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Immunoregulatory Role of the Spleen in Antibody Responses to Pneumococcal Polysaccharide Antigens

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Antibody responses to two structurally different pneumococcal polysaccharides, type 3 (SIII) and type 14 (SXIV), were examined in intact and splenectomized (Sx) A/J mice to determine whether the role of the spleen in immune responses to these antigens varies with respect to the dosage, the antigenic structure, or the interval between immunization and assay. Antibody responses to SIII and SXIV, measured over a 4-week period by radioimmunoassay, differed in antigenic load requirements, kinetics, and extent of dependence upon the spleen. Intact mice given 50 or 100 ng of SIII produced peak antibody responses on day 5, which tapered off by days 14 and 21. Intact mice given SXIV required doses 100 times greater than those of SIII to stimulate high levels of antibody response; antibody responses increased on day 5 and remained elevated through day 28. In Sx mice given 50 or 100 ng of SIII, the peak antibody response on day 5 was obliterated, but extrasplenic sources produced low levels of antibody which peaked by day 14. In Sx mice given SXIV, all anti-SXIV responses were abrogated regardless of the dose or day of assay. Differences between the anti-SIII and anti-SXIV responses in dependence upon the spleen were probably due to structural differences between the two antigens and to the localization of each to different sites in the reticuloendothelial system. These results attest to the importance of the spleen in antibacterial resistance. They show that, even in the presence of extrasplenic antibody synthesis, the spleen is required for early antibody production, the timing of which is critical for the effective clearance of bacteria.

The spleen plays a major role in humans in the prevention of overwhelming sepsis caused by encapsulated bacteria, primarily *Streptococcus pneumoniae*. It functions both as a producer of antipolysaccharide antibody and as a phagocytic filter. The former function is under complex control and is responsive to diverse influences. For example, although polysaccharide antigens are traditionally classified as thymus independent (TI) because nude and neonatally thymectomized mice produce antibody levels comparable to those of normal mice (13, 28), the amplifier, suppressor, and contrasuppressor T cells are known to influence the magnitude of the antibody response (5, 9, 35). While X-linked genes regulate antipolysaccharide antibody responses in an all-or-none manner (3, 32), allotype-linked (1, 26) and autosomal (6) genes are thought to influence the extent of response. Results of short-term (21, 22) and single-point (29) experiments on BALB/c mice suggest that the dosage, timing of antigenic stimuli, and structure of the antigens could also influence antibody responses to polysaccharides; but, partly because of differences in experimental design, the results of these studies differ with regard to the precise effects produced by splenectomy. We show here the extent to which the presence of the spleen modulates antibody responses to polysaccharides and how these antibody responses are affected over a longer interval of time by differences in the molecular structure of the antigens and in the dosage given.

The functions of the spleen in filtration, immune responsiveness, and activation of complement have been well documented (8), but questions persist concerning its role as an essential component of host defense in healthy individuals. Recent reports of improved immune function and prolonged survival after splenosis, partial splenectomy, and retention of splenic fragments in patients treated for traumatic rupture of the spleen (10, 12, 14, 27, 30, 31, 36) have raised the possibility of a critical role for the spleen in host defense, particularly in antibody responses to certain polysaccharides.

The present study was undertaken to investigate splenic influence on antibody responses to graded doses of two structurally different pneumococcal polysaccharide antigens, type 3 (SIII) and type 14 (SXIV), as measured over a 4-week period. We hypothesized that some of the conflicting results reported in the literature could be explained if the role of the spleen in antibody responses to polysaccharide antigens varied with the dosage used, the structure of the antigen, and the interval between immunization and assay. Our results show that in A/J mice the immune responses to SIII and SXIV differed not only in antigenic load, magnitude, and kinetics of response, but also in the extent to which each was affected by splenectomy. While splenectomy ablated an early peak in antibody response to low or optimal doses of SIII, it obliterated antibody responses to all doses of SXIV on all days assayed. Extrasplenic sources were capable of responding to low or optimal doses of SIII with a delayed antibody response, but were unable to respond to any dose of SXIV even 30 days after immunization.

MATERIALS AND METHODS

Mice. A/J male and female mice, purchased at 6 weeks of age from the Jackson Laboratory, Bar Harbor, Maine, were maintained in conventional cages and supplied ad libitum with acidified tap water and mouse chow (no. 5015; Ralston Purina Co., St. Louis, Mo.). Mice were used at 8 to 12 weeks of age, and all experimental groups were studied simultaneously.

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TABLE 1. Influence of dose and day of assay on antibody responses to SIII in intact A/J mice

Dose (ng)	No. of mice immunized[a]	Antibody levels (mean ± SEM in ng/ml)[b]				ANOVA[c] (F, P)
		Day 5	Day 9	Day 14	Day 21	
50	18	1,367 ± 108	1,118 ± 140	916 ± 114[d]	737 ± 10[de]	7.5, 0.001
100	18	1,394 ± 85	900 ± 62[f]	800 ± 69[f]	627 ± 52[fg]	53.7, 0.001
1,000	5	160 ± 0[ij]	352 ± 60[hk]	594 ± 100[hi]	440 ± 69[h]	16.9, 0.001

[a] In response to 1,000 ng, only one of five mice produced a detectable antibody response on day 5.

[b] Multiple range tests ($P = 0.05$). Superscripts: (differences due to day of assay) d, significantly lower than the day-5 response to 50 ng; e, significantly lower than the day-9 response to 50 ng; f, significantly lower than the day-5 response to 100 ng; g, significantly lower than the day-9 and -14 responses to 100 ng; h, significantly greater than the day-5 response to 1,000 ng; and i, significantly greater than the day-9 response to 1,000 ng; (differences due to dose) j, significantly lower than the day-5 response to 50 or 100 ng; and k, significantly lower than the day-9 response to 50 or 100 ng.

[c] ANOVA for differences due to day of assay. ANOVA for differences due to dose: day 5, $F = 4.2$, $P = 0.02$; day 9, $F = 6.01$, $P = 0.005$; day 14, not significant; day 21, not significant.

Antigens. The soluble capsular polysaccharides SIII and SXIV were used as antigens. SIII is a highly acidic linear polymer composed of repeating subunits of cellobiuronic acid. SXIV is a neutral polysaccharide containing short branches and is composed of glucose, galactose, and *N*-acetylglucosamine in a structural arrangement resembling the human ABO blood group antigens. Purified SIII and SXIV were prepared by ethanol and ammonium sulfate fractionation of pneumococcal culture filtrates by the method of Kabat and Mayer (23). The final products contained less than 2% of C polysaccharide, were pyrogen free, and by quantitative precipitation, radioimmunoassay, and inhibition were found to be more than 90% pure immunologically. Stock antigens were diluted in pyrogen-free bacteriostatic sodium chloride (0.9%) (Elkins-Sinn, Cherry Hill, N.J.).

Immunizations. All experimental groups were immunized and assayed simultaneously. Intact or sham-operated and splenectomized (Sx) mice were immunized with a single intraperitoneal 0.5-ml injection containing either 50, 100, or 1,000 ng of SIII or 500, 5,000, or 10,000 ng of SXIV. These doses of SIII were selected because 100 ng had been shown to be the optimal dose for 8- to 12-week-old A/J mice (11). Males and females were grouped together because previous studies with SIII (11) and preliminary unpublished studies with SXIV had shown that, among A/J mice, there were no sex-linked differences in antibody responses either to SIII or to SXIV. Preliminary studies had also shown that sham-operated and intact mice produced similar antibody responses provided that a 3-week recovery period was permitted between surgery and immunization.

Splenectomy. Mice were anesthetized with sodium pentobarbital (0.05 mg/g of body weight) (Henry Schein, Inc., Port Washington, N.Y.). Spleens were removed through an oblique subcostal incision. Peritoneum and skin were closed with 3 interrupted 6-0 silk sutures and wound clips, respectively. Sham-operated mice were exposed to the same dose of anesthesia, and the same incision and closure procedure was used, but their spleens were not excised. A minimum interval of 3 weeks between surgery and immunization was alloted for recovery.

Antibody determinations. Sera from individual mice were assayed 5, 9, 14, 21, and 28 days postimmunization. In selected mouse groups, serum samples were also assayed on days 30 and 58. Samples of blood from the orbital plexus were collected in heparinized microhematocrit tubes. Samples (5 or 20 μl) of plasma were incubated with 0.5 ml of biosynthetically radiolabeled SIII or SXIV and assayed in duplicate by radioimmunoassay as previously described (33). Antibody levels were expressed as nanograms of antibody nitrogen (AbN) per milliliter. The C-polysaccharide impurities in the immunizing antigens could not have resulted in the production of antibodies measurable in our radioimmunoassay. ^{14}C label is not incorporated into C polysaccharide and thus is not a component of the labeled antigens used in this radioimmunoassay. Any antibodies produced in response to C polysaccharide would therefore not bind and precipitate the radiolabeled antigen used in this procedure.

Statistics. The range of results within experimental groups was sufficiently narrow to permit the use of arithmetic means. To determine whether significant differences occurred because of dosage, day of assay, or the presence of the spleen, antibody responses to each antigen were analyzed as follows. Differences in antibody responses due to the day of assay were detected by using an SAS analysis of variance (ANOVA) general linear models procedure with repeated measures and Duncan's multiple range tests to determine significant differences (Statistical Analysis System, Gary, N.C.). To detect differences in response due to dosage, an SPSS-X one-way ANOVA (Statistical Package for the Social Sciences, Inc., Chicago, Ill.) and Duncan's multiple range tests were used. The responses of intact and Sx animals were analyzed by using the Student *t* test (Statistical Package).

RESULTS

Preimmunization assays indicated that none of the mice had detectable levels of antibodies to SIII or SXIV (Fig. 1 and 2).

SIII. (i) Intact mice. To determine the normal pattern of responses to SIII in A/J mice, antibody responses in intact mice were assayed at various intervals after immunization with 50, 100, or 1,000 ng of SIII (Table 1). Immunization with 50 or 100 ng stimulated a peak in response on day 5 that tapered off significantly by days 14 and 21. After immunization with 1,000 ng, only one of five mice produced detectable levels of antibody on day 5, but on subsequent days all of the mice produced antibody responses to the 1,000-ng dose; antibody responses increased gradually and reached a significant but modest peak on day 14. Antibody responses to all doses on day 28 (data not shown) were similar to those produced on day 21. Dose-dependent effects were evident when responses to each dose were compared for each day. Antibody responses to 1,000-ng doses were significantly lower than those to 50- or 100-ng doses on days 5 and 9, but not on days 14 and 21.

(ii) Sx mice. To investigate whether the role of the spleen varied with the dosage given, antibody responses to different doses of SIII were assayed in Sx mice (Table 2). Only 9 of 12 Sx mice given 50 or 100 ng of SIII produced detectable levels

TABLE 2. Antibody responses to SIII in splenectomized A/J mice

Dose (ng)	No. of mice immunized[a]	Antibody levels (mean ± SEM in ng/ml)[b]				ANOVA[c] (F, P)
		Day 5	Day 9	Day 14	Day 21	
50	6	152 ± 35	381 ± 21[d]	533 ± 80[d]	420 ± 52[d]	9.8, 0.001
100	6	116 ± 19	250 ± 32[ef]	503 ± 33[e]	445 ± 160[e]	4.9, 0.01
1,000	6	100 ± 0	130 ± 19[fg]	123 ± 47[h]	116 ± 22[i]	NS

[a] On day 5, four of six mice responded to 50 ng, five of six mice responded to 100 ng, and two of six mice responded to 1,000 ng.
[b] Multiple range tests ($P = 0.05$). Superscripts: (differences due to day of assay) d, significantly greater than the day-5 response to 50 ng; and e, significantly greater than the day-5 response to 100 ng; (differences due to dose) f, significantly lower than the day-9 response to 50 ng; g, significantly lower than the day-9 response to 100 ng; h, significantly lower than the day-14 responses to 50 and 100 ng; and i, significantly lower than the day-21 responses to 50 and 100 ng.
[c] ANOVA for differences due to day of assay. NS, Not significant. ANOVA for differences due to dose: day 5, NS; day 9, F = 22.7, $P = 0.001$; day 14, F = 12.43; $P = 0.001$; day 21, NS.

of antibody on day 5. Among those that responded, the peak on day 5 seen in intact mice was totally obliterated. Subsequently, all mice produced anti-SIII responses that gradually rose to levels which, although modest, were significantly higher than those of the response on day 5. Among Sx A/J mice given 1,000 ng of SIII, only two of six mice responded on day 5. On subsequent days, all of the mice responded, but with no significant increases over the barely detectable responses produced on day 5. Antibody responses to all doses on day 28 (data not shown) were similar to those produced on day 21. The dose-dependent differences in response seen on day 5 in intact mice were obliterated in Sx mice because Sx mice produced low day-5 responses regardless of the dose given. On the other hand, dose-dependent differences were evident on days 9 and 14, because Sx mice produced significantly reduced antibody responses to 1,000 ng than to 50 or 100 ng. When differences in antibody responses due to the presence or absence of the spleen are shown graphically (Fig. 1), it can be seen that the effects of splenectomy varied with the dose. With 50- or 100-ng doses of SIII, the peak antibody response seen in intact mice on day 5 was absent and a lower, delayed peak occurred on day 14. With a 1,000-ng dose of SIII, splenectomy made no difference in antibody response on day 5, but it led to drastically reduced antibody responses on subsequent days.

SXIV. (i) Intact mice. To determine the normal pattern of response to SXIV in intact A/J mice, antibody responses were assayed at various intervals after immunization with 500, 5,000 or 10,000 ng of SXIV. Compared to SIII, much higher doses of SXIV were required to stimulate elevated antibody responses, and an entirely different pattern ensued (Table 3). The peak response to SXIV occurred later than day 5, and the decline that followed was less pronounced than that seen after SIII immunization. On day 5, the levels of antibody produced after mice were immunized with 5,000 or 10,000 ng of SXIV were lower than those produced after immunization with 50 or 100 ng of SIII; but by days 9 and 14, antibody levels to both antigens were within the same range. In response to 500-ng doses, anti-SXIV levels rose modestly on day 9 and then tapered off. After intact mice were immunized with 5,000 or 10,000 ng of SXIV, anti-SXIV levels rose sharply on day 5 and generally remained within the same range through days 21 and 28. Antibody responses persisted through day 58 with levels of 109 ± 53 ng of AbN per ml in mice immunized with 500 ng and 345 ± 13 ng of AbN per ml in mice immunized with 5,000 or 10,000 ng of SXIV. When responses to the different doses of SXIV were compared, antibody levels after 5,000- and 10,000-ng doses were similar on all days assayed and were significantly greater than antibody responses to 500-ng doses.

(ii) Sx mice. To investigate whether the role of the spleen varied with the structure of the polysaccharide as well as with the dosage given, antibody responses to three doses of SXIV were assayed in Sx mice. Splenectomy totally abrogated antibody responses to 500 and 5,000 ng of SXIV on all days assayed (Fig. 2). After a dose of 10,000 ng, no Sx mice responded on day 5, and the few mice that responded on subsequent days produced barely detectable levels of antibody. By day 30 (data not shown) there were still no detectable antibody responses to SXIV. Figure 2 shows that, regardless of the dose or day of assay, extrasplenic sources were unable to produce antibodies to SXIV.

DISCUSSION

The current studies were designed to investigate whether the role of the spleen in antibody responses to polysaccharide antigens varies with the dose, the antigenic structure, or the interval between immunization and assay. First, it was necessary to demonstrate the characteristic pattern of re-

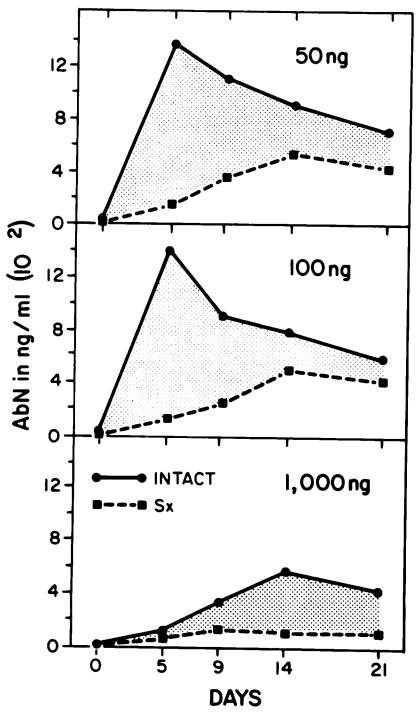

FIG. 1. Antibody responses to SIII in intact and Sx A/J mice after a single intraperitoneal injection of 50, 100, or 1,000 ng. Each point represents the arithmetic mean of 5 to 18 mice. Shaded areas indicate the splenic contribution to total antibody production and are based in part upon data shown in Tables 1 and 2.

TABLE 3. Influence of dose and day of assay on antibody responses to SXIV in intact A/J mice

Dose (ng)	No. of mice immunized	Antibody levels (mean ± SEM in ng/ml)[a]				ANOVA[b] (F, P)
		Day 5	Day 9	Day 14	Day 21	
500	10	196 ± 41	309 ± 51[c]	272 ± 55	193 ± 41	3.3, 0.03
5,000	10	763 ± 96[e]	820 ± 85[f]	717 ± 79[g]	533 ± 65[dh]	9.1, 0.001
10,000	5	743 ± 229[e]	638 ± 178[f]	621 ± 228[g]	469 ± 117[h]	NS

[a] Multiple range tests ($P = 0.05$). Superscripts: (differences due to day of assay) c, significantly higher than the day-5 and -21 responses to 500 ng; and d, significantly lower than the day-5, -9, and -14 responses to 5,000 ng; (differences due to dose) e, significantly higher than the day-5 response to 500 ng; f, significantly higher than the day-9 response to 500 ng; g, significantly higher than the day-14 response to 500 ng; and h, significantly higher than the day-21 response to 500 ng.

[b] ANOVA for differences due to day of assay. NS, Not significant. ANOVA for differences due to dose: day 5, F = 10.2, $P = 0.001$; day 9, F = 9.5, $P = 0.001$; day 14, F = 4.1, $P = 0.02$; day 21, F = 8.4, $P = 0.001$.

sponse in A/J mice over an extended period of time after treatment with different doses of the two polysaccharide antigens, SIII and SXIV. The results showed that immune responses to SIII and SXIV differed in kinetics of response and antigenic load requirements. In intact mice given 50 or 100 ng of SIII, antibody levels peaked on day 5 and tapered off by days 14 and 21. In mice given a supraoptimal dose of SIII, antibody levels did not rise until day 14. While a 50- or 100-ng dose elicited optimal antibody responses to SIII, doses 100 times greater were required to stimulate high antibody responses to SXIV. Anti-SXIV responses also showed different patterns from those produced in response to SIII. After mice were immunized with 5,000 or 10,000 ng of SXIV, antibody levels rose on day 5, but once stimulated, they remained elevated through day 28.

The differences observed in dosage and kinetics of response to SIII and SXIV could be related to structural differences in the two polysaccharides. SIII is a highly acidic linear polysaccharide with a molecular weight ranging from 100,000 to 300,000. It is composed of units of glucose and glucuronic acid in a 1-4 glycosidic linkage (23). Antigens such as SIII have been subclassified as TI type 2 (TI-2) because of the inability of CBA/N mice to generate antibodies against these antigens (3, 32). SXIV is a neutral polymer of somewhat lower molecular weight than SIII which contains short branches and is composed of alternating units of glucose, galactose, and *N*-acetylglucosamine in an arrangement similar to that of the blood group antigens (23).

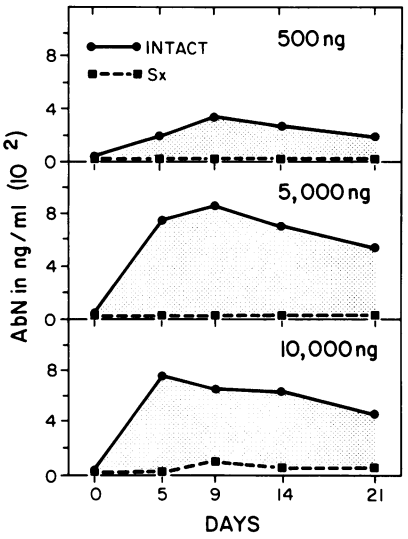

FIG. 2. Antibody responses to SXIV in intact and Sx A/J mice after a single intraperitoneal injection of 500, 5,000, or 10,000 ng. Each point represents the arithmetic mean of 5 to 10 mice. Shaded areas indicate the splenic contribution to total antibody production and are based in part upon data shown in Table 3.

Immune responses to the two polysaccharides also differed in their dependence upon the spleen. Splenectomy obliterated the peak in antibody response on day 5 in mice given 50 or 100 ng of SIII, but by day 14, a low, significant antibody response was produced by extrasplenic sources. No anti-SIII antibodies were produced in Sx mice given 1,000 ng of SIII. In contrast to its effect on the response to SIII, splenectomy totally abrogated antibody responses to SXIV on all days assayed regardless of the dose. The total dependence upon the spleen of antibody responses to SXIV could have been due to the inability of extrasplenic sources to respond to the dosages used in this study. Previous studies of Sx humans showed that antibody responses to SXIV and other pneumococcal serotypes are only minimally affected by removal of the spleen (34) or display different kinetics in Sx individuals (17). Alternatively, the SXIV injected might not have reached the lymph nodes and therefore might not have stimulated extrasplenic antibody production. This, however, seems unlikely since the mice had received an intraperitoneal injection. The peritoneal cavity is known to drain through lymphatics that penetrate the diaphragm and empty into the mediastinal lymph nodes (38). Another possibility is that the dependence of SXIV on the spleen was due to the tendency of the antigen, as a neutral polysaccharide, to localize to a unique site in the spleen, i.e., the marginal zone; SIII, in contrast, localizes to the red pulp. The marginal zone of the spleen is an antigen-independent structure for which there is no equivalent anatomical site in other lymphatic tissues (24). Its lymphocytes and macrophages are considered to be unique (7, 16, 18, 19, 20, 24, 25). Marginal zone B cells contain interleukin-2 receptors (18), surface immunoglobulin M but not immunoglobulin D, and CR1 and CR2 receptors and are not recirculating (25). Although the nature of interleukin-2 influence on B-cell function remains unclear (37), marginal-zone Tac^+ cells could represent activated, partially differentiated, or a discreet lineage of B lymphocytes (7, 18, 24, 25). Marginal-zone macrophages are large, morphologically distinct cells. Unlike those of the red pulp, these macrophages do not resemble the macrophages of other lymphatic tissue (15). Marginal-zone macrophages are found in close association with adherent B lymphocytes and are capable of taking up neutral polysaccharides (16, 20). It has been postulated that these marginal-zone macrophages could function in the transport of antigen-antibody complexes (25) or in the presentation of TI-2 antigens (20).

Other studies of antibody responses to polysaccharide antigens have reported dose-response data and effects of splenectomy which differ from our findings (4, 21, 22, 29).

Previous investigators used short-term or single-point assays, whereas we simultaneously studied at multiple intervals over a 4-week period the effects of splenectomy on antibody responses to three doses of two structurally different polysaccharides. In part, our work confirms the findings of Markham et al. (29) in that an acidic polymer (in our case SIII and in theirs type 3 group B streptococci [GBS3]) could stimulate antibody production in the absence of the spleen; whereas antibody responses to SXIV, a neutral polysaccharide, were totally dependent upon splenic immunocompetent cells. Since the difference between SXIV and GBS3 is simply a terminal sialic acid, the charge on the molecule could be critical. In support of this possibility, other neutral polysaccharides which are TI-2 antigens, such as dinitrophenylated conjugates of Ficoll and hydroxyethyl starch, also require splenic tissue for generating immune responses (2). On the other hand, GBS3 and SIII, although both acidic, are dissimilar both in structure and in the responses that they generate. Markham et al. (29) found no significant differences between Sx and intact BALB/c mice in antibody responses to GBS3 on day 6, the only day for which the responses were assayed. With A/J mice we found that, although Sx mice produced extrasplenic antibodies by day 14, they showed no peak in antibody levels on day 5 in contrast to intact mice and produced antibody responses on days 5, 9, and 14 which were strikingly lower than those of intact mice. The extent of dependence upon the spleen in A/J mice did vary with the antigen.

Jones et al. (21) and Amsbaugh et al. (4) found that antibody responses to SIII were highly dependent upon the spleen, but mainly during the first week postimmunization. Extrasplenic sources produced one-third of the total serum antibody by day 7, but no data were provided beyond day 10. In our study, we observed that extrasplenic sources required 2 weeks to produce significant levels of antibody and that the kinetics of the extrasplenic response were dose dependent. The lowest doses produced detectable levels in the shortest time. Possible explanations for the differences between our findings and those of the two groups cited include differences in the strains of mice, antigen preparations, dosages used, methods of assay, and interval between surgery and immunization. Both of these groups used BALB/c mice given 500 ng of SIII. They assayed for serum antibody levels by using an externally labeled antigen and the supernatant method of Minden and Farr. We used A/J mice given 50, 100, or 1,000 ng of SIII. We assayed for antibody by using a biosynthetically internally labeled antigen in our radioimmunoassay. Although we allocated a minimum 3-week recovery period between surgery and immunization, both of these groups (4, 21) immunized their animals within 7 to 10 days of surgery.

We conclude that the extent of dependence upon the spleen varies with the structure of the antigen. By using multiple assays over an extended period of time, we investigated whether other sources could replace the spleen in antibody production. Clearly, this was possible after 2 weeks in response to SIII, but not in response to SXIV. By giving different doses we investigated whether a larger or smaller exposure to the antigen would overcome the defect produced by splenectomy. Changing the dose made no difference in the ablated antibody responses of Sx animals given SXIV, but in Sx animals given SIII the lower the dose, the greater the extrasplenic response detected.

The 1- or 2-week delay in extrasplenic antibody production and the inability of Sx individuals to respond to SXIV can present serious problems. Host responses to encapsulated bacterial infections depend in part upon an early appearance of type-specific antibody accompanied by a functional reticuloendothelial system. Host defense against the pneumococcus requires clearance of antibody and complement-coated bacteria. The observations in our report clearly indicate that the production of antibody within a short period of time after immunization requires a functional spleen. In the absence of the spleen, either no antibody is produced or its appearance is delayed. The second aspect of the role of the spleen in host defense is its role as a phagocytic filter. In the presence of antibody produced soon after infection, phagocytosis is greatly enhanced. Thus, both functions of the spleen in host defense are served by the prompt production of antibody. This explains in part why, depending upon the organisms involved, individuals lacking a functional spleen are at increased risk of dying from overwhelming sepsis.

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