ANTIGEN- AND RECEPTOR-DRIVEN
REGULATORY MECHANISMS

I. Induction of Suppressor T Cells
   with Anti-idiotypic Antibodies*

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There is considerable evidence that thymus-derived (T) and bone marrow-derived
(B) lymphocytes share similar V_H-region products on the cell surface (1, 2). Nevertheless, the molecular nature of the T-cell receptor still remains essentially unresolved.

Studies by Binz and Wigzell (3, 4) in the rat demonstrated that anti-idiotypic
antiserum raised against T-lymphocyte receptors or against alloantibodies which were
specific for the same alloantigens can inhibit a variety of T-cell functions. It has also
been reported by Eichmann and his colleagues, who investigated the A5A (anti-
streptococcal) immune response in the mouse, that anti-idiotype antibodies raised in
guinea pig, when passively administered to A/J mice, could preferentially activate
suppressor T cells or helper T cells, depending on the isotype of the antibodies used
(5, 6). Another series of experiments exploring the similarity of receptors on T and B
lymphocytes dealt with the response to the hapten, 4-hydroxy-3-nitrophenyl acetyl
(NP). It was found that the primary anti-hapten antibody response to hapten protein
conjugate (NP-chicken globulin), is heteroclitic in mice of the Igh-1^b allotype; i.e.,
these antibodies bind an analogue of the hapten, 4-hydroxy-5-iodo-3-nitrophenyl
acetyl with greater affinity than the immunizing hapten itself (7). In addition,
antigen-binding material isolated from T cells also exhibited similar fine specificity
(8). More germane to the studies reported here are the recent findings (9) that
delayed-type hypersensitivity to the same hapten also demonstrates heterocliticity.
Furthermore, this T-cell-mediated specificity is also genetically linked to the heavy-
chain Igh-1^b allotype, as in the antibody response.

In an attempt to gain more insight into the cellular regulation of T-cell-mediated
immune response and the nature of the cell receptor, we have initiated a series of
studies on delayed-type hypersensitivity to azobenzene-arsonate (ABA) (10–12). This

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Abbreviations used in this paper: ABA, p-azobenzene-arsonate; CRI, cross-reactive idiotype common to anti-ABA antibodies of A/J mice; DTH, delayed-type hypersensitivity; HBSS, Hanks' balanced salt solution; IBC, Idiotype binding capacity; NP, 4-hydroxy-3-nitrophenylacetyl; NRS, normal rabbit serum; TNCB, 2,4,6-trinitrochlorobenzene.
is a system where extensive information on the humoral aspect of the immunity is available (13, 14). The antibody response of A/J mice to ABA conjugates is characterized by the presence of cross-reactive idiotypic (CRI) determinants on 20–70% of anti-ABA antibodies as demonstrated with anti-idiotypic antibodies raised in rabbits.

In this paper, we will present evidence that rabbit antibodies raised against anti-ABA CRI determinants, when passively administered to A/J mice at the time of immunization, suppress the development of DTH to ABA-coupled cells. Furthermore, we have performed adoptive transfer experiments which provide direct evidence that the suppression is a consequence of the activation of suppressor T cells by anti-CRI antibodies. The significance of these findings will be discussed in terms of genetic restrictions of suppressor cells operating in the immune network.

Materials and Methods

Mice. A/J (H-2a, Igh-1e) and BALB/c (H-2d, Igh-1d) female mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All C.AL-20 (H-2a, Igh-1d) female mice were obtained from breeding colonies established at Brandeis University. The C.AL-20 strain was originally produced by Michael Potter at the National Institutes of Health. All animals in these experiments were 8–10 wk of age at the time of the experiment and each experimental group consisted of at least four animals.

Preparation of Antigen and Antigen-coupled Cells. The diazonium salt of p-arsanilic acid (Eastman Kodak Co., Rochester, N.Y.) was prepared as described earlier (10). 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 delayed-type hypersensitivity (DTH) as previously described (10).

Induction and Elicitation of DTH to ABA-coupled Cells. To induce DTH to ABA, a total of 3 x 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 25 μl of 10 mM diazonium salt of p-arsanilic acid into the left footpad.

Measurement of Footpad Swelling. 24 h after the footpad challenge, DTH reactivity was assessed by measuring the swelling of the footpad using a Fowler micrometer (Schlesingers For Tools Ltd., 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. Responses are given in units of 10^-3 in ± SEM. Statistical analysis of the difference of data obtained utilized the two-tailed Student's t test.

Sensitization and Elicitation of Contact Sensitivity. Mice were sensitized with 7% TNCB (2,4,6-trinitrochlorobenzene, Matheson Scientific, Cincinnati, Ohio) by application of 100 μl of this solution on the shaved abdominal skin. Elicitation was done 5 d after sensitization by applying 20 μl of 1% TNCB in olive oil to the dorsal surface of the ears. Ear swelling was measured 24 h later with a Mitutoya engineer's micrometer (Mitutoya/MTI Corp., New York) and is expressed in units of 10^-4 in.

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

Preparation of Anti-idiotypic Antibodies. Anti-idiotypic antibodies against the cross-reactive idioype (CRI) characteristic of the anti-ABA antibodies of A/J mice were prepared and quantitated as described earlier (15). Preparation of the F(ab')2 fragments of the anti-CRI antibodies has also been described elsewhere (12).

Induction of Suppressor Cells with Anti-idiotypic Antibodies. Anti-idiotypic antibodies (2 μg idioype binding capacity (IBC)) were injected intravenously into appropriate animals in a volume of 0.2 ml for 5 successive days. On day 6, the mice were sacrificed and used as donors of suppressor cells.

Transfer of Suppressor Cells. Animals which had received anti-idioype antibodies on 5 successive days were sacrificed. Spleens from such animals were removed and a single cell suspension was prepared. The cells were washed twice in Hanks' balanced salt solution (HBSS)
and counted, and 50 × 10⁶ cells in 0.5 ml were injected i.v. into normal recipients. Control mice received either no cells or cells from normal donors. The recipient and control mice were immunized with ABA-coupled cells 1–2 h after transfer as described earlier.

**Affinity Chromatography of Anti-CRI Antiserum.** Specifically purified A/J anti-ABA antibodies were coupled to Sepharose 4B beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) as described earlier (12). Anti-CRI antibodies (10 μg IBC in 1 ml) were passed through the column and antibodies that remained adsorbed to the column were rapidly eluted with a glycine-HCl buffer, pH 2.8. The eluate was neutralized to pH 7.0 with 1 N NaOH. Both the filtrate and the eluate were concentrated to the original volume by negative pressure dialysis and then assessed for their ability to suppress ABA-specific DTH.

**Anti-θ and Anti-MIg Serum Treatment.** Anti-Thyl.2 hybridoma antiserum donated by Dr. P. Lake, University College, London, and polyvalent anti-mouse immunoglobulin (anti-MIg) serum were used as described earlier (10). Briefly, 1 × 10⁶ cells were incubated with 1 ml of a 1:25 dilution of anti-Thyl.2 hybridoma antiserum or a 1:20 dilution of anti-MIg serum for 45 min at 0°C, washed once in HBSS and then incubated again with 1.0 ml of a 1:10 dilution of Low Tox rabbit complement (Cedarlane Laboratories, London, Ontario) for 30 min. at 37°C. The cells were then washed twice in HBSS and resuspended for cell transfer.

**Results**

**Effect of Anti-CRI Antibodies on the Induction of DTH to ABA.** Experiments were initiated to examine the effect of passively administered anti-CRI antibodies on the induction of ABA-specific delayed-type hypersensitivity. A/J mice were immunized with ABA-coupled syngeneic spleen cells subcutaneously as described in Materials and Methods. Some of these animals received normal rabbit serum (NRS) or anti-CRI antibodies (2 μg IBC per mouse for 5 successive days) in equivalent volumes. On day 5, they were challenged with 25 μl of the diazonium salt of p-arsanilic acid in the footpad, and the increase in footpad swelling was measured 24 h later.

The results of such an experiment are shown in Table I. As can be seen, animals receiving normal rabbit serum were able to manifest significant levels of ABA-specific immunity as compared with untreated control animals. In contrast, A/J mice injected with anti-CRI antibodies previously absorbed with A/J splenocytes failed to develop significant immunity to ABA.

**Dose Response of Inhibition by Anti-CRI Antibodies.** The minimum amount of anti-CRI antibodies necessary to inhibit DTH to ABA-coupled cells was next determined. The results are depicted in Fig. 1. Animals which received 0.1 μg IBC of anti-CRI antibodies still showed significant immunity, as compared to control animals. How-

**Table I**

| Group | Immunization | Treatment* | Units of footpad swelling‡ × 10⁻³ | P value |
|-------|-------------|------------|----------------------------------|---------|
| I     | 3 × 10⁷ ABA-sc (s.c.) | — | 12 ± 1.0 | — |
| II    | 3 × 10⁷ ABA-sc (s.c.) | Anti-CRI antibody (2 μg IBC/mouse/day) | 4.5 ± 0.7 | <0.001 |
| III   | 3 × 10⁷ ABA-sc (s.c.) | NRS | 11 ± 1.2 | NS |
| IV    | — | — | 3 ± 0.6 | <0.001 |

* Anti-CRI antibodies were injected (immediately after immunization) i.v. at 2 μg IBC per day for five successive days (10 μg IBC total).
‡ Challenges were done 5 d after immunization by injecting 25 μl of diazonium salt of p-arsanilic acid in the footpad. Increases in footpad swelling were measured 24 h later.
ever, all the animals that received from 1 to 10 µg IBC of anti-CRI showed significant reduction in ABA-specific DTH. This clearly demonstrated that the ability of anti-CRI antibodies to inhibit DTH is a dose-dependent phenomenon.

Inhibition of DTH by Anti-CRI Antibodies Requires Whole Intact Molecules; Suppressive Activities Can Be Removed by Passage through Idiotype-coupled Sepharose Immunoadsorbents. We next investigated whether inhibition of DTH by passively administered rabbit anti-CRI antiserum requires intact antibody molecules, by examining whether the F(ab')2 fragments of the same antibodies also inhibit ABA-specific DTH. F(ab')2 fragments of the anti-CRI antibodies were therefore prepared precisely as described earlier (12). Animals were immunized with ABA-coupled cells and were then given either anti-CRI antibodies or the F(ab')2 fragments of anti-CRI intravenously. The volumes were adjusted so that the IBC of each antiserum was the same.

The results presented in Fig. 2 indicate that in mice given intact anti-CRI the DTH response was suppressed almost to the background level. Inoculation of an equal amount of F(ab')2 fragments (in terms of IBC) had no significant effect on DTH. Furthermore, anti-CRI antibodies, after passage through an immunoadsorbent prepared using antibodies possessing the CRI, were incapable of suppressing ABA DTH. Nevertheless, the suppressive capacity could be recovered from this immunoadsorbent by elution with acid. From these studies we can conclude that suppression by anti-CRI requires the whole intact molecule and is not a result of contamination with nonspecific antibodies in the rabbit anti-CRI antiserum.

Inhibition of DTH by Anti-CRI Antibody is Antigen Specific. The antigen specificity of the inhibition of DTH by anti-CRI antibody was next examined. Experiments were done to investigate whether anti-CRI will inhibit DTH to an unrelated hapten, TNCB, known to induce T-cell-dependent DTH responses. A/J mice were immunized with ABA-coupled cells subcutaneously on the dorsal flanks, whereas TNCB was applied on the shaved abdomen. Mice were treated either with anti-CRI or normal

![Fig. 1. Dose response of inhibition by anti-CRI antibodies. Normal A/J mice were immunized with 3 x 10^7 ABA-coupled syngeneic spleen cells (ABA-sc) subcutaneously. They were then injected with 2 µg IBC of anti-CRI antibodies in 0.2 ml (i.v.) for 5 successive days (10 µg IBC total). On day 5, they were then challenged in the footpad with diazonium salt of p-arsanilie acid and the increase in footpad swelling was measured 24 h later.](image-url)
rabbit serum for 5 successive days. Challenges were performed by injecting 25 μl of ABA diazonium salt in the right footpad to measure for ABA specific immunity and, in addition, 20 μl of TNCB was applied onto the dorsal side of the ear to assay for TNCB-specific immunity. The increases in footpad and ear swelling were measured 24 h after challenge as described in Materials and Methods.

Fig. 3 presents the results of a representative experiment. Animals that are sensitized with ABA-coupled cells and TNCB developed significant immunity to both ABA and TNCB. As predicted from our earlier results, animals which received anti-CRI failed to develop immunity to ABA. Nevertheless, they were still fully capable of responding to the unrelated hapten, TNCB. Normal rabbit serum had no discernible effect on ABA DTH or on the T-cell-dependent ear swelling in response to TNCB. Thus, suppression of ABA-DTH mediated by anti-CRI antibodies is hapten-specific.

**Activation of Suppressor Cells with Passively Administered aAnti-CRI Antibodies.** Experiments were undertaken to ascertain whether the inhibition induced with anti-CRI antibodies is a result of the activation of suppressor cells or simply a result of direct blockade or elimination of immune T cells that are specific for ABA by anti-CRI antibodies.

Animals were treated with either normal rabbit serum or anti-CRI antibodies for 5 successive days. On day 6, these animals were sacrificed and used as the donors of suppressor cells. Fig. 4 presents the results of a typical experiment. Spleen, lymph node, or thymus cells obtained from anti-CRI-treated animals were able to adoptively transfer suppression to naive recipients. In contrast, the same number of spleen cells...
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Immunization Treatment

ABA-A/J — —
ABA-A/J TNB NRS
ABA-A/J TNB Anti-CRI
Challenge Alone — —
TNB — —
Challenge Alone — —

Footpad Increment (10^3 inches ± SEM) Ear Increment (10^4 inches ± SEM)

Fig. 3. Antigen specificity of suppression induced with anti-CRI antibodies. A/J mice were immunized subcutaneously with 3 × 10^7 ABA-sc and/or contact sensitized with 100 μl of 7% TNCB. Immediately after immunization, groups of mice were injected (i.v.) with 2 μg IBC of anti-CRI antibodies or normal rabbit serum (NRS) for 5 successive days. On day 5, the mice were challenged with diazonium salt of p-arsanilic acid in the footpad and/or with 20 μl of 1% TNCB on the ear. The increase in footpad and ear swelling was measured 24 h after challenge.

obtained from NRS-treated animals were unable to transfer any detectable level of suppression.

From these studies we concluded that anti-CRI antibodies inhibit ABA-DTH by activating suppressor cells. The spleen was found to be the best source of suppressor cells whereas lymph node and thymus were also found to contain significant numbers of suppressor cells.

Failure of anti-CRI Antibody to Inhibit ABA-Specific DTH in Animals Pretreated with Cyclophosphamide. The ability of spleen cells from anti-CRI treated animals to transfer suppression suggested that suppressor cells were involved in this inhibition. Nevertheless this did not rule out the possibility that anti-CRI antibody might also work directly on immune T cells. To address this question, we employed cyclophosphamide pretreatment, which has been shown to be able to selectively eliminate suppressor cell precursors (16-19). A/J mice were pretreated with cyclophosphamide (50 mg/kg) 2 d before immunization with ABA-coupled cells. They were then given either anti-CRI or normal rabbit serum. The results presented in Fig. 5 showed that cyclophosphamide pretreatment prevented anti-CRI antibodies from inhibiting ABA-specific DTH reactivity as compared to animals which were not pretreated with cyclophosphamide. These findings therefore provide evidence that the inhibition of DTH by anti-CRI antibodies is mainly a result of the cyclophosphamide-sensitive induction of suppressor cells.

Suppressor Cells Induced by Anti-CRI Antibodies Are Sensitive to Treatment with Anti-Thy 1.2 Antiserum Plus Complement. The demonstration that anti-CRI treatment induces suppressor cells, before antigen exposure, raised the issue of the phenotype of the cells responsible for the transfer of suppression. To examine this question, suppressor cells were generated by passively administered anti-CRI antibodies, before transfer to naive recipients. They were subjected to negative selection with anti-Thy 1.2 antiserum or anti-MIg serum and complement as described in Materials and Methods. As shown in Fig. 6, the ability of cells to adoptively transfer suppression to ABA was
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| Treatment of Donors | No. of Cells Transferred | Immunization |
|---------------------|--------------------------|--------------|
|                     |                          |              |
| NRS                 | 5x10^7 Spleen Cells      | ABA-SC       |
| Anti-CRI            | 5x10^7 Lymph Node Cells  | ABA-SC       |
| Anti-CRI            | 5x10^7 Thymocytes        | ABA-SC       |
| Anti-CRI            | 5x10^7 Spleen Cells      | ABA-SC       |
| Challenge Alone     |                          |              |

**Fig. 4.** Induction of suppressor cells with anti-CRI antibodies. Normal A/J mice were injected (i.v.) with 2 μg of IBC of anti-CRI or NRS for 5 successive days. On day 6, they were sacrificed and single cell suspensions were prepared from their draining lymph nodes, spleen, and thymus. Fifty million viable cells were then transferred to naive recipients. All recipients were then immunized with 3 × 10^7 ABA-sc subcutaneously within 2 h after cell transfer. 5 d later, they were challenged in the footpad with diazonium salt of p-arsanilic acid and the increase in footpad swelling was measured 24 h after challenge.

| Pretreatment | Immunization | Treatment |
|--------------|--------------|-----------|
| (a) —        | ABA-SC       | NRS       |
| (b) Cy(50mg/kg) | ABA-SC    | NRS       |
| (c) —        | ABA-SC       | Anti-CRI  |
| (d) Cy(50mg/kg) | ABA-SC    | Anti-CRI  |
| (e) —        | Challenge Alone |         |

**Fig. 5.** Failure of anti-CRI antibodies to inhibit ABA-specific DTH in cyclophosphamide pre-treated animals. Normal A/J mice were treated with cyclophosphamide (50 mg/kg) or HBSS 2 d before immunization with 3 × 10^7 ABA-sc subcutaneously. Immediately after immunization, they were then injected with normal rabbit serum (NRS) or anti-CRI antibodies (2 μg IBC/day) for 5 successive days. 5 d later, they were then challenged with diazonium salt of p-arsanilic acid and the increase in footpad swelling was measured 24 h later.
Fig. 6. Sensitivity of suppressor cells to anti-Thy 1.2 anti-serum and complement. A/J mice injected with 2 μg IBC/d of anti-CRI antibodies for 5 successive days were the donors of suppressor cells. On day 6, single cell suspensions were prepared from the spleen. Before transfer to naive recipients, the cells were treated in vitro with either normal mouse serum (NMS), anti-Thy 1.2 antiserum, or anti-Mlg serum and complement. Recipients and appropriate controls were immunized with 3 x 10^7 ABA-SC subcutaneously within 2 h after cell transfer. Challenges were done 5 d later with diazonium salt of p-arsanilic acid and the increase in footpad swelling was measured 24 h later.

sensitive to treatment with anti-Thy 1.2 plus complement. In contrast, spleen cells treated with normal mouse serum or anti-Mlg serum plus complement still retained their ability to transfer suppression. These studies establish that anti-idiotype can be used to induce antigen-specific Thy 1.2-positive suppressor cells which are effective in suppressing DTH induced by ABA-coupled cells.

Genes Controlling the Inhibition of DTH by Anti-CRI Antibodies Are Linked to the Heavy-chain Igh-1 Allotype Locus. It is now well established that genes linked to the heavy-chain allotype locus are required for the expression of idiotypes both in T and B cells (20–22). Experiments were designed to examine whether anti-CRI antibodies raised against A/J (H-2a, Igh-1e) anti-ABA antibodies can suppress ABA immunity in allotype-matched C.AL-20 mice (H-2d, Igh-1d) and allotype-mismatched BALB/c mice (H-2d, Igh-la). It should be noted again that C.AL-20 and BALB/c mice are congeneric, differing only at the Igh-1 locus. C.AL-20 mice, but not BALB/c, produce humoral anti-ABA antibodies with the CRI (20).

The results are shown in Fig. 7. Anti-CRI antibodies significantly inhibited ABA DTH immunity in both A/J and C.AL-20 mice. However, the same amount of anti-CRI antibodies administered to BALB/c mice was unable to detectably inhibit ABA DTH. Thus, inheritance of the ability of anti-CRI antibodies to activate suppressor T cells is also linked to the heavy-chain Igh-1 allotype locus.

Discussion

Delayed hypersensitivity (DTH) to ABA in mice is a T-cell-dependent antigen-specific phenomenon. Recently, we have found that although subcutaneous injection of ABA-coupled cells induces immunity, intravenous injection of the same preparation stimulates the development of antigen-specific suppressor T cells. Suppressor T cells
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Fig. 7. Inhibition of ABA-specific DTH by anti-CRI antibodies is linked to heavy-chain Igh-allotype. Normal BALB/c (H-2b, Igh-1a), A/J (H-2a, Igh-1b) or C.AL-20 (H-2b, Igh-1b) mice were immunized with 3 x 10^7 ABA-coupled syngeneic spleen cells subcutaneously. Immediately after immunization, they were injected with NRS or anti-CRI antibodies (2 μg IBC/day) for 5 successive days. On day 5, they were challenged with diazonium salt of p-arsanilic acid in the footpad and the increase in footpad swelling was measured 24 h later.

upon adoptive transfer render the recipient animals unresponsive to subsequent subcutaneous immunization with ABA-coupled cells.

In this paper we have shown that passively administered anti-CRI antibodies cause the stimulation of ABA suppressor T cells comparable to those induced by antigen-coupled cells. Intravenous injection of minute amounts of anti-CRI antibodies induces immune unresponsiveness, apparently mediated by suppressor T cells, whereas F(ab')2 preparations of the same anti-CRI antibodies were unable to induce discernible suppression. It was therefore concluded that the induction of antigen-specific suppressor T cells by this regimen requires the intact antibody. The contribution of the Fc portion of the anti-idiotype molecule for the activation of regulatory T cell has been previously observed by Hart and his coworkers (23). At present no satisfactory explanation is readily available for this requirement of the Fc piece and experiments to resolve this tissue are in progress.

The ability of passively administered anti-idiotype antibody to induce a state of idiotypic suppression has been reported in many different experimental systems. Such experiments have been carried out in vivo (24) and in vitro (25). Eichmann and his co-workers (5, 6), studying the response of mice to group A Streptococcal polysaccharide demonstrated that passively administered anti-idiotype (anti-A5A) also suppresses the expression of idiotype on anti-A5A antibodies. Further studies from the same group have shown that although the IgG1 fraction of anti-A5A will induce helper T cells, intravenous injection of the IgG2 fraction preferentially induced suppressor T cells. This was considered at the time to reflect different complement activation properties of the isotypes used. Owen et al. found that large numbers of idiotype-specific suppressor T cells can be generated by hyperimmunizing a recipient mouse after administration of anti-idiotypic antibodies and allowing a rest period of 8-12
wk after immunization (26). With this regimen, nearly all the idiotype-specific suppressor T cells that are active in the humoral response could be removed by binding to erythrocytes coated with Fab fragments of antibodies carrying the major idiotype. Thus the suppressor cells had anti-idiotypic receptors. Suppressor cells with idiotypic (27) and anti-idiotypic (28) receptors have been identified by other investigators. Eichmann states that his attempts to lyse suppressor T cells with anti-idiotypic antibodies plus complement have occasionally but inconsistently been successful (23), an observation consistent with the presence of more than one type of idiotype-specific suppressor T cell.

The present studies of the ability of anti-CRI antibodies to completely abrogate the development of ABA-specific DTH also revealed a fundamental difference between the effect of anti-CRI on T-cell-mediated DTH and on antibody responses to ABA-protein conjugates. In the antibody studies it was found that injection of anti-CRI antibodies prevented the expression of idiotype; however there was no concomitant suppression of the formation of anti-ABA antibodies inasmuch as the total level of anti-ABA antibodies remained the same (29). The idiotypes on anti-ABA antibodies of such mice are generally absent or present at very low concentration in other immunized mice, either suppressed or nonsuppressed (30). In contrast, DTH to ABA was almost completely suppressed by anti-CRI antibody. One explanation for this discrepancy is that the T-cell repertoire may be somewhat more restricted than the B-cell counterpart and makes greater use of major cross-reactive idiotypes (8). Alternatively, the suppressor T cells which suppress ABA-specific DTH represent a different regulatory subset than those responsible for the suppression of antibody formation. It is possible that one is antigen specific and hence, idiotypic whereas the other may be idiotype specific and therefore anti-idiotypic. It is also conceivable that the two cell types may be mutually stimulatory. Preliminary results indicate that Ts induced by anti-CRI antibody bear idiotypic determinants on their cell surface because they can be lysed by anti-CRI and complement.

Because anti-CRI antibodies failed to inhibit DTH in animals pretreated with cyclophosphamide in doses known to eliminate suppressor T-cell precursors selectively (16-18) this was interpreted to indicate that the ability of anti-CRI antibodies to inhibit DTH is dependent upon the ability of the antiserum to activate Ts. Anti-CRI antibodies were thus unable to inhibit immune T cells directly. This postulate was further supported by the finding that anti-CRI antibodies given before challenge failed to inhibit DTH (data not shown).

Studies from many different experimental systems have already provided evidence indicating that the expression of particular idiotypes on T and B cells is linked to the presence of distinct heavy-chain allotypes genes (20-22, 24). Because this linkage is observed both at the T- and B-cell levels, it has been suggested that both B and T cells employ the same V_{H} genes to encode constituents of their receptors. These findings are in accord with our observation that anti-CRI antibodies of the A/J strain were able to inhibit the development of DTH in C.AL-20 mice, which have an allotype similar to that of A/J and which produce humoral antibodies with the CRI BALB/c mice. Thus, inheritance of the ability of anti-CRI antibodies to activate suppressor T cells is also linked to the heavy-chain Igh-1 allotype locus. Recent reports from this laboratory (12) have shown that suppressor factors induced by intravenous injection of ABA-coupled cells in A/J mice also bear CRI determinants, the expression
of which was also linked to the allotype-linkage group of genes. The Ts from which these TsF are derived as well as Ts induced by anti-idiotypic have now been shown to bear idiotypic determinants on their surface (M.-S. Sy, A. Nisonoff, B. Benacerraf, and M. I. Greene. Manuscript in preparation.). Therefore, suppressor T cells, induced by antigen or by anti-idiotypic in the absence of antigen, and suppressor factors bear idiotypic determinants similar to the determinants found in antibodies. These studies provide evidence that passively administered anti-CRI antibodies mimic the action of antigen-coupled cells. However, whether the suppressor T cells induced by these two apparently different methods belong to the same T-cell subpopulation or actually represent two different T-cell subpopulations is now under investigation. In conclusion, these studies reveal that antigen-independent stimulation of antigen-specific regulatory T cells by antibodies to idiotypic determinants on B-cell products can be a potent tool to analyze immunological responses, presumably in terms of receptor anti-receptor interactions.

Summary

Delayed-type hypersensitivity (DTH) to the azobenzene arsonate (ABA) hapten can be readily induced in A/J mice injecting ABA-coupled syngeneic spleen cells subcutaneously. To further characterize this T-cell-dependent immunological phenomenon, the effect of passively administered anti-cross-reactive idiotype common to anti-ABA antibodies of A/J mice (CRI) antibodies on the development of ABA-specific DTH was investigated. Animals given daily injections (of minute amounts) of anti-CRI antibodies subsequent to immunization with ABA-coupled cells show significant reduction of ABA specific responses. This inhibition is antigen specific and requires the intact immunoglobulin molecule, as F(ab')2 treatments were ineffective in suppressing the reaction.

Investigations of the mechanism of the anti-CRI-induced suppression of ABA DTH revealed that the observed suppression is a result of the activation of suppressor cells. Spleen cells taken from animals which received anti-CRI antibodies were able to adoptively transfer suppression to naive recipients. This suppression was shown to be mediated by T cells, as anti-Thyl.2 plus complement completely abrogated the transfer of suppression. In addition, animals pretreated with low doses of cyclophosphamide were not suppressed by the administration of anti-CRI antibodies.

The genetic restriction of anti-CRI-induced suppression was demonstrated. Antibodies to the major cross-reactive idiotype, (CRI) associated with anti-ABA antibodies in A/J mice were unable to suppress the development of DTH to ABA in BALB/c mice (H-2d, Igh-1c). Such antibodies were, however, fully active in suppressing ABA DTH in the allotype-congenic C.AL-20 strain which has an allotype (Igh-1b) similar to that of A/J (Igh-1b) on a BALB/c background, and which produces humoral antibodies with the CRI.

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