Immunologic Defect of the Alternate Pathway-of-Complement Activation Postsplenectomy: A Possible Relation Between Splenectomy and Infection

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Total hemolytic complement (CH50) and activation of the alternate mechanism were measured in eight patients before and after splenectomy and compared to similar measurements made in a control group of patients following other abdominal surgery. In the splenectomy group, alternate-pathway-mediated activation of C3 was significantly different from the controls. The mean five-day postsplenectomy value of 16 percent for the immunoelectrophoretic conversion of C3 to C3i was depressed ($p<0.001$) from the presplenectomy value of 85 percent and five-month postsplenectomy level of 71 percent ($p<0.01$). The difference between presplenectomy and five-month postsplenectomy values was not significant. Further, activation of C3 in patients five days postsplenectomy was significantly less ($p<0.01$) than in the five-day postoperative controls. In both the splenectomized patients and control group, five-day postoperative determinations indicated an increase in CH50 values and a decrease in degree of activation of Factor B. The spleen appears to manufacture certain substances required for activation of C3 via the alternate mechanism. That the manufacture is eventually assumed by other immune-competent organs is shown by the eventual increase of activation toward preoperative levels five months postsplenectomy. This defect in C3 activation may account for the tendency of splenectomized patients to have an increased incidence of bacterial infections and sepsis in the postoperative period.

King and Schumacker, in 1952, first reported an increased incidence of serious infections in children following splenectomy.[1] Since that time numerous studies showing an increased incidence of infection postsplenectomy have been published,[2-5] although the exact incidence is still controversial.[6] Reasons for this propensity to develop septicemia postsplenectomy have been evaluated by various investigators. Significantly lower mean immunoglobulin (IgM) levels, as well as a deficiency of the leukocyte-stimulating peptide tuftsin,[8] have been described in a splenectomized population.[7] The role of other immunologically active substances in postsplenectomy vulnerability to infection has not been well studied.

The third component of complement is well known to participate in opsonization of certain types of bacteria.[9] In addition, patients with sickle cell disease have functional asplenia[10,11] and have been described as having a defect in the alternate pathway of the complement system.[12-14] "Alternate pathway" (or alternative pathway) is the term used to describe the activation of the third component of complement not utilizing specific, preformed antibody and bypassing a requirement for fixation of the early components of the classical complement system. This decrease in complement activation is felt to lead to decreased opsonization and a subsequent susceptibility to infections, especially those caused by the pneumococcus.[15-17] Patients, pre and postsplenectomy, were evaluated to determine if alternate-pathway activation of complement were impaired and, if so, the time course of the impairment.

Patients

There were eight individuals in the group of splenectomized patients (Table 1). All but one were white. The group included patients with chronic myelogenous leukemia who were splenectomized according to an approved protocol. Other patients had lymphoreticular disease and underwent splenectomy as part of a diagnostic procedure. One patient did not have an oncological or hematological problem and underwent splenectomy for removal of an echinococcal cyst. Patients used as controls were hematologically normal, but had had various elective or emergency intraabdominal procedures performed excluding splenectomy (Table 2). Consent was obtained from each patient for these studies.

Materials and Methods

Blood was drawn from each patient and allowed to clot. The serum was separated from the clot within two hours and stored at -70C, until the time

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of testing. The serum was then thawed and placed immediately in an icebath.

Total hemolytic complement (CH50) was determined by standard procedures.[18,19] Activation of the alternate mechanism of complement was determined by measuring immunoelectrophonetic conversion of Factor B (C3PA) to Factor $\bar{B}$ (C3A) and C3 to its hemolytically inactive form (C3i) following incubation of the serum with the plant polysaccharide inulin.[20]

The degree of electrophoretic conversion of Factor B and C3 was evaluated by one of the authors who was not aware of the clinical state of the patients. Statistical comparison of the results was performed using the student's t-test.[21]

Results

The effect of splenectomy on CH50 values and the ability of Factor B and C3 to be activated by inulin is detailed in Table 1. Splenectomy significantly increased CH50 levels ($p<0.01$) in the five-day postoperative and distant (five-month) postoperative periods ($p<0.05$) over control values. This was true for all patients except patient 1, the only patient who did not have a malignant disease. A rise in CH50 levels was also seen in postoperative controls not undergoing splenectomy (Table 2), consistent with the fact that the CH50 value may reflect an acute phase which is reactant postoperatively. The degree of activation of Factor B was slightly decreased in both groups of postoperative patients at five days (48 and 64 percent) but the difference between the groups was not significant.

Alternate mechanism-induced activation of C3 is the variable which showed the most significant change in patients undergoing splenectomy. The mean five-day postsplenectomy value of 16 percent was significantly depressed from the preoperative ($p<0.001$) and distant postoperative ($p<0.0$) levels of 85 and 71 percent. There were no consistent differences between preoperative and distant postoperative levels. Patients undergoing abdominal surgical procedures other than splenectomy showed a slight decrease in mean C3 conversion five days postoperatively (65±24 percent), but postsplenectomy patients were significantly lower than the controls at five days ($p<0.01$).

Intraabdominal surgery is associated with an increase in CH50 levels and a decrease in the ability of Factor B to be activated whether or not a splenectomy is performed. Splenectomy itself apparently results in a specific reduction in alternate mechanism-induced C3 conversion five days following the procedure. The ability of C3 to be activated approached preoperative values at five-months postsplenectomy.

Discussion

The spleen appears to perform at least four functions in combating infection. Its role as a filter as part of the reticuloendothelial system is well known. Under ordinary circumstances, phagocytic function is adequately performed by the liver and other reticuloendothelial tissues, so that the splenic contribution is not essential. Splenic phagocytosis appears to be critically important, however, in the face of a quantitative or qualitative deficiency of opsonizing antibody.[22,23] In addition, the spleen seems to be one of the sources of acute-phase IgM, although there have been conflicting reports concerning this.[7,24-28] Tuftsin, a leukocyte-stimulating peptide, has been considered to be synthesized in the spleen.[8,29] The present study indicates that activation of C3 via the alternate pathway also requires certain substances or at least a substance generally dependent on the presence of a spleen.

Whereas patients undergoing abdominal surgery show a decrease in C3 and Factor B activation, those undergoing splenectomy show a total decrease in C3 conversion which is significantly lower than that of postoperative controls. Although the splenectomy population was ill, they were able to activate C3 more readily at five months postsplenectomy despite the fact that the levels in most cases remained depressed when compared to the normal study population. This time course would indicate that the maximum vulnerability to infection postsplenectomy is within five months of the operation. Clinical reports support the contention that approximately 80 percent of overwhelming postsplenectomy infections occur within the first two years postoperatively.[3,22,30] Individual patients may show depressed activation of C3 for periods of longer than five months (eg, patients 2, 3, 6, and 7 in Table 1).

This study was concerned with adults. A similar study is needed in children. Theoretically, children, having been exposed to fewer antigens, would have less induction of the classic pathway of complement activation and, therefore, might remain vulnerable to infection for a longer period of time. Nevertheless, it would appear that postsplenectomy penicillin prophylaxis in adults might be useful for about five months, or until C3 alternate pathway induced activation returns to normal.

Of note is the discrepancy between activation of Factor B and activation of C3. The ability to activate Factor B in postoperative patients and in postsplenectomy patients was diminished to about the same extent. On the other hand inulin-dependent activation of C3 was diminished to a much greater extent postsplenectomy than in the control group; thereby indicating that C3 activation, in the present study, may have been dependent on other means of activation than Factor B. This is consistent with recent data which points to several means by which C3 conversion may be achieved.[31]

The present study documents a vulnerability in the immune system following splenectomy. Whether the deficit in the ability to activate C3 by the alternate mechanism is the primary one which predisposes postsplenectomy patients to infections remains to be determined. Furthermore, whether this abnormality is present in patients with functional asplenia such as sickle cell disease, or with reticuloendothelial blockage occurring in patients receiving repeated transfusions, should be studied. It has recently been noted that total levels of Factor B are decreased about 50 percent in patients with sickle cell disease.[32] If specific factors can be demonstrated to be decreased in patients postsplenectomy or with splenic dysfunction, specific replacement therapy becomes a possibility allowing for the hope that the postoperative vulnerability to infection can be eliminated.

Table 1. Whole Complement Hemolytic Activity (CH_{50}) and Degree of Immunoelectrophoretic Conversion of Factor B and C_3 Following Incubation of Serum with Inulin Before, 5 Days, and 5 Months After Splenectomy

Patient	Age	Sex	Disease	CH_{50}			Conversion of Factor B* (as % of normal control)			Conversion of C_3† (as % of normal control)		
				Before	5 Days	5 Months	Before	5 Days	5 Months	Before	5 Days	5 Months
1	49	M	Echinococcal cyst	98	141	98	50	12	72	50	5	100
2	38	M	Chronic myelogenous leukemia	96	149	171	100	87	30	90	30	60
3	53	M	Lymphoma	152	163	148	100	88	83	90	8	66
4	42	F	Chronic myelogenous leukemia	140	180	156	66	33	65	90	5	100
5	35	M	Lymphoma	151	214	**	100	33	**	90	5	**
6	26	M	Chronic myelogenous leukemia	118	156	168	8	50	90	100	45	56
7	52	F	Chronic myelogenous leukemia	83	173	172	32	32	22	66	16	56
8	43	F	Chronic myelogenous leukemia	72	122	**	87	50	**	100	11	**
Mean	42			114	162	152	68	48	60	85	16	71
± SD	9			31	28	28	35	27	28	17	15	20

*Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (see Methods).

† Immunoelectrophoretic conversion of C3 to C3i used as a measure of the degree of activation of C3 following incubation of serum with inulin.

**Unavailable for follow up.

Table 2. Complement Determinations in Control 5-Day Postoperative Patients

Patient	Age	Sex	Diagnosis	CH_{50}	Conversion of Factor B* (as % normal control)	Conversion of C3** (as % normal control)
1	22	F	Hernia repair	121	45	30
2	52	F	S/P cholecystectomy	214	58	50
3	24	F	Appendectomy	106	95	100
4	48	M	Appendectomy	196	8	83
5	56	M	Exploratory laparotomy	140	94	50
6	38	F	Appendectomy	140	100	70
7	22	M	Appendectomy	117	58	90
8	24	M	Appendectomy	178	57	50
Mean	36			153	64	65
± SD	15			43	31	24

* Immunoelectrophoretic conversion of Factor B to Factor $\bar{B}$ used as a measure of the degree of activation of Factor B following incubation of serum with inulin (see Methods).
** Immunoelectrophoretic conversion of C3 to C3i used as a measure of the degree of activation of C3 following incubation of serum with inulin.

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