# ACTIVATION OF ANTIGEN-SPECIFIC SUPPRESSOR T CELLS BY B CELLS FROM MICE IMMUNIZED WITH TYPE III PNEUMOCOCCAL POLYSACCHARIDE*

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Although there is much evidence to indicate that the magnitude of the antibody response to the capsular polysaccharide of type III *Streptococcus pneumoniae* (SSS-III)[1] is regulated by suppressor as well as amplifier T lymphocytes (1–7), the phenomenon of low-dose paralysis provides an ideal experimental model for examining the properties of suppressor T cells and their interaction with other lymphoid cells engaged in the immune response. In this antigen-specific T-cell-dependent form of unresponsiveness, which persists for several weeks after its induction, mice pretreated (primed) with a single subimmunogenic dose of SSS-III make a greatly reduced antibody response upon subsequent immunization with an optimally immunogenic dose of antigen (3, 6, 8). Recent studies have shown that low-dose paralysis to SSS-III can be transferred with spleen cells from primed mice (4); the transfer of such suppression, which likewise is antigen-specific, requires Thy-1$^+$, Lyt-1$^-$, 2$^+$, I-J$^+$ lymphocytes (5).

The mechanism(s) by which suppressor T cells are activated in mice immunized or primed with SSS-III has not yet been defined. However, we have proposed, based on the results of previous studies, that suppressor T cells are activated in response to the idiotypic determinants of B-cell-associated antibody specific for SSS-III (6, 7). If this is indeed the case, it should be possible to induce significant suppression with B cells transferred from mice either primed or immunized with SSS-III; the kinetics for the induction and expression of such unresponsiveness should resemble those noted for the phenomenon of low-dose paralysis. Also, it should be possible to transfer suppression with T lymphocytes after it has been induced by immune B cells. The present study was designed to provide experimental evidence in support of this hypothesis.

## Materials and Methods

*Mice.* Female BALB/cCum mice (8–10 wk of age), obtained from Cumberland View Farms, Clinton, TN, were used throughout this work.

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[1] *Abbreviations used in this paper:* C′, complement; GAMIg, goat anti-mouse immunoglobulin; HBSS, Hanks' Balanced Salt Solution; Ig$^+$, immunoglobulin bearing; Ig$^-$, nonimmunoglobulin bearing; PFC, plaque-forming cell; SRBC, sheep erythrocytes; SSS-III, type III pneumococcal polysaccharide.

*Antigen and Immunization Procedure.* The immunological properties of the preparations of SSS-III and dextran B-1355 used, as well as the method by which they were prepared have been described (3, 8–12). Mice were given (i.p.) appropriate doses of SSS-III or dextran B-1355 in 0.5 ml saline as stated in the text.

*Immunologic Methods.* Numbers of antibody-producing plaque-forming cells (PFC) specific for SSS-III provided a measure of the magnitude of the antibody response produced at peak, i.e., 5 d after immunization with an optimally immunogenic dose (0.5 μg) of SSS-III. PFC making antibody of the IgM class (>90% of all PFC [9, 11]) were detected by a slide version of the technique of localized hemolysis-in-gel (8, 10–12) using indicator sheep erythrocytes (SRBC) sensitized with SSS-III by the $CrCl_3$ method (13). PFC making antibody specific for dextran B-1355 were detected by a similar procedure (12) 5 d after immunization (i.p.) with an optimally immunogenic dose (100 μg) of this antigen. In all cases, corrections were made by subtraction for numbers of background SRBC-specific PFC found (<200 PFC per spleen) so that only PFC making antibody specific for SSS-III or dextran B-1355 are considered in this work. The values obtained (PFC per spleen), which are log-normally distributed (14), are expressed as the geometric mean of the $\log_{10}$ number of PFC per spleen for groups of similarly treated mice; this also provides a reasonably good measure of the total antibody response produced (15). Previous studies have shown that SSS-III and dextran B-1355, both of which are helper T-cell-independent polysaccharide antigens, do not cross-react serologically and that immunization with one antigen does not influence the magnitude of the antibody response to the other (12).

Student's *t*-test was used to assess the significance of the differences observed. Differences were considered to be significant when probability (*P*) values <0.05, were obtained.

*Treatment of Cells with Monoclonal Antibodies.* Monoclonal antibodies specific for Lyt-2.2 (NEI-006) or Thy-1.2 (NEI-001A) mouse lymphocyte surface antigens were purchased from New England Nuclear, Boston, MA. The procedure used for treating spleen cells with these monoclonal antibodies in the presence of complement (C′), as well as information concerning their specificity and immunologic characteristics, have been given (4). In all cell transfer experiments, mice were injected (i.v.) with known numbers of antibody-treated cells in a volume of 0.2 ml Medium 199.

*Isolation of Spleen Cells by the Plate-separation Procedure.* Immunoglobulin-bearing ($Ig^+$), as well as nonimmunoglobulin-bearing ($Ig^-$) cells, were recovered from spleen cell suspensions by the plate-separation method of Wysocki and Sato (16). Here, affinity-purified goat anti-mouse Ig antibody (GAMIg; 0.5 mg protein/ml) was used. The specificity and method of preparation of GAMIg, which contains anti-IgG1, -IgG2A, -IgG2B in equal proportions, have been described (17). GAMIg was diluted (1:10) in 0.05 M Tris, pH 9.5, and 10 ml of the resulting solution were poured onto plastic petri plates (100 mm × 15 mm; Fisher Scientific Co., Silver Spring, MD). The plates were held for 1 h at room temperature and then overnight at 4°C; they were washed extensively with phosphate-buffered saline, pH 7.4, just before use.

Suspensions of spleen cells in Hanks' Balanced Salts Solution (HBSS) first were treated with ACK lysing buffer (18) to eliminate intact erythrocytes. The remaining cells were washed five times with HBSS and resuspended to a final density of $10^7$ nucleated cells/ml in Medium 199. Then, 10 ml of cell suspension were added to petri plates coated with affinity-purified GAMIg antibody. The plates were kept stationary at 4°C for 40 min, after which they were swirled gently to redistribute unattached cells and held at 4°C for 30 min; then, the remaining unattached ($Ig^-$) cells were decanted.

In order to recover adherent ($Ig^+$) spleen cells from the plates, the procedure described above was modified slightly in that the plates were coated beforehand with a 1:50, rather then 1:10, dilution of affinity-purified GAMIg antibody. After unattached cells were decanted, the plates were washed gently (four times) with HBSS. Then, 5 ml of 10% normal mouse serum in HBSS was added to each plate. After the plates were held for 2–4 min at room temperature (25°C) the attached ($Ig^+$) cells became loose; they were recovered by rinsing the plates several times with HBSS.

The purity of the cell suspensions recovered by the plate-separation procedure was assessed by fluorescence-activated cell sorter analysis (Becton Dickinson FACS-II System,

Mountain View, CA); details concerning the reagents and procedures used for this purpose have been given elsewhere (19). In the experiments to be described, the nonadherent cell suspension used was found to contain <2% $Ig^+$ and 96% Thy-$1.2^+$ cells; the adherent cell suspensions contained >97% $Ig^+$ cells. For convenience, these will be referred to as plate-separated $Ig^-$ and $Ig^+$ cell suspensions, respectively.

*Isolation of Antigen-binding B cells from Suspensions of Immune Spleen Cells.* Antigen-binding B cells specific for SSS-III were isolated by the adsorption, and then elution, of immune spleen cells from a Sepharose 6MB column to which SSS-III had been bound covalently. In order to prepare the column, an amino derivative of SSS-III first was synthesized. Here, 50 mg of SSS-III (10 mg/ml in borate saline buffer, pH 8.0) was placed in a beaker kept in an ice bath. Then, 0.67 ml (0.1 mol) of anhydrous ethylenediamine and 0.25 ml of 0.1 M sodium metaperiodate were added with constant stirring. After 30 min, 1.25 ml of 2.0 M sodium borohydride was added and the mixture was stirred for 1 h; at the end of this time period, the solution was dialyzed against several changes of borate saline buffer. The amino derivative of SSS-III was conjugated directly to cyanogen bromide (CNBr)-activated Sepharose 6MB (Pharmacia Chemicals, Piscataway, NJ), at a ratio of 11 mg SSS-III/ml of beads using a standard procedure for conjugating proteins to CNBr-activated Sepharose. The beads were washed extensively with several volumes of HBSS to remove all traces of unbound SSS-III. Also, a Sepharose 6MB column in which all reactive sites were blocked by the addition of excess 0.2 M glycine—in place of the amino derivative of SSS-III—was prepared for use as a specificity control (glycine-Sepharose-6MB).

Spleen cells from mice immunized or primed with SSS-III were treated with ACK lysing buffer (18), washed (3×) with HBSS, and resuspended in Medium 199 at a density of $10 \times 10^7$ cells/ml. For the recovery of unbound cells, 1.5 ml of spleen cell suspension was applied to a column consisting of 6 ml of either SSS-III or glycine-coated Sepharose 6MB beads in a 12-ml syringe. Then ~10 drops of Medium 199 were added to allow the cells to perfuse further into the column. The column was incubated for 30 min at 4°C; then the unbound cells were eluted (drop by drop) by the addition of 18 ml HBSS. For the recovery of bound cells, the incubation time was reduced to 15 min at 4°C. The column was washed free of all unbound cells with 50 ml HBSS. After a period of agitation in HBSS and allowing the beads to settle, the bound cells which then became loose were decanted.

*Preparation of Amplifier T Cells.* Mice were pretreated (primed) with a subimmunogenic dose (0.005 μg) of SSS-III. 3 d later, spleen cell suspensions were prepared and treated with monoclonal anti-Lyt-2.2 antibody plus C′ to remove suppressor T cell activity (4). The resulting cell suspension has been shown to be a relatively rich source of amplifier T cell activity (5).

*Irradiation Procedure.* Mice were given whole body irradiation by exposure to a $^{137}Ce$ source at a dose rate of 139 rads/min.

## Results

*Induction of Antigen-specific Suppression of the Antibody Response with B Cells from Mice Immunized with SSS-III.* Groups of 5–10 mice were given a single injection (i.p.) of 0.5 μg SSS-III; 48 h later, when few—if any—SSS-III-specific PFC can be detected (9), spleen cell suspensions were prepared from which adherent ($Ig^+$) cells were isolated by the plate-separation procedure. Then, known numbers of immune $Ig^+$ cells were transferred (i.v.) to mice that were immunized (i.p.), 3 d later, with both 0.5 μg SSS-III and 100 μg dextran B-1355; recipient mice were assayed for numbers of SSS-III-specific and dextran-specific PFC 5 d after immunization. The results obtained were compared with those of immunized mice not given $Ig^+$ immune spleen cells, or those of immunized mice given $Ig^+$ spleen cells from either nonimmunized mice or mice immunized only with

dextran B-1355.

The data of Table I show that the transfer of $3.4 \times 10^6$ $Ig^+$ spleen cells from mice immunized with SSS-III greatly reduced ($P < 0.05$) the capacity of recipient mice to make an antibody response to an optimally immunogenic dose (0.5 μg) of SSS-III; the transfer of the same population of such cells had no effect upon the antibody response to dextran B-1355 ($P > 0.05$). In another experiment, the transfer of $Ig^+$ spleen cells from either nonimmunized mice or mice immunized with dextran B-1355 did not influence the capacity of recipient mice to make an antibody response to 0.5 μg SSS-III ($P > 0.05$, in both instances). The transfer of similar numbers of $Ig^-$ spleen cells was without effect (data not shown). These findings indicate that the transfer of B cells from mice immunized with SSS-III suppresses the capacity of recipient mice to make an antibody response to this antigen upon subsequent immunization. Since the suppression induced is antigen-specific, it is not an artifact of the procedure used to isolate $Ig^+$ spleen cells.

*Ability to Induce Suppression with T-Cell–depleted Immune Spleen Cells.* Since it was difficult, as well as time-consuming, to obtain large numbers of adherent $Ig^+$ immune spleen cells by the plate-separation procedure, we wished to determine whether comparable suppression also could be induced with T-cell-depleted immune spleen cells. Groups of 5–10 mice were given a single injection (i.p.) of 0.5 μg SSS-III. Then, 48 h later, spleen cell suspensions were prepared that were either transferred (i.v.) directly to recipient mice, or treated with monoclonal anti-Thy-1.2 antibody + C′ before transfer; the latter treatment has been shown to eliminate effects likely to be produced by transferred suppressor and amplifier T cells (4, 5). Recipients of transferred cells were immunized (i.p.) with both 0.5 μg SSS-III and 100 μg dextran B-1355 3 d after cell transfer; they were assayed for numbers of antigen-specific PFC 5 d after immunization.

The data of Table II show that significient ($P < 0.05$) suppression of the antibody response to SSS-III was induced following the transfer of $20 \times 10^6$

TABLE I

*Specificity of Suppression Induced by B Cells from Mice Immunized with SSS-III*

| Expt. no. | Number and type of B cells transferred | PFC/spleen* vs. | |
|---|---|---|---|
| | | SSS-III | Dextran B-1355 |
| 1 | 0 | 4.058 ± 0.081 (11,442) | 4.613 ± 0.038 (41,042) |
| | $3.4 \times 10^6$ cells‡ | 3.744 ± 0.012 (5,543) | 4.696 ± 0.099 (49,685) |
| 2 | 0 | 4.041 ± 0.051 (10,994) | — |
| | $5 \times 10^6$ cells§ | 4.014 ± 0.087 (10,332) | — |
| | $5 \times 10^6$ cells‖ | 4.000 ± 0.082 (10,000) | — |

* $Log_{10}$ PFC per spleen ± SEM for 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III and 100 μg dextran B-1355; geometric means are in parentheses.

‡ Plate-separated, $Ig^+$ spleen cells from mice, 48 h after immunization (i.p.) with 0.5 μg SSS-III.

§ Plate-separated, $Ig^+$ spleen cells from mice, 48 h after immunization (i.p.) with 100 μg dextran B-1355.

‖ Plate-separated, $Ig^+$ spleen cells from nonimmunized mice.

TABLE II
*Induction of Suppression with T-Cell–depleted Immune Spleen Cells*

| Number of cells transferred* | Treatment of transferred cells | PFC/spleen vs. | |
|---|---|---|---|
| | | SSS-III | Dextran B-1355 |
| 0 | — | 4.228 ± 0.053‡ (16,916) | 4.706 ± 0.069 (50,829) |
| $20 \times 10^6$ | None | 3.638 ± 0.124 (4,347) | 4.718 ± 0.061 (52,254) |
| $20 \times 10^6$ | Anti-Thy-1.2 ± C′ | 3.310 ± 0.109 (2,041) | — |

* Spleen cell suspensions were prepared for transfer, 48 h after donor mice were given (i.p.) 0.5 μg SSS-III.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III and 100 μg dextran B-1355; geometric means are in parentheses. Mice were immunized (i.p.) 3 d after the transfer of cells.

TABLE III
*Dose-response Relationships for the Induction of Suppression with T-Cell–depleted Immune Spleen Cells*

| Number of cells transferred* | SSS-III-specific PFC/spleen | Mean % suppression |
|---|---|---|
| 0 | 4.253 ± 0.079‡ (17,886) | — |
| $0.5 \times 10^6$ | 4.205 ± 0.060 (16,030) | 10 |
| $2.5 \times 10^6$ | 4.045 ± 0.076 (11,081) | 38 |
| $5 \times 10^6$ | 3.809 ± 0.146 (6,442) | 64 |
| $10 \times 10^6$ | 3.642 ± 0.179 (4,387) | 76 |
| $20 \times 10^6$ | 3.509 ± 0.146 (3,230) | 82 |
| $40 \times 10^6$ | 3.674 ± 0.115 (4,725) | 74 |

* Spleen cell suspension, prepared 48 h after mice were given (i.p.) 0.5 μg SSS-III, were treated with anti-Thy-1.2 + C′ as described.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III; geometric means are in parentheses. Mice were immunized, 3 d after the transfer of cells.

immune spleen cells, with or without treatment with monoclonal anti-Thy-1.2 + C′ before cell transfer; such suppression was antigen-specific since the same population of transferred cells did not influence the magnitude of the antibody response to dextran B-1355 ($P > 0.05$). The degree of suppression induced with T-cell-depleted immune cells increased with the number of cells transferred and reached maximal levels in mice given 10–40 × $10^6$ cells (Table III). Since the degree of suppression induced with 5 × $10^6$ T-cell-depleted immune cells (64%) was similar to that obtained with 3.4 × $10^6$ plate-separated $Ig^+$ immune spleen cells (52%; Table I), both types of cell suspension appeared to be comparable

with respect to their ability to induce antigen-specific suppression of the antibody response to SSS-III.

*The Induction of Suppression by Transferred Immune Spleen Cells Requires Antigen-binding B Cells.* Spleen cell suspensions were prepared from mice 48 h after immunization with 0.5 μg SSS-III. The cells were treated with ACK lysing buffer, to remove intact erythrocytes, and then monoclonal anti-Thy-1.2 antibody + C′ to inactivate T cells. A portion of the resulting cell suspension, containing ~150 × $10^6$ nucleated cells, was passed over a 6-ml Sepharose 6MB column to which either SSS-III or glycine had been bound covalently. Cells that did not adhere to the column (unbound cells) were collected, as well as cells recovered by decantation after agitation and rapid settling of the beads (bound cells). Equivalent numbers of bound and unbound cells, as well as cells from the original unfractionated cell suspension, then were transferred (i.v.) and compared for their ability to induce suppression of the antibody response to SSS-III in mice immunized with 0.5 μg SSS-III 3 d after cell transfer.

The data of Table IV show that 5 × $10^6$ spleen cells that did not bind to the SSS-III Sepharose 6MB column did not induce suppression of the antibody response to SSS-III after transfer ($P > 0.05$). However, significant suppression ($P < 0.05$) was induced after the transfer of 5 × $10^5$ bound or 5 × $10^6$ bound cells recovered from the SSS-III-Sepharose 6MB column ($P < 0.05$ in both cases). When the same cell suspension was passed through the glycine-Sepharose-6MB column, significant ($P < 0.05$) suppression was induced only with the unbound cell fraction. It should be noted that the degree of suppression induced by 5 × $10^5$ or 5 × $10^6$ SSS-III-bound cells was similar to that obtained with 20 × $10^6$ unfractionated cells. The foregoing studies indicate that the capacity of immune spleen cells to induce suppression of the antibody response to SSS-III is dependent upon the presence of $Ig^+$ cells capable of binding specifically to SSS-III.

*B Cell-induced Suppression Is Not Due to Antibody-mediated Feedback Inhibition or*

TABLE IV

*Antigen-binding Cells are Required for the Induction of Suppression*

| Column used to isolate cells | No. and type* of cells transferred | Mean % suppression after transfer‡ |
|---|---|---|
| SSS-III-Sepharose-6MB | 5 × $10^6$ Unbound | <7 ± 4 |
| | 5 × $10^6$ Bound | 56 ± 8§ |
| | 5 × $10^5$ Bound | 54 ± 8§ |
| | 1 × $10^5$ Bound | 34 ± 5 |
| Glycine-Sepharose-6MB | 5 × $10^6$ Unbound | 45 ± 11§ |
| | 5 × $10^6$ Bound | 10 ± 7 |
| | 5 × $10^5$ Bound | 12 ± 5 |
| | 1 × $10^5$ Bound | 16 ± 8 |
| — | 20 × $10^6$ Unfractionated | 67 ± 10 |

* Unfractionated cells = original cell suspension, before passage through a Sepharose 6MB column; unbound cells = cells that did not adhere to the Sepharose 6MB column; bound cells = cells recovered after adherence to the Sepharose 6MB column. Mice were immunized with 0.5 μg SSS-III 3 d after cell transfer. PFC/spleen for groups of 10 mice was determined 5 d after immunization.

‡ Calculated with respect to the control i.e. immunized mice not given immune spleen cells.

§ Significant suppression ($P < 0.05$).

*Residual Antigen.* Two experimental approaches were used to determine whether suppression was induced by antibody synthesized and released from transferred immune B cells, i.e., antibody-mediated feedback inhibition (20). In the first, two groups of mice were immunized (i.p.) with 0.5 μg SSS-III. However, one group was irradiated (1,000 rads) 48 h later, just before the removal of spleens and the preparation of spleen cell suspensions; at this time, few, if any, PFC can be detected in the spleens of mice immunized with 0.5 μg SSS-III (9). Cell suspensions from both irradiated and nonirradiated mice were treated with monoclonal anti-Thy-1.2 antibody + C′ before transfer (i.v.) to recipient mice that were immunized (i.p.) with 0.5 μg SSS-III 3 d later. Recipients were assayed for SSS-III-specific PFC/spleen 5 d after immunization.

The data of Table V show that significant suppression ($P < 0.05$) was induced after the transfer of either irradiated or nonirradiated spleen cells. It should be noted that treatment with only 250 rads of irradiation has been found to abolish completely the capacity of mice to make a detectable antibody response to SSS-III and that no serum antibody or SSS-III-specific PFC could be detected in the spleens of *unimmunized* recipient mice after the transfer of irradiated immune spleen cells (data not shown). Earlier studies (21) have shown that large amounts of passively administration high-titer immune serum, containing much more antibody than is likely to be produced under these circumstances, are required to demonstrate only a modest degree, at best, of antibody-mediated feedback inhibition; it is most unlikely that the suppression obtained is dependent upon the contained synthesis and release of antibody by transferred cells. The administration of 1,000 rads X-irradiation also has been shown to abolish suppressor T cell activity in cell transfer experiments (5).

In the second experimental approach, donor mice were given a single injection (i.p.) of a subimmunogenic dose (0.005 μg) of SSS-III which, though capable of inducing low-dose paralysis, does not result in the production of detectable SSS-III-specific PFC or serum antibody (8); 48 h later, spleen cell suspensions were prepared that were treated with monoclonal anti-Thy-1.2 antibody + C′ before

TABLE V

*Effect of Irradiation (1,000 rads) on the Capacity of Transferred Immune Spleen Cells to Induce Suppression*

| Number and type of cells transferred* | SSS-III-specific PFC/spleen‡ | Mean % suppression |
|---|---|---|
| 0 | 4.124 ± 0.042 (13,308) | — |
| $20 \times 10^6$, irradiated§ | 3.661 ± 0.086 (4,584) | 66 |
| $20 \times 10^6$, nonirradiated | 3.365 ± 0.104 (2,315) | 83 |

* Spleen cell suspensions were prepared 48 h after immunization (i.p.) with 0.5 μg SSS-III; the cells were treated with monoclonal anti-Thy-1.2 + C′ before transfer.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III; geometric means are in parentheses. Mice were immunized (i.p.) 3 d after cell transfer.

§ Donor mice were irradiated (1,000 rads) just prior to removal of spleen, i.e., 48 h after immunization (i.p.) with 0.5 μg SSS-III.

transfer to recipient mice. Recipients were immunized (i.p.) with 0.5 μg SSS-III, 3 d after cell transfer and assayed for SSS-III-specific PFC 5 d after immunization.

The data of Table VI show that significant ($P < 0.05$) suppression was induced after the transfer of spleen cells from mice given a subimmunogenic dose (0.005 μg) of SSS-III; in fact, the degree of suppression noted (78%) was at least as great as that obtained when suppression was induced with spleen cells from mice given an optimally immunogenic dose (0.5 μg) of SSS-III (Tables I–III).

The results of other studies (22) have shown that >90% of the SSS-III used in this work is excreted from the body within 48 h after immunization. Also, convincing low-dose paralysis is not induced in mice primed with <0.001 μg of SSS-III (23). In view of these considerations and the fact that spleen cell suspensions are washed several times before transfer, it is extremely unlikely that the ability to induce suppression with spleen cells from mice given a subimmunogenic dose of SSS-III (Table VI) is due to residual antigen in donor cell suspensions.

*The Transfer of Immune B Cells Results in the Activation of Suppressor T Cells.* Two types of experiments were conducted to determine whether transferred immune B cells act directly or indirectly to mediate the suppression observed. The first experiment involved an examination of the kinetics for the induction of suppression *after* the transfer of immune B cells. Donor mice were given a single injection (i.p.) of 0.5 μg SSS-III; 48 h later, spleen cell suspensions were prepared that were treated with monoclonal anti-Thy-1.2 antibody + C′ before transfer to recipient mice. Then groups of recipients, which were given (i.v.) $10 \times 10^6$ immune cells, were immunized (i.p.) with 0.5 μg SSS-III at different times after cell transfer and the magnitude of the PFC response produced was assessed 5 d after immunization. The values obtained were compared with those of immunized mice not given immune spleen cells in order to calculate the degree of suppression induced. The results obtained (Fig. 1) show that an inductive period of ~16–24 h is required before significant ($P < 0.05$) suppression is induced after cell transfer. Thereafter, the degree of suppression expressed increases progressively until maximal levels are obtained 48–72 h after cell transfer;

TABLE VI

*Ability to Induce Suppression After the Transfer of Spleen Cells from Mice Given a Subimmunogenic Dose (0.005 μg) of SSS-III*

| Number of cells transferred* | SSS-III-specific PFC/spleen‡ | Mean % suppression |
|---|---|---|
| 0 | 4.199 ± 0.077 (15,822) | — |
| $20 \times 10^6$ | 3.510 ± 0.162 (3,510) | 78 |

* Spleen cell suspensions were prepared 48 h after donor mice were given a single injection (i.p.) of a subimmunogenic dose (0.005 μg) of SSS-III; cell suspensions were treated with monoclonal anti-Thy-1.2 antibody + C′ before transfer.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III; geometric means are in parentheses. Mice were immunized (i.p.) 3 d after the cell transfer.

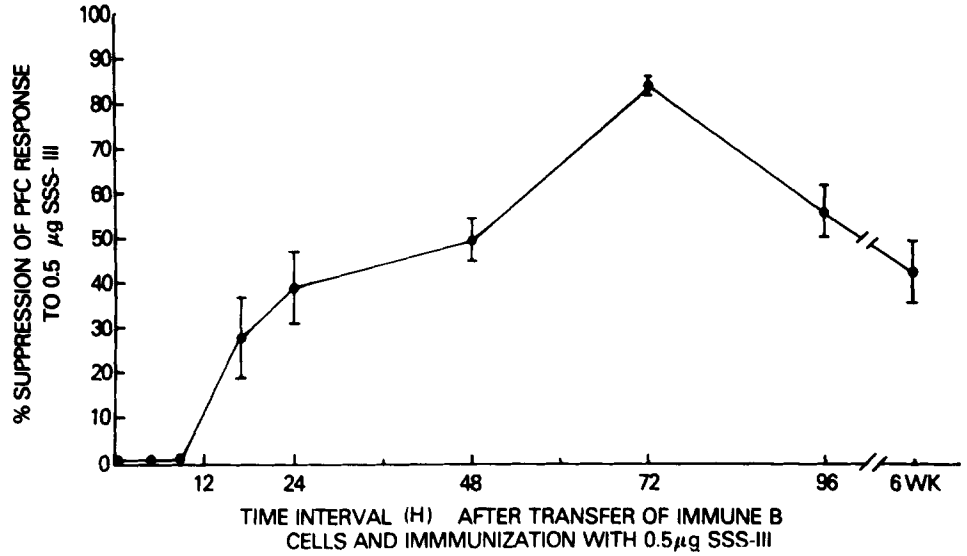


FIGURE 1. Kinetics for the induction of suppression of the PFC response to 0.5 μg SSS-III after the transfer of $10 \times 10^6$ immune B cells. Values shown represent the mean degree of suppression ± SEM for groups of 10–24 mice.

significant suppression is demonstrable for at least 6 wk after cell transfer. This kinetic pattern is similar to that noted for the induction of low-dose paralysis with a subimmunogenic dose of SSS-III (4). The latent period noted for the induction of suppression after the transfer of immune spleen cells suggests that B cells do not act directly to mediate the suppression observed.

In the second type of experiment, a two-step cell transfer procedure was used to identify the type of cell responsible for mediating suppression after the transfer of immune B cells. Donor mice were given a single injection (i.p.) of 0.5 μg SSS-III; 48 h later, spleen cell suspensions were prepared that were treated with monoclonal anti-Thy-1.2 + C′ before transfer to recipient mice. Then, 24 h after the transfer of immune B cells, spleen cell suspensions were prepared and $10 \times 10^6$ T cells, from the nonadherent fraction obtained by the plate-separation method (<2% $Ig^+$ and 96% Thy-$1.2^+$ cells), were transferred to another group of recipient mice that were immunized (i.p.) with 0.5 μg SSS-III at the time of T cell transfer (no inductive period allowed). Mice were assayed for SSS-III-specific PFC 5 d after immunization; the results obtained were compared with those of immunized mice not given T cells derived from immune B-cell-primed mice. The data of Table VIII clearly show that significant ($P < 0.05$) suppression was induced, without the need for an inductive period, in mice given T cells from donors primed with immune B cells. Thus, the suppression induced after the transfer of immune B cells is mediated by suppressor T cells which are activated in response to immune B cells.

*Abrogation of B-cell-induced Suppression by Amplifier T Cells.* The results of previous studies have shown that the antibody response to SSS-III is regulated by the competitive interaction of amplifier and suppressor T cells (1, 2, 6); such regulatory T cells, which differ with respect to their Lyt phenotype and kinetics

TABLE VII

*Suppression of the Antibody Response to SSS-III After the Transfer of T Cells from Mice Given Immune B Cells*

| Number and type of cells transferred* | SSS-III-specific PFC/spleen‡ | Mean % suppression |
|---|---|---|
| 0 | 4.206 ± 0.035 (16,062) | — |
| $10 \times 10^6$ T cells | 3.827 ± 0.131 (6,714) | 58 |

* T cells, isolated by the plate-separation procedure, were obtained from mice 24 h after the transfer of $10 \times 10^6$ immune B cells. Immune B cells were obtained 48 h after immunization (i.p.) with 0.5 μg SSS-III; these cells were treated with monoclonal anti-Thy-1.2 + C′ before transfer.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III; geometric means are in parentheses. Mice were immunized at the time of transfer of $10 \times 10^6$ T cells.

TABLE VIII

*Effect of Amplifier T Cells on the Expression of Suppression Induced by Immune B Cells*

| Number and type of cells transferred* | SSS-III-specific PFC/spleen‡ | Mean % suppression |
|---|---|---|
| 0 | 4.185 ± 0.072 (15,298) | — |
| $10 \times 10^6$ immune B cells | 3.721 ± 0.165 (5,257) | 66 |
| $10 \times 10^6$ immune B cells + $2.5 \times 10^6$ amplifier T cells | 4.332 ± 0.036 (21,491) | 0 |

* Immune spleen cells were obtained 48 h after donor mice were immunized (i.p.) with 0.5 μg SSS-III and treated with monoclonal anti-Thy-1.2 + C′ before transfer. Amplifier T cells were obtained 72 h after donor mice were primed (i.p.) with 0.005 μg SSS-III; these cells were treated with monoclonal anti-Lyt-2.2 antibody + C′ before cell transfer. Amplifier cells were given at the time of immunization.

‡ $Log_{10}$ PFC/spleen ± SEM for groups of 10 mice, 5 d after immunization (i.p.) with 0.5 μg SSS-III; geometric means are in parentheses. Mice were immunized 3 d after transfer of immune spleen cells.

for activation, are known to have profound influences on the results obtained in cell-transfer experiments (5). Consequently, we wished to examine whether amplifier T cell activity could overcome the suppressive effects induced following the transfer of immune B cells. To this end, mice were given $10 \times 10^6$ immune B cells from donor mice immunized (i.p.), 2 d earlier, with 0.5 μg SSS-III to induce suppression as described in the foregoing experiments. Amplifier T cells were obtained from another group of mice 72 h after priming (i.p.) with a subimmunogenic dose (0.005 μg) of SSS-III; these cells were treated with monoclonal anti-Lyt-2.2 antibody + C′ before cell transfer to eliminate suppressor T cell activity (5). 3 d after cell transfer one-half of the mice were immunized (i.p.) with 0.5 μg SSS-III; the other half received $2.5 \times 10^6$ amplifier T cells (i.v.) along with 0.5 μg SSS-III. All mice were assayed for SSS-III-specific PFC 5 d after immunization. The data of Table VIII show that significant suppression ($P < 0.05$) was induced after the transfer of immune B cells as expected. However, the transfer of amplifier T cells completely eliminated the suppression induced

($P < 0.05$); the resulting response, in fact, appeared to be greater ($P < 0.05$) than that of immunized mice not given transfer cells. Aside from providing further evidence that suppression, which is reversible, is not due to antibody-mediated feedback inhibition, these findings further attest to the fact that amplifier and suppressor T cells interact in a competitive manner to regulate the magnitude of the antibody response to SSS-III.

## Discussion

Since it was discovered that suppressor T cells exert their inhibitory effects not only during the course of a normal antibody response to an optimally immunogenic dose of SSS-III (1, 2), but also after the induction of low-dose paralysis to SSS-III (3, 8), much attention has been focused on the mechanism(s) by which suppressor T cells become activated under these experimental conditions. Because the effects produced by suppressor T cells involved in the antibody response to SSS-III are antigen-specific (4, 23), the direct activation of suppressor T cells by antigen was the first possibility considered. However, the inability of SSS-III to elicit (*a*) T-cell-proliferative responses, (*b*) immunological memory, (*c*) helper T cell effects, and (*d*) delayed hypersensitivity reactions, all of which are characteristic of the capacity of T cells to recognize and respond directly to antigen, was inconsistent with such a concept (reviewed in reference 6). Consequently, we proposed that suppressor T cells are activated in response to the idiotypic determinants of B-cell-associated antibody specific for SSS-III (7); such a recognition mechanism would provide an efficient means for regulating the magnitude of the antibody response with a high degree of antigen specificity. The present work was designed to obtain experimental evidence in support of this hypothesis. The results obtained show that antigen-specific suppression of the antibody response to SSS-III can be induced, in a cell-dose-dependent manner, by the transfer of $Ig^+$ spleen cells from mice previously immunized with an optimally immunogenic dose (0.5 $\mu$g) of SSS-III (Tables I and III). The induction of such suppression is not dependent upon the transfer of suppressor T cells activated by prior exposure to antigen; it can be induced using T-cell-depleted populations of immune spleen cells and requires the presence of $Ig^+$, antigen-binding cells from mice immunized with SSS-III (Tables I, II, and IV). These findings indicate that the suppression observed is induced by B cells bearing cell-associated antibody specific for SSS-III. It is most unlikely that such suppression is due to antibody-mediated feedback inhibition (20) since the same degree of suppression could be induced using immune cells from donors irradiated *before* the appearance of detectable PFC or serum antibody, i.e., <48 h after immunization (Table V), as well as with spleen cells from mice pretreated (primed) with a subimmunogenic dose (0.005 $\mu$g) of SSS-III (Table VI); in the latter case, priming with the subimmunogenic dose used does not elicit the development of detectable PFC or serum antibody specific for SSS-III (8). Furthermore, suppression was abrogated with the transfer of cell populations, rich in amplifier T cell activity (Table VIII), a finding that is consistent with the results obtained in other cell-transfer studies (5).

The kinetics for the induction of suppression after the transfer of immune B cells (Fig. 1) are virtually identical to those described for the in vivo induction of

low-dose paralysis to SSS-III (4). In both cases, after a latent or inductive period of ~16–24 h, there is a progressive increase in the degree of suppression expressed until a maximal level is attained, 72 h after the transfer of immune B cells (Fig. 1) or priming with a subimmunogenic dose of SSS-III (4); once induced, suppression persists for several weeks. This kinetic pattern suggests that immune B cells may not be acting directly to mediate the suppression observed; instead, they may provide a signal or stimulus for the activation of suppressor T cells that regulate the magnitude of the antibody response by limiting the extent to which antigen-stimulated B cells proliferate in response to SSS-III (24, 25). This indeed was found to be the case since T cells taken from mice previously given immune B cells were found to produce significant suppression, without the need for a latent period, upon transfer to immunized mice (Table VII). These findings clearly show that B-cell-associated antibody specific for SSS-III plays an instrumental role in the activation of suppressor T cells that regulate the magnitude of the antibody response to SSS-III. Because the suppression observed is antigen-specific (Tables I and II), we assume that the idiotypic determinants of antibody specific for SSS-III are the major recognition units involved in this process and are attempting to obtain direct evidence in this regard. Indeed, work done by several investigators (26–28) shows that T cells can recognize idiotypic determinants.

Although other investigators (29, 30) have reported the induction of suppressive effects with immune spleen cells (presumably B cells), on antibody response to the helper T-cell-dependent antigen, SRBC, the mechanism(s) responsible for producing such suppression remain unclear. Here, one cannot ascribe such effects solely to the activation of suppressor T cells by B-cell-associated antibody since suppression is believed to result from the interference of helper T cell activity by suppressor T cells; this process is H-2 restricted as well as allotype restricted. In contrast the antibody response to SSS-III does not require helper T cells and is not influenced by genes linked to either the major (H-2) histocompatibility or $IgC_H$ locus (6). In this regard the work of Braley-Mullen (31) is particularly significant. Using a system in which tolerance is induced by the administration of SSS-III conjugated to syngeneic spleen cells, this investigator showed that suppressor T cells specific for SSS-III could be eliminated from cell suspensions by absorption to plates coated with anti-SSS-III antibody, a finding that supports our proposed hypothesis. The results of the present work (Table IV) also show that immune spleen cells required for the induction of suppression can be adsorbed to, and then eluted from, Sepharose 6MB columns to which SSS-III is bound covalently; cells not binding to the column were unable to induce suppression.

Although the ability of amplifier T cells to abrogate the suppression induced by immune B cells (Table VIII) once more illustrates the competitive nature of the interaction between suppressor and amplifier T cells (1–6), the recognition unit(s) involved in the activation of amplifier T cells remains to be defined. Two models have been proposed (7) that could explain how such interactions might occur; both are consistent with the results obtained in this work. In the *competitive interaction model*, both suppressor T cells and amplifier T cells possess receptors (antiidiotypic receptors) capable of recognizing the idiotypic determinant of cell-

associated antibody on the surface of antigen-stimulated (immune) B cells; thus, both types of regulatory T cells can interact with a selected clone of B cells in a competitive manner. However, in the *dual inhibition model*, the idiotypic determinant common to cell-associated antibody specific for SSS-III is present on the surface of both antigen-stimulated B cells and amplifier T cells; since suppressor T cells possess a receptor capable of recognizing the idiotypic determinant, they can act to inhibit the functional activity of both B cells and amplifier T cells. Sufficient information is not available at this time to permit one to decide between either of these possibilities.

It has long been known that the antibody response to the capsular polysaccharide of *Streptococcus pneumoniae* provides the principal means for conferring protective immunity against virulent pneumococci (32–36). The experimental model described in the present work, which deals with regulation of the antibody response to SSS-III, is the best documented—if not the only—one now in use that relates directly to T cell regulatory mechanisms involved in an antibody response to a microbial antigen. Obviously, a more complete understanding of these regulatory processes is likely to increase our knowledge concerning the development of effective immunity to other microbial agents of biomedical importance.

## Summary

The transfer of B lymphocytes from mice immunized with type III pneumococcal polysaccharide (SSS-III) results in antigen-specific suppression of the antibody response of recipients immunized with SSS-III. Such suppression shares many features associated with low-dose paralysis, a phenomenon mediated by suppressor T cells; it reaches maximal levels 3 d after the transfer of viable or irradiated immune B cells and can be eliminated by the depletion of SSS-III-binding cells from spleen cell suspensions before transfer. In a two-step cell transfer experiment, purified T lymphocytes, isolated from recipients previously given immune B cells, caused suppression upon transfer to other mice immunized with SSS-III. Also, B-cell-induced suppression could be abrogated in a competitive manner by the infusion of amplifier T lymphocytes, as was previously demonstrated in the case of low-dose paralysis. These findings suggest that B cell surface components, presumably the idiotypic determinants of cell-associated antibody specific for SSS-III, are instrumental in activating suppressor T cells involved in regulating the magnitude of the antibody response to SSS-III.

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