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Enrichment of Suppressor T Cells by Means of Binding to Monophosphoryl Lipid A

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The binding and elution of spleen cells from plastic dishes coated with monophosphoryl lipid A (MPL) resulted in a >1,000-fold enrichment of antigen-specific suppressor T-cell (T_S) activity when spleen cells from mice 18 to 24 h after exposure to a low dose of type III pneumococcal polysaccharide (SSS-III) were used. The removal of MPL-adherent T_S cells resulted in an increase in the degree of amplifier T-cell (T_A) activity present in the remaining MPL-nonadherent cell fraction; however, both T_S and T_A activities were found in the MPL-adherent cell fraction when spleen cells from mice 4 days after immunization with an optimal dose of SSS-III were examined. These findings, as well as others, suggest that both T_S and T_A, once activated, acquire a cell surface receptor that enables them to bind to MPL. Because of differences in the kinetics for the activation of T_S and T_A during the course of the antibody response and the fact that T_S, but not T_A, activity appears as early as 18 to 24 h after exposure to SSS-III, it is possible to use this experimental approach to obtain cell suspensions greatly enriched in T_S activity.

The magnitude of the antibody response to type III pneumococcal polysaccharide (SSS-III) is influenced in both a positive and a negative manner by the activities of two types of T cells having opposing functions; such regulatory T cells have been termed suppressor T cells (T_S) and amplifier T cells (T_A), respectively (reviewed in references 1 and 6). T_S limit the extent to which antigen-stimulated B cells proliferate, whereas T_A drive B cells to proliferate further in response to antigen (1, 6, 11, 21). Neither T_S nor T_A are activated directly by antigen per se; rather, both are activated during the course of a normal antibody response by cell-associated antibody on the surface of immune B cells (14, 26). Since the inhibitory effects produced by activated T_S are antigen specific (12), the idiotypic determinants of cell-associated antibody most likely play a major role in this homeostatic control mechanism (1, 6, 14, 26). T_S and T_A differ with respect to the phenotype of the cell surface Lyt antigens they possess. Since they are T cells, both are Thy-1^+; however, T_S are Lyt-1^-, L3T4$^-$, and Lyt-2^+, whereas T_A are Lyt-1^+, L3T4$^+$, and Lyt-2^- (3, 4, 14, 22, 24, 25). These differences have enabled one, by means of cell depletion procedures with appropriate monoclonal antibodies and complement, to demonstrate the activity of one type of regulatory T cell in the absence of the other in cell transfer experiments (3, 4, 24, 26). This would be difficult to do with cell suspensions containing both T_S and T_A activity because of the competitive nature of the cell interactions involved (reviewed in reference 4).

Recently, we found that treatment with nontoxic monophosphoryl lipid A (MPL), derived from a heptoseless Re mutant of *Salmonella typhimurium*, abrogates T_S activity, as evidenced by a decrease in the degree of T_S-mediated low-dose immunological paralysis expressed, as well as an increase in the magnitude of the antibody response to an optimally immunogenic dose of SSS-III (5). These effects, which could not be attributed to the polyclonal activation of immune B cells by MPL, were dependent on the dose of MPL used, as well as the time when MPL was given relative to low-dose priming or immunization with SSS-III (5). Since neither T_A nor helper T-cell (T_H) activity was decreased by treatment with the same, or larger, amounts of MPL (5), these findings suggest that the effects observed might initially involve the binding of MPL to a receptor present on activated T_S. This possibility was examined in the present work, in which we find that such binding, though not unique to T_S, does indeed occur and can be used with advantage to obtain cell suspensions greatly enriched in T_S activity.

MATERIALS AND METHODS

Mice. Female BALB/cByJ mice (8 to 10 weeks of age), obtained from the Jackson Laboratory, Bar Harbor, Maine, were used in this work.

Antigen and immunization procedure. The immunological properties of the preparation of SSS-III and bacterial (*Leuconostoc*) dextran B-1355 used as well as the methods by which they were prepared have been described previously (2, 6–10), For immunization, mice were given a single intraperitoneal (i.p.) injection of an optimally immunogenic dose (0.5 μg) of SSS-III or 2 μg of dextran B-1355 in 0.5 ml of saline. The magnitude of the antibody response produced was determined at peak, i.e., 5 days after immunization.

Immunological methods. Numbers of antibody-producing plaque-forming cells (PFC) making antibody specific for the immunogen provided a measure of the antibody response produced. PFC making antibody of the immunoglobulin M (IgM) class (>90% of all SSS-III-specific PFC found [2, 9]) were detected in individual mice by a slide version of the technique of localized hemolysis-in-gel, using indicator sheep erythrocytes coated with SSS-III (13) or dextran (8). Corrections were made (by subtraction) for numbers of background sheep erythrocyte-specific PFC found (<200 per spleen) so that only numbers of antigen-specific PFC are considered. The values obtained (numbers of antigen-specific PFC per spleen), which are log-normally distributed

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(16), are expressed as the geometric mean of the $\log_{10}$ number of PFC per spleen for groups of similarly treated mice. This provides a valid measure of the total antibody response produced against SSS-III because PFC making antibody specific for SSS-III can be detected only in the spleen and values for SSS-III-specific PFC per spleen parallel those for serum antibody titers (10, 18). 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.

MPL. MPL was obtained from Ribi ImmunoChem Research, Inc., Hamilton, Mont. It was isolated from the heptoseless Re mutant of *S. typhimurium* G30/C21 as described previously (23). Lyophilized MPL was reconstituted to 1 mg/ml in distilled water containing 0.2% triethylamine. It was warmed to 45°C, mixed thoroughly in a Vortex mixer, and sonicated for 10 to 20 s to obtain an opalescent stock solution which was stored at 4°C until used; the stock solution was diluted with saline to the desired concentration of MPL. Pertinent information on the properties of MPL, as well as of the bacterial lipopolysaccharide (LPS) from which it was derived, has been published elsewhere (5, 23).

Coating culture dishes with anti-IgM antibody. Affinity-purified goat anti-mouse IgM antibody (Southern Biotechnology, Birmingham, Ala.) was diluted to 20 μg of protein per ml in 0.05 M Tris buffer, pH 9.5, and 5 ml of the resulting solution was added to Primaria plastic culture dishes (100 by 20 mm; Becton Dickinson Labware, Oxnard, Calif.). The dishes were kept at room temperature for 1 h and then placed at 4°C overnight (12 to 18 h), after which they were washed several times with phosphate-buffered saline, pH 7.4, before use.

Coating culture dishes with MPL. Primaria culture dishes were flooded with 10 ml of 15% isopropyl alcohol and held at room temperature for 30 min. The alcohol was decanted and, while the dishes were still wet, 10 ml of a solution of MPL (10 μg of MPL per ml in 0.05 M bicarbonate buffer, pH 9.5) was added. The dishes were incubated for 2 h at 37°C and then placed at 4°C overnight (12 to 18 h), after which the MPL was decanted and each dish was washed twice with 10 ml of bicarbonate buffer. About 10 ml of 0.01 M Tris buffer, pH 7.5, containing 0.2% bovine serum albumin and 0.1% Tween 20, was added, and the dishes were incubated for 1 h at 37°C; the solution was decanted from the dishes just prior to use.

The binding of MPL to the surface of coated dishes was affirmed by means of an enzyme-linked immunosorbent assay spot test. Discrete drops (10 μl) containing twofold dilutions of a mouse monoclonal IgG2b antibody specific for lipid A (MoAb-H3) were applied in a linear pattern to the surface of a dish coated previously with MPL. The dish was held at room temperature for 1 h, after which it was rinsed with several volumes of phosphate-buffered saline to remove unbound antibody. Then, the dish was flooded with a dilute solution of affinity-purified goat anti-mouse IgG2b antibody to which was conjugated alkaline phosphatase; the dish was held at room temperature for 1 h, washed several times with phosphate-buffered saline, and then covered with 5 ml of melted (45°C) 0.6% agarose containing appropriate substrate. After incubation for about 1 h at 37°C, colored spots appeared only where monoclonal antibody specific for lipid A was applied. The application of monoclonal antibody not specific for lipid A was without effect. Although details concerning the preparation and specificity of monoclonal antibody MoAb-H3 have not been published, they can be obtained from J. T. Ulrich upon request.

Isolation of MPL-adherent cells. Spleen cell suspensions from mice given (i.p.) either a low (0.005 μg) or an optimally immunogenic (0.5 μg) dose of SSS-III were prepared in medium 199. The cells were washed once by centrifugation and adjusted to contain 10^7 nucleated cells per ml of medium 199. Then, 10 ml of the resulting cell suspension was added to each of 10 dishes coated previously with affinity-purified goat anti-mouse IgM antibody. The dishes were held at 4°C for 40 min, after which the cell suspension was swirled gently and the dishes were held at 4°C for 30 min. The contents were swirled once more, and the suspension of unbound B-cell-depleted cells (26) from one dish was added directly to a second dish coated previously with MPL. Those dishes were held at 4°C for 40 min and then, after swirling the contents, for 30 min at 4°C, after which the unbound cells were decanted and the dishes were rinsed twice with about 10 ml of medium 199 to remove residual unbound cells.

MPL-adherent cells were detached from each dish by the addition of 5 ml of a thymus cell extract containing 15 μg of soluble protein per ml of medium 199. After incubation for 10 min at 37°C, the detached cells were collected, washed once with medium 199, and suspended in a total volume of 2 ml of medium 199. The thymus cell extract, which we assume contains the full array of surface antigens present on all T cells including T_S, was prepared by disrupting a single cell suspension of thymus cells with a Teflon tissue homogenizer. The resulting suspension, which was initially prepared in phosphate-buffered saline, pH 7.4, was centrifuged to remove all insoluble material and then adjusted spectrophotometrically (optical density at 280 nm) to contain 1 mg of soluble protein per ml; this stock solution was stored in small aliquots at −70°C and diluted in medium 199 just before use.

In the experiments described, 39.0 ± 3.7% (mean ± standard error) of cells initially added did not attach to dishes coated with anti-mouse IgM antibody. About 52.8% of such cells, or about 20.6% of the initial spleen cell suspension, bind to MPL-coated dishes. Of the cells binding to MPL-coated dishes, about 15.7% are detached by the addition of thymus cell extract. Thus, the detached cells represent about 2.9% or less of the initial cell suspension. Ten dishes coated with anti-mouse IgM antibody and MPL provide enough donor cells for use in each of the experiments described.

We wish to emphasize the fact that different types of cells may bind to dishes coated with MPL; however, the present work deals with the functional activity of only those adherent cells that are detached after the addition of thymus cell extract. Although this results in a substantial enrichment of regulatory T-cell activity, we are not proposing that MPL binds specifically to such cells.

Monoclonal antibodies and their use. A 50-μl portion of dilute (1:10) monoclonal anti-mouse Thy-1.2, Lyt-1.2, or Lyt-2.2 antibody (NEI-011, NEI-017, or NEI-006, respectively; Dupont NEN Research Products, Boston, Mass.) was added to 2.5 ml of washed MPL-adherent cells (about 5×10^6 to 10×10^6 cells per ml) in medium 199. The mixture was held at 4°C for 30 min, after which the cells were washed by centrifugation and suspended to the original volumes. Then, 1.5 ml of dilute (1:20) rabbit complement (Low-Tox-M; Accurate Scientific, Inc., Westbury, N.Y.) was added and the mixture was placed at 37°C for 30 min. The cells were washed once by centrifugation and adjusted to contain the desired number of cells for injection (intravenously [i.v.]) in 0.2 ml of medium 199. Controls consisted of cell suspensions to which only complement was added.

TABLE 1. Enrichment of T_S activity in populations of MPL-adherent primed spleen cells

Cells examined[a]	No. of cells transferred	SSS-III-specific PFC/spleen[b]	P[c]
Immunized controls		4.322 ± 0.048 (20,944)	
Initial cell suspension	2×10^6	4.334 ± 0.054 (21,587)	>0.05
Initial cell suspension	2×10^7	4.030 ± 0.109 (10,716)	<0.05
MPL-adherent cells	1.5×10^5	4.058 ± 0.100 (11,434)	<0.05
MPL-adherent cells	1.5×10^6	4.063 ± 0.064 (11,567)	<0.01

[a] The initial cell suspension was obtained from mice 18 to 24 h after prior treatment (priming) with 0.005 μg of SSS-III. MPL-adherent cells represent primed spleen cells eluted from plates coated with MPL. Cells were administered i.v. at the time of immunization with SSS-III.

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

[c] *P* values were based on comparisons to immunized controls not given spleen cells.

TABLE 2. Enrichment of T_S activity in populations of MPL-adherent primed spleen cells

Cells examined[a]	No. of cells transferred	SSS-III-specific PFC/spleen[b]	P[c]
Immunized controls		4.200 ± 0.037 (15,854)	
Initial cell suspension	2×10^6	4.145 ± 0.034 (13,974)	>0.05
Initial cell suspension	2×10^7	3.898 ± 0.110 (7,914)	<0.05
MPL-adherent cells	1.5×10^4	3.835 ± 0.067 (6,842)	<0.02
MPL-adherent cells	1.5×10^5	3.775 ± 0.151 (5,951)	<0.05

[a] The initial cell suspension was obtained from mice 18 to 24 h after prior treatment (priming) with 0.005 μg of SSS-III. MPL-adherent cells represent primed spleen cells eluted from plates coated with MPL. Cells were administered (i.v.) at the time of immunization with SSS-III.

[b] Log_{10} SSS-III-specific PFC per spleen ± standard error 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 were based on comparisons to immunized controls not given spleen cells.

RESULTS

Binding of T_S to MPL-coated dishes. A pooled spleen cell suspension was prepared from 10 mice 18 to 24 h after pretreatment (priming) with a single injection (i.p.) of a subimmunogenic (0.005 μg) dose of SSS-III to induce low-dose immunological paralysis, a form of unresponsiveness known to be mediated by T_S (3, 10, 11, 14). Groups of mice were given (i.v.) 2×10^6 or 2×10^7 cells from this initial suspension, whereas other groups of mice were given (i.v.) 1.5×10^5 or 1.5×10^6 B-cell-depleted, MPL-adherent cells derived from the same suspension. All mice were immunized (i.p.) with 0.5 μg of SSS-III at the time of cell transfer. The magnitude of the antibody (PFC) response elicited was determined 5 days after immunization and compared with that of immunized mice not given primed spleen cells.

The magnitude of the SSS-III-specific PFC response was decreased significantly ($P < 0.05$) in mice given 2×10^7, but not 2×10^6, cells from the initial suspension of primed spleen cells (Table 1); this is consistent with the results of previous studies showing that at least 2×10^7 cells are required to transfer suppression under the experimental conditions used and that such suppression is antigen specific and mediated by Lyt-2^+, Thy-1^+ T_S activated following exposure to SSS-III (3, 26). Significant ($P < 0.05$) suppression also was noted in mice given 1.5×10^5 or 1.5×10^6 MPL-adherent cells. Since the degree of suppression obtained with 1.5×10^5 MPL-adherent cells was similar ($P > 0.05$) to that noted with 2×10^7 cells from the initial cell suspension, adherence to MPL-coated dishes results in at least a 130-fold enrichment of T_S activity in the eluted cell suspension. In another experiment (Table 2), significant ($P < 0.02$) suppression was obtained with as few as 1.5×10^4 MPL-adherent primed spleen cells. Since the degree of suppression observed was comparable ($P > 0.05$) to that noted with 2×10^7 cells from the initial suspension of primed spleen cells, the adherence procedure resulted in a >1,300-fold enrichment in T_S activity in this experiment.

Specificity of the suppression produced by MPL-adherent primed spleen cells. MPL-adherent cells were isolated from mice 18 to 24 h after priming with 0.005 μg of SSS-III as described in the preceding experiments (Tables 1 and 2). Then, 5×10^5 MPL-adherent cells were given (i.p.) to mice at the time of immunization (i.p.) with either 0.5 μg of SSS-III or 2 μg of dextran B-1355. The magnitude of the antibody (PFC) response to each antigen was determined 5 days after immunization and compared with that of immunized mice not given MPL-adherent primed spleen cells.

The administration of 5×10^5 MPL-adherent primed spleen cells caused significant ($P < 0.01$) suppression of the SSS-III-specific PFC response (Table 3); however, the PFC response to dextran was not suppressed ($P > 0.05$) in mice given the same cells. Thus, the suppression mediated by MPL-adherent spleen cells from mice primed with SSS-III is antigen specific.

Expression of T_A activity after the removal of MPL-adherent cells. The results of previous studies showed that T_S and T_A are activated during the course of a normal antibody response to an optimally immunogenic dose (0.5 μg) of SSS-III and that the activities of both types of regulatory T cells are maximal 3 to 4 days after immunization; however, because these cells interact with immune B cells in a competitive manner, it is not possible to demonstrate, by means of cell transfer experiments, the presence of one type of cell without first removing the activity of the other

TABLE 3. Specificity of suppression elicited by MPL-adherent primed spleen cells

Immunization	No. of primed spleen cells transferred[a]	PFC/spleen[b] vs:	
		SSS-III	Dextran
SSS-III, 0.5 μg		4.299 ± 0.037 (19,914), *n* = 10	
SSS-III, 0.5 μg	5×10^5 MPL adherent	3.783 ± 0.109 (6,066), *n* = 10	
Dextran, 2 μg			4.995 ± 0.058 (98,809), *n* = 10
Dextran, 2 μg	5×10^5 MPL adherent		5.035 ± 0.062 (108,361), *n* = 9

[a] Primed spleen cells were obtained from mice 18 to 24 h after prior treatment (priming) with 0.005 μg of SSS-III. MPL-adherent cells represent primed spleen cells eluted from plates coated with MPL.

[b] Log_{10} antigen-specific PFC per spleen ± standard error for groups of *n* mice 5 days after immunization (i.p.) with either 0.5 μg of SSS-III or 2 μg of dextran B-1355; geometric means (antilogs) are in parentheses.

TABLE 4. Enrichment of T_A activity in populations of non-MPL-adherent immune spleen cells

Cell population examined[a]	No. of cells transferred	SSS-III-specific PFC/spleen[b]	P[c]
Immunized controls		4.046 ± 0.069 (11,116)	
Initial cell suspension	2×10^7	4.150 ± 0.067 (14,133)	>0.05
MPL-nonadherent cells	2×10^6	4.128 ± 0.059 (13,441)	>0.05
MPL-nonadherent cells	4×10^6	4.447 ± 0.044 (27,960)	<0.001
MPL-nonadherent cells	8×10^6	4.433 ± 0.035 (27,085)	<0.001

[a] The initial spleen cell suspension was obtained from mice 4 days after immunization with 0.5 μg of SSS-III. MPL-nonadherent cells represent immune spleen cells that did not bind to plates coated with MPL. Cells were administered (i.v.) to recipient mice that were immunized, 2 days previously, with 0.5 μg of SSS-III.

[b] Log_{10} SSS-III-specific PFC per spleen ± standard error for groups of 8 mice 5 days after immunization (i.p.) with 0.5 μg of SSS-III; geometric means (antilogs) are in parentheses.

[c] *P* values were based on comparisons to immunized controls not given spleen cells.

(reviewed in reference 4). Therefore, if all or most of the activity of T_S is removed from a cell suspension as a result of binding to MPL-coated dishes, the remaining MPL-nonadherent cell fraction should be greatly enriched for T_A activity. This possibility was examined in the following way. A pooled spleen cell suspension was prepared from 10 mice 4 days after immunization with 0.5 μg of SSS-III. Groups of mice were given (i.v.) various numbers of cells from either this initial cell suspension or the MPL-nonadherent cell suspension 2 days after recipient mice were immunized (i.p.) with 0.5 μg of SSS-III; previous studies showed that donor cells should be given 2 days after immunization with SSS-III to reveal isolated T_A activity best (4, 14, 26). The magnitude of the antibody (PFC) response produced was determined 5 days after immunization and compared with that of immunized mice not given donor cells.

The administration of 2×10^7 spleen cells from the initial cell suspension resulted in no significant change (Table 4; $P > 0.05$) in the magnitude of the PFC response; the transfer of smaller (2×10^6) or larger (8×10^7) numbers of cells likewise was without effect ($P > 0.05$; data not shown). Although the transfer of 2×10^6 MPL-nonadherent cells failed to influence the PFC response ($P > 0.05$), the administration of 4×10^6 and 8×10^6 MPL-nonadherent cells resulted in significant enhancement ($P < 0.001$, in both cases). Although these findings suggest that the binding of T_S to MPL can result in the enrichment of T_A activity in the remaining nonadherent cell fraction, the results of another experiment (Table 5) show that both T_S and T_A, which are activated and present in the spleen 4 days after immunization with an optimal dose of SSS-III (reviewed in reference 4), bind to MPL-coated dishes. Here, because of the competitive interaction between T_S and T_A, the administration of MPL-adherent donor cells treated with complement alone results in no significant change ($P > 0.05$) in the magnitude of the antibody response to SSS-III. Likewise, the inactivation of both types of regulatory T cells by treatment with monoclonal anti-Thy-1.1 plus complement is without effect ($P > 0.05$); however, treatment with monoclonal anti-Lyt-1 antibody plus complement (to remove T_A activity) resulted in the expression of significant ($P < 0.005$) suppression, whereas treatment with monoclonal anti-Lyt-2 antibody plus complement (to remove T_S activity) led to the expression of significant ($P < 0.05$) enhancement. Since T_A activity is not demonstrable in suspensions of MPL-adherent cells collected 18 to 24 h after exposure to SSS-III (Tables 1 and 2), it would appear that

TABLE 5. Binding of both T_A and T_S to MPL-coated dishes

Treatment of MPL-adherent donor cells[a]	SSS-III-specific PFC/spleen[b]	P[c]
Immunized controls	4.415 ± 0.035 (26,000), *n* = 10	
Complement	4.425 ± 0.051 (26,600), *n* = 9	>0.05
Anti-Thy-1.2 + complement	4.405 ± 0.055 (25,400), *n* = 9	>0.05
Anti-Lyt-1.2 + complement	4.194 ± 0.036 (15,600), *n* = 8	<0.005
Anti-Lyt-2.2 + complement	4.564 ± 0.054 (36,600), *n* = 9	<0.05

[a] MPL-adherent donor cells were obtained from mice 4 days after immunization with 0.5 μg of SSS-III. Recipient mice were given (i.v.) 10^5 donor cells at the time of immunization (i.p.) with 0.5 μg of SSS-III. Donor cells were treated with complement, with or without monoclonal antibodies, prior to transfer.

[b] Log_{10} SSS-III-specific PFC per spleen ± standard error 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 were based on comparisons to immunized controls not given spleen cells.

both T_S and T_A, once activated, acquire a cell surface receptor that promotes binding to MPL. Previous studies show that T_A activity can be detected in the spleen 3 days after priming with 0.005 μg of SSS-III (3, 26).

DISCUSSION

Differences between T_S and T_A in Lyt phenotype have enabled us to examine the effects of one type of regulatory T cells in the absence of the other and to establish that such cells act on B cells in an opposing and competitive manner to influence the magnitude of the antibody response to SSS-III (3, 4, 14, 22, 24). T_S are Lyt-2^+, whereas T_A are Lyt-2^-; however, this does not necessarily mean that all Lyt-2^+ lymphocytes are T_S or that T_S are identical to cytotoxic T cells which also are Lyt-2^+ in phenotype (15). Obviously, the ability to obtain cell populations greatly enriched for T_S activity would permit one to determine the frequency of T_S within the Lyt-2^+ subset, as well as to obtain more precise information on the mode of action of T_S.

Treatment with MPL results in a significant decrease in the expression of T_S activity, without altering T_A and T_H function (5). Although the basis for this selective effect of MPL on T_S is not known, the binding of MPL to a cell surface receptor on activated T_S might play an important role. In the present work, the binding and subsequent elution of B-cell-depleted primed spleen cells from MPL-coated dishes result in more than a 100- to 1,000-fold enrichment of T_S activity in the resulting cell suspension (Tables 1 and 2). Since the suppression elicited by MPL-adherent primed spleen cells is antigen specific (Table 3), it is characteristic of that mediated by T_S in other studies conducted under the same experimental conditions, using spleen cells obtained from mice exposed to a low dose of SSS-III (3, 22). If spleen cell suspensions known to contain both T_S and T_A are subjected to the same experimental manipulations, the deletion of MPL-adherent T_S can result in the detection of significant T_A activity in the remaining nonadherent fraction (Table 4); however, under such experimental conditions, one also can demonstrate the presence of both types of regulatory T cells in the MPL-adherent cell fraction obtained 4 days after immunization with an optimal dose of SSS-III, i.e., at a time when the expression of T_A activity is known to be maximal (4). Consequently, the degree and type of regulatory T-cell activity present in either the MPL-adherent

or nonadherent cell fraction is likely to depend on a number of variables such as (i) the number and proportion of activated T_S and T_A present initially, (ii) differences between activated T_S and T_A in the density or affinity of receptors for MPL, and (iii) differences between T_S and T_A in the kinetics for their activation following exposure to antigen. With respect to the last possibility, there is evidence to indicate that T_S are activated as early as 18 to 24 h after exposure to SSS-III, whereas T_A activity is not demonstrable before 2 to 3 days after exposure to SSS-III and maximal levels are not attained until 4 to 5 days after immunization (reviewed in reference 4). Therefore, by using spleen cells obtained 18 to 24 h after priming with SSS-III, one can obtain a substantial (>1,000-fold) enrichment of T_S activity in the MPL-adherent cell fraction. There is no procedure extant which will enable one to accomplish this objective.

The existence of a major bacterial LPS-binding protein (80 kilodaltons; 6.5 pI) on purified splenic B and T cells, as well as on macrophages, has been described before (19). This LPS-binding protein reacts with the lipid A, but not the polysaccharide, portion of the LPS molecular complex (20). It not only appears to be the dominant LPS-binding site on cells but also is present on the splenocytes of both LPS-responsive and LPS-unresponsive strains of C3H mice. In addition to this dominant LPS-binding site, two minor LPS-binding proteins (30 kilodaltons; pI > 6.5) can be detected only on T cells (19); it is not known whether these binding proteins are present on T cells of both LPS-responsive and LPS-unresponsive strains of C3H mice and whether they correspond to the MPL-binding site described in the present work. Other studies showed that LPS-responsive and LPS-unresponsive strains of C3H mice do not differ in the degree of low-dose immunological paralysis induced and expressed upon exposure to SSS-III (17). Since this form of unresponsiveness is mediated by T_S (3, 5), these strains of mice do not differ with respect to T_S function; however, low-dose paralysis is abrogated only in LPS-responsive C3H mice treated with MPL (17). This suggests that, if there indeed is an MPL-specific binding site on T_S, it functions in LPS-responsive, but not LPS-unresponsive, strains of C3H mice. Alternatively, MPL may bind equally to T_S of both strains of mice; however, MPL may decrease the functional activity of T_S present in only LPS-responsive C3H mice. These possibilities are being examined.

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