TWO DISTINCT ANTIGEN-SPECIFIC
SUPPRESSOR FACTORS INDUCED BY THE
ORAL ADMINISTRATION OF ANTIGEN*

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It has been known since the turn of the century that antigen-specific suppression of antibody production can be induced by the oral administration of antigen (1, 2). It was later shown that this mode of antigen administration also suppressed the ability of an animal to respond to the antigen with a delayed-type hypersensitivity response (3). Both of these phenomena have been repeatedly verified with modern techniques using protein (4–7) as well as cellular (8–10) antigens. It has further been shown that antigen-specific suppressor T cells (Ts)1 are formed after the feeding of protein antigens (7, 11), contact sensitizing agents (6), and cellular antigens (9). Nonimmunoglobulin serum suppressor factors (10) and antigen-antibody complexes (8, 12) have also been reported to mediate unresponsiveness after antigen feeding. The present experiments demonstrate that antigen feeding leads to the production of at least two distinct, antigen-specific suppressor factors.

Our previous studies of rats fed sheep erythrocytes (RBC) (SRBC) have shown that both Ts and soluble T cell-derived suppressor factors (TsF) are induced (9).2 Numerous reports characterizing TsF produced by mice have appeared (13–23), including reports of TsF specific for SRBC. Because immunoregulatory circuits and T cell subsets are more fully characterized in mice than in rats, and because congenic mice make genetic experiments accessible in mice, in the present experiments TsF were induced in mice by feeding with SRBC.

We report here that the oral administration of antigen leads to the release of at least two different antigen-specific TsF by spleen cells. These TsF differ from one another in the subsets of T cells they influence as well as in the genetic restrictions they demonstrate in inducing suppression. Their ability to bind to SRBC has also allowed us to prepare antisera specific for each factor in sheep.

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1 Abbreviations used in this paper: BRBC, burro erythrocytes; FrI, fraction I; FrII, fraction II; HRBC, horse erythrocytes; IgH, immunoglobulin heavy-chain gene complex; MHC, major histocompatibility complex; PFC, plaque-forming cell(s); RBC, erythrocytes; SRBC, sheep erythrocyte(s); Ts, suppressor T cells; TsF, T cell-derived suppressor factors.
2 Mattingly, J. A., and B. H. Waksman. Immunologic suppression after oral administration of antigen. II. Antigen-specific helper and suppressor factors produced by spleen cells of rats fed sheep erythrocytes. J. Immunol. In press.

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Materials and Methods

**RBC.** SRBC were obtained (Colorado Serum Co., Denver, Colo.) in sterile Alsever's solution. Before use, they were washed three times in cold RPMI-1640 (Microbiological Associates, Walkersville, Md.) and resuspended to the desired concentration. Horse RBC (HRBC) and burro RBC (BRBC) (Colorado Serum Co.) were similarly prepared.

**Mice.** C57BL/6, BALB/c, SJL/J, and DBA/2 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. C57BL/10, B10.D2, and BALB.B were bred at Yale University (New Haven, Conn.), and were kindly donated by Dr. D. B. Murphy. C.AL20, BAB-14, and CB.20 were from our own colony at Yale University. All mice used were 6–10 wk old.

**Feeding of SRBC.** C57BL/6 mice were ether anesthetized and given 0.3 ml of a 50% SRBC suspension via stomach tube. The feedings were carried out daily for at least 5 d, and the mice were killed 18–24 h after the last feeding. In some experiments, BALB/c and B10.D2 were also treated in this manner.

**Generation of Factors.** Spleen cells from the SRBC-fed mice (and unfed mice as controls) were washed three times in cold RPMI-1640 and were cultured in the same medium in a 5% CO₂ atmosphere at a concentration of 2 × 10⁸ cells/ml in 50-ml vessels (flask 3023; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). In every 50 ml of culture, 0.1 ml of 10% SRBC was added. After 48 h, the suspension was centrifuged at 5,000 g for 20 min, and the supernate was concentrated 10–20 times with an Amicon diafiltration unit (Amicon Corp., Scientific Sys. Div., Lexington, Mass.) using a PM-10 filter. 5 ml of this concentrate was placed on a 2.5 X 100-cm Sephadex G-100 column (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.), which was flowing at a constant rate of 0.2 ml/min with phosphate-buffered saline, pH 7.2, as the column buffer. The void volume that contained the immunoglobulins was discarded, and two distinct peaks were eluted from the column. One peak had a 60–75 × 10⁶ mol wt (fraction I [FrI]), and the other peak had a 30–40 × 10⁶ mol wt (fraction II [FrII]). Each fraction was concentrated to 2 ml, dialyzed against RPMI-1640 for 24 h at 5°C, and stored at −70°C in 0.2-ml aliquots after filtration with a 0.22-μm Millipore filter (Millipore Corp., Bedford, Mass.). Molecular weights were estimated by using a series of molecular weight standards to calibrate the Sephadex G-100 column: aldolase (158,000 mol wt); bovine serum albumin (BSA) (68,000 mol wt); ovalbumin (45,000 mol wt); and chymotrypsinogen A (25,000 mol wt).

**Absorption of Factors.** 0.5 ml of packed SRBC or HRBC was incubated with 1 ml of FrI or FrII for 45 min in a 37°C water bath. The cells were removed by centrifugation, and the resultant supernates were then tested for activity along with a sample from the same batch that had undergone the same incubation treatment without the RBC.

**Preparation of Anti-Factor Antisera.** Samples of blood were collected from two sheep (2318 and 2537) in sterile bottles that contained citrate at the Yale animal facilities. 2 ml of packed freshly drawn cells from sheep 2318 were incubated with 2 ml of FrI at 37°C for 45 min, and the same was done with the cells of sheep 2537 and FrII. After this adsorption step, the cells were washed once and brought up to 3 ml with phosphate-buffered saline and each was mixed with 3 ml of complete Freund's adjuvant. The thick paste that resulted after vigorous mixing was then injected back into the sheep from which the SRBC were drawn. Intramuscular injections of 1.5 ml in each of four sites were given to each sheep. 1 mo later, the procedure was repeated with complete adjuvant. 4 wk after the second injection, the sheep were bled out, and the sera were tested against each of the factors by Ouchterlony gel diffusion and were then stored at −20°C.

**Cell Separation.** In some experiments, purified T and B cells were prepared according to the method of Wysocki and Sato (24). Briefly, plastic Petri dishes (8-757-12, Fisher Scientific Co., Fair Lawn, N. J.) were precoated with goat anti-mouse immunoglobulin, and 3 ml (2 × 10⁷ cells) of spleen cells was incubated on these plates for 70 min at 4°C. The nonadherent cells were removed and were used as a purified T cell population. After additional washes and a 45-min incubation at 37°C, the adherent cells were removed and used as a population of purified B cells. Staining with appropriate antisera showed only a slight (1–3%) cross-contamination.

In some cases, the purified T cells were further treated with anti-Lyt-2.2 serum (a gift of Dr. Fung-Win Shen, Memorial Sloan-Kettering Cancer Center, New York) plus complement. For this treatment, 2 × 10⁷ T cells were suspended in 2.5 ml of a 1:25 dilution of anti-Lyt-2.2 sera and incubated for 30 min at 37°C. After washing, the cells were resuspended in 2.5 ml of a 1:
10 dilution of rabbit complement for 30 min at 37°C. The cells were then washed three times, and staining revealed that ~30% of the treated cells were viable, with only 1-2% showing positive fluorescence with anti-Lyt-2.2 sera (25).

**Cell Culture.** 1 ml of a spleen cell suspension that contained $10^7$ cells was placed in each well of a tissue culture plate (3008; Falcon Labware, Div. of Becton, Dickinson & Co.). A modification of the Mishell and Dutton culture system (26) was used, because the medium consisted of RPMI-1640 plus 10% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.), supplemented with 2 mM 1-glutamine (Grand Island Biological Co.), $5 \times 10^{-5}$ M 2-mercaptoethanol (Matheson, Coleman, & Bell, East Rutherford, N. J.), gentamicin (Schering Corp., Kenilworth, N. J.), and Hepes buffer (Sigma Chemical Co., St. Louis, Mo.) to pH 7.3. All cultures contained 0.025 ml of 1% SRBC, HRBC, or BRBC ($\sim 2 \times 10^8$ RBC), and some also received 0.2 ml of a soluble factor preparation. Control cultures received either 0.2 ml of a control factor preparation, or were simply adjusted to volume with 0.2 ml RPMI-1640. These cultures were incubated for either 3 or 5 d at 37°C in a 5% CO₂ atmosphere with 100% humidity. The use of this modified medium eliminated the need for daily culture nutritive additives.

Triplicate cultures were assayed for direct plaque-forming cells (PFC) (27) using the Cunningham and Szenberg modification (28). Results are reported as mean PFC/culture ± SEM.

**Results**

**Generation of Specific, Nonimmunoglobulin Suppressor Factors.** The supernate from cultures of spleen cells from SRBC-fed mice was always suppressive when assayed in an in vitro anti-SRBC Mishell-Dutton culture. Because antibody is known to be suppressive of this response, the supernate was fractionated on Sephadex G-100. The void volume was discarded; two peaks of approximate molecular weight 60,000–75,000 (Fr I) and 30,000–40,000 (FrII) were obtained. Table I demonstrates that each of these peaks contained active suppressive material, and that suppression was specific for SRBC, because the addition of the factor to in vitro cultures in which HRBC or BRBC were antigens did not suppress. Suppression titered out for both FrI and FrII at >1:10. All subsequent experiments use FrI and FrII at 1:6–1:10.

**Both Factors Bind Specifically to the Antigen.** Because of the specificity for SRBC of both of the factors, we next tested for binding of the factors to specific antigen. Both FrI and FrII bind to SRBC but do not bind to HRBC or BRBC (Table II). Thus, antigen specificity of function reflects a specific antigen binding site on each factor.

**Time-Course of Suppression.** We next examined the time-course of the effect of each factor during primary in vitro responses to SRBC (Table III). Both factors suppress the response when PFC are measured at day 5 of culture. However, FrII added at the initiation of the culture led to marked increases in the number of SRBC PFC found on day 3 of culture, after which responses in the presence of FrII steadily declined to suppressed levels by day 5 of culture. FrI was suppressive at all times in all experiments.

**Genetic Restrictions in the Effects of Suppressor Factors.** Because interactions of T cells or T cell-derived factors with other cells are frequently restricted by genetic differences at various loci, we next examined the ability of our suppressor factors to interact with spleen cells derived from various strains of mice. In particular, we determined the role of major histocompatibility complex (MHC) genes, immunoglobulin heavy-chain gene complex (Igh) genes, and genes in the B10 background on these factors.

As seen in Table IV, studies with three different sets of FrI derived from different strains of mice show no effect of MHC- or Igh-linked genes on the activity of this factor. However, FrI will not suppress responses in any strain with the B10 background.
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Table I
Both FrI and FrII Specifically Suppress Day-5 SRBC-PFC Formation by B6 Spleen Cells In Vitro

| Factor | Final dilution | Antigen | Day-5 PFC |
|--------|----------------|---------|-----------|
| None   | —              | SRBC    | 5,460 ± 380 |
| FrI    | 1:5            | SRBC    | 633 ± 166  |
| FrI    | 1:10           | SRBC    | 800 ± 100  |
| FrI    | 1:25           | SRBC    | 3,660 ± 233 |
| FrI    | 1:50           | SRBC    | 5,100 ± 650 |
| FrI    | 1:100          | SRBC    | 5,220 ± 490 |
| FrII   | 1:5            | SRBC    | 820 ± 180  |
| FrII   | 1:10           | SRBC    | 640 ± 60   |
| FrII   | 1:25           | SRBC    | 2,880 ± 333 |
| FrII   | 1:50           | SRBC    | 5,866 ± 666 |
| FrII   | 1:100          | SRBC    | 5,900 ± 300 |
| None   | —              | HRBC    | 2,120 ± 166 |
| FrI    | 1:10           | HRBC    | 1,880 ± 210 |
| FrII   | 1:10           | HRBC    | 2,280 ± 133 |
| None   | —              | BRBC    | 1,620 ± 180 |
| FrI    | 1:10           | BRBC    | 1,680 ± 100 |
| FrII   | 1:10           | BRBC    | 1,500 ± 133 |

B6 spleen cells were cultured with antigen ± factor and PFC determined on the immunizing RBC after 5 d; values represent mean ± 1 SE.

Table II
Both FrI and FrII Bind Specifically to SRBC

| Cells   | Factor | Adsorption* | SRBC PFC/culture |
|---------|--------|-------------|------------------|
| B6 spleen | None   | —           | 6,430 ± 333      |
| B6 spleen | FrI    | —           | 1,420 ± 180      |
| B6 spleen | FrI    | SRBC        | 6,000 ± 440      |
| B6 spleen | FrI    | HRBC        | 1,640 ± 233      |
| B6 spleen | FrI    | BRBC        | 2,480 ± 200      |
| B6 spleen | FrII   | —           | 2,660 ± 266      |
| B6 spleen | FrII   | SRBC        | 5,140 ± 340      |
| B6 spleen | FrII   | HRBC        | 3,140 ± 140      |
| B6 spleen | FrII   | BRBC        | 2,980 ± 100      |

Underlined values are suppressed. Factors were added to cultures of B6 spleen cells plus SRBC and PFC determined on day 5 of culture.

* Incubated with RBC for 45 min at 37°C. See Materials and Methods for details.

(including B10.A[2R]) (data not shown), even though B10.D2 and B10.A[2R] (not shown) produce active FrI. This is reminiscent of a TsF described by Taniguchi et al. (29), which would also not suppress any B10 strain. This restriction is also strong
### Table III

**Kinetics of Activity of FrI and FrII: Induction of Early Help and Feedback Suppression by FrII**

| Cells          | Factor* | Day 3   | Day 4    | Day 5         |
|----------------|---------|---------|----------|---------------|
| B6 spleen      | None    | 0       | 60 ± 100 | 1,640 ± 220  |
| B6 spleen      | FrI     | 0       | 50 ± 50  | 170 ± 133    |
| B6 spleen      | FrII    | 4,900 ± 666 | 900 ± 133 | 330 ± 160   |

* Factors were added at day 0 of culture.

### Table IV

**Genetic Restriction of Action of FrI TsF on Spleen Cell Anti-SRBC Responses**

| Responding strain | Background | H-2 | Ig-H | Percent suppression using FrI from |
|-------------------|------------|-----|------|-----------------------------------|
|                   |            |     |      | C57BL/6 | B10.D2 | BALB/c |
|                   |            |     |      | %       |        |        |
| C57BL/6           | C57BL/6    | b   | b    | 86      | —       | —      |
| B10               | B10        | b   | b    | 0       | —       | —      |
| B10.D2            | B10        | d   | b    | −4      | −3      | −18    |
| BALB/c            | BALB       | d   | a    | —       | 88      | 70     |
| C.B20             | BALB       | d   | b    | —       | 100     | 100    |
| C.AL20            | BALB       | d   | e    | —       | 100     | 100    |
| DBA/2             | DBA/2      | d   | c    | 84      | —       | —      |
| SJL/J             | SJL        | s   | b    | 94      | —       | —      |

Percent suppression = (1 − response in presence of TsF/response in absence of TsF) × 100. Absence of suppression and matching disparities are boxed.

### Table V

**Genetic Restriction of Action of FrII TsF on Spleen Cell Anti-SRBC Responses**

| Responding strain | Background | H-2 | Ig-H | Percent suppression using FrII from |
|-------------------|------------|-----|------|-----------------------------------|
|                   |            |     |      | C57BL/6 | B10.D2 | BALB/c |
|                   |            |     |      | %       |        |        |
| C57BL/6           | C57BL/6    | b   | b    | 71      | —       | —      |
| B10               | B10        | b   | b    | 85      | —       | —      |
| B10.D2            | B10        | d   | b    | 89      | 68      | −15    |
| BALB/c            | BALB       | d   | a    | —       | 14      | 84     |
| BALB.B            | BALB       | b   | a    | —       | −9      | 74     |
| C.B20             | BALB       | d   | b    | —       | 100     | 0      |
| C.AL20            | BALB       | d   | e    | —       | 13      | 27     |
| DBA/2             | DBA/2      | d   | c    | −19     | —       | —      |
| SJL/J             | SJL        | s   | b    | 82      | —       | —      |

See Table IV legend.
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TABLE VI

Effect of TsF on B Cells, B Cells Plus Ly-1 T Cells, and Spleen Cells

| Responding population | Day-5 PFC response to SRBC with* |
|-----------------------|----------------------------------|
|                       | Experiment 1 | Experiment 2 |
|                       | Control | FrI | FrII | Control | FrI | FrII |
| B cells‡             | 140 ± 80 | 620 ± 120 | 300 ± 66 | 70 ± 66 | 90 ± 40 | 180 ± 133 |
| B cells plus Ly-1 T cells§ | 5,420 ± 433 | 5,800 ± 300 | 9,880 ± 520 | 540 ± 120 | 500 ± 80 | 2,200 ± 233 |
| Whole spleen          | ND      | ND   | ND   | 3,840 ± 300 | 940 ± 100 | 1,960 ± 266 |

* TsF produced by C57BL/6J mice fed SRBC.
‡ Produced by anti-mouse Ig plate separation (24) of spleen cells from C57BL/6J. ND, not done.
§ B cells plus Ly-1 T cells prepared by lysis of plate-purified T cells with anti-Ly-2.2 and complement.

TABLE VII

Cells Receiving the Factors*

| Pretreated cells‡ | Factors | PFC/culture on day 5 on precultured cells added to |
|-------------------|---------|---------------------------------------------------|
|                   |         | B cells | B cells + T cells |
| None              |         | 40 ± 66 | 3,100 ± 300 |
| T cells           | —       | 1,720 ± 333 | 1,720 ± 266 |
| T cells FrI       |         | 480 ± 120 | 0 |
| T cells FrII      |         | 500 ± 66 | 300 ± 120 |
| Ly-1 T cells      | —       | 2,620 ± 366 | 3,380 ± 500 |
| Ly-1 T cells FrI  |         | 2,950 ± 400 | 3,040 ± 40 |
| Ly-1 T cells FrII |         | 5,100 ± 220 | 1,240 ± 133 |

* FrI is accepted by Ly-2⁺ T cells to induce suppression, whereas FrII is accepted by Ly-2⁻ T cells (to induce enhancement in the absence of Ly-2⁺ cells and to induce feedback suppression in the presence of Ly-2⁺ cells).
‡ C57BL/6-pretreated T cells were cultured for 96 h with SRBC and the corresponding factors, harvested, washed, and added to a new 5-d culture of responding cells. Each cell population listed numbered 5 × 10⁶ cells, and no additional factors were added to these cultures after transfer of pretreated cells.

Evidence against trivial explanations for the activity of FrI, such as that it is antigen or anti-SRBC antibody.

The results obtained with FrII (Table V) reveal a different pattern of restriction. Here, MHC-linked genes and genes in the B10 background do not restrict the effect of FrII on the day 5 PFC response. However, in all cases in which the Igh genes of the responding cells differ from those of the FrII producer strain, no suppression is seen. Studies with allotype congenics such as the C.B20, which is a BALB/c mouse with Igh genes derived from C57BL, clearly map this restriction to the Igh-linked genes. Again, this finding negates trivial explanations of the activity of this factor. The finding of Igh-linked restriction in a factor that appears to induce a feedback-type of suppression is reminiscent of the recent finding of a similar restriction in feedback suppression by Eardley et al. (30).

Analysis of the Mode of Action of Suppressor Factors. At present, three facts point to T cells as the producers of these suppressor factors: antigen specificity, non-Ig nature,
and the fact that spleen cells treated with anti-Thy-1.2 and complement do not produce suppressor factors (negative data not shown). However, the T cell nature of the producing cells has not been proven with purified T cells.

On the other hand, the cells activated by each suppressor factor have been partially characterized. First, the factors were added to cultures of B cells alone, or cultures of B cells plus Ly-1 T cells (or whole spleen). As seen in Table VI, neither factor affected (helped) either day-3 (not shown) or day-5 responses of B cells. When added to B cells plus Ly-1 T cells, FrI did not suppress, but FrII led to increased responses on day 3 and day 5 of culture. Because both factors suppress the day-5 response of whole spleen, this suggested that FrI was a suppressor inducer factor similar to that described by Tada (31), which required an Ly-2+ cell (Ly-123 or Ly-23) for its action, whereas FrII induced heightened activity in a cell set that had both helper and inducer of feedback suppression activities, similar to the Ly-1, Qa-1+ T cell set defined by the experiments of Cantor et al. (32) and McDougal et al. (33).

To further explore this possibility, purified T cells or purified Ly-1 cells were cultured for 4 d with SRBC and either FrI or FrII. The cells were then recovered, thoroughly washed, and added to either B cells or B cells plus T cells from normal mice. The results are given in Table VII. FrI has no effect when precultured with anti-Ly-2-treated cells, whether these are added to B cells or B cells plus T cells. However, when it is cultured with unfractionated T cells, it induces potent suppression in these cells for both responses. In contrast, FrII has very pronounced effects on anti-Ly-2-treated cells. When Ly-1 cells precultured with FrII and SRBC are added to B cells, a marked increase in helper activity at day 5 is seen. However, when these same cells are added to B plus T cell cultures, potent suppression is observed. Thus, FrII would appear to activate inducers of feedback suppression that are also Ly-1 helper T cells.

Discussion

These experiments demonstrate that the oral administration of antigen can induce, some days later, the production of at least two suppressive factors by spleen cells. These factors are both antigen specific and nonimmunoglobulin in nature, and their production is sensitive to anti-Thy-1.2 and complement. Thus, it is highly likely that they are produced by T cells. However, this needs to be confirmed by positive-selection experiments now in progress.

It is our goal in these experiments to relate the activity of these factors to the known immunoregulatory circuits defined in several experimental systems. Thus, FrI most closely resembles the TsF of Tada et al. (19, 29, 31), in that it will not work on strains with the B10 background, and does not affect Ly-1 helper cells directly. It rather serves to activate suppression from Ly-2+ T cells (Ly-123 or Ly-23 cells, or both). Preliminary data suggest that FrI is bound by anti-I-J antibody. However, it is not apparently MHC restricted in its activity. In this, it more closely resembles a series of TsF isolated by Benacerraf and his colleagues (14–16), which are antigen specific, I-J positive, and induce suppression in T cell populations that have not been further defined by anti-Ly fractionation. Perhaps these two sets of factors form different parts of the same suppressor-amplification loop, involving signals delivered by an Ly-23 cell to an Ly-123 cell, leading to increased Ly-23 suppressor activity.

FrII appears to be a novel suppressor factor. It apparently acts by inducing a subset
of Ly-1 T cells that has both potent helper activity and the ability to induce feedback suppression. Thus, FrII would appear to activate the Ly-1, Qa-1+ subset defined as containing both a helper T cell and the inducers of feedback suppression by Cantor et al. (32). We believe both activities (help and induction of feedback suppression) derive from a single cell. This hypothesis is now being tested with these factors. The finding that the activity of FrII was restricted only by Igh-linked genes is also similar to the finding of such a restriction in the activity of the inducers of feedback suppression described by Eardley et al. (30). Preliminary studies suggest FrII is I-A positive and I-J negative.

Besides further studies aimed at defining the producing and accepting cells for these factors, their chemical nature is also being investigated. Thus far, we have succeeded in producing sheep anti-factor antisera, each of which will precipitate novel proteins from the surface of radio-iodinated mouse T cells (R. E. Cone, R. W. Rosenstein, and C. A. Janeway. Unpublished data.). Anti-FrI will also give a line in Ouchterlony diffusion with purified anti-trinitrophenyl TsF prepared by the method of Zembala and Asherson (23). These antisera should allow the purification of these activities from a variety of different culture supernates. Finally, our studies will attempt to demonstrate that the incubation of these inducing TsF with their target populations gives rise to a set of effector TsF currently being analyzed by K. Yamauchi and R. K. Gershon (Manuscript in preparation.) at Yale University.

Summary

The feeding of sheep erythrocytes (SRBC) to mice leads to the production of two distinct T cell-derived suppressor factors by spleen cells. Each has been characterized for specificity, genetic restrictions, and cellular interactions.

Fraction I has a 60,000-75,000 mol wt, is specific for antigen, and is suppressive of primary in vitro anti-SRBC responses at all times. It is not restricted by major histocompatibility complex (MHC)- or Igh-linked genes, but it fails to suppress spleen cells derived from any strain of mouse with a B10 background. It acts on an Lyt-2+ T cell to increase suppressive activity. An antiserum has been prepared against this factor that reacts with other, unrelated T cell suppressor factors.

Fraction II has an ~30,000-40,000 mol wt, is specific for antigen, and has a dual effect on in vitro anti-SRBC responses. On day 3 of culture, it leads to augmentation of the response, whereas at day 5 it suppresses the response. It is not restricted by MHC genes, but it is restricted by Igh-linked genes. It acts by activating an Ly-1 T cell to both help and induce feedback suppression.

These factors, and the antisera prepared against them, should allow more precise dissection of the molecular pathways by which immunoregulatory cells communicate with one another.

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