MECHANISMS OF REGULATION OF CELL-MEDIATED IMMUNITY

IV. Azobenzenearsonate-Specific Suppressor Factor(s) Bear Cross-Reactive Idiotypic Determinants the Expression of Which Is Linked to the Heavy-Chain Allotype Linkage Group of Genes*

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The existence of regulatory molecules derived from T cells, which play an important role in the control of immune reactions, has been documented in a number of experimental systems (1-4).

Such T-cell-derived regulatory molecules appear to share many biochemical properties which would argue for their structural and functional similarity (3, 4). These common properties support the notion that similar gene families may be responsible for encoding the structural components of such regulatory factors. In addition to antigenic specificity, many suppressor factors contain determinants which are controlled by the major histocompatibility complex. These H-2-region determinants are usually, (3-5) but not always (6), encoded by genes which map at the I-J subregion. In the companion paper (7) it has been established that p-azobenzenearsonate (ABA)1-specific suppressor T cells elaborate a product which shares many of the known structural features of specific suppressor factors (3-6). ABA-specific suppressor factor(s) are proteins, with a molecular weight between 33 and 68,000 daltons, which do not bear antigenic determinants of the constant regions of conventional immunoglobulin (7) but contain antigenic determinants coded for by the K end of the H-2 major histocompatibility complex (MHC) (7).

The presence of both antigen-binding specificity and major histocompatibility complex gene products in soluble T-cell-derived antigen-specific suppressor material permits analysis of gene products concerned with the structure of antigen-specific molecules derived from T cells. The ABA specificity was chosen because of the extensive data available relating to the nature of the cross-reactive idiotype (CRI) evoked in A/J mice immunized with ABA-keyhole limpet hemocyanin (KLH) (8).

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Abbreviations used in this paper: ABA, p-azobenzenearsonate; anti-Id, anti-idiotype; BBS, borate-buffered saline; CGAT, cross-reactive idiotype GAT; CRI, cross-reactive idiotype; DTH, delayed type hypersensitivity; GAT, L-glutamic-L-alanine-L-tyrosine; H, heavy; IBC, idiotype-binding capacity; KLH, keyhole limpet hemocyanin; MHC, major histocompatibility complex; NMG, normal mouse globulin; PBS, phosphate-buffered saline; SF, suppressor factor; STC, suppressor T cells.
Furthermore, because genes linked to the C_{R} locus are required for the expression of idiotype on antibodies to this antigen (9), genetic studies utilizing appropriate strains of mice should permit a similar analysis of ABA-specific T-cell suppressor products. The experiments presented in this paper indicate that genetic determinants associated with the clonally restricted B-cell product are also shared with suppressor molecules from T cells in this system. The presence of idiotypic determinants in ABA suppressor factor (SF) will be shown to be contingent upon the genes linked to the heavy-chain allotype locus and not upon the MHC haplotype of the strain in which the factor is made. Furthermore, data will be presented indicating the presence of both the ABA C_{R}I and H-2-controlled determinants on a molecular complex capable of suppressing delayed-type hypersensitivity against the ABA-hapten group.

Materials and Methods

**Mice.** A/J (H-2^{a}, Ig-1^{e}) and B10.A (H-2^{a}, Ig-1^{b}) female mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All C.AL-20 (H-2^{a}, Ig-1^{d}) and some B10.A (H-2^{a}, Ig-1^{b}) female mice were obtained from breeding colonies established at Brandeis University. The C.AL-20 strain was originally produced by Michael Potter. All animals in these experiments were 8–10 wk old at the time of the experiment. All experimental groups consisted of five animals per group.

**Antigen and Preparation of Antigen-Coupled Cells.** 10 mM ABA diazonium salt was prepared exactly as described in the accompanying paper (7). The preparation of ABA conjugates of cells from A/J, B10.A, BALB/c, and C.AL-20 mice was performed as described (10).

**Preparation of Suppressor Factor.** Suppressor factor was prepared according to the protocols presented in the accompanying paper. Most of the suppressor factors used in the studies presented here were prepared by the snap-freeze thaw technique (11).

**Preparation of Antiserums.**

**Affinity chromatography of rabbit anti-idiotype antiserum.** Affinity chromatography of rabbit anti-idiotypic (anti-id) antiserum was performed by passing the F(ab')_{2} fragments of anti-id antibodies (IgG fraction) over columns which had been previously coupled with either A/J globulins, precipitated with 18% sodium sulfate, or with antigen-purified A/J anti-ABA antibodies which contain a high concentration of C_{R}I' immunoglobulins. The absorbed material was eluted with 3 M KCl or glycine-HCl, pH 2.8, or a mixture of both.

**Affinity chromatography of ABA-suppressor factor.** Freshly prepared ABA suppressor factor(s) were fractionated on immunoadsorbents in the following manner. 5-cm^{3} plastic columns containing Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.), conjugated with immunoglobulin, were prepared and extensively washed with phosphate-buffered saline (PBS), pH 7.2, immediately before loading the ABA-suppressor factor. The absorption of the factor was performed at 4°C by allowing 5 × 10^{6} cell eq of suppressor factor to enter the gel matrix. The usual volume of solution containing suppressor factor was 1 ml and the Sepharose 4B gel volume was 3 cm^{3} in most columns. The suppressor factor was allowed to remain in the column for at least 60 min at 4°C. The column was then washed with five times its own volume of PBS, pH 7.2. Such effluents were termed filtrates. The columns were washed again with at least 10-bed vol of PBS over a 15-min period. Materials that remained adsorbed to the column were rapidly eluted with 3-bed vol of a glycine-HCl buffer, pH 2.8 (alternatively 3 M KCl or 3 M KCl-glycine HCl, pH 2.8, was used for elution). The collected column eluate was neutralized to pH 7.0 with 1 N NaOH immediately as the material emerged from the column bed. Both the filtrate and the eluate were concentrated by negative pressure dialysis in the cold and were frozen in small portions at −40°C. Such materials were thawed immediately before use.

In experiments using two specific adsorptions, concentrated filtrates and eluates were prepared as above and then refractionated on the appropriate adsorbents after being concentrated to the original volume by negative pressure dialysis in PBS, pH 7.2.
SOLID PHASE RADIOIMMUNOASSAY. F(\(\text{Ab'}\))\(_2\) fragments of rabbit anti-idiotypic antibody were coupled to CNBr-activated Sepharose 4B at a concentration of 2 mg/cm\(^2\) of Sepharose 4B. The coupling efficiency usually exceeded 95% as assessed by OD at 280 nm of the pre- and postcoupling washes. The anti-idiotypic antisera of the highest titer used in this study had an idiotype-binding capacity of 300 \(\mu\)g/10 mg of protein when assayed for its capacity to bind labeled idiotype; an anti-globulin reagent was used to precipitate complexes in a sensitive idiotypic inhibition assay (12). When the F(\(\text{ab'}\))\(_2\) fragments of these antibodies were coupled to Sepharose 4B and then incubated with \(^{125}\text{I}\)-labeled anti-ABA antibodies, specific values of between 30 and 60 ng of idiotype-binding capacity per milligram of gel were obtained. Uncoupled Sepharose 4B was used as a background control. Based on these data, we extrapolated back to the original idiotype-binding capacity of 300 \(\mu\)g. This implies that the average Sepharose 4B column, weighing 3,000 mg, has an idiotype binding capacity between 90 and 180 \(\mu\)g. This indicates that between 30 and 60% of the original binding capacity of the anti-idiotypic serum was available on the immunoadsorbents as seen in the following calculation.

Sample calculation:

(a) Idiotype binding capacity (IBC)/column = gel wt \times IBC/mg
\[
= 3,000 \text{ mg} \times 60 \text{ ng/mg} = 180,000 \text{ ng}
\]
\[
= 180 \mu\text{g}.
\]

(b) Percent uncoupled IBC = \[
\frac{\text{IBC of column}}{\text{IBC coupled to column}} \times 100 \times \frac{180 \mu\text{g}}{300 \mu\text{g}} \times 100 = 60%.
\]

Results

In the accompanying paper (7), the intravenous injection of ABA coupled cells to syngeneic recipients was shown to induce a population of antigen-specific suppressor T cells. This population of suppressor T cells has been shown to yield, upon disruption, suppressor molecule(s) which exhibit both biological specificity for ABA as well as binding specificity for immunoadsorbents which contain ABA. Such suppressor molecules, when administered in the amount of \(2 \times 10^7\) cell eq/mouse per day, lead to the suppression of ABA-specific delayed type hypersensitivity (DTH). These antigen-specific factors could not be mimicked in their biological action by immunoglobulin molecules with similar antigen-binding specificity, nor could such factors be absorbed by reverse immunoadsorbents directed against heavy- and light-chain constant determinants present in constant regions of heavy and light chains of conventional immunoglobulins. In an attempt to characterize the nature of the combining site of such suppressor molecules immunochemically, studies were undertaken to ascertain whether such molecules could be specifically adsorbed by antisera directed against antigenic determinants associated with the idiotype of ABA-binding immunoglobulins. Fig. 1 shows the results of two representative experiments which indicate that F(\(\text{ab'}\))\(_2\) fragments, with specificity for the variable region determinants associated with the CRI of anti-ABA antibodies of A/J mice, are capable of specifically absorbing ABA-suppressor factors. Freshly prepared ABA-suppressor molecules were fractionated on such adsorbents exactly as described in Materials and Methods.

Several points are apparent from the data in Fig. 1. In all cases the suppressor factor was capable of suppressing the development of DTH to a marked extent. As can be seen, a significant fraction of the suppressive activity was removed in the filtrate of such anti-idiotypic columns. However, in no experiment was all the suppressive activity of SF removed by filtration on anti-idiotypic immunosorbents. Eluates of anti-idiotypic columns to which SF had been absorbed were obtained by washing with pH 2.8 glycine HCl, or with 3 M KCl followed by glycine-HCl, pH 2.8.
EXPERIMENT #1

| Material Injected for 5 d | Immunization |
|--------------------------|--------------|
| HBSS Medium              |              |
| Thymus Factor (4678)     |              |
| [Anti-ID #11 (IBC: 33 ng/mg Cell)] |
| Flab2 Anti-Idiotype Filtrate | $P < 0.009$ |
| Flab2 Anti-Idiotype Acid Eluate | $P < 0.008$ |
| Challenge Alone          | $P < 0.002$ |

EXPERIMENT #2

| Material Injected for 5 d | Immunization |
|--------------------------|--------------|
| HBSS Medium              |              |
| Thymus Factor (42078)    |              |
| [Anti-ID #11 (IBC: 33 ng/mg Cell)] |
| Flab2 Anti-Idiotype Filtrate | $P < 0.005$ |
| Flab2 Anti-Idiotype Acid Eluate | $P = 1.00$ |
| Challenge Alone          | $P < 0.002$ |

FIG. 1. Depicted in Fig. 1 are the results of two representative experiments in which ABA cell-induced STC-derived thymus SF was passed through anti-CRI-Sepharose columns. The IBC was assessed before use and are indicated in the figure. The untreated SF, the filtrate after passage, and the acid eluate, were all adjusted to $5 \times 10^6$ cell eq/ml and administered at a dose of $2 \times 10^7$ cell eq mouse/day beginning at the time of immunization with $3 \times 10^7$ ABA-cells subcutaneously. Challenge was performed 5 d later with $25 \mu l$ of $10 \text{mM}$ ABA diazonium salt and the footpad swelling measured 24 h later.

The material obtained in such eluates was able to restore almost completely the suppression which filtration through an anti-idiotypic column had removed. The suppressor activity in such eluates is particularly labile to prolonged acid conditions (11). In rare experiments (data not shown) it was not possible to demonstrate a significant reduction in the suppressive activity of factors which had been adsorbed over the anti-idiotypic columns. However, in all such cases significant suppressive activity was detected in the acid eluates. The failure to remove a significant degree of the total suppressive activity in such filtrates may be a result of the overloading of the binding capacity of the columns or to the presence of some ABA SF lacking the CRI. Thymic extracts from normal mice were passed over the F(ab')2 anti-idiotypic column; neither the filtrate nor the acid eluate were found capable of suppressing ABA-specific DTH reactivity (B. A. Bach and M. I. Greene, unpublished observations). We conclude, therefore, that a significant fraction of ABA-specific A/J SF interacts with antibodies directed against idiotypic determinants on anti-ABA antibodies directed against idiotypic determinants on anti-ABA antibodies of A/J mice, and can be specifically recovered from anti-idiotypic immunoadsorbents in a biologically active form.

Idiotype Specificity of the Rabbit Antiserum. To determine whether A/J ABA suppressor factors bear the idiotypic determinants of the CRI, a careful assessment of the specificity of the rabbit antiserum was performed. It is unlikely that the rabbit anti-idiotypic antiserum recognized any allotypic determinants as such antisera were
### Table I

**Absorption and Elution of Suppressor Factor by Anti-Idiotype Immunoadsorbent**

| Group | Material injected* | Immunoadsorbent column specificity | Immunization | Mean foot-pad increment $10^3$ in + SEM‡ | $P$ value |
|-------|-------------------|-----------------------------------|--------------|---------------------------------------|-----------|
| I     | —                 | —                                 | $3 \times 10^7$ ABA/A/J spleen cells | $8.0 \pm 0.70$ | —                     |
| II    | Filtrate§         | NMG-adsorbed rabbit α Id          | $3 \times 10^7$ ABA/A/J spleen cells | $4.8 \pm 0.86$ | <0.01             |
| III   | Acid eluate§      | NMG-adsorbed rabbit α Id          | $3 \times 10^7$ ABA/A/J spleen cells | $1.9 \pm 0.86$ | <0.005           |
| IV    | Unfractionated suppressor factor | —                                 | $3 \times 10^7$ ABA/A/J spleen cells | $1.6 \pm 0.55$ | <0.005           |
| V     | Filtrate§         | Prebleed normal rabbit serum       | $3 \times 10^7$ ABA/A/J spleen cells | $1.5 \pm 0.70$ | <0.005           |
| VI    | Acid eluate§      | Prebleed normal rabbit serum       | $3 \times 10^7$ ABA/A/J spleen cells | $8.1 \pm 0.65$ | NS               |
| VII   | —                 | —                                 | —            | $0.5 \pm 0.25$ | <0.001           |

* Material obtained from columns, was adjusted to $2 \times 10^7$ cell eq/200 µl per mouse and administered daily for 5 d.

‡ 25 µl of 10 mM activated ABA diazonium salt was injected into the footpad.

§ Suppressor factor was fractionated on Sepharose 4B immunoadsorbents as described in Materials and Methods.

‖ Rabbit F(ab')$_2$ anti-idiotypic globulin was passed over an immunoadsorbent column which was coupled with pooled normal mouse globulins (NMG).

¶ Rabbit F(ab')$_2$ fragments of 7S globulins obtained from prebleed serum from rabbits in which anti-idiotypic serums were raised.

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Extensively adsorbed with insolubilized mouse globulins obtained from A/J mice to remove any nonidiotypic specificities present in the antiserum. Table I shows the results of an experiment performed in exactly the same way as those in Fig. 1 except that before coupling to Sepharose 4B, the rabbit F(ab')$_2$ fragments of anti-idiotypic serum were passed through an immunoadsorbent consisting of normal A/J globulins. The resulting filtrate, after dialysis, was coupled to Sepharose 4B. As can be seen, such an immunoadsorbent is indistinguishable in its activity from those shown in Fig. 1.

To more rigorously demonstrate the appropriate specificity of the anti-CRI antisera, such antisera were affinity purified on immunoadsorbents which consisted of highly purified, anti-arsenate antibody from A/J mice (12), which had been shown to bear the cross-reactive idiotype in high concentration. Fig. 2 demonstrates that F(ab')$_2$ rabbit anti-idiotype was specifically adsorbed by passage through the Sepharose 4B column containing purified anti-arsenate CRI* antibodies. Such an affinity purification generated two fractions of antiserum, a filtrate, which after concentration was coupled directly to activated Sepharose 4B, and an eluate which, obtained with 3 M KCl after negative pressure dialysis, was directly coupled to Sepharose 4B. The ability of such columns to adsorb specifically purified, radiolabeled CRI* -bearing immunoglobulin was tested in a solid phase radioimmunoassay before the fractionation of suppressor factor. As can be seen in Fig. 2, the idiotype absorbed filtrate
Specifically Adsorbed Suppressor Factor Can Be Eluted from, and Readsorbed by, Anti-Idiotypic Columns. Shown in Fig. 3 are the results of an experiment which indicates that suppressor factor, once specifically bound by an anti-idiotypic immunoabsorbent, can
Thymus Suppressor Factor 6978

Affinity Purified Rabbit F(ab')2 Anti-Idioype Coupled to Sepharose 4B

(IBC = 55 ng/mg gel)  (Anti-ID 2)

3 M KCl Elution

Dialysis and Vacuum Concentration

Affinity Purified Rabbit F(ab')2 Anti-Idioype Coupled to Sepharose 4B

(IBC = 57 ng/mg gel)  (Anti-ID 2)

Filtrate

Positive Control (5 x 10^7 ABA Cells SC)

Footpad Challenge Only

Immunization

Immunization

Fig. 3. ABA-cell-induced SF was passed on a rabbit anti-CRI Sepharose 4B column with IBC = 55 ng/mg gel. The 3 M KCl eluate, after concentration and dialysis, was then passed on a second rabbit anti-CRI Sepharose 4B column and the filtrate and eluate similarly gathered. The filtrate from the first rabbit anti-CRI Sepharose 4B was assessed at the same time as the doubly passed ABA SF. Depicted in this figure are the results of the administration of filtrate from the first rabbit anti-CRI, and the filtrate and eluate from the second rabbit anti-CRI Sepharose 4B column. All factors were administered at 2 x 10^7 cell eq/day per mouse for five consecutive days beginning at the time of immunization with 3 x 10^7 ABA-cells subcutaneously. Challenge with 25 μl of 10 mM ABA diazonium salt in the footpad was followed 24 h later by assessment of DTH reactivity.

be eluted and readsobered to another such immunoabsorbant of the same specificity. As data shown in Fig. 1 indicate, most but not all suppressive activity associated with the ABA SF was absorbed by an anti-idiotypic column. After such a filtration, it was possible to elute the suppressor factor from the first anti-idiotypic column and then readsober and subsequently refractionate the suppressor factor in an analogous manner on another anti-idiotypic column. These results demonstrate that biologically active suppressor molecules can be specifically readsobered once they have been isolated on the basis of their idiotypic determinants.

ABA Suppressor Factors Which Exhibit Antigen-Binding Capacity Bear the CRI. Having shown that ABA suppressor factor is adsorbed consistently on anti-idiotypic immunoabsorbents, experiments were performed to demonstrate the concordance between antigen-binding specificity and the presence of idiotypic determinants on the ABA-suppressor factor. Spleen suppressor factor was first fractionated over an antigen column consisting of ABA fowl gamma globulin coupled to Sepharose 4B as previously
Fig. 4. ABA-cell-induced spleen SF was first passed over an ABA-FGG Sepharose 4B immunoadsorbent. The filtrate and the 3 M KCl eluate were concentrated separately. The eluate was then passed on a Rb anti-CRI Sepharose 4B column with IBC = 31 ng/mg gel. The filtrate of this column and its 3 M KCl eluate were gathered, concentrated, and dialyzed. The filtrate from the ABA-fowl gamma globulin Sepharose 4B, and the filtrate and the eluate from Rb anti-CRI Sepharose 4B column assessed by the administration of $2 \times 10^7$ cell eq/day per mouse beginning at the time of immunization with $3 \times 10^7$ ABA cells subcutaneously. Challenge was performed 3 d later and footpads measured 24 h later.

described (7). Such suppressor factor(s) were then concentrated and refractionated over anti-idiotypic immunoadsorbents. As can be seen in Fig. 4, the suppressor factors are specifically adsorbed to the antigen column and such material is then retained by an anti-idiotypic immunoadsorbent. Such suppressive activity was subsequently eluted from the anti-idiotypic column by glycine HCl, pH 2.8, and shown to contain suppressive activity (Fig. 4).

Relationship between ABA Suppressor Molecules Which Bear Idiotypic Determinants and the MHC. As shown in the preceding paper (7), ABA-suppressor molecules may be adsorbed by columns which have specificity for products of the MHC.

The experiments summarized in Table II demonstrate a concordance between MHC determinants and idiotypic specificities on the suppressor molecules. In these experiments the suppressor factor was first fractionated on whole anti-H-2$^a$ (B10 anti-B10.A) Sepharose 4B immunoadsorbents. The antiserum for the immunoadsorbents were obtained by immunizing the congenic B10 (H-2$^b$, Ig-1$^b$) mouse strain with B10.A (H-2$^a$, Ig-1$^b$) spleen cells. The resulting sera were highly cytotoxic for B10.A and A/J spleen cell targets (>1:1,280) but not cytotoxic for spleen cells from B10 (H-2$^b$) mice (data not shown).

After first being fractionated on an immunoadsorbent prepared by coupling anti-
Mice of the C.AL-20 and B10.A strains were chosen as the appropriate test strains. Suppressor factor was produced in C.AL-20 mice by the intravenous administration of ABA-conjugated C.AL-20 spleen cells. Such factors were prepared from the spleen and thymus of such animals. The same procedure was followed in B10.A mice using syngeneic B10.A-ABA spleen cells to induce the factor. As is apparent from Fig. 5, mice of the C.AL-20 (H-2d, Ig-1d) strain make a factor which can be absorbed by the appropriate anti-idiotypic Sepharose 4B immunoadsorbent. Suppressor molecules derived from B10.A (H-2a, Ig-1b) animals, which share the MHC haplotype of A/J mice (H-2a) but differ at the heavy-chain allotype locus, can not be specifically retained on anti-idiotypic immunoadsorbents.

Fig. 6 provides evidence for the importance of the heavy-chain allotype locus in the expression of idiotypic determinants on ABA SF. BALB/c (H-2d, Ig-1a) and C.AL-20 (H-2d, Ig-1b) mice are congenic except for the Ig-1 heavy-chain allotype locus. As can
Fig. 5. ABA-cell-induced SF were induced in B10.A or C.AL-20 mice by the intravenous administration of $3 \times 10^7$ ABA-derivatized syngeneic cells. Spleens and thymuses were the source of B10.A or C.AL-20 SF. B10.A SF or C.AL-20 SF were passed separately on two identical Rb anti-CRI columns with IBC = 54 ng/mg gel. The filtrates and eluates were gathered separately, concentrated, and dialyzed against BBS. The unfractionated SF or the Rb anti-CRI-Sepharose 4B filtrate and eluate of either B10.A or C.AL-20 SF were then assessed for suppressive activity in the strain of origin. The SF were administered daily at doses of $2 \times 10^7$ cell eq/day per mouse beginning at the time of immunization with $3 \times 10^7$ ABA-derivatized syngeneic cells. Footpad challenge was performed 5 d later by the injection of 25 μl of 10 mM ABA diazonium in the footpad. DTH was assessed 24 h later.

As can be seen, the C.AL-20 strain produces idiotypic positive SF, whereas the BALB/c mouse does not. These data are completely analogous to the data concerning the expression of the idiotype on anti-arsonate immunoglobulins in the two strains, where the C.AL-20 is idiotype positive and BALB/c is idiotype negative (13).

Discussion

In the preceding study (7) we reported some of the properties of ABA-specific SF and found that these SF shared many features of T-cell-derived suppressor substances found in many systems. In the present report we have presented data relating to the presence of idiotypic determinants on the SF.

It has been shown that A/J mice, immunized with ABA-KLH, produce anti-ABA antibodies that bear (in up to 70% of the antibodies, a strain-specific idiotype (12–16)). In one of these studies, genetic linkage between loci governing the expression of idiotype and immunoglobulin heavy (H)-chain allotype (Ig $C_H$ locus) was investigated (13). Such genetic linkage has been shown for a number of specific idiotypes (13–15). In the ABA system the cross-reactive idiotype that appears in antibodies to the ABA
hapten in all A/J mice is also expressed by the AL/N strain (13). The C.AL-20 strain, which possess the Cg-region allotype of AL/N on the genetic background of the idiotype-negative BALB/c strain, produces anti-ABA antibodies which bear the cross-reactive idiotype, demonstrating the close linkage of ABA idiotype with allotype. Finally, recent work has also revealed that the light-chain (VK-1) locus, closely linked to the locus controlling the Lyt 3 thymocyte antigen(s), may also contribute to the inheritance of the ABA-idiotype character (16). For these reasons, the ABA system was chosen as being particularly appropriate to explore the genetic structure of T-cell-derived regulatory molecules.

We have now established that anti-idiotypic antibodies interact in a highly specific manner with ABA SF. This interaction was shown to be solely dependent upon antibodies in the anti-idiotypic antisera which could be removed by immunoabsorbents consisting of CR1 + Ig. Passage of F(ab')2 fragments of rabbit anti-idiotype over such an adsorbent removed the component responsible for binding SF. In contrast, the protein that adhered to the CR1 + column could be eluted with 3 M KCl and shown to possess the capacity to bind SF. Furthermore, SF isolated and eluted from an ABA-FGG column could be shown to specifically bind to an anti-idiotypic column. This indicates that the antigen-binding structure was present on a molecule or molecular complex which has the idiotype.
It was also shown that the same molecular species or complex bearing determinants encoded by the H-2 region also bears idiotypic determinants. This indicates that SF bears at least two definable genetically determined structures—one coded by genes on the murine XVIIth chromosome, and the other by genes controlling the expression of the idiotype.

Finally, although all strains assessed to date (A/J (H-2\textsuperscript{a}, Ig-l\textsuperscript{a}), BALB/c (H-2\textsuperscript{d}, Ig-l\textsuperscript{d}), B10.A (H-2\textsuperscript{a}, Ig-l\textsuperscript{b}), C57BL/6 (H-2\textsuperscript{b}, Ig-l\textsuperscript{b}), and C.AL-20 (H-2\textsuperscript{d}, Ig-l\textsuperscript{d})) produce SF active in the strain of origin, we found that A/J SF and C.AL-20 SF, but not B10.A SF nor BALB/c SF, bear the idiotypic determinants characteristic of A/J antibodies; this reflects linkage of the genes controlling idiotypic determinants on SF and CH allotypes. Thus, it would appear that genes linked to the Ig C\textsubscript{a} locus and genes within the H-2 locus are involved in the formation of SF.

The demonstration of the CRI on ABA SF and the linkage of the expression of idiotypic specificities on the T-cell suppressor factor with the Ig-l\textsuperscript{e} (A/J) and (AL/N) heavy-chain linkage groups raises several important issues: First, what is the role of light-chain V regions, if any, in the specificity of the suppressor factor for ABA. Second, is the light chain required for the expression of idiotypic specificities recognized by anti-CRI? Experiments with strains which fail to produce light chains that can contribute to the formation of the CRI (16) may resolve the issue.

The demonstration of CRI determinants on antigen-specific products of suppressor T cells poses important questions about the cellular distribution and expression of the CRI on other T cells specific for the ABA determinant; these would include T-helper, T-cytolytic, or T-DTH effector cells. Recent studies from this laboratory by Sherman et al. (17) have failed to detect the ABA CRI on ABA-specific cytolytic T cells, using both negative selection with anti-idiotypic serum and complement and a sensitive technique applying the fluorescence-activated cell sorter. Experiments are in progress to examine the expression of ABA CRI on ABA-specific suppressor T cells, and on cell-mediating ABA-specific DTH.

Recently Ju et al. (18) have shown the presence of cross-reactive idiotypic structures on antibodies from mice immunized with the synthetic terpolymer L-glutamic\textsuperscript{60}-L-alanine\textsuperscript{30}-L-tyrosine\textsuperscript{10} (GAT). A guinea pig anti-idiotypic serum, recognizing a cross-reactive idiotype (CGAT) on GAT-specific antibodies, was used in recent studies by Germain et al. (19) to demonstrate that GAT-specific SF derived from T cells also bear CGAT determinants. The same molecular complex, GAT SF, which is biologically active in the suppression of antibody responses, was also shown to bear I-J region-encoded products. Together with the result of this study, the data on the FAT system provide two independent instances where immunoglobulin idiotypes are detected together with MHC gene products in T-cell suppressor factors.

The absence of CGAT-negative mouse strains has prevented linkage analysis of the genes coding for CGAT idiotype with Ig-1 genes. However, the results with ABA SF have established the presence of CRI on SF derived only from mice which have the appropriate heavy-chain allotype linkage group of genes and express the ABA CRI in their anti-ABA antibodies.

As shown earlier, although a significant proportion of ABA-specific SF bears the CRI, we cannot conclude from these studies that all ABA SF in the appropriate strains bears CRI. To resolve this issue, we have initiated experiments to examine the presence or absence of CRI\textsuperscript{*} SF in A/J mice immunologically suppressed for the
expression of CRI on their anti-ABA antibodies (15, 20, 21). Such experiments should be informative as to whether, even in the presence of a CRI− antibody response, T cells may still produce CRI+ SF.

Krammer and Eichmann (22) have found that the MHC and the Ig C_H locus affect the idiotypic pattern of alloantigen receptors on murine T lymphocytes, although the presence of either gene product was not reported. The present study suggests an association of H-2 coded products and determinants coded by genes linked to the Ig C_H locus in the ABA STC derived SF. This observation and other relevant data (19, 22) can be explained on the basis that suppressor T cells express V_H gene products as well as those of the MHC gene products in the same molecular complex. The V_H gene product would be responsible for the antigen-binding capacity and specificity of such factors (19), whereas the MHC product portion could be decisive in cell-signalling events, including cell interaction; the most conservative explanation for the structure of such specific suppressor factors would postulate a multichain molecule coded for by different chromosomes. In this model, both H-2 and V_H gene products would be required to permit appropriate function of the ABA STC-derived SF. Thus, the ABA SF derived from suppressor T cells contains the products of at least two distinct but identifiable chromosome regions. Work is in progress to analyze the potential influence of a third, the VK-1 locus. However, definitive biochemical analysis of SF may only be possible through the use of T-cell hybridomas which produce significant amounts of suppressor factors (23).

Summary

T-cell derived suppressor factor(s) (SF) specific for azobenzene arsonate (ABA) were prepared by the mechanical disruption of suppressor cells. Such suppressor factors were adsorbed to and recovered from immunoadsorbents prepared from the F(ab')2 fragments of rabbit immunoglobulin directed against the cross-reactive idiotype of A/J anti-ABA antibodies. These ABA-suppressor factors were not retained on Sepharose 4B immunoadsorbent columns which had been coupled with F(ab')2 fragments of normal rabbit immunoglobulins prepared from prebleeds of rabbits used to make anti-idiotypic antiserum.

The specificity of the F(ab')2 rabbit anti-idiotypic serum was established by direct idiotypic-binding assays and by affinity purification over an immunoadsorbent consisting of CRI+ anti-ABA immunoglobulin from A/J mice. ABA-suppressor factors were shown to be specifically absorbed and eluted from F(ab')2 anti-idiotypic columns. Furthermore, the eluted suppressor factor can be specifically reabsorbed and recovered from a second anti-idiotypic immunoadsorbent. The concordance between antigen-binding specificity and the presence of idiotypic determinants was demonstrated by adsorbing ABA SF to antigen columns and then fractionating the ABA-specific factor on anti-idiotypic immunoadsorbents. ABA-suppressor factors were shown to be specifically retained on immunoadsorbents directed against major histocompatibility complex (MHC) determinants. Factor eluted from anti-MHC columns could then be specifically adsorbed to anti-idiotypic immunoadsorbents. This suggests that the same molecular complex that is recognized by the H-2 alloantisera is specifically adsorbed to an anti-idiotypic immunoadsorbent. Genetic analysis of the expression of CRI+ suppressor factor was performed using the C.AL-20 mouse strain which has the AL/
N allotype and produces CR1\(^{+}\) anti-ABA immunoglobulins. The implication of these findings to the nature of T-cell-derived regulatory molecules is discussed.

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