The Influence of Splenic Weight and Function on Survival after Experimental Pneumococcal Infection

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Splenectomy impairs survival after pneumococcal challenge in rats, while preservation of sufficient splenic tissue can be protective. This study investigated the effects of methylcellulose on stimulation of splenic weight, splenic histology, reticuloendothelial (RE) activity, and survival after pneumococcal infection. Methylcellulose increased spleen weight four- to five-fold but did not improve RE function or survival after infection. These parameters correlated best with the weight of the remnant in animals that did not receive methylcellulose. The functional limitations of splenic autotransplants were not corrected by methylcellulose stimulation of splenic weight. Preservation of a splenic remnant with intact blood supply is preferable to autotransplantation of the spleen to conserve RE capability.

THE SPLEEN PLAYS A VITAL ROLE in the host defense against infection as a component of the reticuloendothelial (RE) system. The importance of the spleen is manifest not only by an increased mortality from an infectious challenge in animals that have had splenectomy, but also by a higher frequency of overwhelming infection and death after splenectomy in children and some adults. While splenectomy impairs survival after pneumococcal infection in the rat, preservation of splenic tissue can restore host resistance to this organism and decrease mortality.[1] VanWyck et al.[2] have reported that preservation of a splenic remnant approximately one-third the size of the normal spleen seems critical to maintain this protective effect in the rat. Our laboratory has recently demonstrated that splenic weight is directly correlated with the phagocytic function of the spleen and has reported that preservation of spleen with intact blood supply maintains more efficient RE function than splenic autotransplants.[3] These observations suggest that the splenic weight is critical to maintain its role as a phagocytic filter.

While autotransplantation of the spleen can be done when splenectomy is necessary, its functional usefulness has been questioned. Splenic autotransplants degenerate and then grow after developing a new capillary blood supply. Their growth limitation has been proposed as an explanation for their inferior protective effect. Previous reports have shown that administration of methylcellulose, glucan, phenylhydrazine or *Cornebacterium parvum* will selectively increase spleen weight.[4–7] We have postulated that the use of methylcellulose chronically would not only stimulate spleen size but may improve survivorship from a bacterial challenge after partial splenectomy or splenectomy with autotransplantation, in animals at risk of death from bacteremia. This study assesses the effect of the administration of methylcellulose upon splenic weight, histology, RE function, and survival after pneumococcal infection.

Methods

Three hundred fifty young female Sprague-Dawley rats weighing 85 to 90 g were housed in our animal care facility and were allowed free access to a standard diet (Purina Rat Chow®) and water. All experimentation satisfied our institution's guidelines for the care and use of laboratory animals. After an adaptation period of 7 days and following an overnight fast, animals were anesthetized with veterinary pentobarbital (50 mg/kg) and had one of the following procedures performed through a midline celiotomy: sham operation (CNTL); hemisplenectomy preserving the lower half of the spleen (50% SX); subtotal splenectomy preserving the extreme lower pole of the spleen (75% SX); total splenectomy with heterotopic intraperitoneal splenic autotransplantation of approximately one-half of the excised spleen within an omental pouch[3] (ATS); or total splenectomy (SX). Remnant size was estimated qualitatively at operation and a quantitative assessment was done retrospectively by subtracting the

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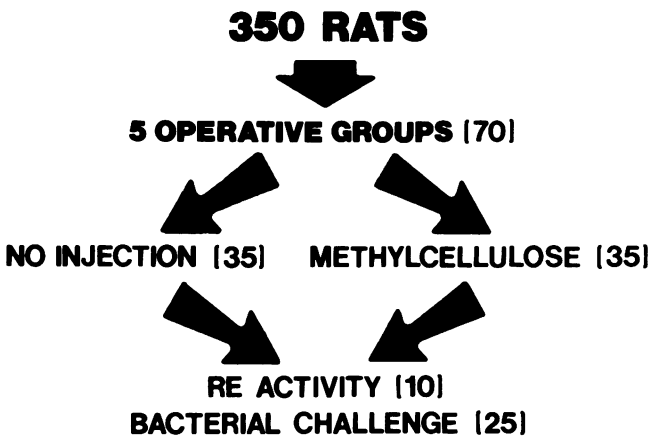

FIG. 1. Experimental outline.

average weight of the excised segments of spleens from the average spleen weight in SX animals.

Methylcellulose, 400 centipoises viscosity (Sigma Chemical, St. Louis, MO) was dissolved in solution by slowly adding 2.5 gm of methylcellulose to 100 ml aliquots of sterile saline that was warmed while stirring constantly. Beginning 2 weeks after operation, 35 rats in each operative group were given one ml of the 2.5% methylcellulose solution by intraperitoneal injection (IP) twice weekly using aseptic technique. Rats were injected for 5 weeks and received 250 mg of methylcellulose.

At 9 weeks after operation, 10 animals in each operative group injected with methylcellulose and 10 animals that were not injected had an assessment of splenic RE activity. These rats were lightly anesthetized and injected intravenously (IV) with 10 microCuries of technetium (Tc^{99m}) sulfur-colloid (Medi-Physics, Inc., Emeryville, CA) per 100 gm body weight. Animals were sacrificed by pentobarbital overdose 15 minutes later, one ml of blood was obtained, and the liver, spleen, kidneys, and lungs were excised and processed for gamma scintillation counting at 140 kev. Total RE activity was expressed as counts per minute (cpm) for each organ and the specific organ activity was expressed as the total activity divided by organ weight. To correct for minor variations in the amount of radionuclide injected, organ RE function was referenced to hepatic activity and expressed as:

$$\frac{\text{cpm organ}}{\text{cpm liver}} \times 100,$$

while the specific organ activity was labelled as organ phagocytic index and was expressed as:

$$\frac{\text{cpm organ/wt of organ}}{\text{cpm liver/wt of liver}} \times 100.^{8}$$

Animal weights and the weights of spleen, liver, lungs, and kidneys removed from sacrificed animals were recorded for comparison. Blood was sampled from rats in each group for hemoglobin, white blood cell and platelet counts. Representative histologic sections of the liver and spleen were stained with hematoxylin and eosin, reticulum stain, and trichrome stain. These were later compared by one of us (U.A.A.) without knowledge of their group of origin.

The remaining animals (24 to 25 per group) were injected IP with 1×10^5 *Streptococcus pneumoniae,* type II (ATCC strain #6302, American Type Culture Collection, Rockville, MD) suspended in one mL sterile saline as previously described.[9] After bacterial challenge, survival was assessed daily for 1 week. The experimental design is summarized in Figure 1. Statistical comparisons of organ weight and RE function were done by two-way analysis of variance. Significant values then were compared by Bonferroni's multiple comparison t-test. Survival between groups was compared by Fischer's exact test.[10,11]

Results

The average weight of the spleens excised from 70 animals in the SX groups was 439 ± 47 mg. Assuming that this weight is representative of spleen weight in all animals, the average spleen weight retained in 50% SX rats was 192 ± 26 mg, and in 75% SX rats it was 80 ± 32 mg. The average weight of spleen reimplanted in ATS animals was 210 ± 19 mg. Body, spleen, and liver weights in animals sacrificed 9 weeks after operation are listed in Table 1. Spleen weights after partial splenectomy or autotrans-

TABLE 1. *Animal, Splenic, and Hepatic Weights at Nine Weeks after Operation*

	No Injection			With Methylcellulose		
Group	Animal (gm ± SD)	Spleen (mg)	Liver (mg)	Animal (gm)	Spleen (mg)†	Liver (mg)
CNTL	201 ± 12	578 ± 104	6032 ± 560	216 ± 7	3258 ± 1282	8786 ± 1324
50% SX	211 ± 10	415 ± 64*	6664 ± 790	215 ± 9	2286 ± 678*	8425 ± 1297
75% SX	214 ± 15	268 ± 68*	6614 ± 1044	214 ± 7	1418 ± 393*	8100 ± 1347
ATS	222 ± 17	110 ± 70*	6481 ± 851	212 ± 10	430 ± 243*	7534 ± 1662
SX	213 ± 10	—	6237 ± 985	209 ± 10	—	7534 ± 1430

* $p < 0.01$ *versus* CNTL.

† $p < 0.001$ *versus* no injection.

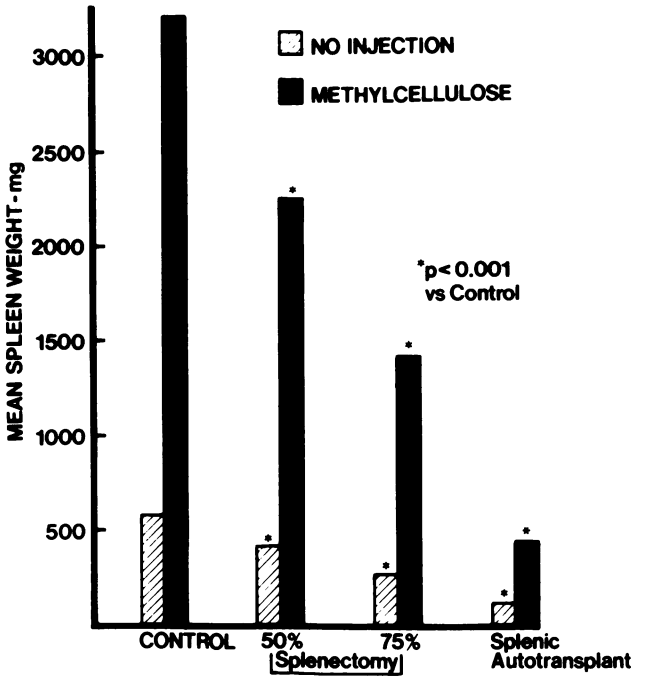

FIG. 2. Graphic representation of comparative splenic weights.

plantation were significantly less than CNTL ($p < 0.01$). Methylcellulose administration increased spleen weight five times in CNTL, 50% SX, and 75% rats and four times in ATS animals compared to animals in each of those groups that were not injected ($p < 0.001$). While the increase in spleen weight ranged from 0.37 to 1.78 times during the 9-week period after operation in CNTL, 50% SX and 75% SX rats, the rate of growth appeared similar between these groups (Fig. 2). Likewise, spleen weight increased from 1.79 to 13 times initial weight in animals that received methylcellulose, with the least rate of growth occurring in ATS rats.

Liver weights were 23 to 58% greater in animals that received methylcellulose. When the observed differences in liver weights were compared between groups, the larger liver size in methylcellulose-injected animals was not statistically significant ($0.05 < p < 0.10$). Kidney and lung weights varied by less than 10% among all groups regardless of operation or methylcellulose injection.

Splenic RE function diminished with decreasing splenic weight and was significantly less in 50% SX, 75% SX, and ATS groups than in CNTL (Table 2). Methylcellulose administration increased splenic RE function slightly within each group, but this increase was not statistically significant. Pulmonary RE function varied from 3.0 ± 0.69 to 4.11 ± 1.12 between groups, a difference that was not statistically significant. The splenic phagocytic index was similar among CNTL, 50% SX and 75% SX rats but was markedly decreased in ATS animals (Table 3). The splenic phagocytic index was less in all methylcellulose-injected

TABLE 2. *Splenic Reticuloendothelial Function (%)*

	No Injection	With Methylcellulose
CNTL	4.3 ± 1.2	7.1 ± 2.1
50% SX	2.9 ± 0.8*	3.4 ± 1.5*
75% SX	1.6 ± 0.8*	1.9 ± 0.8*
ATS	0.4 ± 0.2*	0.9 ± 0.7*

* $p < 0.01$ *versus* CNTL.

rats than in their noninjected counterparts ($p < 0.01$). A comparison of animals that received methylcellulose demonstrates that 75% SX and ATS animals have a significantly lower splenic phagocytic index than CNTL-injected rats.

Methylcellulose-injected animals with residual splenic tissue demonstrated a slight anemia and leukopenia compared to noninjected rats. The platelet count varied considerably. Expectedly, splenectomy resulted in leukocytosis and thrombocytosis even after methylcellulose administration.

Histologic sections of CNTL, 50% SX and 75% SX rats were indistinguishable when the number of germinal follicles, distribution of red and white pulp and thickness of the splenic capsule were compared. Spleens from ATS rats showed evidence of decreased white pulp and increased red pulp with greater collagen deposition within the red pulp. There were areas of microcalcifications and increased fibrosis within ATS spleens.[3]

Spleens from animals injected with methylcellulose over the 5-week period did not demonstrate any change in splenic reticulum, white pulp, or qualitative collagen content. These spleens had capsular thickening with surface deposition of methylcellulose along with increased hemosiderin deposition and foamy histocytes within the spleen. Microscopically, there were no differences between the livers of injected and noninjected rats except for surface deposition of methylcellulose and slight capsular thickening in rats that received methylcellulose.

Survival at 1 week after pneumococcal challenge was decreased in 75% SX, ATS and SX animals compared to CNTL ($p < 0.05$). Survivorship in 75% SX rats remained significantly greater than in SX animals ($p < 0.01$). Animals that received methylcellulose had a survivorship after bacterial challenge that was similar to their noninjected

TABLE 3. *Splenic Phagocytic Index (%)*

	No Injection	With Methylcellulose
CNTL	40.7 ± 8.2	19.7 ± 6.3
50% SX	43.6 ± 4.2	13.7 ± 5.1
75% SX	41.9 ± 14.2	9.2 ± 3.9†
ATS	23.0 ± 16.3*	9.6 ± 4.7†

* $p < 0.01$ *versus* CNTL.
† $p < 0.05$ *versus* CNTL.

TABLE 4. *Survival at One Week after Pneumococcal Challenge (Survivors/Number Challenged)*

	No Injection	With Methylcellulose
CNTL	24/24	23/24
50% SX	21/25	22/24
75% SX	17/25*	12/24*
ATS	9/24†	7/25†
SX	2/24†	3/24†

* $p < 0.05$ *versus* CNTL.
† $p < 0.01$ *versus* CNTL.

counterparts within the same operative group (Table 4). Sporadic cultures of rats dying after bacterial challenge grew *S. pneumoniae,* Type II, from the blood and body fluids, while no bacteria were recovered from survivors.

Discussion

While macromolecular agents such as methylcellulose can induce "hypersplenism" in animals after both acute and more chronic administration, these substances have not been evaluated for their ability to manipulate splenic RE function in a beneficial manner. Heuper[12] reported that methylcellulose administered to animals results in a progression of changes leading to splenomegaly. Palmer et al.[4] gave methylcellulose to rats over a 15-week period and noted that these animals developed a pancytopenia associated with secondary hypersplenism. Most of these changes could be overcome by splenectomy done prior to injection of methylcellulose. We have noted a five-fold increase in spleen weight due to methylcellulose administered to rats after sham celiotomy and partial splenectomy, which is greater than the three-fold increase reported by others.[4]

Aside from increasing splenic weights, methylcellulose leads to higher hepatic weights as well. Using a similar model, Teoh[13] did not observe any change in liver weight. This increase in hepatic weight may be due to a generalized RE system stimulation induced by methylcellulose. The absence of congestive or inflammatory changes within the liver mitigate against postsinusoidal hepatic obstruction as a cause for the higher weight.

The spleens of ATS rats did not increase in weight in the same proportion as those spleens of animals with intact blood supply. Palmer et al.[4] have suggested that the increase in spleen weight may relate to the induction of hemolysis by methylcellulose. Our results suggest that the autotransplanted spleen does not sequester damaged red blood cells or methylcellulose in the same manner as the regenerated spleen after partial resection. Degenerative changes and disorganization within the autotransplanted spleen have been noted previously by our group and others.[1,3,14] There is a reduction in white pulp, which is the principal location of the fixed macrophages[15] and the site of phagocytosis of pneumococci within the spleen,[16] after autotransplantation that may account for reduced animal survival after bacterial challenge. Bradshaw and Thomas[17] postulate that preservation of the reticular framework of the spleen is necessary for functional regeneration of splenic tissue to occur. The lymphoid and phagocytic elements of the spleen, which are the important components of its RE function, seem to depend on this reticulum as a framework on which they can become established. Unlike Palmer's study,[4] we did not observe any histologic changes in the liver in association with methylcellulose injection. This may be due to the lesser total dose that we used and suggests that deposition of methylcellulose within the hepatic parenchyma may only occur after the splenic tissue is saturated.

We have reported previously that splenic weight and splenic RE activity measured by the distribution of technetium Tc^{99m} sulfur-colloid, a colloidal suspension phagocytized primarily by the liver and spleen, are highly correlated.[3] The decrease in splenic RE function associated with resection was compensated by a slight increase in hepatic uptake. Stimulation of splenic weight with methylcellulose has led to a slight increase in splenic RE activity in our study, although the per cent increase in activity is less pronounced after partial resection and after autotransplantation. The corresponding phagocytic index or measure of RE function adjusted for organ weight was decreased in all groups. These structural differences in the spleen noted on microscopy after methylcellulose injection are consistent with congestive splenomegaly and may influence the ability of the fixed phagocytes within the spleen to function.

Our method of measurement of RE function is an assessment of phagocytosis by the major component organs of the RE system. Biozzi et al.[18] and Grover and Loegering[19] have used the disappearance of a radiocolloid that is more slowly cleared from the bloodstream to calculate a dynamic rate of phagocytic function. These two methods are complimentary and measure specific organ sequestration and total systemic phagocytic function, respectively.

Survival after pneumococcal challenge remains the putative test to evaluate the utility of maneuvers to preserve splenic function. In that regard, the use of methylcellulose to stimulate spleen weight was unsuccessful in the manner used and at the time frame studied. Grover and Loegering[20] also have reported that stimulation of the spleen by provision of an excess load of damaged red blood cells may impair its RE function when that same animal is given a septic challenge. They postulate that elimination of splenic RE ability by splenectomy or saturation of the phagocytic cells both result in an increased susceptibility to infection. While methylcellulose increased spleen weight, splenic RE function was not increased significantly

and this parameter accurately predicted the lack of improvement in survivorship after pneumococcal injection.

Cooney and colleagues[7] have shown that *C. parvum* administered to rats results in a significantly increased splenic weight after hemisplenectomy with minimal change in splenic architecture as assessed by light microscopy. This was accompanied by improved survivorship after pneumococcal challenge in hemisplenectomized rats given *C. parvum*. Whether the increase in spleen weight or the "nonspecific" immunostimulation afforded by *C. parvum* is responsible has not been determined. Hebert et al.[21] have demonstrated that *C. parvum* given to totally splenectomized mice also improves survival after pneumococcemia.

Despite the anticipated advantages of stimulation of spleen weight by methylcellulose, our results show that it adds no protection from a pneumococcal challenge. Preservation of splenic tissue with an intact blood supply remains more effective than autotransplantation in maintaining splenic RE activity and protective function even after increase in autotransplant size due to methylcellulose stimulation. Methylcellulose did not improve pulmonary RE function, which may have been one manner to compensate for diminished splenic phagocytic capabilities. Preservation of a splenic remnant with an intact blood supply remains preferable to autotransplantation as a means to conserve functional splenic tissue.

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Discussion

DR. LEON MORGENSTERN (Los Angeles, California): In conjunction with this paper, I thought it would be of interest to show a clinical counterpart of the methylcellulose splenomegaly model.

(Slide) This is the spleen of a 44-year-old woman with Gaucher's disease who had hypersplenism and severe thrombocytopenia. The estimated weight was 2000 g. It has already been partially devascularized.

The technetium 99 scan showed good functional RE activity of the spleen, and 9 days ago this patient underwent a partial (subtotal) splenectomy (Slide), leaving a normal-sized splenic remnant, nurtured by an inferior polar artery.

(Slide) The deposition of glucocerebroside in the spleen is well shown in this final slide. The glucocerebroside deposition is akin to that of the methylcellulose.

Subtotal splenectomy was performed in our patient not only for the preservation of immunologic competence, as demonstrated by Dr. Malangoni, but also to prevent the destructive deposition of glucocerebroside in bone, which had already begun to take place in this patient.

The patient has done well and is ready for discharge with a platelet count in the 300,000 range, as compared with a preoperative count of 20,000, and the postoperative technetium scan done yesterday shows a well functioning splenic remnant.

DR. LARRY C. CAREY (Columbus, Ohio): Thank you, Dr. Rosoff.

It appears that there really are two important aspects of this very nicely done work.

One is, can one enlarge the spleen through this mechanism and have it become a more effective reticuloendothelial organ? The answer to that appears to be a resounding no.

I suppose we can deduce that the important factor is not the size of the dog in the fight, but rather the size of the fight in the dog.

With regard to the second issue, that has to do with how much spleen can a human being lose and still have adequate protection with regard to the phagocytic activity of the residue.

If one examines the data in the manuscript, it appears that the amount of phagocytic activity per unit of weight is preserved in all of the subtotal splenectomies to a significantly different degree than it is in the allotransplanted spleen, and there is no significant difference, apparently, in