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Antigen-Specific Suppressor T Cells Respond to Recombinant Interleukin-2 and Other Lymphokines

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Previous studies have shown that transfer of whole spleen cell populations obtained from primed donors or transfer of purified T cells enriched for suppressor activity (Ts) to recipient mice decreased the antibody response to pneumococcal polysaccharide type III (SSS-III) when the animals were simultaneously immunized with SSS-III. In the present studies, such suppression of the antibody response was transferred with 10- to 100-fold fewer primed spleen cells when the cells were treated in vitro with recombinant interleukin-2 (rIL-2) before transfer; spleen cells from naive mice or mice primed with an unrelated antigen (dextran) and then treated with rIL-2 did not cause suppression of the antibody response to SSS-III, thereby eliminating the possibility of nonspecific carryover effects induced by rIL-2. In vivo administration of rIL-2 at the time of immunization with an optimally immunogenic dose of SSS-III resulted in significant ($P < 0.05$) suppression of the antibody response relative to that of control animals, suggesting that IL-2 augments the clonal expansion of Ts cells in vivo. Further, the ability of passively administered anti-IL-2 receptor antibody to inhibit generation of Ts cells in vivo is consistent with such a view. Spleen cells from primed animals treated with rIL-4, rIL-5, or gamma interferon—but not those from primed animals treated with rIL-6—likewise were able to transfer suppression of the antibody response with fewer cells than those required when primed cells not treated with lymphokines were used. Thus, these studies indicate that Ts cell activity is greatly influenced by lymphokines produced by helper T cells. The studies also suggest that these lymphokines are required during activation and/or clonal expansion of Ts cells.

A number of studies have demonstrated that suppressor T (Ts) cells are capable of down-regulating the antibody response to pneumococcal polysaccharide type III (SSS-III) (1–7, 21–24) and that Ts cells function mainly to limit the expansion of antigen-stimulated B cells. These studies have also shown that Ts cells are activated during the course of a normal immune response not by antigen but by cell-associated antibody on the surfaces of immune B cells (12, 14, 23). However, the events that occur following activation, i.e., clonal expansion of precursor T cells, leading to fully functional Ts cells, have not been examined in sufficient detail. Studies done in vivo show that induction of SSS-III-specific Ts cells can be blocked by velban, a mitotic inhibitor (21), indicating that development of Ts-cell activity requires cell proliferation. It is well established that helper T cells require interleukin-2 (IL-2) during clonal expansion. However, it has not been demonstrated that antigen-specific Ts cells respond in a similar fashion to IL-2. In the present studies, we tested the hypothesis that Ts cells also require IL-2 to proliferate and examined whether expression of Ts-cell activity is influenced by other lymphokines known to affect antibody responses.

MATERIALS AND METHODS

Mice. Female BALB/cByJ mice (8 to 10 weeks old) obtained from the Jackson Laboratory, Bar Harbor, Maine, were maintained in accordance with the guidelines established by the National Institutes of Health, Animal Care Committee.

Antigens and immunization procedure. The immunological properties of the SSS-III and dextran B-1355 preparations used and the method by which they were prepared have already been described (2, 11, 23). For immunization, mice were given a single intraperitoneal (i.p.) injection of an optimally immunogenic dose (0.5 μg) of SSS-III in 0.5 ml of saline. The magnitude of the antibody response produced was determined 5 days postimmunization.

Immunological methods. Numbers of plaque-forming cells (PFCs) making antibody specific for SSS-III were detected by means of a well-established slide version of the technique of localized hemolysis in gel at 5 days postimmunization (i.p.) with SSS-III, i.e., at the peak of the antibody response (8–11). PFCs making antibody of the immunoglobulin M class (≥90% of all PFCs found) were detected by using indicator sheep erythrocytes coated with SSS-III by the $CrCl_3$ method (8–11). Polyethylene glycol (average molecular weight, 6,000 to 7,500; J. T. Baker Chemical Co., Phillipsburg, N.J.) was added to the reaction mixture (melted agarose) at a final concentration of 0.25% (wt/vol) to improve the quality of the plaques obtained. Corrections were made (by subtraction) for the small number of background sheep erythrocyte-specific PFCs present, so that only values for PFCs making antibody specific for SSS-III (SSS-III-specific PFCs) were considered in this work. The values obtained, which are log-normally distributed (15), are expressed as the geometric mean (antilog) of the $\log_{10}$ number of PFCs per spleen for groups of similarly treated mice. This provides a reasonably good measurement of the magnitude of the total antibody response produced, since SSS-III-specific PFCs are detected only in the spleens of immunized mice (1, 9, 17) and the magnitude of the PFC response produced is directly related to the antibody titer in serum (1, 9, 17).

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TABLE 1. Effect of rIL-2 on the antibody response to SSS-III

Amt (U) of rIL-2[a]	Log_{10} SSS-III-specific PFCs/spleen ± SEM[b]	P value[c]
None	4.202 ± 0.092 (15,928) [8]	
2×10^3	4.247 ± 0.094 (17,667) [9]	>0.05
2×10^4	3.990 ± 0.109 (9,767) [9]	<0.05
2×10^5	3.800 ± 0.130 (6,302) [9]	<0.05

[a] rIL-2 was given i.v. at the time of i.p. immunization with 0.5 μg of SSS-III (i.p.)
[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.
[c] Relative to controls immunized with SSS-III but not given rIL-2.

TABLE 2. Effect of aIL-2R antibody on expression of low-dose paralysis induced by SSS-III

Donor pretreatment with SSS-III[a]	aIL-2R[b]	Log_{10} SSS-III-specific PFCs/spleen ± SEM[c]	P value[d]
−	−	3.960 ± 0.056 (9,128) [10]	
+	−	3.298 ± 0.106 (1,988) [9]	<0.001
+	+	3.826 ± 0.155 (6,698) [7]	>0.05

[a] Mice were primed i.p. with 0.005 μg of SSS-III 72 h before immunization (i.p.) with 0.5 μg of SSS-III.
[b] One group of animals received (i.p.) monoclonal aIL-2R (4 μg per mouse) at 24, 48, and 72 h after priming with 0.005 μg of SSS-III.
[c] For groups of [n] mice 5 days after immunization with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.
[d] Relative to control mice immunized with SSS-III but not pretreated with SSS-III or aIL-2R.

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

Preparation and treatment of spleen cells. Spleen cells were obtained from donor mice 16 to 24 h after prior exposure (priming) of the animals (see below) to a subimmunogenic dose (0.005 μg) of SSS-III; they were suspended at a density of 10^8 cells per ml in medium 199. Aliquots (2.5 ml) were then incubated in the presence of each lymphokine for 30 min at 4°C. In all cases, spleen cells were washed three times with medium 199 after lymphokine treatment and then suspended at appropriate densities for intravenous (i.v.) injection into recipient mice. Recipient mice were immunized (i.p.) simultaneously with 0.5 μg of SSS-III. Previous studies have consistently shown that spleen cells harvested 16 to 24 h after priming with SSS-III are rich in Ts activity (3–5, 20–22). Suppression can be transferred with at least 20 $\times$ 10^6 whole spleen cells or 10^5 purified T cells recovered from plates treated with monophosphoryl lipid A (4). We observed in the present studies, as well as in the past (4), that 5×10^6 or 2×10^6 primed spleen cells not treated with recombinant IL-2 (rIL-2) did not transfer significant suppression.

Lymphokines and antibodies. rIL-2, rIL-4, rIL-5, and gamma interferon (IFN-γ) were obtained from Genzyme Corp., Boston, Mass. Human (rIL-6) was a generous gift of Jin Kim (Genentech Inc., San Francisco, Calif.). Anti-IL-2 receptor antibody (aIL-2R) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.).

RESULTS

Effect of rIL-2 on the antibody response to SSS-III. First we examined the effects of rIL-2 on the antibody response to SSS-III in vivo. Mice were given different amounts of rIL-2 at the time of immunization with SSS-III. At 5 days postimmunization, the antibody response produced was determined and compared with that of immunized mice not given rIL-2. Administration of 2×10^4 or 2×10^5 U of rIL-2 resulted in significant ($P < 0.05$) suppression of the antibody response relative to that of controls that did not receive rIL-2, whereas injection of 2×10^3 U of rIL-2 caused no significant ($P > 0.05$) alteration of the antibody response to SSS-III (Table 1).

Earlier studies have shown that administration of a subimmunogenic dose of SSS-III leads to a state of unresponsiveness known to be mediated by antigen-specific Ts cells (2, 3, 7). In view of the results obtained in the preceding experiment, we conducted an experiment to examine whether Ts-cell generation in vivo could be inhibited by treatment with monoclonal aIL-2R antibody. Two groups of animals were primed with 0.005 μg of SSS-III, and a control group received saline. One of the primed groups received aIL-2R antibody at 24, 48, and 72 h after priming. All animals, including those in the control group, were then immunized with an optimally immunogenic dose of SSS-III (i.e., 0.5 μg of SSS-III) at 72 h after priming with 0.005 μg of SSS-III. At 5 days postimmunization, the antibody response was assessed. Mice primed with 0.005 μg of SSS-III alone gave a significantly ($P < 0.001$) lower antibody response than control animals, as expected (Table 2). On the other hand, the antibody response of mice primed with SSS-III and then treated with aIL-2R did not differ significantly ($P > 0.05$) from that of the controls, although the response of the treated mice was lower in magnitude than that of the controls. These findings suggest that IL-2 plays an important role in the generation of Ts cells.

Effect of rIL-2 on the ability of spleen cells from primed animals to transfer suppression. Although administration of rIL-2 significantly decreased the antibody response to SSS-III in vivo (Table 1), one could argue that such suppression might be due to a direct effect of rIL-2 on the function of antigen-stimulated B cells or that rIL-2 may act to increase the activity of Ts cells, which are known to be activated early during the course of a normal antibody response to SSS-III (1–3, 20–22), and that Ts cells, in turn, suppress clonal expansion of antibody-producing cells. Consequently, experiments were conducted to examine whether rIL-2 can alter the capacity of spleen cells from primed animals (with enriched Ts-cell activity) to transfer suppression.

In this experiment, spleen cells from donors primed 16 to 24 h earlier with 0.005 μg of SSS-III were treated in vitro with rIL-2 before cell transfer. All mice were immunized simultaneously with 0.5 μg of SSS-III at the time of cell transfer and assayed for numbers of PFCs at 5 days postimmunization. As expected, transfer of 20×10^6 primed spleen cells not treated with rIL-2 caused significant ($P < 0.001$) suppression of the antibody response to SSS-III relative to that of controls that did not receive transferred cells (Table 3). Although significant ($P < 0.001$) suppression of the antibody response was noted in mice that received 2×10^6 or 2×10^5 spleen cells treated with rIL-2 (Table 3), transfer of 2×10^6 primed cells had no effect without rIL-2 treatment; the latter finding is consistent with those of previous studies in which $<10 \times 10^6$ primed cells did not cause suppression of the antibody response. Significant suppression ($P < 0.05$) of the antibody response was also noted with 2×10^6 cells when they were treated with 250, 500, or 1,000 U of rIL-2.

Inability of spleen cells from naive animals or dextran-primed mice to cause suppression after rIL-2 treatment. It

TABLE 3. Effect of rIL-2 on the ability of primed spleen cells to transfer suppression

Expt no.	No. of spleen cells (amt of rIL-2 [U])[a]	Log_{10} SSS-III-specific PFCs/spleen ± SEM[b]	P value[c]
1	NCT	4.333 ± 0.017 (21,542) [8]	
	20×10^6 (none)	3.966 ± 0.057 (9,254) [8]	<0.001
	2×10^6 (10^4)	3.910 ± 0.013 (8,122) [7]	<0.01
	2×10^5 (10^4)	4.036 ± 0.073 (10,855) [8]	<0.01
2	NCT	4.275 ± 0.082 (18,821) [10]	
	10×10^6 (none)	4.005 ± 0.099 (10,115) [10]	<0.05
	2×10^6 (none)	4.299 ± 0.038 (19,907) [10]	>0.05

[a] Spleen cells were obtained from donor mice primed earlier with 0.005 μg of SSS-III for 16 to 24 h; in some cases,the cells were treated with rIL-2 for 30 min at 4°C. Spleen cells were then injected (i.v.) into recipient mice immunized simultaneously (i.p.) with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] Based on comparisons with control mice immunized with SSS-III but not given donor cells.

was possible that portions of the rIL-2 used to treat spleen cells in vitro was nonspecifically adsorbed to the donor cells. Thus, the following experiments were done to obtain information relevant to this issue. In the first case, spleen cells were obtained from either SSS-III-primed or naive (given an equivalent volume of saline) donors and treated separately in vitro with rIL-2. A quantity (2×10^6) of each type of cells was transferred to recipient mice at the time of immunization with 0.5 μg of SSS-III. Whereas rIL-2-treated spleen cells from primed donors caused significant ($P < 0.001$) suppression of the antibody response, rIL-2-treated cells from naive mice did not cause suppression of the antibody response to SSS-III compared with controls that did not receive transferred cells (Table 4). These experiments suggest that transfer of suppression of the antibody response with smaller numbers of rIL-2-treated spleen cells is not due to residual IL-2 on the transferred cells. In a separate experiment, donor mice were primed with 0.005 μg of either SSS-III or dextran (a dose that has been shown to generate dextran-specific Ts cells). As before, 2×10^6 cells were then

TABLE 4. Specificity of transfer of suppression

Expt no.	Amt (U) of rIL-2 used to treat transferred cells[a]	Treatment of donor mice	Log_{10} SSS-III-specific PFCs/spleen ± SEM[b]	P value[c]
1	NCT		4.319 ± 0.037 (20,838) [10]	
	500	SSS-III	3.973 ± 0.040 (9,390) [9]	<0.001
	250	SSS-III	3.909 ± 0.073 (8,107) [9]	<0.001
	250	Saline	4.257 ± 0.031 (18,057) [9]	>0.05
2	NCT		3.963 ± 0.099 (9,183) [10]	
	500	SSS-III	3.702 ± 0.053 (5,034) [9]	<0.05
	500	Dextran	3.970 ± 0.121 (9,331) [10]	>0.05

[a] Spleen cells were obtained 16 to 24 h after priming of donor mice with 0.005 μg of SSS-III, 0.005 μg of dextran, or saline. Spleen cells were treated with rIL-2 for 30 min at 4°C, and then 2×10^6 cells were injected i.v. into recipient mice immunized simultaneously with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] Based on comparisons with mice immunized with SSS-III but not given primed spleen cells.

TABLE 5. Effect of rIL-4 on the ability of primed spleen cells to transfer suppression

Amt (U) of rIL-4 used to treat transferred cells (no. of cells [10^6])[a]	Log_{10} SSS-III-specific PFCs/spleen ± SEM[b]	P value[c]
NCT	4.112 ± 0.049 (12,940) [9]	
None (20)	3.350 ± 0.128 (2,240) [8]	<0.001
250 (2)	3.789 ± 0.078 (6,146) [6]	<0.01
500 (2)	3.505 ± 0.077 (3,198) [10]	<0.001
1,000 (2)	3.630 ± 0.125 (4,267) [10]	<0.001

[a] Spleen cells were obtained from donor mice 16 to 24 h after priming with 0.005 μg of SSS-III and then treated with rIL-4 for 30 min at 4°C. Later, 2×10^6 rIL-4-treated or 20×10^6 untreated cells were injected into mice at the time of immunization with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] Based on comparisons with control animals immunized with SSS-III but not given primed spleen cells.

transferred to recipient mice at the time of immunization with 0.5 μg of SSS-III. While the antibody response of mice that received rIL-2-treated spleen cells from SSS-III-primed donors was lower than that of controls, the response of mice that received dextran-primed spleen cells was not altered (Table 4). Thus, these experiments indicate that the effect of rIL-2 on transfer of SSS-III-primed spleen cells is not due to nonspecific adsorption of rIL-2 or its elution after cell transfer (i.e., carryover effects).

Effects of other recombinant lymphokines on the ability of primed spleen cells to transfer suppression. We also examined the effects of rIL-4, rIL-5, rIL-6, and IFN-γ on the ability of spleen cells to transfer suppression, since these lymphokines have been shown to be involved in the generation of an antibody response. The design of these experiments was similar to that of the other cell transfer experiments involving rIL-2 (Table 4). Similar numbers of cells treated with rIL-4 (Table 5), rIL-5 (Table 6), or IFN-γ (Table 7) caused significant ($P < 0.01$) suppression of the antibody response to SSS-III, whereas cells treated with rIL-6 did not (Table 8).

DISCUSSION

Several studies have shown that Ts cells can regulate the antibody response to SSS-III. However, the mechanisms by which these cells are activated and expanded remain to be

TABLE 6. Effect of rIL-5 on the ability of primed spleen cells to transfer suppression

Amt (U) of rIL-5 used to treat transferred cells (no. of cells [10^6])[a]	Log_{10} SSS-III-specific PFCs/spleen ± SEM[b]	No. of mice/group
NCT	4.112 ± 0.047 (12,938)	8
None (20)	3.848 ± 0.086 (7,041)[c]	9
250 (2)	3.916 ± 0.046 (8,236)[c]	9
500 (2)	3.956 ± 0.047 (9,225)[c]	9
1,000 (2)	3.930 ± 0.066 (8,517)[c]	10

[a] Spleen cells were obtained from mice 16 to 24 h after priming with 0.005 μg of SSS-III, and then portions were treated with 1,000, 500, or 250 U of rIL-5 for 30 min at 4°C. Later, 2×10^6 rIL-5-treated or 20×10^6 untreated cells were injected per recipient mouse at the time of immunization with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] Measured 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] $P < 0.05$ on the basis of comparisons with control mice immunized with SSS-III but not given primed spleen cells.

TABLE 7. Effect of recombinant IFN-γ on the ability of primed spleen cells to transfer suppression

Amt (U) of IFN-γ used to treat transferred cells (no. of cells [10^6])[a]	Log_{10} SSS-III-specific PFCs/ spleen ± SEM[b]	P value[c]
NCT	4.154 ± 0.086 (14,251) [10]	
None (20)	3.856 ± 0.111 (7,183) [9]	<0.05
250 (2)	4.171 ± 0.050 (14,817) [10]	>0.05
500 (2)	4.121 ± 0.072 (13,211) [10]	>0.05
1,000 (2)	3.672 ± 0.073 (4,702) [10]	>0.001

[a] Spleen cells were obtained from donor mice 16 to 24 h after priming with 0.005 μg of SSS-III, and then portions were treated with 1,000, 500, or 250 U of IFN-γ. Later, 2×10^6 IFN-γ-treated cells or 20×10^6 untreated cells were injected into recipient mice at the time of immunization with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] Based on comparisons with control mice immunized with SSS-III but not given primed spleen cells.

elucidated. We previously showed that B cells taken from animals immunized with SSS-III can induce Ts cells in vivo and that this can occur in the absence of residual antigen (12–14, 23); here, the kinetics for induction of Ts-cell activity is similar to that noted for induction of Ts cells after priming with antigen (14, 21). Because the suppression induced is antigen specific, these findings have been interpreted as indicating that Ts cells are activated by recognition of idiotypic determinants of cell-associated antibody on the surface of antigen-stimulated B cells. Recent studies have shown that antigen-stimulated B cells must be metabolically active to induce Ts cells (14). Despite these advances, little is known concerning the involvement of lymphokines in either the activation process or the clonal expansion of Ts cells. Therefore, in the present studies we examined the effects of a few selected lymphokines (mainly rIL-2) on the antibody response in vivo and on the ability of Ts cells to transfer suppression of the antibody response to recipient mice immunized with SSS-III.

We found that in vivo administration of rIL-2 at the time of immunization resulted in substantial suppression of the antibody response to SSS-III (Table 1). This suppression required at least 2×10^4 U of rIL-2, and in subsequent experiments we observed that the effect was highly reproducible. It is conceivable that the suppression of the antibody response observed was the result of either direct interaction of rIL-2 with antigen-stimulated B cells or an indirect effect mediated by Ts cells whose activity was enhanced following treatment with rIL-2. Indeed, prior studies have shown that Ts cells are activated early (5 to 24 h postimmunization) during the course of a normal antibody response to SSS-III (3, 5, 22); thus, it is possible that administration of rIL-2 at the time of immunization, as in this work (Table 1), may have resulted in expansion of a population of activated Ts cells. In other experiments, in which rIL-2 was given at 2 days postimmunization with SSS-III (i.e., at peak amplifier T-cell activity) the antibody response was enhanced (22a). These data suggest that IL-2 acts directly on both types of regulatory T cells (Ts and amplifier T cells), which in turn influence the magnitude of the antibody response to SSS-III.

TABLE 8. Effect of rIL-6 on the ability of primed spleen cells to transfer suppression

Amt (U) of rIL-6 used to treat transferred cells (no. of cells [10^6])[a]	Log_{10} SSS-III-specific PFCs/ spleen ± SEM[b]	P value[c]
NCT	4.208 ± 0.055 (16,155) [10]	
None (20)	3.948 ± 0.085 (8,865) [9]	<0.05
500 (2)	4.133 ± 0.071 (13,591) [10]	>0.05
1,000 (2)	4.090 ± 0.047 (12,297) [10]	>0.05

[a] Spleen cells were taken from donor mice primed with 0.005 μg of SSS-III 16 to 24 h earlier, and then portions were treated with rIL-6 for 30 min at 4°C. Later, spleen cells (treated or untreated) were injected i.v. into recipient mice immunized simultaneously with 0.5 μg of SSS-III. NCT, No cells transferred.

[b] For groups of [n] mice 5 days after immunization with SSS-III; geometric means (antilogs) are in parentheses.

[c] Based on comparisons with control mice immunized with SSS-III but not given primed spleen cells.

Earlier studies done in this laboratory have shown that one can induce an antigen-specific form of unresponsiveness known to be mediated by Ts cells by low-dose priming (i.e., prior exposure to a subimmunogenic dose of 0.005 μg of SSS-III). Because it was also demonstrated that mitotic inhibitors can abrogate the generation of such Ts cells, it appears that cell proliferation is required for expression of optimal Ts-cell activity (21). Indeed, administration of aIL-2R at the time of low-dose priming with SSS-III resulted in partial reversal of the reduced antibody response normally expected (Table 2). In effect, this finding (Table 2) is consistent with the observation that mitotic inhibitors abrogate generation of Ts cells in vivo after low-dose priming. The partial reversal of the low-dose priming effect by aIL-2R suggests that other lymphokines are required for induction of full expression of Ts-cell activity in vivo. In the present studies, we examined this idea further by testing the effects of rIL-2 and a few selected lymphokines on the capacity of spleen cells from SSS-III primed mice to transfer suppression of the antibody response to SSS-III.

We found that transfer of suppression with rIL-2-treated spleen cells could be carried out by using 100-fold fewer primed spleen cells than in cell transfer experiments using primed spleen cells not treated with rIL-2 (Table 3); this indicates a substantial increase in Ts-cell activity. In earlier studies, we observed that because of the low frequency of putative Ts cells, approximately 20×10^6 spleen cells from SSS-III-primed mice were required to transfer suppression. In fact, only under conditions of enrichment, such as use of monophosphoryl lipid A-adherent T cells, was it possible to transfer suppression with such small numbers of cells (4, 5). These studies therefore suggest that precursor Ts cells, once activated, express IL-2 receptors that are capable of binding available IL-2; this results in clonal expansion of precursor Ts cells, as reflected by an increase in Ts-cell activity. Because it was also possible that the added IL-2 is nonspecifically adsorbed to normal or activated spleen cells during in vitro treatment, we also treated spleen cells from naive animals or mice primed with a non-cross-reactive antigen (dextran) with rIL-2. The results show that transfer of similar numbers of such rIL-2 treated spleen cells did not suppress the antibody response to SSS-III in recipient animals (Table 4). Our present studies also showed that, in addition to rIL-2, in vitro treatment of spleen cells from primed mice with rIL-4, rIL-5, or IFN-γ also resulted in significantly increased Ts-cell activity; however, treatment with rIL-6 was without effect. Although some of these lymphokines appear to influence the development of Ts cells from inactive precursors, their role in the induction of Ts cells remain to be determined. Experiments are in progress to determine which lymphokines are produced by cloned

amplifier T or Ts cells and how these lymphokines affect the overall antibody response to SSS-III.

In other experimental systems, it has been shown that IL-2 can modulate Ts-cell activity (16, 19, 25–27). For instance, it has been demonstrated that T cells capable of inhibiting cytotoxic T lymphocyte (CTL) activity can adsorb IL-2 from preparations containing IL-2 activity (26). Also, it has been demonstrated that high concentrations of IL-2 can induce nonspecific Ts cells in mixed-lymphocyte reactions (19). Furthermore, deficient Ts-cell activity was found in old animals and addition of exogenous IL-2 restored functional Ts-cell activity (25). The above-described studies and the experiments reported here show that the ability of Ts cells to respond to IL-2 has important clinical implications for IL-2-based immunotherapeutic procedures. In some of these procedures, IL-2 is used in vitro to expand lymphokine-activated killer cells or tumor-infiltrating lymphocytes; in other instances, IL-2 is given in vivo with other lymphokines to boost the immune response (20). Recently, it was observed that a considerable improvement in the immune response was possible when cyclophosphamide was used to eliminate Ts-cell activity (18). Studies conducted in our laboratory show that Ts activity can be removed from populations of spleen cells by adherence to monophosphoryl lipid A-coated plates. Thus, it seems likely that experimental approaches to expand the pool of lymphokine-activated killer cells or tumor-infiltrating lymphocytes might be improved if suspensions containing such cells are first treated to remove Ts-cell activity, which can also expand in the presence of IL-2.

REFERENCES

1. **Baker, P. J.** 1990. Regulation of the magnitude of the antibody response to bacterial polysaccharide antigens by thymus-derived lymphocytes. Infect. Immun. **58**:3465–3468.
2. **Baker, P. J., D. F. Amsbaugh, P. W. Stashak, G. Caldes, and B. Prescott.** 1981. Regulation of the antibody response to type III pneumococcal polysaccharide. Rev. Infect. Dis. **3**:332–341.
3. **Baker, P. J., D. F. Amsbaugh, P. W. Stashak, G. Caldes, and B. Prescott.** 1982. Direct evidence for the involvement of thymus-derived (T) suppressor cells in the expression of low-dose paralysis to type III pneumococcal polysaccharide. J. Immunol. **128**:1059–1062.
4. **Baker, P. J., K. R. Hasløv, M. B. Fauntleroy, P. W. Stashak, K. Myers, and J. T. Ulrich.** 1990. Enrichment of suppressor T-cell activity by means of binding to monophosphoryl lipid A. Infect. Immun. **58**:726–731.
5. **Baker, P. J., J. R. Hiernaux, M. B. Fauntleroy, B. Prescott, J. L. Cantrell, and J. A. Rudbach.** 1988. Inactivation of suppressor T-cell activity by nontoxic monophosphoryl lipid A. Infect. Immun. **56**:1076–1083.
6. **Baker, P. J., B. Prescott, P. W. Stashak, and D. F. Amsbaugh.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. III. Studies on the average avidity of the antibody produced by specific plaque-forming cells. J. Immunol. **107**:719–724.
7. **Baker, P. J., N. D. Reed, P. W. Stashak, D. F. Amsbaugh, and B. Prescott.** 1973. Regulation of the antibody response to type III pneumococcal polysaccharide. I. Nature of regulatory T cells. J. Exp. Med. **137**:1431–1441.
8. **Baker, P. J., and P. W. Stashak.** 1969. Quantitative and qualitative studies on the primary antibody response to pneumococcal polysaccharide at the cellular level. J. Immunol. **103**:1342–1348.
9. **Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. I. Dose-response studies and the effect of prior immunization on the magnitude of the antibody response. Immunology **20**:469–481.
10. **Baker, P. J., P. W. Stashak, D. F. Amsbaugh, and B. Prescott.** 1971. Characterization of the antibody response to type III pneumococcal polysaccharide at the cellular level. II. Studies on the relative rate of antibody synthesis and release by antibody-producing cells. Immunology **20**:481–493.
11. **Baker, P. J., P. W. Stashak, and B. Prescott.** 1969. The use of erythrocytes sensitized with purified pneumococcal polysaccharides for the assay of antibody and antibody-producing cells. Appl. Microbiol. **17**:422–426.
12. **Baker, P. J., C. E. Taylor, M. B. Fauntleroy, P. W. Stashak, and B. Prescott.** 1985. The role of antigen in the activation of regulatory T cells by immune B cells. Cell. Immunol. **96**:376–385.
13. **Elkins, K. L., P. W. Stashak, and P. J. Baker.** 1987. Transferred B cells from autoimmune NZB/N mice fail to activate T suppressor cells. Cell. Immunol. **110**:14–27.
14. **Elkins, K. L., P. W. Stashak, and P. J. Baker.** 1990. Metabolic activity is necessary for activation of T suppressor cells by B cells. J. Immunol. **144**:2859–2864.
15. **Gottlieb, C. F.** 1974. Applications of transformation to normalize the distribution of plaque-forming cells. J. Immunol. **113**:51–57.
16. **Holda, J. H., T. Varies, and H. N. Claman.** 1986. Natural suppressor activity in graft-vs-host spleen and normal bone marrow is augmented by IL2 and interferon-gamma. J. Immunol. **137**:3538–3543.
17. **Jones, J. M., D. F. Amsbaugh, P. W. Stashak, B. Prescott, P. J. Baker, and D. W. Alling.** 1976. Kinetics of the antibody response to type III pneumococcal polysaccharide. III. Evidence that suppressor cells function by inhibition of the recruitment and proliferation of antibody-producing cells. J. Immunol. **116**:647–656.
18. **Mitchell, M. S., R. A. Kempt, W. Harel, H. Shau, W. D. Boswell, S. Lind, and E. C. Bradley.** 1988. Effectiveness and tolerability of low dose cyclophosphamide and low dose intravenous interleukin-2 disseminated melanoma. J. Clin. Oncol. **6**:409–424.
19. **Oh-Ishi, T., C. K. Goldman, J. Mishti, and T. A. Waldmann.** 1989. The interaction of interleukin 2 with its receptor in the generation of suppressor T cells in antigen-specific and antigen-nonspecific systems in vitro. Clin. Immunol. Immunopathol. **52**:447–459.
20. **Pulley, M. S., V. Nagendran, J. M. Edwards, and D. C. Dumonde.** 1986. Intravenous, intralesional and endolymphatic administration of lymphokines in human cancer. Lymphokine Res. **5**:157–163.
21. **Stashak, P. W., C. E. Taylor, G. Caldes, B. Prescott, and P. J. Baker.** 1983. Cyclic expression of low-dose paralysis. Cell. Immunol. **77**:143–149.
22. **Taylor, C. E., D. F. Amsbaugh, P. W. Stashak, G. Caldes, B. Prescott, and P. J. Baker.** 1983. Cell surface antigens and other characteristics of T cells regulating the antibody response to type III pneumococcal polysaccharide. J. Immunol. **130**:19–23.

22a. **Taylor, C. E., M. B. Fauntleroy, P. W. Stashak, and P. J. Baker.** Unpublished data.

23. **Taylor, C. E., P. W. Stashak, G. Caldes, B. Prescott, T. E. Chused, A. Brooks, and P. J. Baker.** 1983. Activation of antigen-specific suppressor T cells by B cells from mice immunized with type III pneumococcal polysaccharide. J. Exp. Med. **158**:703–717.
24. **Taylor, C. E., P. W. Stashak, G. Caldes, B. Prescott, B. J. Fowlkes, and P. J. Baker.** 1984. Lectin induced modulation of the antibody response to type III pneumococcal polysaccharide. Cell. Immunol. **83**:26–33.
25. **Thoman, M. L., and W. O. Weigle.** 1983. Deficiency in suppressor T cell activity in aged animals. Reconstitution of this activity by interleukin 2. J. Exp. Med. **157**:2184–2189.
26. **Ting, C. C., S. S. Yang, and M. E. Hargrove.** 1984. Induction of suppressor T cells by interleukin 2. J. Immunol. **133**:261–266.
27. **Volk, H. D., and T. Diamantstein.** 1986. IL-2 normalizes defective suppressor T cell function of patients with systemic lupus erythematosus in vitro. Clin. Exp. Immunol. **66**:525–531.