PROPERTIES OF ANTIGEN-SPECIFIC SUPPRESSIVE
T-CELL FACTOR IN THE
REGULATION OF ANTIBODY RESPONSE OF THE MOUSE
I. In Vivo Activity and Immunochemical Characterizations*

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Our previous publications (1, 2) indicated that thymocytes and spleen cells of mice that had been immunized with a high dose of carrier antigen, when transferred into syngeneic host, could suppress the antibody response against a hapten coupled to the homologous carrier. This suppressive effect was found to be mediated by a population of thymus-derived lymphocytes, whose activity was completely abrogated by the treatment with anti-\( \theta \) and complement. Moreover, the specificity of the observed suppression has been established by the fact that neither normal T cells nor those obtained from mice immunized with an unrelated antigen could suppress the antibody response. These results indicate that immunization with a relatively high dose of carrier antigen generated a subpopulation of T cells that specifically inhibits the antibody response against hapten on the same carrier. Similar antigen-specific T-cell-mediated suppression has recently been demonstrated in a number of other experimental systems (3–10), and is now considered to be an important regulatory mechanism in various forms of immune responses (reviewed in 11).

A possible molecular mechanism of the antigen-specific suppression is that certain primed T cells liberate a suppressive factor(s) which then gives rise to an "off" signal to other cell types via combination with antigen. There are several examples of soluble T-cell factors that influence the magnitude and quality of the immune response (12–24). Thus, the above findings prompted us to explore the subcellular component of T cells which can mediate the antigen-specific suppression of the antibody response. We have attempted to separate such a soluble component by simple sonication followed by ultracentrifugation of the thymocytes and spleen cells possessing the suppressor activity, since we were successful in obtaining an antigen-specific suppressive factor from sonicated T-cells of the rat, which had shown a strong inhibitory effect on an ongoing IgE antibody response of the same species (19, 25). This paper will describe some of the properties of the antigen-specific suppressive T-cell factor obtained in mice, which was found to suppress mainly IgG antibody response of syngeneic mice in an in vivo experimental system.

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Materials and Methods

**Antigens.** Keyhole limpet hemocyanin (KLH)\(^1\) was purchased from Calbiochem, San Diego, Calif. Bovine serum albumin (BSA) and bovine gamma globulin (BGG) were obtained from the Nutritional Biochemical Corporation, Cleveland, Ohio. The following 2,4-dinitrophenyl (DNP) conjugates of these proteins were prepared by the method of Eisen et al. (26): DNP\(_{30}\)-KLH (assuming the average mol wt of KLH as 7,000,000); DNP\(_{35}\)-BGG, and DNP\(_{35}\)-BSA. Subscripts refer to the numbers of DNP groups per molecule of carrier protein. Bordetella pertussis vaccine was purchased from the Chiba Serum Institute, Chiba, Japan.

**Animals.** Randomly bred BALB/c AnN and DBA/2 mice were raised in our animal facility. C57BL/6J mice were supplied from the National Institute of Radiological Science, Chiba, Japan. All mice were used at 8- and 12-wk old.

**Immunization.** To test the primary antibody response, mice were immunized with an intraperitoneal injection of 100 \(\mu\)g of DNP-KLH or DNP-BGG mixed with 1 \(\times\) \(10^9\) \(B.\) pertussis vaccine. The antibody response was estimated by enumerating the DNP-specific plaque-forming cells (PFC) in their spleen 6 days after the immunization by the method of Cunningham and Szenberg (27) using sheep erythrocytes coated with DNP\(_{35}\)-BSA by chromium chloride. Direct PFC were considered to be IgM antibody-producing cells, and indirect PFC only developed with a 1:250 dilution of rabbit antimouse IgG antiserum to be IgG antibody producers.

To obtain carrier-primed thymocytes and spleen cells, mice were immunized with two intraperitoneal injections of 100 \(\mu\)g of soluble KLH or BGG without adjuvant at a 2 wk interval. Animals were killed 2 wk after the second injection, and their thymuses and spleens were removed and processed as below.

**Preparation of Cell-Free Extracts from Primed Thymocytes and Spleen Cells.** The thymuses and spleens of KLH- or BGG-primed mice were placed in a small quantity of chilled Eagle’s minimal essential medium (MEM) in Petri dishes. They were minced and teased with forceps, and then gently pressed between two glass slides to release the cells from fibrous tissues. The cells were washed three times with cold MEM, and were resuspended in a small amount of borate-buffered saline at a concentration of 5 \(\times\) \(10^9\) cells/ml. The cell suspensions were then subjected to sonication for 2 min in ice water. Cell-free extracts were obtained by ultracentrifugation at 40,000 \(g\) for 1 h. These extracts will be designated as KLH- or BGG-primed thymocyte (T) extract and spleen cell (S) extract.

**Antisera.** A polyvalent antiserum against mouse immunoglobulins (Ig’s) was raised by repeated injections of washed precipitates of diphtheria toxoid and mouse antitoxoid antibodies in rabbits. Antisera specific for mouse IgG, IgM, and Fab fragment were prepared by immunization of rabbits with purified proteins followed by appropriate absorption. The specificity of these antisera was confirmed by immunodiffusion and immunoelectrophoresis.

Antimouse thymocyte serum (ATS) was produced in rabbits by repeated immunizations with washed mouse thymocytes in complete Freund’s adjuvant (CFA). The pooled serum was absorbed with mouse erythrocytes and bone marrow cells. The resulting antiserum killed 100% of the thymocytes, 35% of the spleen cells and less than 5% of the bone marrow cells at a dilution of 1:64.

The maximal dilution of antiserum for 50% cytotoxicity for thymocytes was 1:256.

Three alloantisera against the H-2 complex were utilized: antisera against the products of whole H-2\(^d\) (B10 anti-B10.D2) and left-hand side (K end) of H-2\(^d\) (B10.A anti-B10.D2) were kindly provided by Dr. B. Benacerraf of the Department of Pathology, Harvard Medical School, Boston, Mass. An antiserum reactive with only the products of right-hand side (D end) of H-2\(^d\) [(B10 \(\times\) LP.RIII)F, anti-B10.A(5R)] (serum designation, D-13) was provided by the Jackson Laboratories through the courtesy of the National Institute of Health, Bethesda, Md.

**Preparation of Immunoabsorbents.** Insoluble immunoabsorbents composed of antigens and antibodies were prepared by the method of Axén et al. (28). 2 ml of Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) were activated by cyanogen bromide and allowed to react with 20–40 \(\mu\)g of antigens or gamma globulin fraction of antisera for 6 h. Residual active sites of

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\(^1\) *Abbreviations used in this paper: AEF, allogeneic effect factor; ATS, antimouse thymocyte serum; BGG, bovine gamma globulin; BSA, bovine serum albumin; CFA, complete Freund’s adjuvant; EA, hen’s egg albumin; KLH, keyhole limpet hemocyanin; PFC, plaque-forming cells; S extract, spleen cell extract; T extract, thymocyte extract.*
Sepharose were blocked by adding excess amounts of BSA. They were washed thoroughly with borate-buffered saline, and were packed in 0.7 x 15.0 cm columns. The absorption of the T-cell factor was performed by gradually passing the extract through the column at 4°C.

**Gel Filtration.** The T or S extract was dialysed against borate-buffered saline, pH 8.0, and applied to a column (2.5 x 90 cm x 2) of Sephadex G-200 (Pharmacia Fine Chemicals, Inc.). The elution was performed with a constant upward flow of 10 ml/h at 4°C. Fractions of 2.6 ml were collected and monitored with a spectrophotometer at 280 nm. For an estimation of the molecular weight of the active component, the column was calibrated by passing marker proteins of known molecular weights (mouse IgM, IgG, albumin, and hen's egg albumin [EA]) immediately after the extract had been excluded. Fractions corresponding to the marker proteins were pooled as shown in the results, concentrated by pressure dialysis, and then tested for their activity. The molecular weight ranges of fractions were calculated by the method of Andrews (29).

**Digestion with Enzymes.** The T extract was digested with DNase (from beef pancreas; 2,000 Kunitz U/mg, Sigma Chemical Co., St Louis, Mo.), RNase (50 Kunitz U/mg, C. F. Boehringer and Sons, Mannheim, Germany), and Pronase (Pronase P from Streptomyces griseus, 1,200 tyrosine U/mg; Kaken Chemical Co., Tokyo, Japan) as follows: 1 ml of the T extract obtained from 1 x 10⁸ cells was incubated with 50 μg of DNase or RNase at room temperature for 1 h. The digestion with Pronase was performed at 37°C at a concentration of 100 μg of enzyme/1 ml of the extract. All digested materials were immediately tested for their activity.

**Experimental Procedure.** The T or S cell extract from carrier-primed mice was injected intravenously into syngeneic (in some cases allogeneic) mice that were concomitantly immunized with 100 μg of DNP-KLH or DNP-BGG plus pertussis vaccine. Direct and indirect PFC responses were estimated 6 days after the immunization at the time when control animals were producing the greatest number of DNP-specific indirect PFC. All the absorbed materials, fractions, and digests were tested for their activity by a similar procedure. The geometric means and standard deviations were calculated from the logarithmically transformed PFC numbers of at least six similarly treated animals. P values were determined by the student's t test.

### Results

**Suppression of Hapten-Specific Antibody Response by Carrier-Specific T and S Extract.** It has previously been shown that the passive transfer of thymocytes and spleen cells from KLH-primed mice into syngeneic normal mice suppresses antibody response of the host against DNP-KLH (1). Thus in the present experiment, the cell-free extracts of thymocytes and spleen cells obtained from KLH-primed BALB/c mice were tested for their suppressive activity in the syngeneic recipient. The extracts corresponding to 1 x 10⁸ original thymus or spleen cells were inoculated intravenously into normal BALB/c mice that were subsequently immunized with DNP-KLH and pertussis vaccine. As a control, groups of mice were given T or S extracts from donors immunized with an unrelated antigen (BGG), and were similarly immunized with DNP-KLH. DNP-specific PFC response was examined 6 days after the immunization, since the previous study indicated that KLH-primed suppressor T cells caused maximal suppression of IgG (indirect PFC) response on day 6 (1).

The upper part of Table I shows the result of one experiment in which one can see a significant suppression of indirect (IgG) PFC response in groups given KLH-primed T and S extracts. BGG-primed T or S extracts produced no such suppression. Direct (IgM) PFC response was not significantly suppressed in repeated experiments.

The lower part of Table I shows the result of a reverse experiment in which DNP-BGG was used as immunizing antigen. In this case, the inoculation of BGG-primed T and S extracts produced a statistically significant (P < 0.01)
suppression of PFC response of the IgG class, whereas KLH-primed T or S extracts displayed no such activity. Thus, the results indicate that the suppression is specific for the carrier of the immunizing antigen.

**Affinity of the Suppressive T-Cell Factor for Antigen.** Since the suppression of antibody response caused by T and S extracts was found to be carrier specific, the possibility that the suppressive factor possesses an affinity for antigen was examined by absorption with antigens. The T extract from KLH-primed donors was absorbed by passing it through a column of immunoabsorbent composed of KLH or BGG, and the effluent corresponding to the $10^8$ original cell number was injected into mice that were subsequently immunized with DNP-KLH. As shown in Table II, the KLH-primed T extract after absorption with KLH immunoabsorbent lost most of its suppressive activity, while that absorbed with BGG immunoabsorbent well retained the suppressive activity.

**Absence of Ig Determinants on Suppressive T-Cell Factor.** Since the suppressive T-cell factor had specificity and affinity for carrier antigens, it could be an Ig. This possibility was tested by absorbing the T-cell extract with various
immunoabsorbents composed of anti-Ig antibodies. The suppressive T-cell extract was passed through anti-Ig immunoabsorbent columns, and then likewise injected into test animals which then were immunized with DNP-KLH.

As shown in the lower part of Table II, none of the anti-Ig columns, i.e. polyvalent antimouse Ig's, anti-Fab, anti-\( \mu \), and anti-\( \gamma \) could remove the suppressive activity. These results exclude the possibility that the suppressive T-cell factor belongs to any known classes or fragments of Ig.

**Absorption of Suppressive T-Cell Factor by Antithymocyte Serum and Anti-H-2 Alloantisera.** To determine the antigenic characteristics of the suppressive T-cell component, the KLH-primed T extract was absorbed with immunoabsorbents composed of gamma globulin fractions of rabbit ATS and anti-H-2 alloantisera. As shown in Table III, absorption with ATS largely removed the suppressive activity, indicating that the suppressive factor contains antigen which is shared with normal thymocytes. The alloantiserum reactive with the products of the whole \( H-2 \) complex (anti-\( H-2^d \)) was also capable of absorbing the suppressive activity. Thus, in order to determine whether the suppressive factor contains antigen(s) coded for by genes in the \( K \) or \( D \) half of the \( H-2 \) complex, two alloantisera directed to either the \( K \) or \( D \) end of \( H-2^d \) were tested for their absorbing capacity. B10.A anti-B10.D2 contains antibodies against the expression of the \( K^d \), \( I-A^d \), and \( I-B^d \) subregions, while (B10 \( \times \) LP.RIII)F\(_1 \) anti-

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**Table II**

Absorption of the Suppressive T-Cell Factor of KLH-Primed Mice with Antigens and Antibodies

| Extract injected | Absorbed with * | Anti-DNP PFC/spleen \( \text{†} \) |
|-----------------|-----------------|--------------------------|
|                 |                 | Direct                   |
| —               | —               | 7,550 \( \pm \) 1.75     |
| —               | Anti-DNP        | 5,860 \( \pm \) 2.02     |
| —               | KLH             | 7,050 \( \pm \) 1.42     |
| —               | BGG             | 8,360 \( \pm \) 1.54     |
| KLH-primed T extract | —               | 7,640 \( \pm \) 1.60     |
| KLH-primed T extract | KLH             | 820 \( \pm \) 3.65 §     |
| KLH-primed T extract | BGG             | 5,830 \( \pm \) 1.78     |
| KLH-primed T extract | Anti-Igs        | 640 \( \pm \) 3.77     |
| KLH-primed T extract | Anti-Fab        | 330 \( \pm \) 5.12     |
| KLH-primed T extract | Anti-\( \mu \)   | 910 \( \pm \) 3.25 §     |
| KLH-primed T extract | Anti-\( \gamma \) | 540 \( \pm \) 5.03     |
| KLH-primed T extract | Anti-\( \gamma \) | 200 \( \pm \) 2.84     |

* Absorption was carried out by passing the T extract through a column of immunoabsorbent composed of each antigen or antibody.
† Geometric means and standard deviations.
§ \( P < 0.01 \) as compared with the control response.
### TABLE III
Absorption of the Suppressive T-Cell Factor with ATS and Anti-H-2 Antibodies

| Extract injected                  | Absorbed with* | Anti-DNP PFC/spleen† |
|-----------------------------------|----------------|---------------------|
|                                   |                | Direct              |
|                                   |                | 7,420 ± 2.37        |
| KLH-primed T extract              | —              | 7,420 ± 2.37        |
| KLH-primed T extract              | ATS            | 8,640 ± 1.43        |
| KLH-primed T extract              | Anti-H-2d      | 7,260 ± 2.02        |
|                                   | [B10.A(5R) anti-B10.D2] | 7,080 ± 2.29        |
| KLH-primed T extract              | Anti-H-2d(I-Cd, Sd, Dd) | 9,280 ± 3.32        |
|                                   | [(B10 × LP.R1IDF1) anti-B10.A(5R)] | 6,400 ± 2.30        |
|                                   |                | 15,000 ± 2.32       |
|                                   |                | 2,300 ± 2.49§       |
|                                   |                | 13,400 ± 1.43       |
|                                   |                | 16,000 ± 1.21       |
|                                   |                | 12,700 ± 2.18       |
|                                   |                | 936 ± 5.21‖         |

* Absorption was carried out by passing the extract through a column of immunoadsorbent composed of gamma globulin fraction of each antiserum.

† Geometric means and standard deviations.

§ P < 0.01.

‖ P < 0.02.

B10.A(5R) contains antibodies reactive with antigens coded for by genes in the I-Cd and Dd subregions. The results in Table III clearly indicate that the former antiserum specific for the K end of H-2d is capable of removing the suppressive activity while the latter (anti-D end) is ineffective in absorbing the activity. Hence, it was concluded that the suppressive factor contains antigen(s) coded for by genes in the left-hand side of the H-2 complex in addition to an antigen present on mouse T cells in general.

**Failure of the Suppressive T-Cell Factor to Suppress the Antibody Response of Allogeneic Mice.** Since the suppressive T-cell component contains antigen coded for by genes in the H-2 complex, we examined whether the factor obtained from one strain of mice could suppress the antibody response of other strains having different H-2 histocompatibility. KLH-primed suppressive T extract was obtained in BALB/c (H-2d) and C57BL/6 (H-2b) mice. These extracts were capable of suppressing the antibody response against DNP-KLH of syngeneic recipient (Table IV). However, the T extract from C57BL/6 mice failed to suppress the response of BALB/c mice. This cannot be ascribed to the induction of nonspecific stimulation due to allogeneic effect, since the T extract from unprimed C57BL/6 mouse produced no such stimulation. Similarly, the extract from BALB/c mice having strong suppressive activity in syngeneic hosts produced no suppression in C57BL/6 mice. In contrast, when the BALB/c T extract was given to DBA/2 mice, which have the same H-2d histocompatibility as BALB/c mice, a statistically significant suppression was observed (Table IV). Thus, it appeared that the suppressive T-cell factor could not act across the barrier of H-2 histocompatibility.
TABLE IV

Failure of Suppression of Antibody Response against DNP-KLH by the T-Cell Factor
Obtained from Histo-incompatible Donors

| Recipient strain | Donor strain | Primed with* | Anti-DNP PFC/spleen† |
|------------------|--------------|--------------|----------------------|
|                  |              | Direct       | Indirect             |
| BALB/c           | —            | 6,340 ± 1.59 | 7,710 ± 1.38         |
| BALB/c           | BALB/c       | 2,030 ± 3.19 | 580 ± 3.348          |
| BALB/c           | C57BL/6      | 6,530 ± 2.05 | 6,870 ± 1.31         |
| BALB/c           | C57BL/6      | 6,810 ± 1.27 | 8,370 ± 1.26         |
| C57BL/6          | —            | 10,400 ± 1.81| 10,700 ± 1.67        |
| C57BL/6          | C57BL/6      | 3,030 ± 1.58 | 1,560 ± 3.63         |
| C57BL/6          | BALB/c       | 14,900 ± 1.99| 11,100 ± 2.03        |
| C57BL/6          | BALB/c       | 8,830 ± 2.04 | 10,300 ± 1.75        |
| DBA/2            | BALB/c       | 7,710 ± 1.69 | 11,100 ± 1.25        |
| DBA/2            | BALB/c       | 7,740 ± 1.32 | 3,520 ± 2.19         |

* Donor animals were either unprimed or primed with two injections of soluble KLH.
† Geometric means and standard deviations.
§ P < 0.001.
|| P < 0.05.
¶ P < 0.01.

The Molecular Weight of the Suppressive T-Cell Factor. The S extract from KLH-primed mice was fractionated by gel filtration with Sephadex G-200. Fractions corresponding to the known marker proteins were separated as shown in Fig. 1. Fraction I is the protein peak eluted with the void volume, fraction II corresponds to mouse IgG, fraction III to serum albumin, and fraction IV contains the second protein peak which is eluted slightly faster than EA. The pooled fractions were concentrated and likewise tested for their suppressive activity.

As presented in Table V, the suppressive activity was exclusively contained in fraction IV, which was eluted slower than serum albumin and slightly faster than EA. The approximate mol wt range of fraction IV was calculated to be between 35,000 and 60,000, being definitely smaller than the values for usual Ig's.

Chemical Nature of the Suppressive T-Cell Factor. The suppressive S extract was digested with nucleases and proteinase, and then immediately injected into mice that were then immunized with DNP-KLH. As shown in Table
FIG. 1. Elution pattern of S extract obtained from KLH-primed mice from a Sephadex G-200 column. Arrows indicate the positions of the marker proteins eluted at the identical condition. Four fractions (Fr) were separated as indicated, and were tested for the presence of suppressive activity after concentration.

TABLE V
Suppressive Effect of Sephadex G-200 Fractions of KLH-Primed S Extract

| Fraction* injected | Anti-DNP PFC/spleen†        |
|-------------------|-----------------------------|
|                   | Direct                      | Indirect                   |
| —                 | 9,320 x 1.55               | 7,490 x 1.38               |
| Fr. I             | 7,680 x 1.67               | 7,440 x 1.65               |
| Fr. II            | 14,160 x 1.33              | 7,130 x 1.82               |
| Fr. III           | 8,000 x 1.68               | 6,380 x 1.56               |
| Fr. IV            | 2,430 x 4.65               | 252 x 3.24§                |

* Fractions of KLH-primed S extract separated by gel filtration through a Sephadex G-200 column (see Fig. 1).
† Geometric means and standard deviations.
§ P < 0.001.

VI, the treatment with DNase and RNase did not affect the suppressive activity, while the digestion with Pronase completely destroyed the suppressive activity. The activity was found to be labile by heating the extract at 56°C for 1 h. When the extract was kept in a refrigerator at 4°C for a week, the suppressive activity disappeared completely (Table VI). These results indicated that the active component is an extremely labile protein.

Discussion

Our previous publications (1, 2) reported that passive transfer of thymocytes and spleen cells from mice primed with a carrier antigen suppresses the anti-
body response of normal syngeneic hosts against a hapten coupled to the homologous carrier. This suppression was found to be clearly antigen specific, and mediated by T cells primed by the carrier antigen. We had attempted to separate a subcellular component of such antigen-specific suppressor T cells, since we were successful in obtaining an antigen-specific soluble component from primed T cells of rats which specifically inhibited the IgE antibody response of rats immunized with the same antigen (19, 25).

In the present communication, we have reported the presence of a similar carrier-specific soluble factor that suppresses antibody response of mice against a hapten coupled to the homologous carrier. This factor was extractable from physically disrupted thymocytes and spleen cells of mice immunized with carrier antigen, and upon inoculation into syngeneic hosts, suppressed the antibody response against a hapten on the homologous carrier. Although the suppressive effect was not as strong as that of live suppressor T cells, the injection of the factor invariably caused a statistically significant suppression of indirect (IgG) PFC response of the host.

The specificity of suppression was warranted by two independent experiments. In the present studies, the factor obtained from KLH-primed mice could suppress the response to DNP-KLH, while failing to suppress the response to DNP-BGG. The reverse was true for the factor obtained from BGG-primed mice. Furthermore, the suppressive activity of KLH-primed T-cell extract was completely removed by the immunoadsorbent composed of KLH but not that of BGG. The results clearly indicate that the specificity of suppression is based on the specific binding of the suppressive T-cell factor to the determinant present on the carrier molecule.
Despite its definite specificity and affinity for the carrier molecule, attempts to remove the suppressive T-cell factor with anti-Ig immunoadsorbents were always unsuccessful. None of the antibodies directed to Ig's and their fragments were able to remove the suppressive activity from the extract. The results indicate that the suppressive T-cell factor is not Ig in nature. This conclusion was further supported by its physicochemical properties. The factor has an approximate mol wt between 35,000 and 60,000, which is considerably less than those of conventional Ig's. These immunochemical and physicochemical properties of the mouse T-cell factor are much the same as those of the antigen-specific suppressive T-cell factor of the rat, which had produced a strong suppressive effect on an ongoing IgE antibody response of the species (19, 25).

The suppressive factor described in the present study undoubtedly originated from carrier-primed T cells. It was previously shown that suppressor activity of parental live cells was associated with thymocytes and θ-bearing spleen cells, their activity being abrogated by treatment of the cells with anti-θ or ATS and complement. Furthermore, in the present experiment it was shown that the suppressive activity of thymocyte extract was completely removed by passing it through a column of immunoabsorbent composed of ATS. The latter finding indicates that the suppressive T-cell factor contains a common antigenic determinant shared by the thymocytes and splenic T cells of various mouse strains, since the ATS used in this study is reactive with these cell types of different mouse strains.

The availability of alloantisera directed to the products of genes in the H-2 complex in the mouse enabled us to study further the antigenic characteristics of the suppressive T-cell factor. It was found that the alloantiserum against H-2d could absorb the suppressive activity with great efficiency. Furthermore, alloantisera containing antibodies directed to the K end (H-2K, I-A, and I-B) of H-2d were capable of absorbing the suppressive activity, while the serum reactive only with the D end (I-C, SsSlp, and H-2D) had no absorbing capacity. The results suggest that suppressive T-cell factor contains a product of genes in either the K or I region of the H-2 complex. It will be reported in another paper that the T-cell factor is, in fact, an I region gene product. It is most interesting that the antibody response to DNP-KLH is definitely regulated by the product of I region genes, since the response to KLH per se is not under Ir-1 gene control (30).

In accordance with the above genetic characteristics of the T-cell factor, it appeared that the suppressive factor could only suppress the response of H-2 histocompatible strains. The factor derived from BALB/c mice could suppress the response of BALB/c and DBA/2 strains possessing the same H-2d haplotype, while failing to suppress the response of C57BL/6 (H-2b) mice, in which the syngeneic C57BL/6 factor exerted a significant suppressive influence. This apparent failure of suppression by the H-2 histoincompatible T-cell factor is not due to the induction of nonspecific stimulation by the given T-cell extract, since the normal allogeneic T-cell extract did not enhance or suppress the response of the host.
The immunochemical and physicochemical properties of the suppressive T-cell factor are much like those of the antigen-specific cooperative T-cell factor reported by Taussig and his associates (22, 31-33), although the effect is quite opposite. Their factor is released from educated T cells by a short-term culture with antigen, and cooperates with bone marrow cells to induce antibody response to the same antigen in irradiated hosts. Although their factor could cooperate with H-2 histoincompatible bone marrow cells (32), the factor contains antigen coded for by genes in the left-hand side of the H-2 complex (33). It has specificity and affinity for the immunizing antigen, but is not an Ig in nature (22, 31). The molecular size of their cooperative factor is apparently very close to that of the presently described suppressive T-cell factor (33). Thus, the most important question is to what differences in the molecular structure can the opposite activity be ascribed. Although some important differences in the mode of release and the target of the T-cell factor will be presented in a subsequent paper, it is apparent that both factors may share an identical structure while the other molecular characteristics may be different. Furthermore, the suppressive T-cell factor seems to have considerable structural similarity to the allogeneic effect factor (AEF) described by Armerding and his associates (23, 34) with respect to the physicochemical and antigenic properties, although the AEF has no specificity for immunizing antigen. Elucidation of the interrelationship between these multiple T-cell factors seems to be of crucial importance.

The chemical nature of the suppressive T-cell factor is still unclear. It has only been demonstrated so far that the active factor is a protein with extreme lability. Heating at 56°C for 1 h and storage at 4°C for 1 wk completely destroyed the suppressive activity. This lability as well as the large quantity required for in vivo suppression restricted further analysis in the present experimental system.

Summary

An antigen-specific suppressive T-cell factor was extracted from physically disrupted thymocytes and spleen cells of mice that had been immunized with soluble protein antigens. The factor, when inoculated into syngeneic normal mice, could induce a significant suppression of IgG antibody response against a hapten coupled to the carrier protein by which the donor of the suppressor factor was immunized. The suppressor factor was found only effective in suppressing the antibody response of syngeneic or H-2 histocompatible recipients. The suppressive T-cell factor was removed by absorption with immunoadsorbent composed of the relevant antigen, but not with any of those of anti-immunoglobulin antibodies. The factor was successfully removed by alloantibodies with specificity for the K end (H-2K, I-A and I-B) of the H-2 complex of the donor strain, but not by those for the D end (I-C, SsSlp, and H-2D). The activity was removed by absorption with a heterologous antithymocyte serum. The mol wt of the suppressive T-cell factor was between 35,000 and 60,000 as determined by Sephadex G-200 gel filtration. The suppressive T-cell factor was found to be a heat-labile protein.

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