The Effect of the Immunomodulator Corynebacterium Parvum on Hemisplenectomized Mice

By Donald R. Cooney, Allan D. Lewis, Wayne Waz, Abdur R. Khan, and Melvyn P. Karp
Buffalo, New York

● **The immunomodulator *Corynebacterium parvum* stimulates the reticuloendothelial system and causes splenic hypertrophy. The ability of *C parvum* to stimulate splenic regeneration in hemisplenectomized mice and decrease susceptibility to intranasal pneumococcal challenge was studied. Mice were divided into two groups, control sham-operated (n = 48) and hemisplenectomized (n = 47) animals. Ten days later, each group was divided into two subgroups, those injected with *C parvum* (700 mcg IVP) and those injected with an equivalent volume of saline. The animals were challenged with *Streptococcus pneumoniae*, which was injected into one nostril three weeks postoperatively, and mortality was assessed. Four and one-half weeks postoperatively, splenic tissue was removed, measured, weighed, and submitted for histologic examination. The number of spleen cells per gram of tissue was assessed. Treatment with *C parvum* resulted in a significant increase in the splenic weight in both hemisplenectomized and control animals compared to similar saline-injected mice ($P < 0.001$). The percentage increase in spleen weight of hemisplenectomized mice (106%) was significantly greater than that for control animals (56%; $P < 0.01$). There was no significant difference among experimental groups in the number of spleen cells per gram of splenic tissue or in the histologic characteristics, indicating that *C parvum* stimulated relatively normal splenic growth. Survival following pneumococcal challenge was significantly increased for hemisplenectomized mice by *C parvum* treatment to a level that did not significantly differ from control mice. This study demonstrates that in mice the deficits related to resistance to infection and spleen size following hemisplenectomy are significantly improved by treatment with *C parvum*.**

INDEX WORDS: Immunomodulation; Corynebacterium parvum; overwhelming postsplenectomy infection (OPSI); partial splenectomy.

From the Departments of Pediatric Surgery and Pathology, The Children's Hospital of Buffalo, Buffalo, NY.

Presented before the 15th Annual Meeting of the American Pediatric Association, Marco Beach, Florida, May 9–12, 1984.

Address reprint requests to Donald R. Cooney, MD, Department of Pediatric Surgery, The Children's Hospital of Buffalo, 219 Bryant Street, Buffalo, NY 14222.

0022-3468/84/1906-0034$03.00/0

A NUMBER of methods have been proposed to avoid the tragic loss of life following splenectomy. *Streptococcus pneumoniae* is the most frequent cause of postsplenectomy sepsis, and prophylatic immunization with polyvalent pneumococcal vaccine has become standard clinical practice. Despite immunization, significant numbers of patients remain at risk. Overwhelming postsplenectomy infection (OPSI) may be caused by organisms other than the pneumococcus, and long-term antibiotic prophylaxis has the disadvantages of lack of patient compliance and organism susceptibility.

Partial splenectomy is an alternative surgical method that experimentally has resulted in a decreased risk of postsplenectomy infection. However, since little if any splenic regeneration occurs, the partially splenectomized subject remains at significant risk from overwhelming sepsis.

Corynebacterium parvum, an immunomodulator, has demonstrated a potential for reducing susceptibility to infection by stimulating the reticuloendothelial system and increasing the phagocytic ability of macrophages. The intent of this study was to determine if the administration of *C parvum* to partially splenectomized mice could produce sufficient regeneration of the splenic remnant which would result in increased survival following *S pneumoniae* challenge.

MATERIALS AND METHODS

Ninety-five adult male Swiss-Webster mice, weighing 25 to 30 grams were randomly divided into four groups: (Group 1) Control/Sham operation and Saline injection (CS/SA), n = 23; (Group 2) Control/Sham operation and *C parvum* inoculation (CS/CP), n = 25; (Group 3) Hemisplenectomy and Saline injection (HS/SA), n = 23; and (Group 4) Hemisplenectomy and *C parvum* inoculation (HS/CP), n = 24.

The mice were kept in a controlled temperature environment, six to a cage, on a 12-hour light/dark cycle. Standard rat chow and water was provided ad libitum.

To establish the accuracy of hemisplenectomy, prior to this study, 10 mice were anesthetized with ether and the spleen exteriorized through a midline incision. The caudal half of the spleen was removed after the blood supply to that section

was interrupted by ligating the corresponding portion of the splenic pedicle with 5–0 silk. The excised portion of the spleen was weighed. The mice were then immediately killed and the remainder of the spleen remnant was removed and weighed to determine the accuracy of the hemisplenectomy technique. This method proved to be a satisfactory technique for removing one half of the spleen with a ± 7% error in achieving an exact hemisplenectomy. All experimental animals to be hemisplenectomized had the caudal portion of the spleen removed through a midline laparotomy utilizing this technique. In the control/sham operations, a midline incision was also used, the spleen palpated, withdrawn from the abdominal cavity, replaced, and the incision closed with 4–0 silk.

S pneumoniae, Type 1 (ATCC 6301), maintained at 4 °C on Trypticase Soy Agar plates containing 5% sheep red blood cells was used in all experiments. Every two weeks, the organism was passaged through mice to ensure its virulence and encapsulation. A new set of stock cultures were prepared after the purity of the culture and the type had been confirmed with type-specific antiserum. All cultures were grown in brain-heart infusion broth with 10% fresh-frozen rabbit serum added (BHI-RS).

For preparation of log-phase Type 1 pneumococci for challenge, 0.2 mL of an overnight culture of *S pneumoniae* in BHI-RS was inoculated in a tube containing 10 mL of BHI-RS and incubated at 37 °C. After six hours, the suspension was spun at 2,000 rpm for 10 minutes at 4 °C, to obtain a pellet of bacteria. The pellet was resuspended in 10 mL of 0.15m, pH 7.2 phosphate-buffered saline (PBS), washed twice in a similar manner and kept on ice.

Bacterial challenge doses were determined using spectrophotometric turbidity measurement at 620 nm compared against a standard curve and confirmed by back-plating serial dilutions.

C parvum (Wellcome strain CN.6134) was provided by Burroughs Wellcome Co. and consisted of washed, formalin-killed organisms at 7 mg of dry weight per mL in 0.9% saline solution with 0.01% thiomersal. *C parvum* (700 mcg) was administered intraperitoneally 10 days postoperatively, at a dose previously demonstrated to produce significant reticuloendothelial system stimulation.[1]

Three weeks postoperatively (11 days after inoculation with *C parvum* or saline) all 95 mice were challenged with pneumococcus. Injected into one nostril was 0.01 mL of a 3×10^9 CFU/mL phosphate buffered saline solution of *S pneumoniae.* The mice were lightly anesthetized with ether during the short period required for intranasal inoculation.

Mortality was assessed for 10 days. Lung cultures were obtained from all animals succumbing during this period to verify that *S pneumoniae* was the cause of death. Those mice that survived past 10 days were killed 4½ weeks postoperatively. Their spleens were removed, weighed, and specimens were submitted for histologic examination and were used for assessing the number of spleen cells present per gram of splenic tissue.

Splenic specimens were weighed then placed in 2 mL of Eagle's medium (Gibco, Grand Island, NY). The spleen was then forced through a 200-mesh screen into a Petri dish by gently grinding. The screen was washed with 10 mL of Eagle's into the dish. The spleen cell suspension was filtered through a 400-mesh screen into a test tube and centrifuged at 2,000 rpm for 10 minutes. The supernatant was discarded and the pellet resuspended in 1 mL of Eagle's medium. Proper dilutions were made and spleen cell counts (excluding red blood cells) were determined using a Coulter Counter ZBI. Sixty-eight spleens (21 CS/SA, 16 CS/CP, 14 HS/SA, and 17 HS/CP) were assayed.

Sixty-nine splenic specimens removed 4½ weeks postoperatively (15 CS/SA, 22 CS/CP, 16 HS/SA, and 16 HS/CP) were randomly numbered, bisected in a fresh state, and immediately fixed in 10% buffered formalin. After fixation, the splenic tissue was sectioned at 6 μm and stained with hematoxylin and eosin. Each specimen was interpreted by a pathologist who did not have prior knowledge of the group to which the spleen belonged. The following criteria were used for evaluation: (a) Prominence of white pulp (5 + most prominent, 1 + lease prominent); (b) Number of follicles per 4 mm field; (c) Diameter of the largest lymphoid follicle; (d) Diameter of the largest germinal center. The width of each spleen specimen was also measured, and the tissue was weighed at the time of autopsy.

Ten days postoperatively, groups 2 and 4 (CS/CP and HS/CP) were inoculated with 700 mcg of *C parvum* (IP). Groups 1 and 3 (CS/SA and HS/SA) were injected with an equivalent volume of sterile saline. The experimental design is summarized in Table 1.

Analysis of mortality data was performed using the Cox proportional hazard method to compare the experimental groups and the relative effect of *C parvum* inoculation. In the Cox method, the conditional probability of death per 12 hours for those animals still alive at time t, was compared, and a ratio that differs from unity at the $P < 0.05$ level was considered significant. Spleen weight and spleen cell assay data were analyzed by one-way analysis of variance with Bonferroni's multiple comparison *t*-test. Statistically significant differences between groups for the percent increase in spleen weight was determined by the use of chi-square analysis.

RESULTS

Gross Morphology

The size of the intact whole spleen and the remaining splenic remnants from hemisplenectomized mice was increased by treatment with *C parvum.* This was evidenced by the mean width

Table 1. Experimental Design Summary

Time	Experimental Animals
0	Hemisplenectomy or control/sham surgery
10 days	*C parvum* or saline inoculation
21 days	*S pneumoniae* challenge
21 to 31 days	Assess mortality
31 days	Sacrifice challenge survivors and use spleens for SCA-Coulter, histology, splenic weight, and width determination

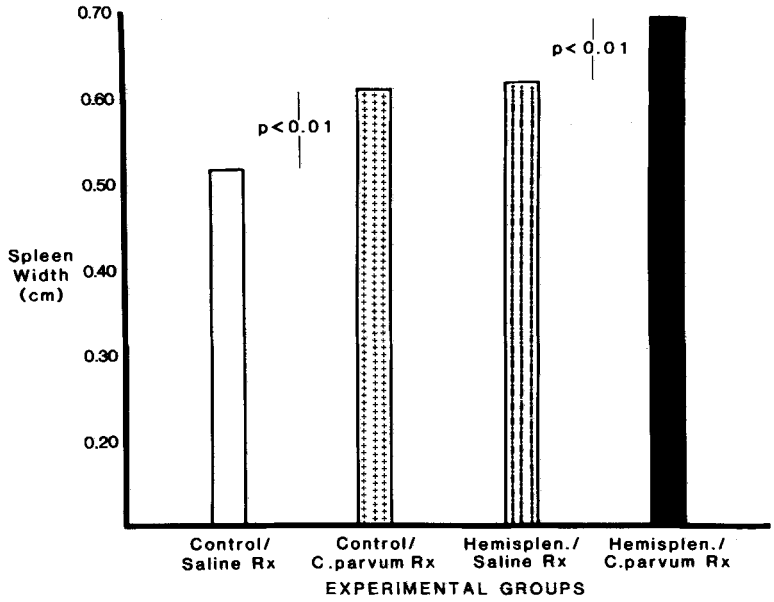

Fig 1. Mean spleen width of mice 21 days following *C parvum* or saline administration and 31 days following control-sham operation or hemisplenectomy.

of the spleen of *C parvum*-treated animals which was significantly greater than saline treated mice ($P < 0.01$; Fig 1).

The largest spleens in terms of weight were found in unsplenectomized control animals treated with *C parvum*. Hemisplenectomized *C parvum*-treated mice had the next greatest splenic mass, followed by saline inoculated unsplenectomized controls, and then the saline treated hemisplenectomized animals (Table 1; Fig 1). Therefore *C parvum* treatment resulted in a significantly greater percentage increase in spleen weight as compared to saline-treated animals (Fig 2). The effects of *C parvum* on splenic growth in sham operated unsplenectomized control mice was characterized by a 56% increase in spleen weight. In contrast, there was a significantly greater increase (106%) in spleen weight in mice who had undergone hemisplenectomy and *C parvum* treatment (X^2, $P < 0.01$).

The final mean width and weight of *C parvum*-treated hemisplenectomized animals was greater than saline-inoculated unsplenectomized control mice (Table 2).

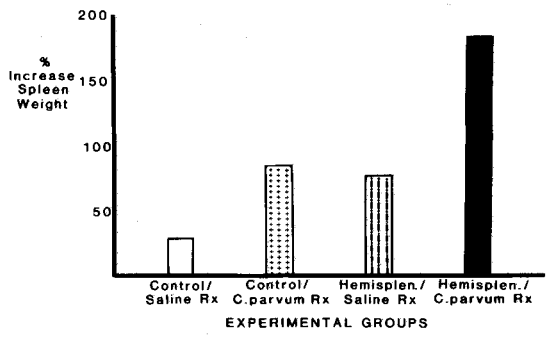

Fig 2. Percentage increase in spleen weight calculated as (mean spleen weight at sacrifice − mean spleen weight at time of operation) *mean* spleen weight at time of operation × 100.

Histology and Spleen Cell Assay

The increase in splenic growth or regeneration observed in *C parvum*-inoculated animals appears to proceed in a relatively normal histologic manner, as evidenced by a comparison of saline- and *C parvum*-treated spleen specimens. No significant microscopic differences in the relative prominence of white pulp, number of lymphoid follicles, the diameter of the lymphoid follicles, or the germinal centers could be detected between any of the experimental groups (Table 3).

In support of these histologic findings is the further observation that the number of spleen cells per gram of splenic tissue did not differ significantly between *C parvum*- and saline-treated animals (Fig 3).

Survival

After exposure to intranasal pneumococcal challenge, the best survival was observed in *C parvum*-inoculated hemisplenectomized mice

Table 2. Statistical Comparison of Splenic Weight and Width*

	Control/Saline	Control/*C Parvum*	Hemisplen/Saline	Hemisplen/*C Parvum*
Initial Weight (g)	0.131 ± 0.02	0.131 ± 0.02	0.067 ± 0.02	0.067 ± 0.02
Final Weight (g)	0.174 ± 0.093†	0.248 ± 0.081†‡	0.119 ± 0.037	0.190 ± 0.089†‖
Width (cm)	0.52 ± 0.08	0.61 ± 0.11‡	0.62 ± 0.12‡	0.69 ± 0.14†‡§

Note: All data are from mice five weeks following operation and treatment with 700 mcg *C parvum* (IP) or saline injection.

*All results are mean ± SD.

†$P < 0.01$ versus hemisplenectomy/saline.

‡$P < 0.01$ versus control/saline.

§$P < 0.01$ versus control/*C parvum*.

‖No significant difference versus control/saline.

Table 3. Splenic Histology

	Control/Saline	Control/*C Parvum*	Hemi/Saline	Hemi/*C Parvum*
Number of specimens	15	22	16	16
Relative prominence of white pulp (least prominent = 1; most prominent = 5)	4.0 ± 0.8*	4.0 ± 0.8	3.9 ± 0.7	3.8 ± 0.9
Number of lymphoid follicles/4 mm section	13.5 ± 6.4	12.6 ± 3.8	14.4 ± 4.2	14.0 ± 4.3
Diameter of lymphoid follicles (mm)	0.37 ± 0.08	0.38 ± 0.11	0.39 ± 0.08	0.40 ± 0.09
Diameter of germinal centers (mm)	0.18 ± 0.06	0.19 ± 0.08	0.22 ± 0.08	0.20 ± 0.07

*Data presented represents mean ± SD.

(91.7%). As predicted, hemisplenectomized animals inoculated with saline had a significantly lower survival rate (34.8%) as compared to the survival of saline-treated unsplenectomized control mice (69.6%; $P < 0.03$; Fig 4). Most striking was the significant difference in survival observed between *C parvum*-treated (91.7%) and saline-inoculated (34.8%) hemisplenectomized animals ($P < 0.001$; Fig 4). The higher survival rate observed for *C parvum*-treated unsplenectomized subjects is not statistically different from the saline-inoculated unsplenectomized control mice when the data is compared using the Cox proportional hazard method ($P = 0.24$; Fig 4).

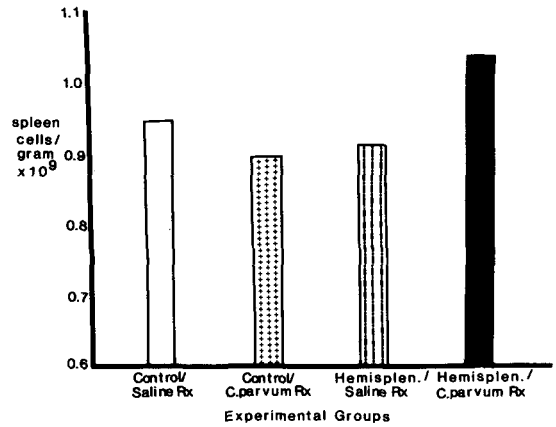

Fig 3. Number of spleen cells per gram of spleen 31 days following control-sham operation or hemisplenectomy.

All mice that died had gross evidence of pneumonia, confirmed bacteriologically to be due to *S pneumoniae* Type 1.

DISCUSSION

The problem of overwhelming postsplenectomy sepsis in children was recognized and reported over 30 years ago.[2] Since that time, a growing body of information has accumulated indicating that adults are also at increased risk from infection following splenectomy.[3–5] A more individualized and selective approach has been advocated for children with splenic injuries.[6] A number of surgical alternatives to total splenectomy for children and adults have been proposed that have included splenorrhaphy or repair, splenic artery ligation or embolization, autoimplantation, and partial splenectomy.[7–13] The relative merits of these techniques have been compared experimentally.[8,12] Based on these investigative studies, partial splenectomy appears to be the most effective splenic preservation operation. Partially splenectomized animals have the highest pneumoccocal antibody titers, the best bacterial bloodstream clearance, and survival following bacterial challenge.[8,12] Clinically, partial splenectomy has been reported in staging lapo-

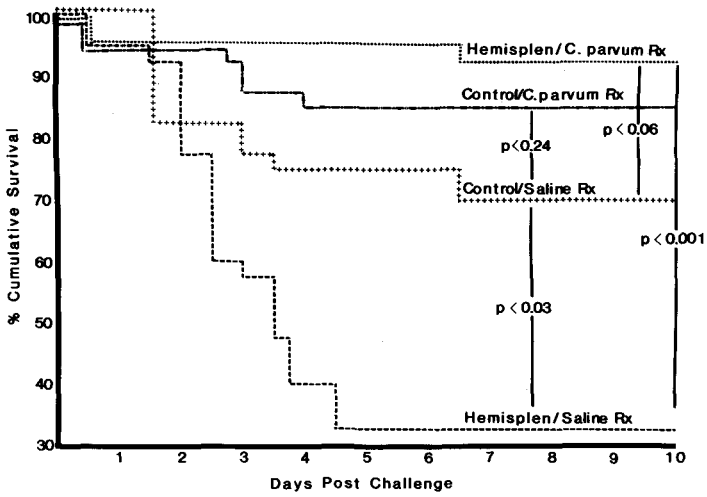

Fig 4. Cumulative percentage survival versus time in days after intranasal challenge with 3 × 10^7 CFU of *S pneumoniae* Type 1. Hemisplen indicates hemisplenectomy.

rotomy for Hodgkin's disease, for the control of hypersplenism, in preparation for renal transplantation, and for repair of splenic lacerations.[14–17] The size of the splenic remnant remaining following partial splenectomy in cases of trauma is dependent on the degree of injury and extent of resection necessary to control hemorrhage.

In an experimental study of regeneration or growth of the splenic remnant following partial splenectomy, Bradshaw et al observed that final regenerated splenic mass was directly related to the initial size of the splenic remnant remaining following the initial partial splenectomy.[18] Similar results were obtained in experimental studies by Cooney et al and Okinaga et al and in our own laboratory.[12,19]

The ability of the remaining splenic tissue to decrease the mortality rate following experimental pneumococcal challenge appears to be directly related to the size or amount of the splenic tissue remaining.[19,20] Okinaga's experiments indicated that partial resections of the spleen, which leave one third or less of the splenic mass intact, may not provide adequate protection. These studies also suggest that opsonic activity may be normal even when only small amounts of splenic tissue remain. However, the filtering function of the spleen appears to be size dependent and is a major determinant of survival.[19,20]

A drug that stimulates regeneration would be useful in cases in which extended partial resection is necessary. Immunomodulators are compounds that have a number of biologic effects, which include lymphocyte (T-cell) function depression, tumor suppression, decrease in thymic weight, and reticuloendothelial system stimulation.[21–25] Splenomegaly, hepatomegaly, and an increase in macrophage function characterizes the effects on the reticuloendothelial system.

A number of immunomodulators have been identified, which include (1) Microbial Sources: *Corynebacter parvum,* (Propionibacterium acnes), *Propionibacterium Granulosum, Propionibacterium Avidum,* BCG, Clugan, and Lipopolysaccharides; (2) Synthetic Sources: Lipoidal Amines (CP-46665, CP-20961), Muramyl Dipeptide (MDP), Thymopoietin Pentapeptide (TP-5), Levamisole, Lithium Carbonate, Methyl Extraction Residues, and Dinitrochlorobenzene; and (3) Animal or Human Sources: Interferon, Lymphokines with macrophage activation factors, Adenosine Triphosphate-Magnesium Chloride, and Tuftsin.[26–30]

Studies have led to the conclusion that some of these compounds may have a beneficial role in decreasing the incidence of experimental infection or sepsis.[31–33] Hebert's experiments using splenectomized mice indicate that *C parvum* results in an improved clearance of pneumococcus from the bloodstream and better survival following aerosol challenge.[1]

In the current study, the use of *C parvum* resulted in survival rates in hemisplenectomized animals that were comparable to the survival rates of unsplenectomized mice. It is interesting to note that the administration of *C parvum* did not statistically improve the survival of unsple-

nectomized control animals. This result is in contrast to the work of Hebert, who demonstrated a *C parvum* mediated improved survival for unsplenectomized control mice. The impressive improvement in survival of *C parvum*-hemisplenectomized animals is probably related to enhanced activity and increase in splenic mass. Although whole spleens of control animals increased in size, *C parvum* administration resulted in twice the regenerative mass effect in hemisplenectomized mice. The rather dramatic *C parvum*-induced regeneration is in contrast to previous observations of Bradshaw et al and Cooney et al—namely, that significant regeneration does not naturally take place following partial splenectomy.[12,18] Touloukian et al and Kovacs et al have pointed out that the ability of splenic tissue to regenerate may be related to the age of splenectomized animals. Their experiments suggest that regeneration may be even more complete in younger subjects.[11,34] Based on Okinaga's studies, one can assume that the large regenerated splenic remnants in these animals cleared pneumococci more effectively from the bloodstream and resulted in better survival.

Waymack et al have demonstrated that the reticuloendothelial stimulatory effects of individual immunomodulators may vary and be host-species specific.[33] The current study does suggest that the use of immunomodulators may be a very attractive therapeutic adjunctive measure for patients requiring partial splenectomy. However, because of the question of species specificity and individual potency of various immunomodulators, one cannot draw any definitive conclusion regarding a recommendation for the clinical use of *C parvum* for humans requiring subtotal splenectomy at the present time.

REFERENCES

1. Hebert JC, Gamelli RL, Foster RS, et al: Improved survival after pneumococcus in splenectomized and nonsplenectomized mice with Corynebacterium parvum. Arch Surg 118:328–332, 1983
2. King H, Shumaker HB, Jr: Splenic studies, susceptibility to infection after splenectomy performed in infancy. Ann Surg 136:239–242, 1952
3. Robinette CD, Fraumeni JF, Jr: Splenectomy and subsequent mortality in veterans of the 1939–1945 war. Lancet 2:127–129, 1977
4. Standage BA, Goss JC: Outcome and sepsis after splenectomy in adults. Am J Surg 143:545–548, 1982
5. Francke EL, Neu HC: Postsplenectomy infection. Surg Clin North Am 61:135–155, 1981
6. King DR, Lobe TE, Haase GM, et al: Selective management of injured spleen. Surg 90:677–682, 1981
7. Sherman R: Rationale for and methods of splenic preservation following trauma. Surg Clin North Am 61:127–134, 1981
8. Cooney DR, Dearth JC, Swanson SE, et al: Relative merits of partial splenectomy, splenic reimplantation, and immunization in preventing postsplenectomy infection. Surg 86:561–569, 1979
9. Patel J, Williams JS, Naim JO, et al: Protection against pneumococcal sepsis in splenectomized rats by implantation of splenic tissue into an omental pouch. Surg 91:638–641, 1982
10. Velcek FT, Jongco B, Shaftan GW, et al: Post-traumatic splenic replantation in children. J Pediatr Surg 17:879–882, 1982
11. Touloukian RJ, Dang CV, Carida VJ: Splenic function following experimental dearterialization injury in the suckling rat. J Pediatr Surg 13:131–135, 1978
12. Cooney DR, Michalak WA, Michalak DM, et al: Comparative methods of splenic preservation. J Pediatr Surg 16:327–338, 1981
13. Pringle KC, Spigos DG, Tan WS, et al: Partial splenic embolization in the management of thalassemia major. J Pediatr Surg 17:884–891, 1982
14. Boles ET, Jr, Haase GM, Hamoudi AB: Partial splenectomy in staging laparotomy for Hodgkin's disease: An alternative approach. J Pediatr Surg 13:581–586, 1978
15. Witte CL, Van Wyck DB, Witte MH, et al: Ischaemia and partial resection for control of splenic hyperfunction. Br J Surg 69:531–535, 1982
16. Guzzetta PC, Stolar CH, Potter BM, et al: Partial splenic ablation in preparation for renal transplantation in children. J Pediatr Surg 18:800–803, 1983
17. Buntain WL, Lynn HB: Splenorrhaphy: Changing concepts for the traumatized spleen. Surg 86:748–760, 1979
18. Bradshaw PH, Thomas AB, Thomas CG, Jr: Regeneration of splenic remnants after partial splenectomy in rats. J Surg Res 32:176–181, 1982
19. Okinaga BK, Giebink GS, Rich RH, et al: The effect of partial splenectomy on experimental pneumococcal bacteremia in an animal model. J Pediatr Surg 16:717–724, 1981
20. Wyck DB, Whitte MH, Whitte CL, et al: Critical splenic mass for survival from experimental pneumococcemia. J Surg Res 28:14–17, 1980
21. Castro JE: The effect of *Corynebacterium parvum* on the structure and function of the lymphoid system in mice. Europ J Cancer 10:115–120, 1974
22. Scott MT: Depression of delayed-type hypersensitivity by *Corynebacterium parvum:* Mandatory role of the spleen. Cellular Immunology 13:251–263, 1974
23. Milas L, Gutterman JU, Basic I, et al: Immunoprophylaxis and immunotherapy for a murine fibrosarcoma with *C granulosum* and *C parvum*. J.Cancer 14:493–503, 1974
24. Tuttle RL, North RJ: Mechanisms of antitumor action of Corynebacterium parvum: The generation of cell-

mediated tumor specific immunity. J Reticuloendothelial Soc 20:197–208, 1976

25. Chapes SK, Haskill S: Evidence for granulocyte-mediated macrophage activation after *C parvum* immunization. Cellular Immunology 75:367–377, 1983

26. Fox AD, Chaudry IH, Kupper TS, et al: Restoration of the depressed immune response after burn therapy by administration of ATP-$MgCl_2$ or tuftsin. Surgical Forum 33:69–70, 1982

27. Browder W, Rakinic J, McNamee R, et al: Protective effect of nonspecific immunostimulation in postsplenectomy sepsis. J Surg Res 35:474–479, 1983

28. Fogler WE, Talmadge JE, Fidler IJ: The activation of tumoricidal properties in macophages of endotoxin responder and nonresponder mice by liposome encapsulated immunomodulators. J of Reticuloendothelial Soc 33:165–174, 1983

29. Stinnett JD, Loose LD, Miskell P, et al: Synthetic immunomodulators for prevention of fatal infections in a burned guinea pig model. Ann Surg 198:53–57, 1983

30. Willmott N, Pimm MV, Baldwin RW: Quantitative comparison of three *C parvum* strains for immunotherapy of transplanted rat tumours. Cancer Immunology 7:117–122, 1979

31. Calhoun K, Trachtenberg L, Hart K, et al: *Corynebacterium parvum:* Immunomodulation in local bacterial infections. Surg 87:52–58, 1980

32. Adlam C, Broughton ES, Scott MT: Enhanced resistance of mice to infection with bacteria following pretreatment with *Corynebacterium parvum.* Nature New Biology 235:219–220, 1972

33. Waymack JP, Miskell P, Gonce S, et al: Immunomodulators in the treatment of peritonitis in burned-malnourished animals. In Press J of Surg Res

34. Kovacs KF, Caride VJ, Touloukian RJ: Regeneration of splenic autotransplants in suckling and adult rats. Arch Surg 116:335–336, 1981

Discussion

Denis R. King (*Columbus*): This is one of a series of studies from Dr Cooney and his colleagues in which he has attempted to carefully dissect the various elements of splenic function in the experimental model. I am not convinced that the increased survival demonstrated in this report is a result of splenic regeneration, however. Certainly, survival is better with *C parvum* treatment and the splenic weights increased significantly; but this may well be a true-true but unrelated phenomenon.

Splenic function depends upon a number of elements, both specific and nonspecific with regard to opsinization, particle trapping, which is blood flow, and antibody dependant; and then, of course, you have specific antibody elaboration. The experimental design should have included a group of animals that were completely splenectomized in order to evaluate whether the *C parvum* treatment was, in fact, an entirely nonspecific serum effect rather than the result of splenic regeneration.

I would have these questions for Dr Cooney. Do you have any evidence that the improved survival is a direct result of splenic regeneration, or is this merely a nonspecific stimulation of the entire reticuloendothelial system? Are you trying to equate weight with function? Should you have done a quantative analysis of the antibody-forming cells after an intravenous challenge of sheep red blood cells? How long does the effect of the *c parvum* last? Is this regeneration permanent or is it temporary? And finally, a clinical correlate perhaps, do you believe that there is any role for hemisplenectomy in the clinical situation other than in patients who are the victims of trauma?

R. Touloukian (*New Haven*): Dr Cooney and his group have again provided us with new and useful information on splenic preservation as we redirect our attention from the more straightforward technical aspects of operative repair to other options directed at enhancing regeneration of the splenic remnant and the host's response to systemic sepsis. As indicated today, administration of the immunomodulator *C parvum,* while appearing to achieve these goals in mice, does not answer questions about the potency of this agent in humans and other species. Dr Cooney has specifically made this disclaimer in his manuscript, and I am sure that you will appreciate that when it comes to print.

Perhaps you will tell us more about the long-term protection afforded by this immunomodulator, in other animal studies, and how long splenic hypertrophy persists following a single intraperitoneal innoculation. Another question concerns the use of adult rather than growing mice in a splenic regeneration model. It is known that spontaneous hypertrophy of autologous implants is more pronounced in the juvenile or immature subject that it is in the adult. Would a similar enhancement of regeneration in hemisplenectomized younger animals blunt or actually

accentuate the influence of this particular immunomodulator? In experiments using a dearterialization hemisplenectomy suckling rat model, we found that hypertrophy returning the weight of the splenic remnant to normal continued into the second month following injury. Would the authors comment on how they chose 4 to 5 weeks as the appropriate time to challenge their animals and whether or not a further delay of perhaps 3 or 4 weeks might reduce or eliminate the differences in splenic weight observed in their study groups. In closing, Could you also tell us what fraction of your clinical patients with splenic injury undergo any operation and, of those that do, how frequently hemisplenectomy is necessary because of the severity of the injury. In our experience, hemisplenectomy is rarely performed.

Donald Cooney (*closing*): Dr King questioned our exclusion of a splenectomized group in these experiments. A review of the literature indicates that *C parvum* does have a relatively nonspecific effect on increasing the survival in this same mouse model. We were not interested in comparing the effects of total splenectomy to hemisplenectomy. Our aim was to determine the effect of *C parvum* on hemisplenectomy. *C parvum* has a specific effect on the spleen, resulting in trapping T-lymphocytes and increasing the numbers of macrophytes. The major determinate of survival in animals and probably in human beings is not opsinozation of bacteria, but the trapping of these organisms in the spleen by the macrophages induced by *C parvum*. Regarding other uses for hemisplenectomy, it has been used for palliating the effects of hypersplenism, in resection of cystic lesions of the spleen, and also Dr King, is very much aware of his own institution's interest in hemisplenectomy for staging of Hodgkin's disease patients. In that situation, one should be very careful about suggesting the use of immuno-modulators because of the complex and very carefully balanced relationship between T-cells, macrophages, and the tumor-suppressive abilities of immunomodulators.

Dr Touloukian, we do not know the effects of these immunomodulators on younger animals. In terms of the long-term effects of *C parvum's* ability to stabilize these larger size splenic remnants, preliminary work in our laboratory indicates that the larger size of the splenic remnant remains stable for an extended period of time. Finally, we have not operated on a single child with a splenic injury at our hospital in the last two years.