ANALYSIS OF THE INTERACTIONS BETWEEN TWO MOLECULES THAT ARE REQUIRED FOR THE EXPRESSION OF Ly-2 SUPPRESSOR CELL ACTIVITY

Three Different Types of Focusing Events May Be Needed to Deliver the Suppressive Signal

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We have described (1) a biologically active antigen-specific Ly-1−; 2+ T cell product that has the capacity to suppress the ability of Ly-2 cell-depleted spleen cells to make primary responses in Mishell-Dutton cultures. It has been presumed that this material, referred to as "Ly-2 suppressor factor from T cells (TsF),"1 is a direct suppressor molecule because (a) it requires no other cells with a suppressor cell phenotype in the culture to work, and (b) its suppressive effect can be seen when the assay cultures are harvested early during the course of an in vitro immune response. Inducers of suppression require a longer latent period before their suppressive activity is expressed (2). An interesting feature of this Ly-2 TsF is that it does not express any I-J or other major histocompatibility complex (MHC)-controlled specificities that can be detected with the use of a large battery of antisera. Nonetheless, the biological activity of the molecule is restricted by the MHC polymorphisms expressed by the producer:acceptor cell partners.

This latter finding might be interpreted to indicate that the Ly-2 TsF must react with an MHC-controlled gene product in the acceptor cell population to express its biological activity. We tested this proposition by treating normal Ly-1 T cells with an anti-I-J serum before adding these cells to T cell-depleted spleen cells (B cells) and determining whether the anti-I-J treatment of the normal Ly-1 cells had affected either (a) the ability of the Ly-1 cells to help the T cell-depleted spleen cells or (b) changed the ability of the Ly-2 TsF to suppress the cultures.

We found that the anti-I-J treatment of the Ly-1 T cells did not affect their ability to help T cell-depleted spleen cell cultures but completely abrogated the ability of the Ly-2 TsF to suppress the helper activity in such cultures. We also found that the cultures that lack I-J+ Ly-1 cells can be suppressed by the Ly-2 TsF when the eluate from an anti-I-J immunoabsorbent through which an I-J+ Ly-1 T cell suppressor-

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Abbreviations used in this paper: BSS, balanced salt solution; FCS, fetal calf serum; MHC, major histocompatibility complex; PFC, plaque-forming cells; SRBC, sheep erythrocytes; TSF, suppressor factor from T cells; TsiF, suppressor inducer-factor from T cells.

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inducer factor had been passed is added back to the cultures. The eluted material neither binds antigen nor must it come from Ly-1 cells that have the relevant antigen specificity (3). Interestingly, the Ly-1 cell that makes the I-J+ material used to return the suppressive capacity to the Ly-2 TsF must match the I-J haplotype of the cell that makes the Ly-2 TsF and also must share an Igh-V-linked polymorphism with the Ly-2 TsF target cell but not the Ly-2 TsF itself.

These results indicate that two separate macromolecules are required for effecting suppression as well as for inducing suppression (3). They can also give us some insight into the biological roles of the two macromolecules involved in effecting the suppression.

Materials and Methods

Mice. C57BL/6, BALB/c, AKR B10.Br, C57BL/10J, and CB6F1 mice, 6–10 wk of age, were obtained from The Jackson Laboratory, Bar Harbor, ME. BALB/B and BALB/K mice were from the Yale University School of Medicine. BALB/c nu/nu mice were maintained at the National Institutes of Health animal facility. Preparation of bone marrow chimeras and thymus grafted nu/nu mice is detailed elsewhere, and they were supplied to us by Alfred Singer, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Antisera. Monoclonal anti-Lyt sera were generously supplied by F.-W. Shen, Memorial Sloan-Kettering Cancer Center, New York. Monoclonal anti-Thy-1 reagents were generously provided by Dr. Jonathan Sprent, University of Pennsylvania, Philadelphia, PA. Anti-I-J serum was prepared by hyperimmunizing B10.A (5R) recipients with a mixture of B10.A (3R) spleen and lymph node cells (antiserum ASM-20) (we thank D. Murphy for preparing these antisera). Depletion of cells bearing Lyt or I-J markers was achieved by incubating 1 × 10^7 cells/ml of antibody appropriately diluted in balanced salt solution (BSS) (1:1000 for anti-Lyt monoclonal antibody, 1:5 for anti-Thy-1 monoclonal antibody hybridoma supernatant, or 1:5 for anti-I-J serum), washing, and incubating with complement for 45 min at 37°C (1 × 10^7 cells/ml of rabbit serum diluted 1:10 for anti-Lyt or anti-I-J antibody or 1 × 10^7 cells/ml of guinea pig complement for anti-Thy-1 antibody). Complement used in these experiments was serum from animals selected for low natural cytotoxicity to mouse spleen cells.

Antigens. Sheep erythrocytes (SRBC) were obtained from Colorado Serum Company Laboratories, Denver, CO.

Method of Cell Preparation. Spleen cells were washed in BSS and suspended in RPMI 1640 supplemented with antibiotics, 10% fetal calf serum (FCS), 100 mM glutamine, 25 mM Hepes, and 5 × 10^-5 M 2-mercaptoethanol for tissue culture. T cells were prepared by adding unprimed spleen cells to plastic petri dishes coated with goat anti-mouse immunoglobulin and harvesting the nonadherent fraction(s). B cells were prepared by treating the cells with monoclonal anti-Thy-1 and complement.

Preparation of Lyt 1+-derived Suppressor-Inducer (Ly-1 TsiF) and the Lyt 2+-derived Suppressor-Effector (Ly-2 TsF) Materials. Preparation of Ly-1 TsiF and Ly-2 has been previously described (1, 2). Briefly, a suspension of spleen cells from mice hyperimmunized with SRBC was treated with anti-Lyt-2 (for Ly-1 TsiF) or anti-Lyt-1 (for Ly-2 TsF) and rabbit complement and subsequently cultivated in vitro for 48 h in RPMI 1640 plus 10% FCS at a concentration of 10^5 cells/ml. After 48 h, supernatant fluids were cleared and passed through millipore filters.

Absorption of Soluble Factors. Absorption with erythrocytes was done by mixing 1 ml of culture supernatants with 0.1 ml of a 50% suspension of sheep erythrocytes for 1 h on ice. The erythrocytes were removed by centrifugation. For absorption with anti-I-J sera, supernatant fluids were passed over an anti-I-J immunoabsorbent (ASM-20) prepared by conjugation of antisera to cyanogen bromide-activated Sepharose.

Assay Cultures. Suppressor activity of the Ly-1 TsiF or the Ly-2 TsF was determined by
adding these materials to cultures of unprimed spleen cells that had been treated with various test reagents. All cells were suspended in culture medium at a concentration of $2 \times 10^6$ T cells and $2 \times 10^8$ B cells in 1 ml and cultured with 0.05 ml of a 1% SRBC suspension in Falcon 3008 plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) in a 5% CO$_2$, 95% air incubator at 37°C. The number of plaque-forming cells (PFC) was determined on day 5 by using the Cunningham modification of the Jerne-Nordin plaque assay, as previously described (4). Results are given as the mean of three individual calculations from each culture condition.

Isolation of I-J$^+$ Molecule from Ly-1 TsiF. Supernatants containing Ly-1 TsiF were passed over the appropriate immunosorbent column made from anti-I-J antisera coupled to Sepharose 4B. After extensive washing, the column was eluted with 0.2 M sodium carbonate (Na$_2$CO$_3$), pH 11.0, and immediately neutralized in 0.3 M borate buffer, pH 8.3. The eluted material was then concentrated to original volume and dialyzed overnight against, first, PBS, then RPMI 1640.

Results

Removal of I-J$^+$ Ly-1 Cells from Assay Cultures Prevents Suppression by Ly-2 TsF (Table I). Supernatants from cultured immune Ly-2 cells had a potent suppressive activity when added to assay cultures composed of Ly-1 T cells and Thy-1-depleted spleen cells (compare group 1 with group 2; Table I). Removal of I-J$^+$ Ly-1 T cells from the assay cultures did not significantly alter their activity in the absence of the suppressive Ly-2 TsF (group 3). However, even though the response in the cultures depleted of I-J$^+$ Ly-1 cells showed no significant change in the absence of Ly-2 TsF, there was a marked difference in the response of these cultures when Ly-2 TsF was added (group 4). Under these conditions the Ly-2 TsF failed to manifest any suppressive activity. Thus, an I-J$^+$ Ly-1 cell in the assay culture must interact with the Ly-2 TsF to give the TsF biological activity.

Return of I-J$^+$ Material from Ly-1 Cells Allows the Ly-2 TsF to Suppress Assay Cultures Depleted of I-J$^+$ Ly-1 Cells (Table II). Appropriately immunized Ly-1 cells release a suppressor-inducer factor (Ly-1 TsiF) (2) that is composed of two separable macromolecules; one of these macromolecules bears an I-J-controlled determinant and is the molecule that imparts an Igh-V-linked restriction on the factor's activity (3). We therefore asked the question of whether I-J$^+$ material isolated from an Ly-1 TsiF would allow the Ly-2 TsF to manifest suppressive activity in cultures depleted of I-J$^+$ Ly-1 cells. The results of such an experiment (Table I) recapitulate the findings in the experiments reported in Table I (i.e., they show that anti-I-J treatment of the Ly-1 T cells in the assay culture does not inhibit helper activity but completely inhibits the ability of the Ly-2 TsF to effect suppression). The key finding is that in group 5, where it is shown that the addition of the eluate from an I-J column through which

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| Table I |
|---|
| **Removal of I-J$^+$ Ly-1 Cells from Assay Cultures Prevents Suppression by Ly-2 TsF** |
| Group | Cells added to cultures | Ly-2 TsF added$\ddagger$ | PFC/culture | Percent suppression |
|---|---|---|---|---|
| 1 | Ly-1 T cells | – | 3,000 | Standard |
| 2 | Ly-1 T cells | + | 500 | 83 |
| 3 | I-J$^+$ Ly-1 T cells | – | 2,600 | Standard |
| 4 | I-J$^+$ Ly-1 T cells | + | 2,200 | 15 |

$^*$ All cultures contained anti-Thy-1-treated spleen cells and SRBC.
$\ddagger$ SRBC specific.
Return of I-J⁺ Material Isolated from Ly-1 Cells Allows Ly-2 TsF to Suppress B6 Assay Cultures Depleted of I-J⁺ Ly-1 Cells

| Group | Cells added to cultures* | Ly-2 TsF added† | Ly-1 I-J added | PFC/culture | Percent suppression |
|-------|--------------------------|------------------|----------------|-------------|-------------------|
| 1     | Ly-1 T cells             | -                | -              | 3,400       | Standard          |
| 2     | Ly-1 T cells +           | +                | -              | 1,100       | 68                |
| 3     | I-J⁻ Ly-1 T cells -      | -                | -              | 3,000       | Standard          |
| 4     | I-J⁺ Ly-1 T cells +      | +                | -              | 3,000       | 0                 |
| 5     | I-J⁻ Ly-1 T cells +      | +                | +              | 1,000       | 67                |

* See footnotes, Table I.
† See footnotes, Table I.

Genetic Requirements That Allow Ly-1 I-J⁺ Material to Assist Ly-2 TsF in Suppressing B6 Assay Cultures Depleted of I-J⁺ Ly-1 Cells; I-J⁺ Material Must Match Ly-2 TsF at H-2 and the Acceptor Cell at Igh-linked Polymorphisms

| Source of I-J⁺ molecule | Source of Ly-2 TsF | None | B6 | BALB/c | AKR | C.B20 |
|-------------------------|-------------------|------|-----|--------|-----|-------|
| None                    | 7,500*            | 6,500| 7,000| 6,200  | 5,900|       |
| B6                      | 8,000             | 1,600§ | 5,800| 7,000  | 4,600|       |
| BALB/c                  | 7,500             | 5,600| 5,700| 6,600  | 6,800‡|       |
| C.B20                   | 7,400             | 5,800| 2,200| 7,200  | 2,100|       |
| BALB/B                  | 7,100             | 6,400| 6,000| 6,700  | 5,800|       |

* (PFC/culture) all assay cultures contained B6 Thy-1⁺ spleen cells and B6 I-J⁺ Ly-1 cells and SRBC.
‡ Important negative control for reciprocal combination.
§ Italicized numbers indicate cultures suppressed by appropriate combination of Ly-2 TsF and I-J⁺ molecule.

The fact that the Ly-2 TsF, which is H-2 restricted, needs to interact with the I-J⁺ molecule in the assay cultures to manifest its suppressive activity, suggested that its H-2 restriction might be anti-I-J and that it was this anti-I-J specificity that allowed it to act in concert with the I-J⁺ material from the Ly-1 TsF to perform suppression. To test this hypothesis, we took B6 spleen cells that had been depleted of Thy-1⁺ cells and added back to them B6 Ly-1 T cells that had been treated with anti-I-J. To these assay cultures, we added Ly-2 TsF from four different mouse strains and I-J⁺ molecules from the same four strains. The results of such an experiment show that only three of these molecular mixtures returned suppressive activity to the Ly-2 TsF in the cultures of B6 spleen cells that lacked I-J⁺ Ly-1 cells. One was the homologous mixtures (B6 with B6). This was simply a repeat of the results reported in Table II. The more interesting results were those obtained with material from C.B20 mice. C.B20 Ly-2 TsF could interact appropriately with C.B20 I-J⁺ material and return suppression. Thus, two molecules that match each other at H-2 can form
a suppressive unit that will work on cells that share Igh-V-linked gene products with the cell that made the I-J\textsuperscript{+} material. The mapping of the Igh-V-linked restriction to the I-J\textsuperscript{+} chain's interaction with the B6 acceptor cell is substantiated by the observation that a BALB/c Ly-2 TsF could replace the C.B20 Ly-2 TsF in restoring suppression but that the reverse was not true. That is, the C.B20 Ly-2 TsF could not be restored with I-J\textsuperscript{+} material from BALB/c. These results indicate that I-J is involved in the interaction between the I-J\textsuperscript{+} and I-J\textsuperscript{-} macromolecules and forms a molecular complex that interacts with the acceptor cell via an Igh-V-linked receptor. In the absence of an H-2 match between the two chains and an Igh match between the I-J\textsuperscript{+} chain and the acceptor cell, no suppression occurred. These findings and conclusions are consistent with the previous report that the I-J\textsuperscript{+} molecule was responsible for the Igh-V-linked restriction of the Ly-1 TsF (3).

**Genetic Requirements That Allow the Ly-1-derived I-J\textsuperscript{+} Material to Interact with the Ly-2 TsF in Suppressing Cultures Depleted of I-J\textsuperscript{+} Ly-1 Cells; the I-J\textsuperscript{+} Material Must Share I-J-linked Polymorphisms with the Ly-2 TsF and Igh-V-linked Polymorphisms with the Acceptor Cell.** (Summary) (Table IV). The results of two more experiments looking at the genetic requirements for functional reconstitution of Ly-2-TsF in cultures that lack I-J\textsuperscript{+} Ly-1 cells are presented in Table IV, using B6 spleen cells as assay cells. In Table IV, it can be seen that in every circumstance save one (17 of 18 determinations) where there was an H-2 match between the two molecules that formed the suppressive complex, suppression occurred.

### Table IV

| Source of I-J\textsuperscript{+} molecule\* | Source of Ly-2 TsF | None | B6 | BALB/c | AKR | C.B20 | B6 into CB6F\textsubscript{1} |
|-------------------------------------------|------------------|------|-----|--------|-----|-------|-----------------------------|
| None                                      | I    | II   | I    | II   | I    | II   | I    | II   | I    | II   | I    | II   |
| B10 or B6\$                               | 0    | 0    | 0    | ++   | 0    | 0    | 0    | ++   | 0    | ++   | 0    | ++   |
| B10.Br                                    | 0    | 0    | 0    | 0    | 0    | ++   | 0    | ++   | 0    | ++   | 0    | ++   |
| B10.A(3R)                                 | 0    | 0    | ++   | 0    | 0    | 0    | 0    | ++   | 0    | ++   | 0    | ++   |
| B10.3A(5R)                                | 0    | 0    | 0    | 0    | ++   | 0    | ++   | 0    | ++   | 0    | ++   |
| BALB/c                                    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| C.B20                                     | 0    | 0    | 0    | 0    | ++   | ++   | ++   | ++   | ++   | ++   |
| Bab.14                                    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| BALB.B                                    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| BALB.K                                    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| BALB/c nude + CB6F\textsubscript{1}T.G.  | 0    | 0    | 0    | 0    | ++   | 0    | ++   | ++   | ++   |

* I and II refer to separate experiments. The I-J\textsuperscript{+} nonantigen-binding material from Ly-1 TsF obtained by affinity chromatography over an anti-I-J immunosorbent in experiment labeled I and by absorption of Ly-1 TsF with SRBC in experiment labeled II.

\$ Results are from assay cultures that contained Thy-1\textsuperscript{-} B6 spleen cells and I-J\textsuperscript{-} B6 Ly-1 cells and SRBC. Results are presented as percent suppression of culture that served as positive control (3,400 PFC/culture in experiment I and 3,100 PFC/culture in experiment II). 0, <25% suppression; ±, 25-49% suppression; +, 50-74% suppression; ++, 75-100% suppression; —, not done.

\| We have no explanation as to why this result was negative; perhaps a technical error was made. We repeated the experiment and the positive result achieved in experiment II is correct.
complex and an Igh-V match between the I-J+ portion of that complex and the B6 acceptor cells, suppression was manifest. In all cases where these two criteria were not met, no significant suppression was seen.

Additional information found in these series of experiments is: (a) the H-2 matching requirement that helps the two chains to form an appropriate molecular complex maps to I-J as the B6 Ly-2 TsF could react with the 3R (I-Jb) and not the 5R (I-Jb), whereas the AKR Ly-2 TsF could react with the 5R and not the 3R; and (b) these results also map the Igh linkage in the present studies to Igh-V (as previous studies had done) (3) because the BALB/c Ly-2 TsF could work appropriately with a C.B20 I-J+ molecule but not with a BAB.14 I-J+ molecule (BAB.14 and C.B20 mice differ genetically only in a portion of 12th chromosome that is linked to Igh-V).

An added interesting observation was made using factors from chimeric mice. We have previously shown that parental cells that differentiate in an F1 thymus graft can suppress the response of cells from the reciprocal parent in the F1 cross. In the experimental results presented in Table IV, we used an Ly-2 TsF from a CB6F1 radiation chimera that had been repopulated with B6 bone marrow cells. Under these circumstances, the B6 Ly-2 TsF from the chimeric mice could interact with I-J+ material from the C.B20 and form a suppressive unit. Thus, the B6-derived Ly-2 TsF in the chimera had “learned” to see the MHC-linked polymorphism on the I-J+ C.B20 molecule and could act to suppress B6 cells because the C.B20 I-J+ molecule matched the acceptor cell at Igh-V.

In addition, the Ly-2 TsF from the B6 into CB6 F1 chimera could interact with an I-J+ molecule derived from a BALB/c nude mouse that had been engrafted with a CB6F1 thymus. Previous studies (6) have shown that the molecules of the latter type of chimera express the I-Ja polymorphisms, so it can be concluded that the B6 cells have learned in the F1 chimera to see the I-Ja-linked polymorphisms on the Ly-1 TsiF from BALB/c nude mice with CB6F1 thymus grafts, and the I-J+ molecule from these latter chimeras have learned to interact with the Igh-V-linked receptor on the B6 acceptor cell. Note: the B6 Ly2 TsF could not form an active molecular complex with the I-J+ material from the BALB/c nude with the F1 thymus graft, whereas the BALB/c Ly-2 TsF could. Thus, it seems quite clear that the Ly-2 TsF must combine with an I-J+ molecule to achieve its biological activity and that there are two restrictions imposed upon its functional interaction with that molecule: (a) it must recognize an I-J-controlled polymorphism on the molecule itself, and (b) the I-J+ molecule with which it interacts must recognize an Igh-V-linked polymorphism on the acceptor cell.

Discussion

We previously described (1) a product found in supernatants of immunized Ly-2 T cells (Ly-2 TsF) that is antigen specific and acts as a direct suppressor of Ly-1 cells when the Ly-1 cells express the same H-2 polymorphisms as the cells that have made the Ly-2 TsF. We also previously described a suppressor-inducer molecule (Ly-1 TsiF) that can induce Ly-2 cells to express suppressor activity when the Ly-2 cells share Igh-V-linked polymorphisms with the cells that made the Ly-1 TsiF (2) (these genetic restrictions can be overcome in parent into F1 chimeras) (6). In the case of the Ly-1 TsiF, we have shown that biological activity requires the interaction of two separate
macromolecules, one of which sees antigen and the other of which expresses I-J and does not see antigen. The Igh-V-linked restriction maps to this I-J+ molecule (3).

In the present series of experiments, we attempted to determine the significance of the genetic restrictions described above. We learned that (a) the H-2 restriction of the Ly-2 TsF stems from its need to interact with an I-J+ molecule and, to interact with that molecule, it must come from mice that share I-J polymorphisms with the I-J+ molecule; and (b) for this molecular complex to influence the target cell, the I-J+ molecule with which the Ly-2 TsF interacts must share genes linked to Igh-V with the acceptor cell. Therefore, the H-2 restriction in these conditions is not one that prevents the Ly-2 TsF from interacting with its acceptor cell, but rather it inhibits its interaction with another molecule that interacts with the receptor for the molecular complex on the acceptor cell. The second molecule’s interaction with the acceptor cell is restricted by Igh-V polymorphisms and thus is likely to be a VH anti-VH type of reaction.

Thus, both the Ly-2 TsF and the Ly-1 TsiF, previously reported (3), require two separate macromolecules for biological function to be seen (a similar situation has been reported with another suppressor factor under study in our laboratory, which acts to inhibit the activity of immune Ly-1 cells that transfer contact hypersensitivity) (5). Taniguchi et al. (6) and Taussig and Holliman (7) also found that antigen-specific TsF are composed of at least two separate macromolecules.

From these data, a general working model by which biologically active T cell-derived factors function can be put forth. A specific model for the mechanism of the Ly-2 TsF we described is illustrated in Fig. 1. We tentatively assigned the functional information to the molecule that is antigen specific. There are two reasons for this assignment. (a) When the I-J+ material that we used to form molecular complexes with the Ly-2 TsF is mixed together with an antigen-binding chain from an Ly-1 TsiF, the functional activity of the molecular complex changes (2). In this situation, the molecular complex does not have the ability to directly suppress T or B cells. Rather, it acts as a suppressor-inducer factor, and thus it will not suppress Ly-2-depleted spleen cell cultures. Thus, if the I-J+ molecule used by the antigen-binding chains of the Ly-1 TsiF and the Ly-2 TsF is the same and the two molecular complexes have different activities, it follows that the activity, per se, is inherent in the antigen-binding chain. Although we have no direct proof that it is the same I-J+ molecule that interacts with both the Ly-1- and Ly-2-derived antigen-specific functional molecules, there is no reason to suspect that they are different. Most importantly, in both cases, the I-J+ molecules impart an Igh-V-linked restriction for functional activity that suggests that if they are not the same, they are quite similar. (b) Fresno and his colleagues (8, 9) have shown that functional activity is in the antigen-binding chain of a clonal T suppressor factor. The functional signal is in the C terminal piece and is not part of the antigen-recognizing mechanisms.

Based on this evidence for assigning biological activity to the antigen-binding chain and a lack of biological activity in the I-J+ chain, we tentatively assign a “schlepper” function to this latter chain. The evidence suggests that the I-J+ chain brings (schlepps) the molecule with biological activity to the appropriate receptor on the target cell. The interaction between the I-J+ chain and the molecule on the acceptor cell is Igh-V linked. The I-J portion of the schlepper chain appears to be the “hook-
Fig. 1. Composition and functional makeup of an antigen-specific T suppressor factor. Molecule 1 (schlepper chain): the Igh-V-linked moiety (anti-Igh-V?) focuses the functional chain on the appropriate cell surface receptor that must also be Igh-V linked; I-J is the receptor for anti-I-J specificity on the functional chain. Molecule 2 (functional chain): the anti-nominal antigen recognition unit brings the chain to the acceptor cell that recognizes the same antigen specificity; the anti-I-J connects to the schlepper chain; and the suppressor signal inhibits the helper activity of target cell.

up" site that joins the schlepper chain to the functional antigen-specific chain. This places an H-2 restriction on the interaction of the two chains.

The functional chain recognizes "nominal" antigen (SRBC in these studies). As a consequence, it will only suppress cells that see the same nominal antigen, indicating that a second interaction site between the molecular complex and the acceptor cell is required for functional activity, and this interaction is most likely brought about by an antigen bridge. The antigen-specific molecule also must carry an anti-I-J (linked) specificity for it to hook up to the I-J piece of the schlepper chain. Lastly, for the reasons listed above, somewhere in the C terminal portion of the antigen-binding chain is the biologically active information signal.

The question of whether the situation we described is unique for the suppressor molecule(s) we described, or whether it can act as a general model for other antigen-specific T cell informational molecules, must be addressed. With a very important caveat, we think that a generalization is possible for two reasons: (a) with three of three different suppressor molecules that we analyzed, two separate macromolecules are required for the deliverance of a biologically active signal (3, 5). Other workers (6, 7) also report requirements for two separate chains to achieve antigen-specific biological activity; (b) the similarity between the mechanism by which the factor we described works and the way diphtheria and other toxins work (10) indicates to us that the mechanism of action is an example of a biological generalization. In the diphtheria model there are two chains, as there are in our model. One of these chains (the B
chain) acts as a schlepper molecule in that it brings the A chain to the appropriate receptor site on the target cell. Without this focusing event, the A chain, which contains the information signal, cannot enter the cell. In the case of the diptheria toxin, the schlepper chain is linked to the functional unit by a disulfide bond; in the system we described, the linkage appears to be an I-J anti-I-J immune-like interaction, but it is possible that the I-J anti-I-J reaction is simply helping the formation of a disulfide linkage. In any case, the mechanism by which the two chains link up to one another is not the important issue, as there might be more than one mechanism by which this could happen. There is evidence in some studies on T cell molecules that there might be disulfide bonding of the two chains also (8). The important generalization we wish to make is of a two chain model where one chain acts as a schlepper molecule to focus the functional molecule on the appropriate cell receptor so that it may enter the cell and perform its function somewhere in the cytoplasmic or nuclear portion of the acceptor cell. (The caveat mentioned above is that we do not propose that all immunological schlepper chains use the same gene products to perform their functions as the one we described herein.)

The model we propose is somewhat more sophisticated than the diptheria model. This is because of the antigen specificity that is imparted in our system by an antigen-specific receptor on the informational molecule. Thus, it could be said that the T suppressor informational molecules are analogous to diptheria toxin except that in the immune situation there is a dual recognition.

A number of very important questions have been brought up by this work, for which we have no firm answers as yet, so rather than discussing alternatives, we will just list the questions raised. (a) How does a product of the 17th chromosome (e.g., I-J) get onto the schlepper chain that is likely to be a product of the 12th chromosome (Igh) for two reasons: (i) it has an Igh-V-linked specificity and this specificity is not H-2 linked, indicating that it is separate from the 17th chromosome; and (ii) in other studies, we raised an antiserum that reacts with this schlepper chain and, using somatic cell hybrids to absorb the antisera, have been able to show that this chain contains determinants that are structural gene products of the 12th chromosome (11). (b) Where is the gene(s) that imparts anti-I-J activity to the antigen-specific informational chain encoded, and what is its relationship to the anti-nominal antigen specificity of that chain? Are the two recognition units (one for nominal antigen and the one for I-J) on different domains of a single molecule, and, if so, how do they get there? Clearly, this molecule can see antigen quite well, and its ability to see antigen is in no way dependent upon its anti-MHC specificity.

These important, as yet unanswered, questions are presently being investigated. However, at the present time, we would like to put forth the notion that all antigen-specific T cell informational molecules require an interaction between two separate molecules (at least), one a schlepper molecule and one a functional molecule, and that the molecular complex has a dual recognition. The schlepper chain sees a cell surface receptor ("self" in immunological parlance) necessary for focusing the functional molecule. (In these studies we have reported that the receptor is Igh-V linked or encoded but, as stressed, need not be in other circumstances. It is quite possible that I-J+ T cell informational molecules that have schlepper chains that impart an H-2 restriction interact with different cell surface receptor molecules, most likely regulated by the H-2 complex itself.)
The second important point is that the molecule that is being schlepped has an antigen-specific receptor on it, and, when that receptor is present, the schlepped molecule will only work on cells with which it can form an antigen bridge (perhaps if the antigen-specific portion of the molecule were to be cleaved by an appropriate protease, the molecular complex would then become antigen nonspecific but would still function in the same way) (8, 9).

To sum up, we should return to the title of this manuscript. The three focusing events required to deliver the biologically active signal are (a) an I-J anti-I-J hook up of a schlepper molecule and a functional molecule; (b) an Igh-V-linked (anti-Igh/V?) hook up of the molecular complex with a target cell's surface receptor; and (c) an antigen bridge that focuses the informational molecule on the target cell with the appropriate specificity for nominal antigen.

Summary

We described a T suppressor factor made by an I-J + Ly-2 T cell (Ly-2 TsF) that expresses biological activity only when its acceptor cell shares H-2-linked polymorphic genes with the cells that made the Ly-2 TsF (or when the producer cell had differentiated in a thymic environment where the gene products of the acceptor cell were expressed). The Ly-2 TsF requires the presence of I-J + Ly-1 cells in the assay culture to express its suppressive activity, although removal of the I-J + Ly-1 cells from the assay cultures does not affect the ability of those cultures to respond to antigen in the absence of the Ly-2 TsF. We have been able to replace the I-J + Ly-1 cells in the assay cultures with an I-J + soluble factor derived from them. This I-J + molecule not only fails to bind antigen but is also antigen nonspecific in that it can come from Ly-1 cells making factors of irrelevant specificities. For the I-J + molecule to replace the activity of the I-J + Ly-1 cell in the assay population, in restoring suppressive function in cultures depleted of I-J + Ly-1 cells, it must share genetic polymorphisms linked to the I-J subregion with the Ly-2 TsF and genetic polymorphisms linked to Igh-V with the target cell.

These results indicate that an I-J + antigen-nonspecific molecule combines with an antigen-specific Ly-2 TsF via an I-J anti-I-J "type" of interaction. The resultant molecular complex is focused on a cell surface receptor of the acceptor cell. This focusing event is controlled by the antigen-nonspecific I-J + molecule, and the precise interaction with the receptor on the acceptor cell is controlled by Igh-V-linked polymorphic gene products. The antigenic specificity of the interaction is controlled by a receptor for antigen on the I-J component of the complex.

Thus, three focusing events are required for Ly-2 TsF to express biologic activity: (a) the Ly-2 TsF must be focused on an acceptor cell that has the same antigenic specificity (most likely via an antigen bridge); (b) it must also be focused onto an I-J + antigen-nonspecific molecule that we refer to as a "schlepper" molecule (most likely via an I-J anti-I-J bridge); and (c) the schlepper molecule must focus the molecular complex on an Igh-V-controlled receptor on the antigen-specific target cell.

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References

1. Yamauchi, K., D. B. Murphy, H. Cantor, and R. K. Gershon. 1981. Analysis of an antigen-specific H-2-restricted, cell-free product(s) made by "I-J" Ly-2 cells (Ly-2 TsF) that suppresses Ly-2 cell-depleted spleen cell activity. Eur. J. Immunol. 11:913.
2. Yamauchi, K., D. B. Murphy, H. Cantor, and R. K. Gershon. 1981. Analysis of antigen-specific, Ig-restricted cell-free material made by I-J+ Ly-1 cells (Ly-1 TsiF) that induces Ly-2+ cells to express suppressive activity. Eur. J. Immunol. 11:905.

3. Yamauchi, K., N. Chao, D. B. Murphy, and R. K. Gershon. Molecular composition of an antigen-specific, Ly-1 T suppressor inducer factor: one molecule binds antigen and is I-J+; another is I-J-, does not bind antigen, and imparts an Igh-variable region-linked restriction. J. Exp. Med. 155:655.

4. Cunningham, A. J., and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody. Immunol. 14:599.

5. Ptak, W., R. W. Rosenstein, and R. K. Gershon. Interactions between molecules (subfactors) released by different T cell sets that yield a complete factor with biological (suppressive) activity. Proc. Natl. Acad. Sci. U. S. A. 79:2375.

6. Taniguchi, M., T. Saito, I. Takei, and T. Tokuhisa. 1981. Presence of interchain disulfide bonds between two gene products that compose the secreted form of an antigen-specific suppressor factor. J. Exp. Med., 153:1672.

7. Taussig, M. J., and A. Holliman. 1979. Structure of an antigen-specific suppressor factor produced by a hybrid T-cell line. Nature (Lond.). 277:308.

8. Fresno, M., G. Nabel, L. McVay-Boudreau, H. Furthmayr, and H. Cantor. 1981. Antigen-specific T lymphocyte clones. I. Characterization of a T lymphocyte clone expressing antigen-specific suppressive activity. J. Exp. Med. 153:1246.

9. Fresno, M., L. McVay-Boudreau, G. Nabel, and H. Cantor. 1981. Antigen-specific T lymphocyte clones. II. Purification and biological characterization of an antigen-specific suppressive protein synthesized by cloned T cells. J. Exp. Med. 153:1260.

10. Pappenheimer, A. M., Jr., and M. Moynihan. 1981. Diptheria toxin: a model for translocation of polypeptides across the plasma membrane. In Receptor-Mediated Binding and Internalization of Toxins and Hormones. J. L. Middlebrook and L. D. Kohn, editors. Academic Press, Inc., New York. 31.

11. Flood, P., A. B. DeLeo, L. J. Old, and R. K. Gershon. The relation of cell surface antigens on 3-methylcholanthrene-induced fibrosarcomas to Igh-V linked T cell interaction molecules. Proc. Natl. Acad. Sci. U.S.A. In press.