



Increased eosinophilic responses in splenectomized patients

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ABSTRACT

Background: The spleen is a key organ within the immune system. Its removal is known to bring about adverse effects such as an increased susceptibility to overwhelming infection. Few reports have suggested that the spleen may play a role in controlling eosinophilic responses, mostly based on animal models.

Objectives: To examine whether the human spleen impacts eosinophil numbers in the blood.

Methods: We have retrospectively analyzed eosinophil counts and medical records of 29 patients who had undergone splenectomy between 2000 and 2010. Statistical comparison was performed between post-splenectomy blood counts and both pre-splenectomy and control values. Data regarding the clinical settings around hypereosinophilia events were obtained from patient charts.

Results: An increased rate of eosinophilia was observed after splenectomy as compared with normal individuals. Furthermore, a considerable proportion of patients who had undergone splenectomies (8/29) presented peak eosinophil numbers exceeding 1,000/mm³, reaching a maximum of 3,070/mm³. These values were mostly encountered perioperatively or during episodes of acute infection.

Conclusions: Our data indicate that impaired control of eosinophilic responses is a long-term post-splenectomy effect and is evident in the context of acute stress. We suggest that the spleen plays a significant role in controlling eosinophil levels and that these cells may mediate some of the harmful consequences observed after removal of the spleen.

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Introduction

For many years, the spleen was thought to be a redundant organ and splenectomy a safe procedure and a potential cure for several diseases. Historically, trauma has been the most common indication, followed by hematological disorders such as hemolytic anemia, immune thrombocytopenic purpura (ITP), β -thalassemia, and sickle cell disease. However, because of improvements in conservative medical treatment modalities, and the realization that the lack of a spleen may adversely impact the immune system, the number of surgical procedures has been consistently declining.

King and Schumacker (1952)¹ were the first to observe an increased rate of infections in infants whose spleens had been removed because of congenital hemolytic anemia. This report was followed by others showing an increased susceptibility to infections after splenectomy, especially in children.² Consequently, severe sepsis, primarily with encapsulated bacteria, has become the most acknowledged complication, currently designated as the “overwhelming post-splenectomy infection” (OPSI). OPSI is traditionally explained by the critical role that the spleen plays in the identification and elimination of opsonized bacteria.

Subsequently, other short- and long-term effects of splenectomy have emerged, shedding light on additional aspects of spleen physiology. Examples for these abnormalities include hypercoagulability^{3–5} and, according to sporadic reports, susceptibility to develop anti-phospholipid syndrome⁶ as well as other autoimmune manifestations.^{7,8} The latter may manifest as elevated titers of autoantibodies such as antinuclear, anti-Sm, anti-Ro, and Anti-La.⁹

Quite intriguingly, even though the spleen is a pivotal lymphatic organ, the bona fide immunomodulatory impact of its removal remains largely obscure. Several changes have been described in both the humoral and cellular arms of the immune system in splenectomized individuals. The levels of immunoglobulins are altered,^{10–13} and T and B lymphocyte numbers appear to increase¹⁴ along with a reduced CD4⁺/CD8⁺ ratio.¹⁵ Furthermore, cell-mediated responses¹⁶ and secretion of cytokines¹⁷ were shown to be compromised. The aberrations in the cellular compartment, as well as in immunoglobulin and cytokine production, all may contribute to the compromised defense that these patients exhibit against severe infections, thus leading to OPSI.

In this study, we set out to investigate the possible impact of splenectomy on the regulation of eosinophilic responses. We hypothesized that if elevated eosinophil counts exist, they may potentially account for some of the aforementioned complications observed in these individuals. Interestingly, screening of the liter-

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Table 1
Indications for splenectomy in the study group

Indication	No. patients	Percentage
Trauma	18	62.1
ITP	3	10.3
Lymphoma	2	6.9
Ca of pancreas	2	6.9
HCL	1	3.4
Hemolytic anemia	1	3.4
Sarcoidosis	1	3.4
Splenomegaly	1	3.4

Abbreviations: ITP, immune thrombocytopenia; Ca, carcinoma; HCL, hairy cell leukemia.

ature showed a striking paucity of data in this regard, including a report by Sevitt (1955)¹⁸ that the spleen plays a role in the physiological reduction of eosinophil counts after injury, a process thought to be induced by adrenocortical hormones. We retrospectively screened the files of patients admitted to the Department of Surgery, Meir Medical Center, during the past 11 years, using the key words *total splenectomy*. A total of 32 records were retrieved, of whom 3 did not meet the inclusion criteria because of postoperative mortality (2 patients) or a preexisting hypereosinophilic state (1 patient). Blood count results disclosed a high post-splenectomy incidence of elevated eosinophils (14.6%) as compared with pre-splenectomy (2%) or control individuals (1.1%). Furthermore, documentation of eosinophil values exceeding 1,000/mm³ (maximum, 3,070/mm³) was found in 8 post-splenectomy patients, but in none of the pre-splenectomy blood counts or control individuals. These exaggerated responses were observed starting from the perioperative setting up to 5 years later, and may be associated with stress such as surgery or acute bacterial infections.

Methods

Patient selection and data retrieval

The database of the Meir Medical Center (Kfar Saba, Israel) was screened for patients admitted to the Department of Surgery between January 2000 and December 2010, with the keywords *total splenectomy* appearing in their problem list. Exclusion criteria included perioperative death (within 1 month) or a preexisting hypereosinophilic state. Consequently, 7 female and 22 male patients were included in the study. Having characterized the patient population, a control group was retrieved using the keywords *abdominal pain* and defining the age range as patient population age average $\pm$ 2 SD. From this list, 7 female and 22 male patients were randomly selected. Patient medical records were screened for eosinophilia events as well as the clinical settings pertaining to removal of the spleen and postoperative eosinophilia. The use of database and patient records for this study was approved by the Ethics Committee of the Meir Medical Center.

Statistical analysis

Nonparametric tests were chosen to analyze the differences between groups, after observing that the distribution of the quantitative data is not normal using the Kolmogorov-Smirnov test for normality (data not shown). The differences between the *post-splenectomy* and *abdominal pain* groups were analyzed by independent nonparametric tests—Mann-Whitney and Kruskal-Wallis. The differences between the *post-splenectomy* and *pre-splenectomy* groups were analyzed by related nonparametric tests—Wilcoxon Signed and Friedman. A *P*-value < .05 was defined as statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (SPSS 17.0 for Windows, SPSS Inc., Chicago, Illinois).

Results

Demographic data

A total of 32 patient files were retrieved using the keywords *total splenectomy*. Of these, 3 did not meet the inclusion criteria: 2 because of perioperative mortality and 1 because of a preexisting hypereosinophilic state secondary to a parasitic infection (visceral leishmaniasis). In the remaining 29 cases, trauma was the leading indication for surgery (62.1%) followed by hematological diseases (24%), carcinoma of the pancreas (6.9%), and sarcoidosis and splenomegaly of uncertain cause (3.4% each) (Table 1). The group of patients admitted because of trauma consisted of 18 individuals, 17 (94%) of whom were males, thus skewing the total male-to-female ratio in the splenectomized population to 3.1:1 (Table 2). The proportion of eosinophilia-positive blood counts, defined as any number exceeding 500/mm³, was considerably higher after splenectomy than before the procedure (14.6% and 2%, respectively). A similar trend was observed with regard to the percentage of individuals exhibiting eosinophilia (69% and 6.9%, respectively). The average pre-splenectomy follow-up time, number of blood count results, and, by definition, patient ages were all lower than those of post-splenectomy. We therefore compared the findings to a control group retrieved by the key words *abdominal pain*. In this group, the rates of both eosinophilia events (1.1%), and of individuals exhibiting eosinophilia (20.1%) were considerably lower than the post-splenectomy data, thus lending further support to the conclusion that removal of the spleen is associated with episodes of elevated eosinophil numbers. Some variation existed between the pre-splenectomy and abdominal pain groups, in terms of the proportion of patients with eosinophilia (6.9% and 20.1%, respectively). That these two populations differ is not entirely unexpected, because, although the former are ostensibly healthy individuals, the latter are hospitalized patients.

Extent of eosinophil count elevation in the post-splenectomized state

The observation that blood counts from splenectomized patients disclosed eosinophilia in a relatively high percentage raised two main questions: First, because of the heterogeneity of the study population, a confounding effect of preexisting conditions (eg, hematological diseases) on eosinophil counts needed to be ruled out. Second, we set out to assess the actual values of these results to determine the extent of exaggerated responses. For this purpose, we identified the highest pre- and post-splenectomy eosinophil count for each of the trauma patients and compared them with the control individuals. Thereby, only splenectomized individuals with no remarkable medical history were included in this analysis. However, the lack of prior diseases was also associated with a low rate of blood counts (*n* = 8) before splenectomy (Fig 1 A). When comparing these 2 sets of values, a statistically significant elevation was noted post-splenectomy (*P* < .05) (Fig 1 A). A similar difference was observed when comparing the abdominal-pain (ie, control) with that of the post-trauma splenectomy group (*P* < .001) (Fig 1 B), thus leading to the conclusion that higher peak-values occur because of

Table 2
Population data

	Splenectomy		Abdominal pain
Gender ratio (M:F)	3.1:1		3.1:1
Age	42.31 $\pm$ 17.22		44.83 $\pm$ 9.97
Follow-up data	Presplenectomy	Postsplenectomy	Abdominal pain
Years	4.48 $\pm$ 3.33	6.21 $\pm$ 3.55	11
Total CBC	202	795	580
Eosinophilia ^a events	4 (2%)	116 (14.6%)	6 (1.1%)
Patients with eosinophilia ^a	2 (6.9%)	19 (69%)	6 (20.1%)

^aEosinophilia indicates any value that exceeds 500 per mm³.

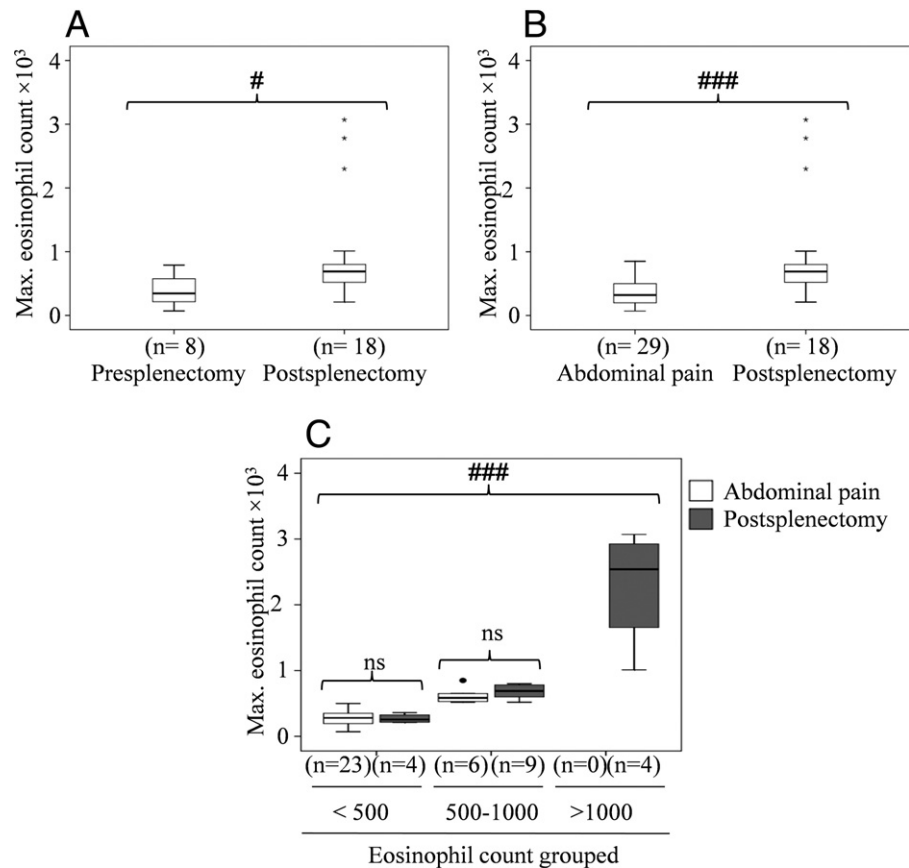


Fig. 1. Peak eosinophil counts increase after posttrauma splenectomy. For each subject, the single highest value of eosinophil count during the follow-up period was obtained, and the various groups were statistically compared. Maximal eosinophil counts are represented as box plots comparing the following groups of individuals: (A) Pre- and post-splenectomy (medians, 0.345 and 0.69, respectively); (B) abdominal pain and splenectomized (medians, 0.32 and 0.69, respectively); (C) abdominal pain and splenectomized subdivided into 3 ranges: 0–500 per mm^3 ; 500–1,000 per mm^3 ; >1,000 per mm^3 . # $P < .05$; ### $P < .001$.

the absence of a spleen. The graphs presented in Figure 1A and 1B may suggest that the data are skewed because of the 3 highest eosinophil values. However, statistical significance was retained even after exclusion of these individuals (not shown). For each group, the numbers were then divided into 3 categories: less than 500/ mm^3 ; 500 to 1,000/ mm^3 ; and greater than 1,000/ mm^3 (Fig 1C). Consequently, both groups included individuals whose maximal value during the follow-up period was in the lower and middle range. However, higher-range eosinophilia was found in patients who had undergone splenectomy but in none of the controls. In the splenectomized population, the values that reached eosinophilia (ie, >500/ mm^3) were detected invariably after removal of the spleen, and not at the pre-splenectomy follow-up period. Furthermore, results similar to those presented in Figure 1 were also obtained after the substitution of posttrauma patients with the entire population of splenectomized subjects, including those with hematological and other diseases (not shown). Taken together, these observations indicate that splenectomy per se and not accompanying diseases impact eosinophil levels.

To further characterize the difference between the splenectomized and the control groups, we calculated the ratio of individuals whose maximal eosinophil count reached the lower, middle, and higher range (Fig 2). As expected, in control subjects, most values were normal, followed by middle-range elevation with no highly increased counts (79%, 21%, and 0, respectively). A similar pattern was noted with the pre-splenectomy values (83.3%, 16.7%, and 0, respectively). Conversely, in the posttrauma splenectomized population, the normal-range fraction was smaller than middle-range and equal to the higher-range values (21%, 58%, and 21%, respec-

tively). We repeated this calculation using data from nontrauma splenectomized patients only. Again, the distribution resembled the posttrauma group (27%, 46%, and 27%, respectively) rather than the normal group. Hence, the inclusion of splenectomized patients with preexisting medical conditions does not skew the data, implying that these diseases do not contribute to the observed hypereosinophilia. A sex- and age-unmatched control population retrieved from the Department of Medicine database recapitulated the features of the control group depicted in Figure 2 (data not shown).

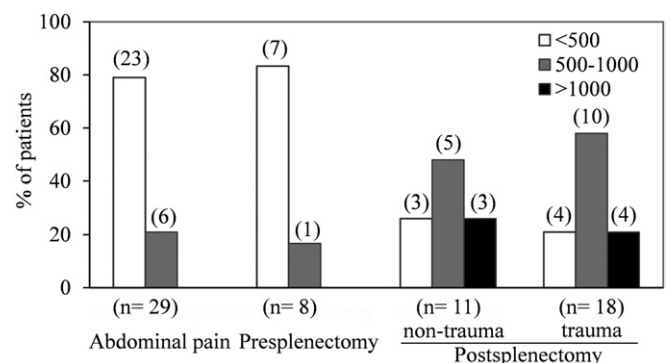


Fig. 2. Distribution of peak eosinophil counts within each group of patients, as indicated, according to eosinophil value ranges. The absolute number of subjects in each column is indicated in parentheses.

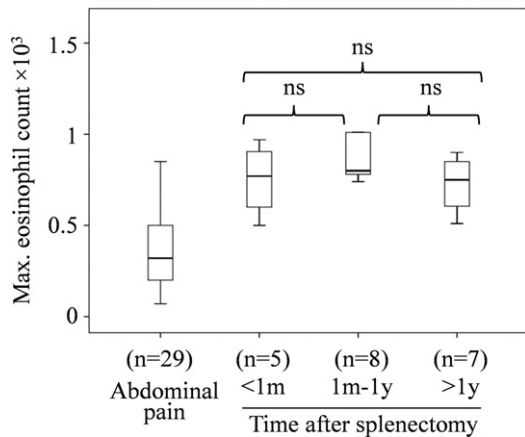


Fig. 3. Posttrauma splenectomy exerts a long-term effect on eosinophil levels. Box plots represent maximal eosinophil counts in patients who had undergone splenectomies divided into three time ranges after splenectomy, as indicated. Patients whose eosinophil levels were less than 500 per mm³ throughout the follow-up period were excluded from this graph. As a reference, the abdominal pain group shown in Figure 1B was added to this graph. No significant changes were observed between the 3 post-splenectomy groups.

The impact of splenectomy on eosinophil responses is long-term and is accentuated by acute stress

At this point we investigated whether the aforementioned events of eosinophilia occurred immediately after removal of the spleen or, alternatively, were a long-term feature of spleen-devoid individuals. For this purpose, the peak eosinophil counts obtained after posttrauma splenectomy were divided into 3 periods according to the time elapsed from surgery: up to 1 month; 1 month to 1 year; beyond 1 year (Fig 3). Consequently, no statistically significant difference was found in peak eosinophil values over time ($P > .05$). This finding strongly suggests that the increased probability to observe eosinophilia in splenectomized patients remains well over the perioperative stage and may very well last for years.

Finally, we sought to identify potential triggers that could precipitate eosinophilic reactions in the entire study population, including trauma and nontrauma patients. To enhance the specificity of this search, we focused on the 8 counts that reached the high range ($>1,000/\text{mm}^3$) and tried to characterize the clinical settings in which these events occurred (Table 3). These results were obtained from 3 days to 5 years after splenectomy. In 4 of the cases, eosinophilia occurred in the postoperative period, another three experienced an acute infection, and in one the clinical circumstances could not be determined. Half of the patients lost their spleens because of trauma, and, interestingly, the three highest numbers (3,070, 2,840, and 2,300 per mm³) were detected in this group. These data prompted us to conclude that, after splenectomy, patients acquire a long-term tendency to mount significant eosinophilic reactions, possibly in response to acute stress, such as trauma or infectious episodes.

Discussion

The spleen is widely accepted as being important for maintaining adequate immune responses. However, many of its functions remain obscure. This is mostly because of the fact that, unlike vital organs such as the liver or kidneys, removal of the spleen does not usually compromise survival in the short term. Furthermore, splenectomy is becoming increasingly uncommon, and thus studying its outcome is more difficult.

In this communication, we are suggesting that splenectomy brings about a predisposition to develop hypereosinophilia. This conclusion is based on several findings. Retrospective screening of complete blood counts shows a high prevalence of elevated eosin-

ophil levels as compared with the same individuals before surgery, or compared with an age- and sex-matched group. These elevated counts may reach values over 1,000 cells/mm³ (maximum, 3,400 cells/mm³), which are beyond the range seen in control individuals (100–900 cells/mm³). Finally, we observed that exaggerated eosinophilic reactions may occur as long as several years after removal of the spleen. The fact that most blood counts are normal with respect to eosinophil counts (only 14.6% of the values were greater than 500/mm³) (Table 2) indicates that this phenomenon is episodic, and our data imply that acute stress, such as infection or surgery, may trigger hypereosinophilia. We should note that bacterial sepsis, in the setting of an intact spleen, is typically associated with low eosinophil counts rather than eosinophilia.¹⁹ Therefore, sepsis-associated eosinophilia in the absence of a spleen may be regarded as paradoxical.

The spleen has been proposed as a regulator of eosinophilic responses in animal models. Screening of the literature reveals that this hypothesis was already suggested almost a century ago by Krumbhaar et al (1916),²⁰ who showed that ligation of the splenic vein recapitulates the results obtained from splenectomy in dogs. They conclude that after this procedure, “there is an initial leukocytosis ... as the total leukocytosis diminishes, there is both a relative and actual increase of small lymphocytes and usually of eosinophils. This may either be temporary or last during the rest of the period of observation and differs from the ordinary postoperative leukocytosis.” Similarly, Basten et al (1970)²¹ observed that, in splenectomized rats, a mild elevation occurs in basal eosinophil levels along with an exaggerated increase after parasitic infection with *Trichinella spiralis*. Some evidence that the spleen may play a similar regulatory role in human subjects as well was provided by Andersen et al,²² in a study of 14 children who underwent splenectomy. The subjects, 3 to 13 years of age, had their spleens removed because of congenital spherocytosis, splenomegaly, or thrombocytopenia and were followed for 1 to 2.5 years.²² The long-term alterations observed in this report included a decrease in immunoglobulin M levels, mild lymphocytosis, and a significant increase in eosinophils, lasting for at least 1 year, albeit well under the 500/mm³ limit. Although this report is in line with our results, the authors did not eliminate potential confounding effects such as preexisting hematological diseases that might impact the levels of blood eosinophils.

We suggest that the abnormal control of eosinophilic responses may play a role in some of the previously described post-splenectomy sequelae. Eosinophils are potent inflammatory cells that produce and secrete a variety of mediators, leading to cytotoxic activity and tissue damage.^{23,24} Therefore, their inappropriately exaggerated increase during acute infections may play a role in the pathogenesis of overwhelming reactions and organ damage. Our data did not provide evidence that individuals who had undergone splenectomies are more prone to develop eosinophil-associated

Table 3
High-range ($>1,000$ per mm³) eosinophilia events

Time Post-splenectomy	Abs. Eosinophil Count	Clinical Setting	Indication for Splenectomy
3 d	1,090	Perioperative	Carcinoma of pancreas
8 d	1,560	Perioperative	Lymphoma
26 d	1,200	Perioperative	Lymphoma
32 d	2,840	Perioperative	Trauma, gunshot
2 m	3,070	Abdominal abscess	Trauma, stab wound
3 m	1,010	Urinary tract infection	Trauma, work accident
2.5 y	1,380	Unknown	Hairy cell leukemia
5 y	2,300	Diverticulitis of colon	Trauma, stab wound

diseases. However, the relatively small number of subjects in this study is also not sufficient to rule out this possibility.

Finally, levels of blood eosinophilia are considered as an imperfect correlate of the potential for eosinophil-mediated tissue damage²⁵ and that their recruitment into tissues is a prerequisite for the propagation of this process. Nevertheless, hypereosinophilia of 1,500/mm³ is the threshold above which tissue toxicity is more likely to take place.²⁵ Here we show that such levels are more expected to occur in the absence of a spleen than in control individuals. However, additional data are needed to determine whether splenectomy can instigate tissue infiltration by these cells in a manner that could account for significant organ damage.

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