# ANTIGEN- AND RECEPTOR-DRIVEN REGULATORY MECHANISMS

## III. Induction of Delayed-Type Hypersensitivity to Azobenzenearsonate with Anti-Cross-Reactive Idiotypic Antibodies*

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It is now well established that B lymphocytes use immunoglobulins as their surface receptors. In contrast, the nature of antigen recognition structures on T cells still remains unresolved. Recent results obtained from many different experimental systems have provided supportive evidence suggesting that T and B cells have certain similar structures in their receptor components (1–3). Most of this evidence is derived from studies using anti-idiotypic antibodies to probe the structure of T-cell receptors. It was found that exogenous administration of anti-idiotypic antibodies could suppress or induce T- and/or B-cell function (4–9). In addition, in vitro treatment of two subpopulations of T cells, namely suppressor T cells and cytolytic T cells with anti-idiotypic antiserum and complement results in a complete loss of their suppressive activity and cytolytic ability, respectively (10–12). Thus, it has been postulated that T and B lymphocytes may use the same genetic information for the construction of part of their receptors.

Many lines of evidence have provided information that within the T-cell family, discernible heterogenicity exists with respect not only to cell surface markers but also to effector functions (13). In the murine system, at least five different subpopulations of T cells have been identified by functional and cell surface characterization. These are suppressor T cells ($Lyt2^+3^+$, $I\text{-}a^+$), cytotoxic T cells ($Lyt2^+3^+$, $I\text{-}a^-$), T cells which mediate delayed-type hypersensitivity (DTH)[1] ($Lyt1^+$), feedback inducer T cells ($Lyt1^+2^+3^+$), and helper T cells ($Lyt1^+$). In addition it has also been demonstrated that different T-cell subpopulations recognize antigenic determinants in association with different regions of their own major histocompatibility complexes (MHC).

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[1] *Abbreviations used in this paper:* ABA, *p*-azobenzenearsonate; ABA-SC, ABA-coupled syngeneic spleen cells; CFA, complete Freund's adjuvant; CRI, cross-reactive idiotypic antibodies; Cy, cyclophosphamide; DNFB, 2,4-dinitro-1-fluorobenzene; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salt solution; IBC, idiotype binding capacity; LN, lymph node; MHC, major histocompatibility complexes; $T_c$, cytotoxic T cells; $T_{DH}$, T cells that mediate DTH; $T_s$, suppressor T cells; $T_s$-aux, auxiliary T cells.


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Cytotoxic T cells ($T_c$) interact optimally with antigen and K and/or D regions of the MHC (14, 15) on relevant target cells. T cells which mediate delayed-type hypersensitivity ($T_{DH}$) (16) and helper T cells (17) recognize antigen in association with I region-encoded determinants. Furthermore, Lonai and co-workers (18) had reported that antigen binding to Lyt-$1^+$ cells was inhibited by anti-$V_H$ antiserum, whereas antigen binding to Lyt$2^+3^+$ cells were inhibited by anti-$V_L$ specifically.

Based on these observations, we have undertaken to investigate the expression of receptors in these various T-cell subpopulations using a simple and well-defined antigen. We have studied DTH reactivity to *p*-azobenzenearsonate (ABA) in mice and its regulation. Extensive information is available concerning the humoral immune response to ABA (19). In our earlier studies, we have demonstrated that anti-idiotypic antibodies to the major cross-reactive idiotype (CRI) associated with anti-ABA antibodies in A/J mice, when injected intravenously into normal A/J mice will stimulate ABA-specific suppressor T-cell responses (20). Moreover, these suppressor T cells bear CRI idiotypic determinants on their surface.[2]

In this report, we will present evidence that the anti-CRI antibodies when passively administered to A/J mice under appropriate conditions will result in induction of T-cell-mediated immunity rather than suppression. Thus, anti-CRI antibodies also can activate immune $T_{DH}$ cells. The importance of this finding will be discussed in terms of the role of idiotype and anti-idiotype interaction in the regulation of T-cell responses.

## Materials and Methods

*Mice.* A/J (H-$2^a$, Igh-$1^a$) and BALB/c (H-$2^d$, Igh-$1^a$) female mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. C.AL20 (H-$2^d$, Igh-$1^d$) female mice were obtained from breeding colonies established at Brandeis University. The C.AL20 strain was originally produced by Michael Potter at the National Institutes of Health, Bethesda, Md. All animals used in these experiments were 8–10 wk of age at the time of the experiment and each experimental group consisted of at least four mice.

*Preparation of Anti-Idiotypic Antibodies.* Anti-idiotypic antibodies against the CRI characteristic of the anti-ABA antibodies of A/J mice were prepared and quantitated as described earlier (21). Preparation of the $F(ab)_2$ fragments of the anti-CRI antibodies has also been described elsewhere (22).

*Preparation of Antigen and Antigen-coupled Cells.* The diazonium salt of *p*-arsanilic acid (Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y.) was prepared as described earlier (23). A 40-mM solution was activated as previously described and conjugated to single cell suspensions of erythrocyte-free splenocytes at a final concentration of 10 mM. After washing in Hanks' balanced salt solution, the ABA-coupled cells were used to induce DTH or suppressor T cells as previously described (23).

*Induction of DTH to ABA with Anti-CRI Antibodies.* To induce DTH to ABA, various amount of anti-CRI antibodies in a 0.2-ml volume were injected subcutaneously into two separate sites on the dorsal flanks of mice. Challenge was performed 5 d later by injecting into the left footpad 25 μl of 10 mM diazonium salt of *p*-arsanilic acid. 24 h after footpad challenge, DTH reactivity was assessed by measuring the swelling of the footpad using a Fowler micrometer (Schlesinger's Tool, Brooklyn, N. Y.). The magnitude of the DTH reaction was expressed as the increment of thickness of the challenged left footpad as compared with the untreated right footpad. Response are given in units of $10^{-4}$ inches ± SEM.

Elicitation with 2,4-dinitro-1-fluorobenzene (DNFB) (Sigma Chemical Co., St. Louis, Mo.) was done by applying 20 μl of a 0.2% of DNFB in acetone-olive oil (4:1) solution on the dorsal

[2] Sy, M. S., and M. I. Greene. Manuscript in preparation.

surface of each ear and increased ear swelling was measured 24 h later with a Mitutoya engineer's micrometer (Schlesinger's Tool) and is expressed in units of $10^{-4}$ inches ± SEM.

Statistical analysis of the data obtained used the two-tailed Student's $t$ test.

*Transfer of Immunity.* Animals which had been immunized with ABA-coupled syngeneic spleen cells (ABA-SC) or anti-idiotypic antibodies 4 d earlier were sacrificed. Draining lymph nodes from such animals were removed and a single cell suspension was prepared.

The cells were washed twice in HBSS and counted, and $30 \times 10^6$ viable cells in 0.5 ml were injected intravenously into normal recipients. Control mice received either no cells or cells from normal donors. The recipients and control mice were then challenged in the footpad as described earlier within 1 h after cell transfer.

*Anti-θ and Anti-CRI Serum Treatment.* Anti-Thy 1.2 hybridoma antiserum were obtained from Dr. P. Lake, University College, London. Briefly, $1 \times 10^8$ cells were incubated with 1 ml of 1:20 dilution of anti-Thy 1.2 hybridoma antiserum or 25 μg of idiotype binding capacity (IBC) of the anti-CRI antibodies in 1 ml 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 for cell transfer.

*Induction of Suppressor T Cells, with ABA-SC.* ABA-SC were prepared as described earlier (23). $5 \times 10^7$ ABA-SC were injected intravenously in a 0.5-ml volume into naive A/J recipients. 7 d later spleens from such animals were removed and a single cell suspension was prepared in HBSS. The cells were washed twice in HBSS, counted and $5 \times 10^7$ viable cells were then injected intravenously into normal recipients. The recipients and control animals were then immunized with anti-CRI antibodies subcutaneously within 1 h after cell transfer as described earlier.

*Cyclophosphamide Treatment.* Mice were injected intraperitonally with 50 mg/kg of cyclophosphamide (Mead Johnson and Co., Evansville, Ind.) diluted in distilled water.

## Results

*Induction of DTH to ABA in Cyclophosphamide-pretreated Animals with Anti-CRI Antibodies.* In earlier studies, we had demonstrated that intravenous injection of anti-CRI antibodies preferentially activates suppressor T cells (20). Furthermore, pretreatment of animals with low doses of cyclophosphamide (Cy) eliminated the ability of the anti-CRI antibodies to induce suppression (20). However, it is now well established that the outcome of many immunological responses depends upon the doses of antigen employed, the manner it is administered, and the status of the host. We investigated whether by lowering the doses of anti-CRI antibodies, changing the route of administration or by pretreating the animals with low doses of Cy we could induce immunity with anti-CRI antibodies.

Animals were either pretreated with 50 mg/kg Cy or saline, and then 2 d later they were injected with various doses of anti-CRI antibodies intravenously. 5 d after immunization, the animals were challenged in the footpad with diazonium salt of *p*-arsanilic acid, and DNFB on the ears as described in Materials and Methods. Increase in footpad and ear swelling was measured 24 h after challenge.

The results of such an experiment were shown in Fig. 1. Animals not pretreated with Cy failed to manifest any significant immunity over a wide range of anti-CRI antibodies used for priming. However, in animals pretreated with Cy 2 d earlier, as little as 0.1 μg of IBC induced significant DTH reaction. In addition, because both groups of animals failed to respond to challenge with DNFB it can be concluded that the immunity induced with anti-CRI antibodies is antigen-specific.

*The Ability of the $F(ab')_2$ Fragments of the Anti-CRI Antibodies to Induce Immunity in Normal A/J Mice.* Even though anti-idiotypic antibodies have been shown to induce

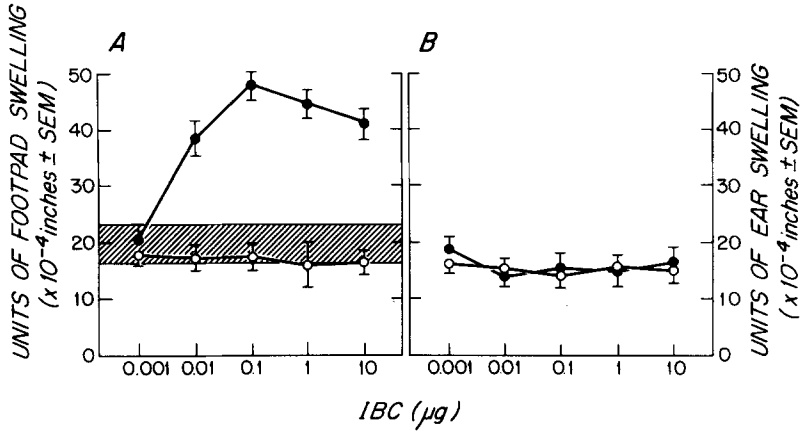


FIG. 1. Induction of DTH to ABA in Cy-pretreated animals with anti-CRI antibodies. Normal A/J mice were either pretreated with Cy (50 mg/kg) (●) or saline (○) 2 d before intravenous injection of various doses of anti-CRI antibodies. 5 d after injection, the mice were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad (A) and with 20 μl of 0.2% DNFB on the ear (B). The increase in footpad and ear swelling was measured 24 h after challenge. Shaded area represents the footpad swelling of animals challenged alone.

suppressor T cells in many different experiments (6, 8, 20), the $F(ab')_2$ fragments of such antibodies were unable to induce suppression (6, 8, 20). We had observed that anti-CRI antibodies injected intravenously led to the rapid induction of suppressor T cells whereas, in the absence of suppressor T-cell activation (for example Cy-pretreated mice), anti-CRI antibodies as shown above will then induce immunity. Experiments were then designed to investigate whether $F(ab')_2$ fragments of the anti-CRI antibodies stimulated immunity in normal A/J mice. Animals were either pretreated with Cy or saline and 2 d later, were injected with various doses of the intact anti-CRI antibodies or their $F(ab')_2$ fragments intravenously. Challenge was performed 5 d later as described above.

The results of a representative experiment are depicted in Fig. 2. As can be seen, the intact molecules of anti-CRI antibodies only induced immunity in Cy-pretreated animals. However, the $F(ab')_2$ fragments of the anti-CRI antibodies induced significant ABA-specific DTH in both normal and Cy-pretreated animals.

*Induction of ABA-specific DTH by Subcutaneous Administration of Anti-CRI Antibodies.* The route of antigen presentation plays a decisive role on the development of an immune response. Although subcutaneous immunization favors immunity, intravenous injection of certain antigens induces specific nonresponsiveness to subsequent immunization (24). We next investigated the ability of anti-CRI antibodies to induce immunity when administered subcutaneously.

Normal A/J mice were immunized with various doses of anti-CRI antibodies either subcutaneously or intravenously. Challenge was administered 5 d later and the increase in footpad swelling measured 24 h after challenge as described above.

The result of such an experiment is shown in Fig. 3. Whereas intravenous injection of anti-CRI antibodies failed to induce any significant immunity in the absence of pretreatment with Cy, in contrast, subcutaneous injection of anti-CRI antibodies stimulated significant levels of DTH reactivity to ABA in normal A/J animals. Thus,

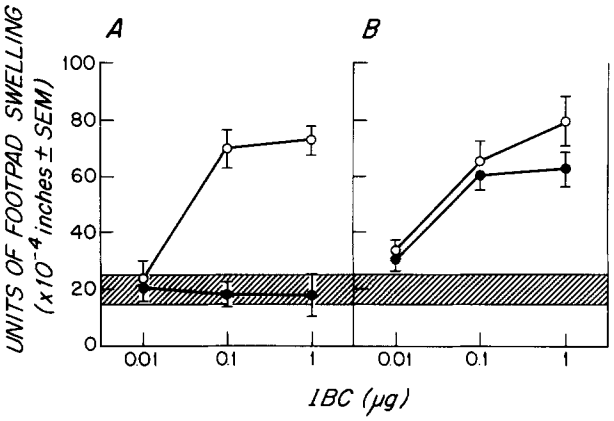


FIG. 2. The ability of the F(ab')$_2$ fragments of the anti-CRI antibodies to induce immunity in A/J mice. A/J mice were either pretreated with Cy (50 mg/kg) (○) or saline (●) 2 d before intravenous injection of various doses of anti-CRI antibodies (A) or the F(ab')$_2$ fragments of the anti-CRI antibodies (B). 5 d after injection, the mice were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad. The increase in footpad swelling was measured 24 h after challenge. The shaded area represents the footpad swelling of animals challenged alone.

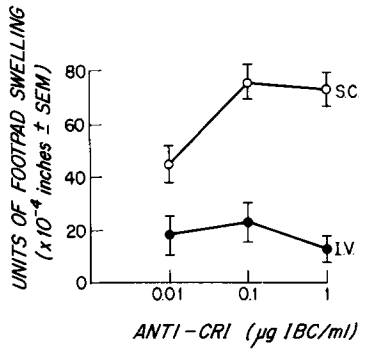


FIG. 3. Induction of ABA-specific DTH by subcutaneous administration of anti-CRI antibodies. Normal A/J mice were injected with various doses of anti-CRI antibodies either intravenously or subcutaneously. 5 d later, the mice were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad. The increase in footpad swelling was measured 24 h after challenge.

if the anti-CRI antibodies are administered subcutaneously it is not necessary to pretreat the animals with Cy to induce ABA-specific DTH.

*Transfer of Immunity from Animals Immunized with Anti-CRI Antibodies to Naive Recipients is a T-Cell-dependent Phenomenon.* To gain additional evidence that the immunity induced with anti-CRI is a form of delayed-type hypersensitivity we investigated the ability of lymph node cells taken from animals sensitized with anti-CRI antibodies to adoptively transfer immunity to naive recipients.

The results depicted in Fig. 4 revealed that lymph node cells taken from animals sensitized with anti-CRI antibodies 4 d earlier indeed can transfer immunity to naive recipients. In addition, pretreatment of immune lymph node (LN) cells with anti-$\theta$ serum plus complement completely abrogated their ability to transfer immunity. Thus, successful transfer of DTH immunity induced by anti-CRI is a T-cell-dependent phenomenon.

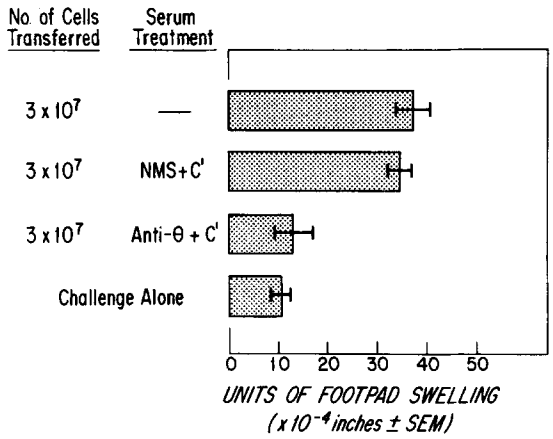


FIG. 4. Transfer of immunity from animals immunized with anti-CRI antibodies to naive recipients, is a T-cell-dependent phenomenon. A/J mice were immunized with 1 μg IBC of anti-CRI antibodies subcutaneously. 4 d later, draining lymph nodes were removed and single cell suspensions were prepared. Before transfer to normal recipients, some of the cells were treated with either normal mouse serum or anti-θ antiserum and complement. All recipients were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad within 1 h after cell transfer. The increase in footpad swelling was measured 24 h after challenge.

*Expression of Idiotype on Immune T Cells Induced with Anti-CRI Antibodies.* To further characterize the immune T cells ($T_{DH}$) responsible for the passive transfer of immunity, we examined the expression of idiotype on their surface.

Immune $T_{DH}$ were induced in normal A/J mice by subcutaneous administration of anti-CRI antibodies or ABA-SC. The results are shown in Fig. 5; as can be seen, anti-CRI serum and complement treatment of LN cells taken from animals immunized with anti-CRI completely abrogated their ability to transfer immunity. However, the same antiserum treatment did not interfere with the ability of LN cells taken from animals immunized with ABA-SC to transfer immunity. From these we concluded that a substantial fraction of immune $T_{DH}$ cells induced with anti-CRI bear idiotypic structures on their surface, whereas in contrast most of the immune $T_{DH}$ induced with ABA-SC seem to lack detectable idiotypic determinants.

*Suppressor T Cells Induced with ABA-SC Suppress the Development of DTH Induced with Anti-CRI Antibodies.* To delineate whether immunity induced with anti-CRI is also subjected to negative regulatory mechanisms, we investigated if suppressor T cells induced with ABA-SC inhibit the development of DTH induced with anti-CRI antibodies.

Suppressor T cells were induced by intravenous injection of ABA-SC as described previously (23). 7 d later they were transferred to normal A/J mice. The recipients were then immunized by the subcutaneous administration of anti-CRI antibodies within 1 h after cell transfer. The results of such an experiment are depicted in Fig. 6. Animals that received normal spleen cells, when immunized with subcutaneous administration of anti-CRI still exhibited significant immunity. However, in animals that received suppressor T cells, anti-CRI antibodies failed to induce significant immunity. Thus immunity induced with anti-CRI antibodies is also subject to negative regulatory signals mediated by idiotype-positive suppressor T cells.

*The Ability of Anti-CRI Antibodies to Induce Immunity is Linked to Igh-1 Heavy-Chain*

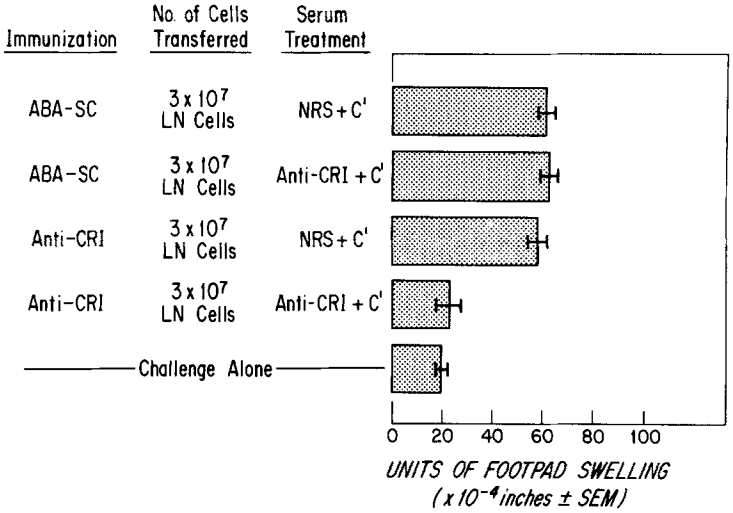


FIG. 5. Expression of idiotype on immune T cells induced with anti-CRI antibodies. Normal A/J mice were immunized with $3 \times 10^7$ ABA-SC or 1 μg IBC anti-CRI antibodies subcutaneously. 4 d later, draining lymph nodes were removed and single cell suspensions were prepared. Before transfer to naive recipients, the cells were treated with either normal rabbit serum (NRS) or anti-CRI antiserum and complement. All recipients were challenged with 25 μl of diazonium salt of *p*-arsanilic acid within 1 h after cell transfer. The increase in footpad swelling was measured 24 h after challenge.

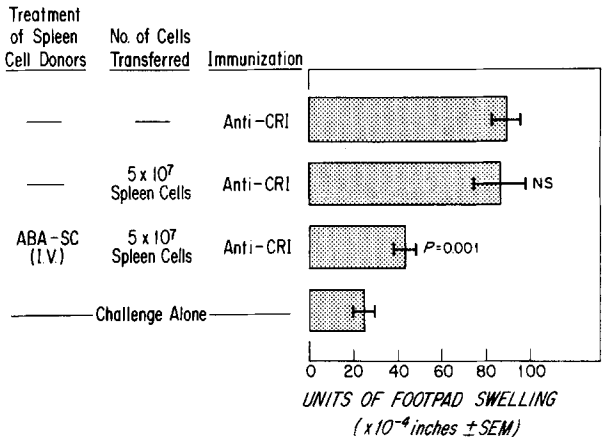


FIG. 6. Suppressor T cells induced with ABA-SC suppress the development of DTH induced with anti-CRI antibodies. A/J mice were injected intravenously with $5 \times 10^7$ ABA-SC. 7 d later, they were the donors of suppressor T cells. $5 \times 10^7$ spleen cells from such animals and $5 \times 10^7$ spleen cells from normal A/J mice were injected intravenously into two different groups of naive recipients. All recipients and control animals were immunized subcutaneously with 1 μg IBC of anti-CRI antibodies within 2 h after cell transfer. 5 d later, the mice were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad and increase in footpad swelling was measured 24 h after challenge.

*Allotype.* The ability of animals to manifest an anti-ABA antibody response which bears CRI determinants is linked to the heavy-chain Igh-1 allotype (25). Furthermore, the ability of anti-CRI antibodies to activate suppressor T cells is also linked to the heavy-chain Igh-1 allotype (20). Experiments were designed to examine whether the

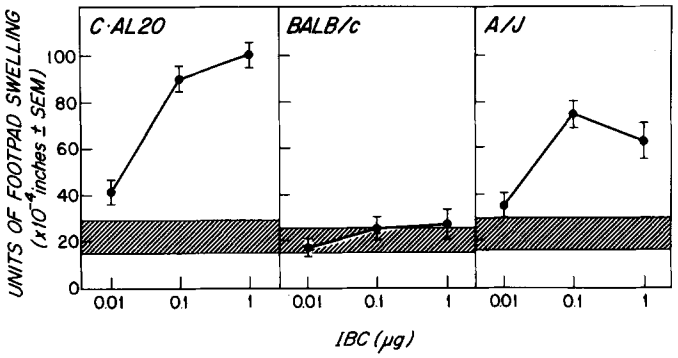


FIG. 7. The ability of anti-CRI antibodies to induce immunity is linked to Igh-1 heavy-chain allotype. Normal A/J (H-$2^a$, Igh-$1^e$), BALB/c (H-$2^d$, Igh-$1^a$), and C.AL20 (H-$2^d$, Igh-$1^d$) mice were immunized with various doses of anti-CRI antibodies subcutaneously. 5 d after immunization, the mice were challenged with 25 μl of diazonium salt of *p*-arsanilic acid in the footpad. The increase in footpad swelling was measured 24 h after challenge. The shaded area represents the footpad swelling of animals challenged alone.

capacity of anti-CRI antibody to induce immunity is also linked to the same genetic locus.

The results are shown in Fig. 7. Anti-CRI antibodies induce significant immunity in both A/J and C.AL20 mice. However, the same amount of anti-CRI antibodies administered to BALB/c mice was unable to induce any detectable immunity. Thus, similar to the observation that the activation of suppressor T cells is genetically restricted (20), the inheritance of the ability to respond to anti-CRI antibodies with the development of ABA-specific DTH is also linked to the heavy-chain Igh-1 allotype locus.

## Discussion

The results presented in this and a previous communication (20) address the issue of activation of distinct subsets of hapten-specific T cells using anti-idiotypic antibodies. In summary, it is possible to induce different effector T-cell functions namely suppressor T cells ($T_s$) and $T_{DH}$ with anti-idiotypic antibodies under appropriate experimental conditions. The observation that intravenous injection of anti-idiotypic antibodies induces immunity only in Cy-pretreated animals is in accordance with our earlier findings that anti-idiotypic antibodies were unable to manifest their suppressive effect in Cy-pretreated animals (20). These results were interpreted to reflect that under normal conditions the intravenous injection of anti-idiotypic antibodies at the appropriate dose always preferentially activated suppressor T cells. However, in the relative absence of an essential Cy-sensitive cell in the suppressor T-cell circuit, pre-$T_{DH}$ effector cells will be activated. This observation in some respects is very similar to the recent findings of Ptak et al. (26). These investigators observed in the trinitrophenol contact sensitivity system, that intravenous injection of hapten-coupled cells will preferentially activate suppressor T cells as shown previously (23, 27). However, if the animals had been pretreated with Cy, the intravenous injection of hapten-coupled cells was found to induce immunity rather than suppression.

Furthermore, from the dose-response studies described herein, it became obvious that the optimal dose of anti-idiotypic antibodies required for the induction of DTH is 10-fold lower than the one necessary for the induction of suppressor T cells (20).

The ability of exogenous anti-idiotypic antibodies to induce effector T and/or B cells has been investigated in many experimental systems (4–9). The elegant works of Eichmann (6, 7) demonstrated that depending on the isotype of anti-idiotypic xeno-antibodies employed, one can either activate suppressor T cells or helper T cells. Passive administration of the $IgG_1$ fraction of the guinea pig anti-idiotypic antibodies was found to induce helper T cells, whereas injection of the $IgG_2$ fraction induced suppressor T cells in mice. However, in our studies intravenous injection of anti-idiotypic antibodies was always found to activate suppressor T cells unless suppressor T-cell precursors had been eliminated first. It should be noted that the anti-idiotypic antibodies employed in our studies are raised in rabbit, whereas the anti-idiotypic antibodies used in Eichmann's study were of guinea pig origin. The ability of anti-idiotypic antibodies to induce DTH has also been suggested earlier. Julius and his co-workers (28) found that the injection of low doses of anti-T15 antibodies to $T15^+$ mice is able to induce phosphorylcholine-specific DTH.

One of the most interesting observations documented herein is derived from the studies using $F(ab')_2$ fragments of the anti-idiotypic antibodies to induce DTH. It was found that the intravenous injection of the $F(ab')_2$ fragments of anti-idiotype induced immunity in normal or Cy-pretreated animals. As previously reported, this reflects the inability of the $F(ab')_2$ fragment to induce suppressor T cells (6, 8, 20). Once again in the absence of suppressor T cells, $T_{DH}$ will be activated by the appropriate signal. The mechanism responsible for activation of suppressor T cells possibly requires a signal mediated by intact $F_c$-bearing molecules, whereas induction of $T_{DH}$ can be achieved with the $F(ab')_2$ fragment. Moreover, recent results from our laboratory have provided indirect evidence that activation of suppressor T cells probably requires the participation of yet another undefined population of lymphocytes, whereas activation of $T_{DH}$ does not.[2] These disparate observations may then reflect a fundamental difference in the activation of suppressor T cells and T cells which mediate DTH. This differential induction of distinct effector T-cell functions has also been reported in the A5A system. Eichmann and his colleagues (29) had found that the intact molecules of $IgG_2$ anti-A5A antibodies will induce suppressor T cells, whereas the $F(ab')_2$ fragments of the same antibodies induce helper T cells.

In addition to pretreatment with Cy or using the $F(ab')_2$ fragments of anti-idiotypic antibodies, we also found that subcutaneous injection of anti-idiotypic antibodies induces immunity in normal mice. It is now well established that the outcome of an immunological response depends critically on the route of immunization and the status of the host (30). The mechanisms that favor the induction of suppressor T cells when antigen is given intravenously is most likely related to the anatomical segregation of antigen in the spleen after antigen administration. Because the spleen has been the major source of suppressor T cells (31, 32), it is logical to postulate that intravenous injection of antigen will preferentially activate suppressor T cells. On the other hand, subcutaneous immunization tends to favor the accumulation of antigen in the draining lymph nodes, which thus predispose to the generation of DTH immunity (24).

The observation that immunity induced with anti-idiotypic antibodies can be transferred to naive recipients and successful transfer of immunity is a T-cell-dependent phenomenon provides direct evidence that immunity induced with anti-idiotypic antibodies is a form of DTH. In addition, it was found the $T_{DH}$ cells induced with

anti-idiotypic antibodies can be lysed with anti-idiotypic antiserum and complement treatment. In contast, $T_{DH}$ cells induced with ABA-SC are resistant to similar treatment within the sensitivity of our methods. Thus immune $T_{DH}$ cells induced with ABA-SC appear to lack idiotypic structures, whereas $T_{DH}$ induced with anti-idiotypic antibodies do bear idiotype on their surface.

Therefore, it is possible to induce ABA-specific $T_{DH}$ cells either expressing or lacking idiotypes depending on the form of antigen administered. The observation that certain types of immune $T_{DH}$ induced by hapten-coupled cells lack idiotype is in accord with the recent findings of Sherman et al. (33). Using an identical immunization protocol, it was found that killer T cells ($T_c$) induced with subcutaneous injection of ABA-SC also failed to exhibit idiotypic markers. However, other $T_{DH}$ cells functionally expressing idiotype were observed in a recent study from our laboratories (34). In another system, it was found that immune $T_{DH}$ induced in Cy-pretreated animals with hapten protein conjugates (NP-BGG) in complete Freund's adjuvant (CFA) do exhibit specificities controlled by $V_H$ genes. Similarly, preliminary studies using ABA-KLH in CFA to induce ABA-specific DTH with identical protocols have revealed that $T_{DH}$ induced in A/J mice in such a manner may also bear idiotypic markers on their surface.[2] Other than the form of antigen used in immunization, we would also like to emphasize that the expression of idiotype-positive T cells also depends critically on the particular T-cell subpopulation being examined. For example, it was found that intravenous injection of ABA-SC will activate suppressor T cells, which express idiotype determinants on their surface.[2] The suppressive function can be completely abrogated by in vitro treatment with anti-idiotypic antibodies and complement.[2] Furthermore, Binz et al. (12) had also provided evidence that two subgroups of T lymphocytes involved in the induction of cell-mediated lympholysis may also express entirely distinct spectra of idiotypes.

The observations that idiotype positive $T_{DH}$ can be suppressed by suppressor T cells induced with ABA-SC which also bear idiotype raised a fundamental question regarding the role of idiotype-governed interactions in the regulation of immune response. In terms of T- and B-cell cooperation, it has been shown that idiotype-positive B cells can be activated by idiotype-positive T cells by virtue of anti-idiotype antibody or antigen bridging (28, 35). Furthermore, there is also evidence indicating that T-T interactions in certain circumstances are also governed by Ig-linked genes that control expression of $V_H$-like structures on T cells (36). However, we must account for the fact that idiotype$^+$ suppressor T cells induced with ABA-SC suppress ABA-specific DTH reactivity irrespective of whether the effector $T_{DH}$ is idiotype-positive or negative; it appears that the suppression is antigen-specific rather than idiotype-specific. Nevertheless, recent experiments suggest that the ability of these $T_s$ to inhibit ABA DTH is allotype restricted (M.-S. Sy and M. I. Greene. Unpublished results.).

Moreover, we have documented in the ABA system that anti-idiotype-specific suppressor T cells induced by the administration of CRI-coupled spleen cells can suppress ABA-specific DTH elicited by immunization with ABA-SC (37), although we have observed that the effector $T_{DH}$ cells resulting from this response are idiotype-negative. These apparent contradictions could be explained by (*a*) the presence of idiotype in the precursors of the DTH effector cells, (*b*) the requirement for idiotype-specific helper T cells which could be the target of the suppression, (*c*) the ability of

small amounts of idiotype-positive anti-ABA antibody concomitantly produced to bind with the $T_{DH}$ cells after their interaction with antigen.

In addition, it is also possible that the suppressor circuit requires the participation of another population of T cells. The requirement for the presence of another T-cell subpopulation other than suppressor T cells and $T_{DH}$ cells in order for suppression to occur has been demonstrated in a very similar DTH system. In contact sensitivity to DNFB, it was found that in order for DNP-specific suppressor T cells to manifest their suppressive activities, the participation of another population of T cells termed auxiliary T cells ($T_s$-aux) is required. These $T_s$-aux could be distinguished from $T_{DH}$ cells by their sensitivity to adult thymectomy and the presence of I-J region markers on the cell surface (38). It is possible that the interaction between $T_s$ and $T_s$-aux may be idiotype-specific whereas the expressed suppression is antigen specific, a notion similar in many respects to that discussed by Eardley et al. (36). Experiments are now in progress to determine whether the expression of ABA-specific suppressor T cells also require $T_s$-aux, and furthermore if the interaction between $T_s$ and $T_s$-aux is idiotype-specific.

In terms of idiotype anti-idiotype interactions, we would also like to stress that anti-idiotypic antibodies appear to induce two distinct types of $T_s$ in vivo (39). In previous studies, Nisonoff and colleagues have found that A/J mice hyperimmunized with antigen and suppressed with anti-idiotypic antibodies develop $T_s$ with putative anti-idiotypic structures (19). That is, these $T_s$ bind idiotype-coupled erythrocytes. In similar studies we found that anti-idiotype induced a suppressor T cell with idiotypic structures on its surface.[2] To address this issue in more detail we have recently established that suppressor factors which are idiotype positive and obtained from idiotype bearing $T_s$, when injected into normal mice, induce a second order suppressor (termed $T_{s2}$) (40), which cannot be lysed by anti-idiotypic antibodies.[2] Hence, mutually stimulating sets of T cells consisting of those bearing idiotype or anti-idiotype structures are generated in the murine host responsive to ABA. With this in mind it is indeed possible that other T-cell participants generated as a consequence of anti-idiotype stimulation occur but have not as yet been evaluated.

Furthermore, $T_{DH}$ can only be induced with anti-idiotypic antibodies in animals which possess the relevant heavy chain allotype (Igh-1). Antibodies to the major cross-reactive idiotype associated with anti-ABA antibodies in A/J mice were unable to induce ABA-specific DTH in BALB/c mice (H-$2^d$, Igh-$1^a$). Such antibodies were, however, fully capable of inducing ABA DTH in the allotype congeneic C.AL20 mice which has an allotype (Igh-$1^d$) similar to that of A/J (Igh-$1^e$) on a BALB/c background and which produced humoral antibodies with CRI. Another issue which must be addressed is the well-studied specificity for I region-coded antigens in addition to their specificity for the immunizing antigen displayed by antigen-induced $T_{DH}$ cells, which reflects the specificity of antigen presentation. It is important to determine whether anti-idiotype-induced $T_{DH}$ also display similar type of MHC restriction, both at the stage of sensitization and elicitation of the reaction. This raises the issue whether the anti-idiotype antibody stimulates $T_{DH}$ directly or after presentation by Ia-bearing macrophage. This is an area for further exploration.

In conclusion, these results stress the possibility of activating distinct subsets of T cells by using anti-receptor antibodies capable of binding to serologically definable antigen-recognitive units on their cell surfaces.

## Summary

Delayed-type hypersensitivity (DTH) to *p*-azobenzenearsonate (ABA) can be induced in A/J mice with intravenous injection of minute amounts of anti-cross-reactive idiotypic (CRI) antibodies, providing that the animals have been pretreated 2 d earlier with low doses of cyclophosphamide (50 mg/kg). However intravenous injection of the $F(ab')_2$ fragments of the anti-CRI antibodies or subcutaneous administration with anti-CRI antibodies induces comparable immunity in both cyclophosphamide-pretreated and normal nontreated animals. Furthermore adoptive transfer experiments indicate that lymph node cells taken from animals sensitized with anti-CRI 4 d earlier can adoptively transfer immunity to naive recipients. Transfer of immunity is mediated by a population of thymus-dependent (T) cells, which express idiotypic structures on their surface. Treatment of effector cells with either anti-$\theta$-serum or anti-idiotypic antibodies plus complement completely abrogated their ability to transfer immunity. In addition idiotype-bearing suppressor T cells induced with ABA-coupled spleen cells inhibit the development of ABA-specific DTH induced with anti-CRI antibodies.

Genetic analysis revealed that the ability of anti-CRI antibodies to induce ABA-specific DTH was linked to Igh-1 heavy-chain allotype. Anti-idiotypic antibodies to the major CRI associated with anti-ABA antibodies in A/J mice failed to induce significant immunity in BALB/c mice ($H\text{-}2^d$, $Igh\text{-}1^a$). Nevertheless, they were able to induce significant immunity in C.AL20 mice ($H\text{-}2^d$, $Igh\text{-}1^d$) which possess a heavy-chain allotype similar to that of A/J mice.

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