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Inactivation of Suppressor T Cell Activity by the Nontoxic Lipopolysaccharide of *Rhodopseudomonas sphaeroides*

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Antibody responses of mice immunized with type III pneumococcal polysaccharide were examined with and without treatment with nontoxic lipopolysaccharide from *Rhodopseudomonas sphaeroides* (Rs-LPS). The results obtained were similar to those described previously for mice treated with monophosphoryl lipid A (MPL) except that lower amounts of Rs-LPS were needed. Both were without effect when given at the time of immunization with type III pneumococcal polysaccharide but elicited significant enhancement when given 2 to 3 days later. Such enhancement was T cell dependent and not due to polyclonal activation of immunoglobulin M synthesis by B cells. Treatment with either Rs-LPS or MPL abolished the expression but not induction of low-dose paralysis, a form of immunological unresponsiveness known to be mediated by suppressor T cells (Ts). The in vitro treatment of cell suspensions containing Ts with extremely small amounts of Rs-LPS or MPL completely eliminated the capacity of such cells to transfer suppression to other mice. These findings indicate that the immunomodulatory effects of both MPL and Rs-LPS are mainly the result of eliminating the inhibitory effects of Ts; this permits the positive effects of amplifier T cells to be more fully expressed, thereby resulting in an increased antibody response. The significance of these and other findings to the use of Rs-LPS as a pharmacotherapeutic agent for gram-negative bacterial sepsis is discussed.

Although bacterial lipopolysaccharides (LPSs) or endotoxins possess antitumor and adjuvant activity and also provide protection against X irradiation and various bacterial infections, these beneficial effects have not been exploited to their fullest extent mainly because LPS, even at very low doses, is extremely toxic and pyrogenic for most animal species (33, 34). Both the beneficial and the harmful effects of LPS are elicited by the diphosphoryl lipid A (DPLA) portion of the molecule (33, 34); however, removal of a single phosphate group from the reducing end of DPLA yields monophosphoryl lipid A (MPL). MPL elicits all of the beneficial effects produced by native LPS but is relatively nontoxic and nonpyrogenic, even at high doses (33, 34). Recent studies have shown that treatment of mice with MPL results in an increase in the magnitude of the antibody response to type III pneumococcal polysaccharide (SSS-III) as well as the synthesis of significant amounts of immunoglobulin G (IgG) antibody, not usually made after immunization with SSS-III alone (5, 6). These adjuvant or immunomodulatory effects have been attributed to the ability of MPL to negate the inhibitory effects of thymus-derived (T) suppressor cells (Ts) without altering the expression of amplifier T cell (T_A) or helper T cell (T_H) function (5, 6).

The LPS of *Rhodopseudomonas sphaeroides* ATCC 17023 (Rs-LPS) is nontoxic and nonpyrogenic and has a lipid A moiety similar in structure to that found in toxic enterobacterial and *Salmonella* LPSs (24, 28, 29, 31, 35–37, 39). Because of similarities in structure, the DPLA of nontoxic Rs-LPS can be used as an antagonist to block, in a concentration-dependent and competitive manner, the induction of cachectin or tumor necrosis factor (TNF) (3) and the release of interleukin-1 (N. Quareshi, K. Takayama, R. Kurtz, L. Chen, R. Wong, and R. J. Cotter, submitted for publication) by the toxic deep-rough-chemotype LPS (Re-LPS) of *Escherichia coli* (38), as well as to block LPS-induced immunoglobulin synthesis by 70Z/3 pre-B cells (T. H. Kirkland, N. Qureshi, and K. Takayama, J. Immunol., submitted for publication). This suggests that the DPLA of nontoxic Rs-LPS can compete effectively with toxic LPS for attachment to the cellular binding sites involved in triggering many of the pharmacological and immunological effects elicited by LPS. In the present work, we examined some of the immunomodulatory effects produced by Rs-LPS to determine whether, in addition to the aforementioned antagonistic effects, it is also similar to MPL in its ability to abrogate the expression of Ts activity.

MATERIALS AND METHODS

Mice. Female BALB/cByJ mice (age, 8 to 10 weeks; Jackson Laboratory, Bar Harbor, Maine) were used in most of the experiments described. Female athymic nude (*nu/nu*) mice and their corresponding thymus-bearing (*nu/+*) littermate controls (age, 7 to 8 weeks) were obtained from the Frederick Cancer Research Center (Frederick, Md.); although these mice have the same genetic background and have been maintained in a closed colony for many years, their pedigree is not known. They were kept in sterile cages placed in a laminar flow hood throughout these experiments and were given sterile food and water ad libitum. They appeared to be in excellent health at the time the experiments were conducted.

MPL. MPL (average molecular size, 1,718) was obtained from Ribi ImmunoChem Research, Inc. (Hamilton, Mont.).

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It was isolated from the heptoseless Re mutant, *Salmonella typhimurium* G30/C21, as described previously (33). Lyophilized MPL was reconstituted to 1 mg/ml in distilled water containing 0.2% triethylamine. It was mixed thoroughly and sonicated briefly to obtain an opalescent solution, which was stored at 4°C until use; the stock solution was diluted in medium 199 to yield the desired amount of MPL to be added to cell suspensions. Information on the toxic and immunological properties of MPL is given elsewhere (5, 6, 33).

Antigens and immunization procedure. The immunological properties of the preparation of SSS-III used and the method by which it was prepared have been described (1, 7, 8, 10–12). 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 after immunization.

R. sphaeroides ATCC 17023 was grown photoheterotrophically in medium 550 (American Type Culture Collection, Washington, D.C.) at 27°C as described previously (28). The cells were first extracted with ethanol and normal butanol to remove pigments; then, the LPS was extracted by the method of Galanos et al. (20) with modification (29). The resulting LPS was treated with 0.1 M EDTA (pH 7.0) as described previously (30) and purified by using reverse-phase Sep-Pak cartridges (Waters Associates, Inc., Milford, Mass.) to yield the Rs-LPS. This purified Rs-LPS has the following structure, as deduced by previous studies (28; N. Qureshi, K. Takayama, L. Chen, and R. J. Cotter, unpublished results) and plasma desorption mass spectrometry: threonine-(glucuronic acid)$_3$-(3-deoxy-D-*manno*-octulosonic acid)-DPLA.

LPS derived from *E. coli* O113 was purchased from Ribi ImmunoChem.

Immunological methods. Numbers of antibody-producing plaque-forming cells (PFCs) specific for SSS-III detected in individual mice provided a measure of the antibody response produced at the peak, i.e., 5 days after immunization (i.p.) with SSS-III. PFCs making antibody of the IgM class (>90% of all PFCs found [7, 8]) were detected by a slide version of the localized hemolysis-in-gel technique (14), using indicator sheep erythrocytes coated with SSS-III by the $CrCl_3$ method (14). Polyethylene glycol 6000 (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 found. Corrections were made (by subtraction) for the small number (<200 per spleen) of background sheep erythrocyte-specific PFCs present, so that only values for PFCs making antibody specific for SSS-III (SSS-III-specific PFCs) are considered in this work. The values obtained (SSS-III-specific PFCs per spleen), which are log normally distributed (21), are expressed as the geometric mean (antilog) of the $\log_{10}$ number of PFCs per spleen ± the standard error of the mean (SEM) for groups of similarly treated mice. This provides a reasonably good measure of the magnitude of the total antibody response produced since SSS-III-specific PFCs are detected only in the spleens of immunized mice (8, 25, 32).

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

Assessment of polyclonal activity of LPS. Cells secreting non-antigen-specific immunoglobulin of the IgM class were detected by a modification of the protein A plaque assay (22), in which indicator sheep erythrocytes were coated with protein A (Pharmacia) in the presence of 66 μg of $CrCl_2$ per ml as the coupling agent. The affinity-purified rabbit anti-mouse IgM used for the detection of non-antigen-specific IgM-secreting PFCs was the same preparation as that used in previous studies (5). A dilution (1:200 in saline) known to reveal maximal numbers of IgM-secreting PFCs was added (50 μl) to the soft agarose reaction mixture before the addition of spleen cells. Results are expressed as $\log_{10}$ numbers of IgM-secreting PFCs per spleen ± SEM for groups of LPS-treated or untreated (control) mice.

Transfer of Ts activity with primed spleen cells. Ts activity was generated and transferred to unirradiated recipient mice by a standard procedure described in detail elsewhere (2, 40). Briefly, donor mice were pretreated (primed) with a single injection (i.p.) of a subimmunogenic dose (0.005 μg) of SSS-III to activate Ts; 18 to 24 h later, a pooled spleen cell suspension was prepared from several primed donor mice and was adjusted to contain 10×10^7 nucleated spleen cells per ml in medium 199. Then, groups of mice were given (intravenously [i.v.]) 20×10^6 primed cells in a volume of 0.2 ml at the time of immunization with 0.5 μg of SSS-III; the magnitude of the SSS-III-specific PFC response produced was determined 5 days after immunization. The results of prior studies have established that the transfer of primed cells results in significant suppression (>70%) of the SSS-III-specific PFC response of immunized recipient mice; such suppression is antigen specific and is known to be mediated by $CD8^+$ Ts (2, 40).

RESULTS

Effect of treatment with Rs-LPS on the antibody response to SSS-III. Groups of mice were given (i.p.) different amounts of Rs-LPS 2 days after immunization (i.p.) with an optimally immunogenic dose (0.5 μg) of SSS-III; the magnitude of the antibody (PFC) response elicited was determined 5 days after immunization and compared with that of immunized control mice not given Rs-LPS. The results obtained indicate that treatment with a single injection of 0.5 to 10 μg of Rs-LPS had no effect ($P > 0.05$) on the magnitude of the SSS-III-specific PFC response (data not shown); however, a significant increase (about twofold; $P < 0.05$) was noted for mice given 20 μg of Rs-LPS. Here, the magnitude of the SSS-III-specific PFC response was increased from $\log_{10}$ 4.379 ± 0.053 (mean = 23,933; $n = 8$) to $\log_{10}$ 4.614 ± 0.045 (mean = 41,069; $n = 8$). In another experiment, mice were given (i.p.) a single injection of 20 μg of Rs-LPS on the day of, or on different days after, immunization (i.p.) with 0.5 μg of SSS-III; the magnitude of the PFC response produced was assessed 5 days after immunization and compared with that of control immunized mice not given Rs-LPS. Treatment with Rs-LPS had no effect on the magnitude of the SSS-III-specific PFC response when given either on the day of immunization (day 0) or 1 day after immunization (day +1) with SSS-III (data not shown); however, a significant increase ($P < 0.05$) in the SSS-III-specific PFC response was observed when Rs-LPS was given 2 days after immunization; the magnitude of the SSS-III-specific PFC response increased from $\log_{10}$ 4.233 ± 0.063 (mean = 17,116; $n = 10$) to $\log_{10}$ 4.446 ± 0.055 (mean = 27,972; $n = 10$). A greater increase was noted when Rs-LPS was given 3 days after immunization with SSS-III, when the magnitude of the SSS-III-specific PFC response increased from $\log_{10}$ 4.233 ± 0.063 (mean = 17,116; $n = 10$) to $\log_{10}$ 4.608 ± 0.048 (mean = 40,590; $n = 10$). Although significant enhancement of the

TABLE 1. Effect of treatment with a single injection of Rs-LPS on expression of low-dose Immunological Paralysis to SSS-III

Exptl group	Treatment[a]			SSS-III-specific PFC/spleen[b]
	SSS-III		Rs-LPS (μg)	
	Priming (0.005 μg)	Immunization (0.5 μg)		
A	−	+	0	4.346 ± 0.072 (22,159)
B	+	+	0	3.563 ± 0.064 (3,654)
C	+	+	0.1	3.817 ± 0.078 (6,566)
D	+	+	1	4.057 ± 0.047 (11,416)
E	+	+	10	4.008 ± 0.055 (10,191)

[a] Mice were pretreated (primed) with a single injection (i.p.) of 0.005 μg of SSS-III 3 days before immunization (i.p.) with 0.5 μg of SSS-III. Rs-LPS was given (i.p.) at the time of immunization with 0.5 μg of SSS-III.

[b] Log_{10} SSS-III-specific PFCs per spleen ± SEM for groups of seven or eight BALB/cByJ mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.

TABLE 2. Effect of treatment with two injections of Rs-LPS on expression of low-dose immunological paralysis to 0.5 μg of SSS-III

Exptl group	Treatment[a]			SSS-III-specific PFC/spleen[b]
	SSS-III		Rs-LPS (μg)	
	Priming (0.005 μg)	Immunization (0.5 μg)		
A	−	+	0	4.082 ± 0.051 (12,089)
B	+	+	0	3.148 ± 0.153 (1,406)
C	+	+	0.01	3.648 ± 0.078 (4,450)
D	+	+	0.1	3.839 ± 0.090 (6,906)
E	+	+	1	4.279 ± 0.099 (19,014)

[a] Mice were pretreated (primed) with a single injection (i.p.) of 0.005 μg of SSS-III 3 days before immunization (i.p.) with 0.5 μg of SSS-III. Rs-LPS was given (i.p.) at the time of immunization (day 0) and 1 day after immunization (day +1) with 0.5 μg of SSS-III.

[b] Log_{10} SSS-III-specific PFCs per spleen ± SEM for groups of nine BALB/cByJ mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.

PFC response also was noted when mice were given 20 μg of Rs-LPS 4 days after immunization with 0.5 μg of SSS-III, the degree of enhancement obtained was no greater than that for mice given the same amount of Rs-LPS 3 days after immunization with SSS-III (data not shown). The results of these experiments attest to the fact that a significant increase in the magnitude of the antibody (PFC) response to SSS-III can be demonstrated routinely in mice given 20 μg of Rs-LPS 3 days after immunization with SSS-III. The effect of giving larger amounts of Rs-LPS was not examined.

Effect of Rs-LPS on induction and expression of low-dose immunological paralysis. Previous studies showed that prior exposure (priming) to a single injection of a marginally immunogenic dose of SSS-III results in the development of an antigen-specific form of unresponsiveness termed low-dose immunological paralysis (12). Such unresponsiveness, which requires at least 3 days to be induced fully, persists for several weeks or months after priming and is known to be mediated by Ts (2, 11). Since treatment with MPL has been shown to abolish the inhibitory effects of Ts (5, 6), mice were given a single injection (i.p.) of different amounts of Rs-LPS either at the time of priming or 3 days after priming to determine if treatment with Rs-LPS alters the induction or expression of low-dose paralysis, respectively.

Although priming with a single injection of 0.005 μg of SSS-III resulted in the development of significant unresponsiveness 3 days later (Tables 1 and 2 and several previously published studies [2, 11, 12]), treatment with 0.1 to 10 μg of Rs-LPS at the time of priming resulted in only a slight reduction in the degree of unresponsiveness induced (data not shown). Thus, treatment with Rs-LPS appears to have only a slight effect at best on the induction of low-dose paralysis. The effects of treatment with 0.1 to 10 μg of Rs-LPS on the expression of fully induced low-dose paralysis were more impressive (Table 1). Here, treatment with increasing amounts of Rs-LPS 3 days after priming, i.e., when primed mice were immunized with 0.5 μg of SSS-III, resulted in a corresponding decrease in unresponsiveness (group B versus group C, $P < 0.05$; group B versus group D or E, $P < 0.001$). Although unresponsiveness was substantially reduced, it was not eliminated, even in primed mice given 10 μg of Rs-LPS (group A versus group E, $P < 0.001$).

In view of the results obtained in a previous study with multiple injections of smaller amounts of MPL (5), we decided to examine whether treatment with more than one injection of Rs-LPS might be more effective than one large dose in abolishing the expression of low-dose paralysis. This indeed appeared to be the case (Table 2). Treatment with two injections (i.p.) of 0.01 or 0.1 μg of Rs-LPS at the time of immunization (day 0) and 1 day after immunization (day +1) with 0.5 μg of SSS-III increased the response of primed mice to within 30 to 50% of unprimed-control values (group B versus group C or D, $P < 0.02$). More important, treatment with two injections of only 1 μg of Rs-LPS completely abolished unresponsiveness; here, the resulting PFC response did not differ significantly from that of unprimed immunized controls (group A versus group E, $P > 0.05$).

Polyclonal activation of B-cell IgM synthesis by Rs-LPS. Groups of mice were given a single injection (i.p.) of different amounts of Rs-LPS or *E. coli* O113 LPS. Numbers of non-antigen-specific IgM-secreting PFCs per spleen were determined 3 days later, and the results obtained were compared with the baseline values for IgM-secreting PFCs per spleen in unimmunized mice not given LPS.

The data in Table 3 show that *E. coli* O113 LPS is a very potent activator of polyclonal IgM synthesis, since treatment with 10 μg of this preparation of LPS caused a significant increase (about threefold; $P < 0.001$) in numbers of IgM-secreting PFCs per spleen; in this case, all plaques detected were rather large and well defined. In contrast, treatment with 10 to 50 μg of Rs-LPS resulted in no significant change ($P > 0.05$) in numbers of IgM-secreting PFC detected. Although the administration of 100 μg of Rs-LPS caused a significant ($P < 0.001$) increase in IgM-secreting PFCs, it should be noted that all plaques detected, though in greater numbers, were faint and not as well defined as those found after the administration of *E. coli* O113 LPS; this suggests a lower rate of IgM synthesis by such PFCs. These findings indicate that Rs-LPS, even at high doses, is a very weak activator of polyclonal IgM synthesis. In view of these findings it appears that the augmented SSS-III-specific PFC response noted in the preceding experiments, in which mice

TABLE 3. Numbers of PFCs secreting non-antigen-specific IgM in the spleens of nonimmunized mice given LPS

LPS (μg/mouse)	IgM-secreting PFC/spleen[a]	P[b]
Control (0)	5.112 ± 0.038 (129,394)	
E. coli O113 LPS (10)	5.471 ± 0.041 (295,674)	<0.001
Rs-LPS (10)	5.063 ± 0.040 (115,603)	>0.05
Rs-LPS (20)	5.186 ± 0.050 (153,483)	>0.05
Rs-LPS (50)	5.244 ± 0.105 (175,363)	>0.05
Rs-LPS (100)	5.489 ± 0.059 (306,919)	<0.001

[a] Log_{10} IgM-secreting PFCs per spleen ± SEM for groups of 10 BALB cByJ mice 3 days after the administration (i.p.) of LPS; geometric means (antilogs) are in parentheses.
[b] Probability (*P*) values relative to control mice not given LPS.

were given 10 or 20 μg of Rs-LPS, cannot be attributed simply to the polyclonal activation of IgM synthesis.

Requirement for T cells to obtain Rs-LPS-induced enhancement of the antibody response to SSS-III. Athymic nude (*nu/nu*) mice, as well as the genetically similar thymus-bearing controls (*nu/+* mice), were given a single injection (i.p.) of 20 μg of Rs-LPS 3 days after immunization (i.p.) with 0.5 μg of SSS-III. The magnitude of the SSS-III-specific PFC response produced was determined 5 days after immunization with SSS-III, and the results obtained were compared with those for immunized *nu/nu* and *nu/+* mice not given Rs-LPS.

Treatment with 20 μg of Rs-LPS caused a significant increase (about fourfold; $P < 0.001$) in the SSS-III-specific PFC response of thymus-bearing *nu/+* mice (Table 4); however, no enhancement ($P > 0.05$) was noted for immunized athymic *nu/nu* mice given Rs-LPS. These results are similar to those obtained in a previous study in which *nu/nu* and *nu/+* mice were given MPL 2 days after immunization with SSS-III (5). Thus, the ability of both Rs-LPS and MPL to augment the antibody (PFC) response to SSS-III is T cell dependent.

The results presented in Table 4 also indicate that in the absence of treatment with Rs-LPS, *nu/nu* mice make a better antibody response to SSS-III than thymus-bearing *nu/+* mice. This is not an unusual finding since it has been noted in other studies (9, 23). It is a reflection of the fact that B cells involved in the antibody response to SSS-III respond more effectively in the absence of the inhibiting effects of Ts present in *nu/+* mice.

TABLE 4. Effect of treatment with 20 μg of Rs-LPS on the magnitude of the 5-day PFC response of *nu/nu* and *nu/+* mice to 0.5 μg of SSS-III

Mice	Treatment[a]		SSS-III-specific PFC/spleen[b]
	SSS-III (0.5 μg)	Rs-LPS (20 μg)	
nu/+	+	−	3.247 ± 0.134 (1,767)
	+	+	3.883 ± 0.130 (7,636)
nu/nu	+	−	3.721 ± 0.119 (5,265)
	+	+	3.649 ± 0.103 (4,453)

[a] Mice were given (i.p.) 20 μg of Rs-LPS 3 days after immunization (i.p.) with 0.5 μg of SSS-III.
[b] Log_{10} SSS-III-specific PFCs per spleen ± SEM for groups of 10 *nu/nu* or *nu/+* mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.

TABLE 5. Effect of in vitro treatment with MPL on ability of primed spleen cells to transfer suppression

No. of primed cells transferred	MPL treatment[a]	SSS-III-specific PFC/spleen[b]	P[c]
0	0	4.133 ± 0.066 (13,598)	
20×10^6	0	3.952 ± 0.035 (8,947)	<0.05
20×10^6	5 ng	4,162 ± 0.075 (14,505)	>0.05
20×10^6	0.5 μg	4.030 ± 0.080 (10,716)	>0.05
20×10^6	5 μg	4.058 ± 0.086 (11,430)	>0.05

[a] Primed spleen cells were obtained from mice 18 to 24 h after the administration (i.p.) of 0.005 μg of SSS-III; they were treated in vitro with different amounts of MPL prior to transfer. Cells were transferred (i.v.) at the time of immunization (i.p.) with 0.5 μg of SSS-III.
[b] Log_{10} SSS-III-specific PFCs per spleen ± SEM for groups of eight mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.
[c] *P* values based on comparisons with immunized controls not given primed cells.

Inactivation of Ts activity after in vitro treatment with MPL or Rs-LPS. A pooled spleen cell suspension was prepared from mice 18 to 24 h after prior exposure (priming) to a single injection (i.p.) of 0.005 μg of SSS-III. The cell suspension was adjusted with medium 199 to contain 10×10^7 nucleated cells per ml and dispensed in 2.5-ml portions among several tubes. To each tube was added a known amount (0.005 ng to 5 μg) of either MPL or Rs-LPS in a volume of 50 μl; the contents were held at 4°C for 30 min after mixing. Then, groups of mice were given (i.v.) 20×10^6 cells in a volume of 0.2 ml at the time of immunization (i.p.) with 0.5 μg of SSS-III; the magnitude of the SSS-III-specific PFC response elicited was determined 5 days after immunization and compared with that of (i) immunized mice not given primed spleen cells and (ii) immunized mice given primed spleen cells not treated in vitro with either MPL or Rs-LPS.

The transfer of 20×10^6 primed spleen cells not treated with MPL caused significant ($P < 0.05$) suppression of the PFC response, as expected (Table 5); such suppression has been demonstrated to be antigen specific and mediated by $CD8^+$ $CD4^-$ Ts, activated following exposure to SSS-III (2, 40). Treatment with all amounts of MPL tested, including as little as 5 ng, abrogated the ability of primed cells to transfer suppression. Similar results were obtained when primed spleen cells were treated in vitro with Rs-LPS before transfer (Table 6); here, treatment with as little as 5 pg of Rs-LPS eliminated the suppressive effects of primed spleen cells. These findings attest to the fact that treatment with extremely small amounts of MPL or Rs-LPS is very effective in inactivating the inhibitory effects produced by transferred Ts. In all of these experiments, cells treated with MPL or Rs-LPS were not washed prior to transfer to remove residual MPL or Rs-LPS. In view of the extremely small amounts of MPL and Rs-LPS used, this was not believed to be necessary since the administration of 10 to 50 μg of MPL or 20 μg of Rs-LPS at the time of immunization with 0.5 μg of SSS-III

TABLE 6. Effect of in vitro treatment with Rs-LPS on ability of primed spleen cells to transfer suppression

No. of primed cells transferred	Rs-LPS treatment[a]	SSS-III-specific PFC/spleen[b]	P[c]
0	0	4.532 ± 0.064 (34,068) (n = 9)	
20×10^6	0	4.271 ± 0.064 (18,662) (n = 10)	<0.01
20×10^6	5 pg	4.689 ± 0.059 (48,862) (n = 10)	>0.05
20×10^6	5 ng	4.564 ± 0.041 (36,635) (n = 10)	>0.05
20×10^6	5 μg	4.388 ± 0.057 (24,453) (n = 10)	>0.05

[a] Primed spleen cells were obtained from mice 18 to 24 h after the administration (i.p.) of 0.005 μg of SSS-III; they were adjusted to contain 100×10^6 nucleated cells per ml. Different amounts of Rs-LPS in a volume of 50 μl were added to 2.5 ml of the resulting cell suspension; the mixture was held at 4°C for about 30 min, after which 20×10^6 cells were transferred (i.v.) at the time of immunization (i.p.) with 0.5 μg of SSS-III.

[b] Log_{10} SSS-III-specific PFCs per spleen ± SEM for groups of n mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.

[c] P values based on comparisons with immunized controls not given primed cells.

does not influence the magnitude of the SSS-III-specific PFC response produced (5; data not shown).

DISCUSSION

The present works shows that, except for the doses used, the effects of treatment with Rs-LPS on the antibody response to SSS-III were similar to those described previously in a report of a study conducted with MPL (5). Both were without effect when given at the time of immunization with an optimally immunogenic dose of SSS-III; however, they elicited significant enhancement when given 2 to 3 days after immunization (5). In both cases, such enhancement was T cell dependent and not due to the polyclonal activation of IgM synthesis by B cells (5; Tables 3 and 4). Treatment with Rs-LPS (Table 2) or MPL (5) abrogated the expression, but not the induction, of low-dose immunological paralysis, a form of antigen-specific unresponsiveness mediated by Ts (2, 11, 13, 15). Other studies have established that the magnitude of the antibody response to SSS-III is regulated in a negative and positive manner by the competitive interaction of Ts and T_A, respectively (for reviews see references 3 and 7). Since treatment with MPL eliminates Ts activity, without altering the expression of T_A and T_H function (5), it appears that the immunomodulatory effects elicited by Rs-LPS, like those of MPL, are mainly the result of eliminating the inhibitory effects produced by Ts, which are activated after exposure to SSS-III; this permits the positive effects of T_A to be more fully expressed, thereby resulting in an increased (enhanced) antibody response to an optimally immunogenic dose of SSS-III or the abrogation of low-dose immunological paralysis (5).

It should be noted that under the same experimental conditions, comparable immunomodulatory effects could be demonstrated with smaller amounts of Rs-LPS than MPL (5). Although the reasons for this are not known, differences in molecular size may be a contributing factor. In contrast to Rs-LPS, which is a complex macromolecule (28, 35), MPL is a small molecule with an average molecular size of 1,718 (5, 33). Consequently, one might expect MPL to be cleared from the circulation within a relatively short period of time after injection, thereby requiring larger amounts to produce a detectable effect. Alternatively, differences between Rs-LPS and MPL in their specific activity may be related to subtle differences in their chemical composition and/or structure. Unfortunately sufficient information is not available to clarify this matter.

The ability to transfer antigen-specific suppression with $CD8^+$ lymphocytes derived from mice previously exposed to SSS-III provides unequivocal proof that such unresponsiveness is indeed mediated by Ts, which play an active role in regulating the magnitude of the antibody response to SSS-III (2, 3, 40). The fact that prior treatment in vitro with minute amounts of nontoxic MPL (Table 5) or Rs-LPS (Table 6) abolished the capacity of such cells to transfer suppression indicates that both MPL and Rs-LPS are extremely effective in abrogating Ts activity. Although the mechanism(s) by which this occurs remains to be defined, it surely must involve more than just the binding of Rs-LPS or MPL to the surface of Ts; other studies have shown that the binding and subsequent elution of antigen-primed spleen cells from plastic dishes coated with MPL results in >1,000-fold enrichment, not a decrease, of Ts activity (4). Since neither T_A nor T_H activity is impaired by treatment with large amounts of MPL (5), it appears that MPL, and perhaps Rs-LPS, after binding to Ts, either (i) decreases their metabolic activity or (ii) alters their distribution in tissues after cell transfer so that they can no longer influence the magnitude of the antibody response to SSS-III. Here, we assume that the former possibility requires MPL to be internalized, a process which may not occur in the reaction between Ts and MPL attached to an insoluble matrix, e.g., plastic dishes. These issues are now being examined.

There is compelling evidence to indicate that cachectin or TNF is the principle mediator of the lethal effects of endotoxin during gram-negative bacterial infections (17, 18, 27). In this context, the administration of recombinant TNF has been shown to mimic many of the toxic effects ascribed to endotoxin (16, 26, 32, 41), whereas the infusion of polyclonal antibody specific for TNF neutralizes or blocks the expression of such effects (19). Thus, TNF appears to be an ideal target for pharmacotherapeutic intervention during severe endotoxemia. It has been reported that the nontoxic DPLA of Rs-LPS not only fails to induce the synthesis and release of TNF by macrophages but also competes successfully with toxic LPS to block the induction of TNF in a dose-dependent manner (38). This suggests that nontoxic DPLA, as well as ReLPS, might be used prophylactically to prevent the development of endotoxic shock (38). Furthermore, the ability of Rs-LPS to augment the antibody response by abrogating the inhibitory effects of Ts suggests that Rs-LPS might also be effective in enhancing host immunity, thereby resulting in a significant reduction of the amount of endotoxin elaborated during gram-negative sepsis. Obviously, systematic studies using various animal models will be required to confirm this view.

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