REGULATION OF THE ANTIBODY RESPONSE TO TYPE III PNEUMOCOCCAL POLYSACCHARIDE

I. Nature of Regulatory Cells*

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Cooperation between thymic-derived cells (T cells) and bone marrow-derived precursors of antibody-forming cells (B cells) is not thought to be required for an antibody response to Type III pneumococcal polysaccharide, or SSS-III[1] (1–3). Yet treatment with antilymphocyte or antithymocyte serum (ALS or ATS), which causes a depletion of T cells (4–10), produces a significant increase in the magnitude of both the plaque-forming cell (PFC) and the serum antibody response to this antigen (11–14) and an increase in the serum antibody response to keyhole limpet hemocyanin (15) and polyvinylpyrolidone (16). With respect to the PFC response to SSS-III, such enhancement can be abrogated by the infusion of syngeneic thymocytes; however, the infusion of peripheral white blood cells, a population reported to contain 60–90% T cells (17, 18), results in additional enhancement (12).

On the basis of these findings, we hypothesized that two functionally distinct types of cells (suppressor and amplifier cells[2]), presumably both thymic-derived, act in an opposing manner to regulate the magnitude of the antibody response to SSS-III by B cells; the enhancement produced after treatment with either ALS or ATS is apparently due to the inactivation of cells that normally exert a negative, rather than a positive, influence on the magnitude of the antibody response elicited after immunization (12).

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[1] *Abbreviations used in this paper:* ALS, antilymphocyte serum; ATS, antithymocyte serum; HRBC, horse erythrocytes; PFC, plaque-forming cell(s); SRBC, sheep erythrocytes; SSS-III, Type III pneumococcal polysaccharide.

[2] The term "amplifier cell" is used in this report to distinguish between T cells that are required for the development of an antibody response to some antigens, e.g., "helper" T cells involved in the antibody response to sheep erythrocytes (SRBC) (19), and those types of cells which, although not required for the establishment of an antibody response, are capable of augmenting the magnitude of the response of antigen-stimulated B cells. The mechanism by which both types of cells produce their effects is not known.

The object of the work here reported was to provide more definitive information on the origin and nature of such regulatory cells. This was accomplished by examining the effects of treatment with ALS on the magnitude of the PFC response to SSS-III in congenitally athymic mice, i.e., mice homozygous for the autosomal recessive mutation "nude" (nu/nu mice); phenotypically normal littermates that were either heterozygous or homozygous for the presence of thymus (nu/+ or +/+, respectively) served as controls. The results of such studies showed that, while nude mice gave a PFC response to SSS-III greater than that produced by thymus-bearing control mice, significant enhancement was demonstrable only in the case of control mice treated with ALS. These findings are consistent with the view that both suppressor and amplifier cells represent subpopulations of T cells present in thymus-bearing mice, but absent in nude mice.

Materials and Methods

Animals.—Congenitally athymic nude mice (nu/nu mice) and phenotypically normal littermate controls, i.e., mice heterozygous or homozygous for the presence of thymus (nu/+ or +/+, respectively), were derived by a previously described procedure (20); these mice were bred and maintained at the Department of Botany and Microbiology, Montana State University, Bozeman, Mont. Pertinent information concerning the lack of thymic tissue and normal immunological functions in nude mice have been reported (20–26). Mice 8–12 wk old, of both sexes, were used.

Female BALB/cAnN mice, 8–10 wk old, were obtained from the Rodent and Rabbit Production Unit of the National Institutes of Health, Bethesda, Md. These mice, which are normal with respect to the presence of thymus and thymic function, were used in only one experiment described in this study (Table II).

Antigens and Immunization Procedure.—The immunological properties of the Type III pneumococcal polysaccharide (SSS-III) used and the method by which it was prepared have been described (27–30). Mice were given a single intraperitoneal injection of an optimally immunogenic dose (0.5 μg) of SSS-III in 0.5 ml saline; the magnitude of the antibody response was assessed at peak, 5 days later.

Horse erythrocytes (HRBC) were obtained from the Ungulate Unit of the Animal Center Section, National Institutes of Health. Mice were given a single intraperitoneal injection of 0.2 ml of a 10% suspension (vol/vol) of washed HRBC in saline; the magnitude of the antibody response was assessed 5 days later.

Detection of Antibody-Producing or Plaque-Forming Cells.—Splenic PFC specific for SSS-III were detected by the technique of localized hemolysis-in-gel (27, 28); sheep erythrocytes (SRBC) sensitized with SSS-III by the chromium chloride coupling procedure (31) were used as indicator cells.

PFC specific for HRBC were detected by the same method, except that native washed HRBC were used as indicator cells. Only values for direct γM-producing PFC were considered in this work.

Antilymphocyte Serum.—Horse antimouse lymphocyte serum (ALS), lot 13162, was purchased from Microbiological Associates, Inc., Bethesda, Md. ALS was given as a single intraperitoneal injection at the time of immunization with SSS-III; the amounts of ALS used are given at appropriate places in the text. Data supplied by the manufacturer indicate that the mean survival time for DBA mouse skin grafts applied to C57BL/6 mice was increased from 10.4 $\pm$ 1.3 days to 21.3 $\pm$ 3.0 days after treatment with this preparation of ALS.

Statistics.—Student's t test (32) was used to evaluate the significance of the differences observed. Differences were considered to be significant when probability (P) values <0.05 were obtained.

RESULTS

Effect of Treatment with ALS on the Magnitude of the PFC Response to SSS-III in Nude and Littermate Control Mice.—The data of Table I show that, without ALS treatment, the 5-day PFC response to an optimally immunogenic dose of SSS-III was slightly, but significantly, higher (1.6-fold) in nude mice than in thymus-bearing littermate controls ($P < 0.02$); in other studies both groups of mice produced similar numbers of facilitated γM-producing PFC in response to the same dose of antigen (33). Treatment with 0.3 ml of ALS had no effect on the magnitude of the PFC response of nude mice ($P > 0.05$). However, the same amount of ALS produced considerable enhancement (150-fold) when given to thymus-bearing littermate controls ($P < 0.001$).

TABLE I

Effect of Treatment with ALS on the Magnitude of the PFC Response to SSS-III in Nude and Littermate Control Mice

Treatment of mice	SSS-III-specific PFC/spleen*	
	Nude mice	Littermate controls
0.5 μg SSS-III	3.537 ± 0.043‡ (3,440)§	3.331 ± 0.049 (2,140)
0.5 μg SSS-III + 0.3 ml ALS	3.603 ± 0.123 (4,020)	5.522 ± 0.096 (333,000)

* Numbers of PFC detected 5 days after immunization with SSS-III.

‡ Log_{10} ± standard error of the mean for 8–12 similarly treated mice.

§ Geometric mean.

To determine whether nude and littermate control mice differ greatly with respect to the amount of ALS required to produce enhancement, both groups of mice were treated with various doses (0.01 ml–0.5 ml) of ALS; the magnitude of the PFC response to 0.5 μg of SSS-III was assessed 5 days after immunization. The data of Fig. 1 show that for littermate control mice, significant enhancement was demonstrable with all doses of ALS used; such enhancement was ALS dose-dependent and maximal with 0.3 ml of ALS. In contrast, none of the tested doses of ALS produced enhancement in nude mice.

Overt signs of cytotoxicity were noted only for groups of mice treated with the largest dose of ALS used in this study (0.5 ml). Both nude and littermate control mice became noticeably ill after treatment with this dose and gave PFC responses lower than those obtained with 0.3 ml of ALS. Nevertheless, the PFC response for control mice given 0.5 ml of ALS was still significantly higher than that of mice not treated with ALS.

Effect of Treatment with ALS on the Magnitude of the PFC Response to SSS-III and HRBC.—Conventionally reared thymus-bearing BALB/c mice were immunized with (*a*) 0.5 μg of SSS-III, (*b*) 0.2 ml of 10% HRBC, or (*c*) both antigens given together; one group of mice immunized with both antigens received a single injection of 0.3 ml of ALS. The magnitude of the PFC response to each immunizing antigen was assessed 5 days later.

The data of Table II show that the administration of HRBC to non-ALS-treated mice produced no significant change in the magnitude of the PFC response to SSS-III ($P > 0.05$); similarly, immunization with SSS-III had no effect on the numbers of HRBC-specific PFC detected. However, for mice given SSS-III and HRBC together, treatment with ALS reduced the magnitude of the PFC response to HRBC by about 50% ($P < 0.01$); the same mice exhibited about a 20-fold increase in SSS-III-specific PFC ($P < 0.001$). In other studies, three injections of ALS were found to produce almost complete suppression of the antibody response to SRBC (34); however, the same regimen—using the same preparation of ALS—gave 8- to 10-fold enhancement of the antibody response to SSS-III (11, 12). These findings illustrate that enhancement of the antibody response to SSS-III is demonstrable under conditions in which "helper" T cells, required for an antibody response to SRBC or HRBC, are inactivated by treatment with ALS.

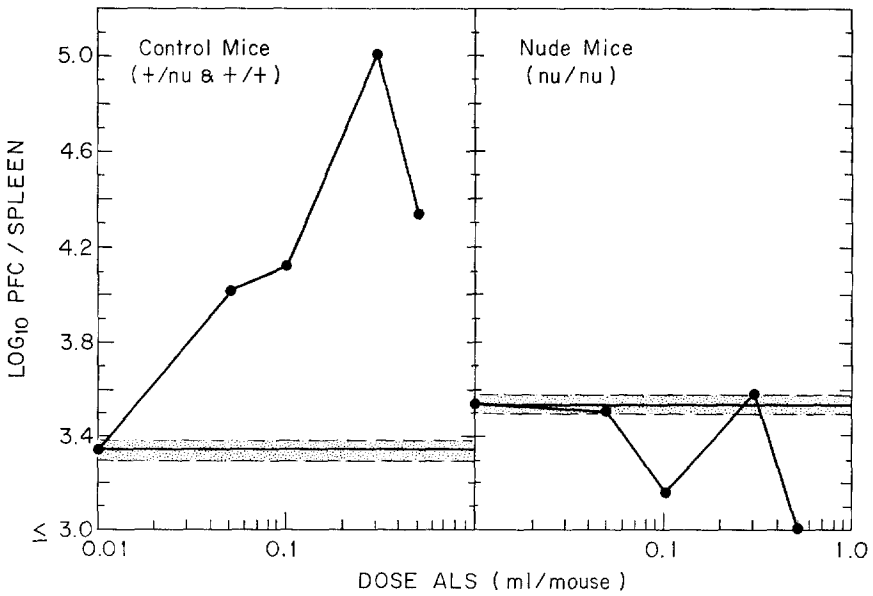

FIG. 1. Effect of administering different doses of ALS on the magnitude of the 5-day PFC response to 0.5 μg of SSS-III in nude and littermate control mice. Solid and broken horizontal lines represent the mean ± standard error of the mean, respectively, for mice not given ALS. Pooled spleen cell suspensions from five similarly treated mice were used for all determinations.

TABLE II

Effect of Treatment with ALS on the Magnitude of the PFC Response to SSS-III and HRBC in BALB/c Mice

Treatment of mice	PFC/spleen*	
	SSS-III specific	HRBC specific
0.5 μg SSS-III	4.230 ± 0.060‡ (16,987)§	None detected
0.2 ml 10% HRBC	None detected	4.552 ± 0.054 (35,685)
0.5 μg SSS-III + 0.2 ml 10% HRBC	4.206 ± 0.048 (16,079)	4.492 ± 0.063 (31,013)
0.5 μg SSS-III + 0.2 ml 10% HRBC + 0.3 ml ALS	5.494 ± 0.053 (311,608)	4.216 ± 0.048 (16,429)

* Numbers of PFC detected 5 days after immunization.

‡ Log_{10} ± standard error of the mean for five similarly treated mice.

§ Geometric mean.

DISCUSSION

Treatment with several other preparations of ALS or ATS has been shown to produce a significant increase in the magnitude of the antibody response to SSS-III in different strains of inbred and hybrid mice (14). Nonimmunized mice given various amounts of ALS or ATS fail to produce antibody specific for SSS-III (unpublished observations). These findings affirm that the ability of ALS to enhance the antibody response to SSS-III is a general phenomenon. Furthermore, the results of recent studies show that enhancement of the γM antibody response to SSS-III is also accompanied by a corresponding increase in both γG and γA antibody (to be published); enhancement, therefore, cannot be attributed to either a reversal or an alteration of antibody-mediated feedback inhibition (35).

The present work was conducted primarily to obtain information concerning the types of cells involved in mediating ALS-induced enhancement. This was accomplished by comparing the effects of treatment with various doses of ALS on the magnitude of the γM PFC response to SSS-III in athymic nude and thymus-bearing littermate control mice. The results obtained show that all doses of ALS employed gave significant enhancement in thymus-bearing mice; such enhancement was ALS dose-dependent (Fig. 1). In contrast, no enhancement was produced in nude mice over the same range of test doses. Since the ability of ALS to induce enhancement can be removed by adsorption with mouse thymocytes (11, 12), these findings clearly establish that (*a*) thymic-derived lymphocytes (T cells) are required to obtain ALS-induced enhance-

ment, and (*b*) enhancement is not the result of a stimulatory effect of ALS upon B cells.

At least three possible mechanisms might account for ALS-induced enhancement. In all instances it is assumed that ALS acts directly upon T cells to produce the effects described:

First, ALS-induced enhancement may be solely the result of a stimulatory effect of ALS upon T cells, and is similar to the type of enhancement produced after the administration of allogeneic cells (36, 37). This appears to be quite unlikely for a number of reasons. The well-known immunosuppressive effects of ALS have been attributed largely to an extensive depletion of not only circulating T cells but also thymic-dependent areas of lymphoid organs (4–10); yet ALS-induced enhancement of the antibody response to SSS-III is demonstrable under conditions in which there is an almost complete (34), or a substantial, loss (Table II) in the capacity to make antibody specific for "helper" T cell-dependent antigens. Also, this mechanism fails to account for the fact that ALS-induced enhancement can be abrogated by the infusion of normal syngeneic thymocytes (12).

Second, enhancement may be solely the result of the inactivation of T cells that have been reported to exert a negative influence on the magnitude of the antibody response (38–45). According to such a view, the magnitude of the antibody response to SSS-III in athymic nude mice, as well as in neonatally or adult thymectomized mice, should be much greater than that produced by intact normal mice, and similar—if not identical—to that of thymus-bearing mice treated with ALS; this does not occur (1–3, 33). Instead, the PFC response to SSS-III in athymic mice is only 1/100 of that produced by thymus-bearing littermate controls given an optimal dose of ALS (Fig. 1) and is only slightly greater than that of non-ALS-treated controls (Table I). The preceding findings also argue against a mechanism in which enhancement is due merely to T cell depletion and the creation of additional "metabolic space," thereby permitting a greater expansion of populations of B cells (46).

In view of the foregoing considerations, the following model provides the best explanation for ALS-induced enhancement; it is in complete agreement with all of the observations cited above. Nude mice lack a population of T cells that normally exert a negative influence on the magnitude of the antibody response to SSS-III; the absence of such suppressor T cells permits nude mice to give an antibody response slightly greater than that produced by thymus-bearing mice (Table I). However, the lack of suppressor T cells per se does not nearly permit nude mice to give a response comparable with that produced by thymus-bearing controls treated with ALS (Table I; Fig. 1). One must therefore postulate that nude mice also lack an additional population of T cells capable of increasing the magnitude of the antibody response of antigen-stimulated B cells; these have been termed amplifier T cells (12). Thus SSS-III, which does not appear to require cooperation between "helper" T cells and B cells to elicit a normal antibody response (1, 3), is not truly a "thymic-independ-

ent" antigen, since the magnitude of its antibody response is influenced by the activities of at least two functionally distinct types of regulatory T cells; most likely, soluble products released from such cells are responsible for mediating their effects (37). Furthermore, the results of these and of other studies (Table II; 11, 34) suggest that the activities of "helper" T cells and regulatory T cells are independent of one another and are mediated by different subpopulations of T cells. This is contrary to the views of others who propose that the product(s) of a single, rather than more than one, type of T cell can—under certain circumstances that are still to be defined—exert either a positive or a negative influence on the magnitude of the immune response (48).

It should be noted that the model proposed in this work does not necessarily imply that the activities of amplifier and suppressor T cells are always counterbalanced. In the normal antibody response to some antigens, e.g., SRBC, amplifier rather than suppressor cells may play a more dominant role; in this case, treatment with ALS would result in suppression. For other antigens, e.g. SSS-III, the converse situation may apply, and enhancement would be obtained. Alternatively, both types of regulatory cells could differ significantly with respect to tissue distribution and access to ALS, minimal numbers required to produce an effect, and the density or type of surface antigens present. Such differences between subpopulations of T cells have already been described (49, 50), and could determine the ease with which amplifier and suppressor T cells are inactivated by ALS. Also, amplifier and suppressor T cells may not necessarily act at the same level of control for the immune response. The results of current studies indicate that suppressor cells appear to regulate the proliferation of antibody-forming B cells (to be published). Although the mode of action of amplifier cells is less clear at this point, it is conceivable that such cells could act primarily by increasing the rate of antibody synthesis by B cells that would otherwise be only minimally stimulated by contact with antigen alone. Either or both types of control processes would have a decisive effect upon the magnitude of the antibody response. These issues are now being investigated.

SUMMARY

The effect of treatment with antilymphocyte serum (ALS) on the magnitude of the plaque-forming cell (PFC) response to Type III pneumococcal polysaccharide (SSS-III) was assessed in athymic nude mice and thymus-bearing littermate controls. Without ALS treatment, the PFC response was slightly higher in nude than in control mice. Treatment with ALS had no effect on the response of nude mice; however, considerable enhancement was noted in thymus-bearing controls. Such enhancement was ALS dose-dependent and demonstrable under conditions in which there was substantial inactivation of thymic-derived "helper" cells required for an antibody response to erythrocyte antigens.

These findings suggest that amplifier and suppressor cells, which have been

reported to regulate the magnitude of the antibody response to SSS-III, represent populations of thymic-derived cells (T cells) that are not present in nude mice. The activities of "helper" T cells and regulatory T cells appear to be independent of one another and mediated by separate subpopulations of T cells.

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