell reactivities. We have confirmed, and extended the earlier works of Battisto and Bloom (23), and also of Claman and his colleagues (17, 24), in which antigen-coupled cells administered by the intravenous route were found to be efficient tolerogens. In addition, we have observed that idiotypes, when coupled to normal spleen cells, can induce immunological unresponsiveness; whereas normal A/J immunoglobulin, when coupled to normal spleen cells, were unable to cause similar suppression. Thus, the determinants which are required for the induction of this unresponsiveness are probably determinants associated with the cross-reactive idiotypic antigen-binding sites. Recently, Dohi and Nisonoff (25) have also coupled idiotypes to thymocytes to induce idiotype-specific suppression and suppressor T cells of the production of CRI\textsuperscript{+} antibody. As compared to passively administered free idiotypes, idiotype-coupled cells administered in vivo are much more efficient in suppressing the production of anti-ABA antibodies which bear the CRI. In contrast, we were unable to demonstrate any significant suppression using varied doses of anti-ABA antibodies in an attempt to suppress ABA-specific DTH (data not shown).

Because only 20–70% of the anti-ABA antibodies from A/J mice bear CRI determinants (22), one of the more critical questions was to determine whether CRI-positive components are solely necessary for the induction of ABA tolerance. The observation that anti-ABA antibodies obtained from HIS animals which lack CRI\textsuperscript{+} antibodies (15), when coupled to normal spleen cells, were unable to cause any significant inhibition provides direct experimental evidence that CRI\textsuperscript{+} antibodies are required for the induction of this form of T-cell unresponsiveness. The failure of private idiotype-coupled cells to induce tolerance suggests that the ability of idiotype-
coupled cells to induce tolerance is not simply a result of a neutralizing effect of the antibody-coupled cells for antigen. This possibility can be excluded based upon the above mentioned data. In addition, the latter explanation could not account for the suppression of the humoral idiotypic response (25), because there was little or no effect on the overall anti-ABA response, although the CRI was completely suppressed.

In addition, the observation that anti-ABA antibodies from HIS animals, when coupled to normal spleen cells, failed to inhibit ABA DTH also implies that not all idiosyncratic antibodies can initiate this form of immunosuppression. This, however, may simply reflect a quantitative effect. For example, HIS antibodies, although lacking the CRI components, nevertheless still bear private idiotypes. It is known that there are a large number of subsets of private idiotypes (15). Therefore it is possible that each individual private idiotype may not be present in sufficient concentration to induce detectable suppressor cells. Alternatively, the private idiotype may have induced regulatory cells, which would comprise only a small population of Ts. These private idiotype-specific Ts would be predicted capable of interacting only with those immune cells which use the corresponding private idiotype. Hence, it is not surprising that HIS antibodies do not stimulate discernible suppression.

Further evidence supporting the importance of CRI components in tolerance induction came from the studies showing that the ability of CRI-coupled cells to induce unresponsiveness is also linked to heavy chain Igh-1 allotype. Only mice which bear similar Igh-1 allotype genes to that of A/J (H-2^d, Igh-1^a), in this case, C.AL-20 (H-2^d, Igh-1^a), were sensitive to suppression with idiotype-coupled cells. BALB/c mice (H-2^d, Igh-1^a) which bear a different Igh-1 allotype were not suppressed with idiotype-coupled cells. These observations are reminiscent of the results obtained with anti-idiotype antibodies (14) where it was found that the ability of anti-idiotype antibodies to inhibit ABA-specific DTH was also linked to Igh-1 heavy chain allotype. The restriction in the ability to respond to CRI-coupled cells may actually reflect the possibility that the receptor repertoire of cells operative in the network of the immune response is itself genetically determined (11, 13). Only those mice which utilize Igh-1-linked genes to make CRI receptors at either the T- or B-cell level can recognize these elements once expressed, and the receptor for these idiotypic structures (i.e., anti-idiotype) may be similarly genetically linked. Therefore, BALB/c (H-2^d, Igh-1^a) mice lacking the VH genes to make CRI were unable to recognize CRI elements in a way which would generate a discernible anti-idiotypic reaction.

There is an alternative explanation. CRI-mouse strains, e.g., BALB/c, indeed will respond to CRI-coupled syngeneic cells, because the CRI element is probably antigenic. The Ts so generated will be able to suppress cells utilizing this CRI element as a component of their receptor. However, the CRI strain will never use CRI elements for their receptors on any immune cell because it is absent from the genome. Hence, despite the possible presence of putative anti-idiotypic regulatory cells in these animals, suppression will never be observed. Experiments to resolve this issue are in progress. Furthermore, because A/J idiotype CRI antibodies coupled to BALB/c cells were still capable of suppressing ABA-specific DTH in A/J^d mice, it appears that the cell surface phenotype does not play any significant role in the induction of this allotype-restricted phenomenon. A similar lack of genetic restriction imposed by the carrier portion (i.e., cell phenotype) have also been found in companion studies of Dohi and Nisonoff (25). It was reported that idiotype-coupled allogeneic
thymocytes can also induce significant suppression of the CRI\(^+\) antibody response.

The ability of either spleen cells or thymocytes from animals injected with CRI-coupled cells to adoptively transfer suppression, and the T-cell dependence of this phenomenon provide evidence that intravenous injection of CRI-coupled cells can readily induce suppressor T cells (13). Furthermore, because these suppressor T cells only suppress the development of ABA-specific DTH but not contact sensitivity to DNFB, they display functional antigen specificity. The exact mechanisms by which idioype-coupled cells induce such suppressor T cells in the absence of antigen still remains to be resolved. However, it is possible that the activation of these suppressor T cells reflects a direct interaction between the idioype on such cells and suppressor T-cell precursors bearing anti-idioype receptors. In this regard, idioype-specific suppressor T cells which inhibit the production of CRI-positive anti-ABA antibodies have been demonstrated by rosette formation (9). Experiments are now in progress to determine if these suppressor T cells, induced with CRI-coupled cells, indeed bear detectable anti-idioype determinants on their surface, using either negative selection procedures such as lysis with idioype and complement or suicide with heavily radioactively labeled idioype, or a positive selection, enrichment on idioype-coated plates.

We would like to develop a model previously suggested elsewhere (13) dealing with receptor-stimulated regulatory pathways in DTH to ABA. This model is based on our observation that antigen-induced suppressor T cells and their factors bear idioype determinants (20, 21). In terms of current understanding of T-cell regulatory circuits as suggested by Benacerraf and Germain (26). Antigen-induced Ts are a first order subset of inhibitory cells denoted as Ts\(_i\). These Ts\(_i\) probably act on the generation of CMI by the stimulation of Ts\(_i\) cells (i.e., afferent inhibition). The activation of idioype bearing Ts belonging to the Ts\(_i\) subset either with antigen-coupled cells or anti-idioype antiserum and the release of idioype-bearing Ts,F probably complexed with antigen then stimulates the development of a second order (Ts\(_s\)) regulatory cell, whose function is to further limit the immune response. Thus, Ts\(_i\) and Ts\(_s\) may be mutually stimulatory. However, Ts\(_s\) could, as suggested herein, bear either antigen receptors or anti-idioype receptors capable of binding directly to idioype. It is known, for example, that Ts\(_i\) or Ts,F can lead to the generation of secondary suppressor T cells in other systems (27, 28). To test this hypothesis we have recently verified that ABA cell-induced Ts,F stimulates the development of Ts\(_s\) in normal A/J mice (7, 13, and M. I. Greene, B. A. Bach, M.-S. Sy, and B. Benacerraf, unpublished material), although it has not been determined whether these Ts\(_s\) are antigen or idioype specific. Ts\(_s\) with anti-idioype structures could act on immunocytes which utilize CRI\(^+\) elements for their receptor. Suppressor cells with idioype (8, 29) and anti-idioype (11, 12) receptors have been identified by many other investigators.

The system we have utilized herein utilizing large amounts of CRI\(^+\) antibodies present on the lymphocyte surface as inducers of a suppressor T cell is somewhat unphysiological. Its use, however, has permitted the demonstration of a similar Ts population active in CMI previously reported to be present in CRI regulation at the humoral level (10, 15, 25). Under normal circumstances the appearance of anti-idioype Ts would be expected to occur with an entirely different kinetics. We are presently investigating the role of anti-idioype regulatory cells which arises as a consequence of the immune response.
Summary

Anti-p-azobenzenearsonate (ABA) antibodies, coupled covalently to normal syngeneic spleen cells and then given intravenously to normal animals, were found to be potent tolerogens for delayed-type hypersensitivity (DTH) to ABA. The ability of the antibody-coupled cells to induce tolerance was determined to be a result of the cross-reactive idiotype (CRI⁺) fraction of the antibodies, because anti-ABA antibodies lacking the CRI⁺ components when coupled to spleen cells were unable to cause any significant inhibition. Furthermore, genetic analysis revealed that the ability of CRI⁺-coupled cells to inhibit ABA-specific DTH is linked to Igh-1 heavy chain allotype, inasmuch as much animals which possess heavy chain allotypes similar to that of A/J were sensitive to this inhibition. Adoptive transfer experiments provided evidence that CRI⁺-coupled cells induce suppressor cells, and spleen cells or thymocytes from animals received CRI⁺-coupled cells were able to transfer suppression to naive recipients. In addition, treatment with anti-Thy1.2 serum plus complement completely abrogated their ability to transfer suppression. Thus, this active suppression is a T-cell-dependent phenomenon. In investigating the specificity of these suppressor T cells, it was found that they functioned in an antigen-specific manner and were unable to suppress the development of DTH to an unrelated hapten 2,4-dinitro-1-fluorobenzene.

Received for publication 24 July 1979.

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