High-Dimensional Analysis of Postsplenectomy Peripheral Immune Cell Changes


*ImmuNoHorizons* 2020, 4 (2) 82-92
doi: https://doi.org/10.4049/immunohorizons.1900089
http://www.immunohorizons.org/content/4/2/82

This information is current as of June 15, 2021.

Supplementary Material
http://www.immunohorizons.org/content/suppl/2020/02/17/4.2.82.DCSupplemental

References
This article cites 49 articles, 10 of which you can access for free at:
http://www.immunohorizons.org/content/4/2/82.full#ref-list-1

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://www.immunohorizons.org/alerts
High-Dimensional Analysis of Postsplenectomy Peripheral Immune Cell Changes


*Department of Surgery, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; †Radboud University Medical Center, 6525 GA Nijmegen, the Netherlands; ‡University of Colorado Cancer Center, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; §Department of Anesthesiology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; ||Department of Medical Oncology, Department of Medicine, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045; and †Department of Immunology and Microbiology, School of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO 80045

ABSTRACT
Although the consequences of splenectomy are well understood in mice, much less is known about the immunologic changes that occur following splenectomy in humans. We sought to characterize the circulating immune cell populations of patients before and after elective splenectomy to determine if these changes are related to postsplenectomy survival outcomes. Retrospective clinical information was collected from 95 patients undergoing elective splenectomy compared with 91 patients undergoing pancreaticoduodenectomy (Whipple procedure). We further analyzed peripheral blood from five patients in the splenectomy group, collected before and after surgery, using single-cell cytometry by time-of-flight mass spectrometry. We compared pre- and postsplenectomy data to characterize both the major and minor immune cell populations in significantly greater detail. Compared with patients undergoing a Whipple procedure, splenectomized patients had significant and long-lasting elevated counts of lymphocytes, monocytes, and basophils. Cytometry by time-of-flight mass spectroscopy analysis demonstrated that the elevated lymphocytes primarily consisted of naive CD4+ T cells and a population of activated CD25+CD56+CD4+ T cells, whereas the elevated monocyte counts were mainly mature, activated monocytes. We also observed a significant increase in the expression of the chemokine receptors CCR6 and CCR4 on several cellular populations. Taken together, these data indicate that significant immunological changes take place following splenectomy. Whereas other groups have compared splenectomized patients to healthy controls, this study compared patients undergoing elective splenectomy to those undergoing a similar major abdominal surgery. Overall, we found that splenectomy results in significant long-lasting changes in circulating immune cell populations and function. ImmunoHorizons, 2020, 4: 82–92.
INTRODUCTION

Since Quittenbaum performed the first reported splenectomy in 1826, it has now become a well-known surgical procedure with ~27,000 splenectomies performed annually in the United States (1, 2). The indications for splenectomy include splenic rupture from trauma and a broad range of benign and malignant hematological, immunological, and oncological diseases (3, 4). Although solid primary tumors of the spleen are rare, because of the anatomical proximity to the pancreas, stomach, and colon, splenectomy is often performed during the resection of malignant pancreatic tail, gastric, and splenic flexure colon tumors to achieve complete oncologic resections (5–7).

Along with the complications associated with the surgical procedure itself, one of the main concerns of asplenia is the loss of important hematological and immunological functions (8, 9). The spleen accounts for 25% of the total lymphoid tissue mass and is separated by the perifollicular zone into two regions: the red and white pulp (10, 11). The macrophages in the red pulp filter blood and recycle iron from erythrocytes. The various immune cells in the white pulp, including B cells, T cells, NK cells, macrophages, and dendritic cells, are able to initiate and regulate innate and acquired immune responses (10, 12). It has been speculated that because of the altered hematological and immunological profiles postoperation, asplenic patients have an increased mortality risk regardless of the initial splenectomy indication, compared with the general population (13). Previous studies have shown that although the mortality risk substantially decreases after the first 90 d, this risk remains elevated more than 1 y postoperation (13, 14).

There are several possible explanations for the increased mortality risk of patients undergoing splenectomy. First, the spleen plays an essential role in the host defense against viruses, fungi, and, most importantly, encapsulated bacteria (10). Because of the severe impairment in their ability to recognize and clear these pathogens from the bloodstream, asplenic patients develop sepsis more easily, and infections are more likely to have a fulminant course (15). Although only occurring in a small fraction of patients, with a mortality rate of ~50%, overwhelming postsplenectomy infection is a potentially serious complication for asplenic patients (15). Furthermore, several vascular complications have been observed in asplenic patients due to activation of thrombocytes and hypercoagulability, including partial or total obstruction of arterial and venous blood vessels, pulmonary hypertension, and coronary artery disease (16–18). Finally, there is an association between splenectomy and cancer. Sun et al. (19) showed that after splenectomy, patients have a significantly higher risk of developing head and neck, gastrointestinal tract, and hematological malignancies. This finding is supported by a large study of American veterans, which also showed that splenectomy is associated with an increased risk of cancer (20).

Because the increased risk of death in asplenic patients is often immune related, we sought to determine how splenectomy alters peripheral immune cell populations in the years following splenectomy. Although several studies in animal models have examined the effects of splenectomy, detailed postsplenectomy peripheral immune cell changes have not been fully elucidated in humans. Therefore, we used both clinical laboratory testing and high-dimensional, single-cell mass cytometry (cytometry by time-of-flight mass spectrometry [CyTOF]) analysis to characterize the changes in frequency and phenotype of peripheral immune cells in the first years following splenectomy (21).

MATERIALS AND METHODS

Patient selection and information

A total of 95 patients who underwent an elective, standard-of-care splenectomy either in isolation or in conjunction with adjacent organ resection at the UCH Health University of Colorado Hospital (Aurora, CO) between 2011 and 2017 were identified. Reasons for these major abdominal surgeries included a broad range of benign and (pre)malignant indications. Patients who underwent surgery in an acute or trauma setting were excluded. Ninety-one patients who underwent a pancreatectoduodenectomy (Whipple procedure) without splenectomy at the same institution between 2011 and 2016 were selected as a comparator group. Retrospectively, patient characteristics (including age at surgery, sex, body mass index, indication for surgery, follow-up after surgery, and overall survival) were collected from their electronic medical record (Table I).

For the high-dimensional immune cell profiling, five patients undergoing splenectomy were recruited to participate in this study: four females and one male, with a median age of 58 y (47–70 y). The indications for surgery included various benign and malignant tumors, including a metastatic gastrointestinal stromal tumor, an adenocarcinoma of the pancreatic tail, a mucinous cystic neoplasm of the pancreatic body, a solid and pseudopapillary tumor of the pancreatic neck, and a stage IV high-grade serous ovarian carcinoma with a splenic infarction. Blood was collected from these patients prior to surgery and between 19 and 241 d postsurgery.

Written informed consent was obtained from all patients according to the Colorado Multiple Institutional Review Board (no. 11-1820).

Laboratory tests

Standard-of-care blood draws were used to obtain the grouped WBC count, RBC count, platelet count, and both the percentage and absolute number of neutrophils, lymphocytes, monocytes, eosinophils, basophils, and immature granulocytes at various time points. These time points include preoperation; 1–3 and 7–30 d postoperation; 1–2, 2–6, and 6–12 mo postoperation; and 1–2, 2–5, and >5 y postoperation (Supplemental Table I). Healthy normal blood count ranges were determined by the University of Colorado Hospital Clinical Laboratory.

Mass cytometry

For immune cell profiling using CyTOF, blood was collected in BD Vacutainer ACD tubes from five patients undergoing splenectomy. Prior to storage at ~80°C, RBCs were lysed from whole blood using the BD Biosciences Lyse/Fix kit according to the manufacturer's
protocol. To decrease intrasample variability and allow analysis of
cryosensitive granulocytic cells, all blood samples were prepared
and cryopreserved immediately after collection. This precluded
the use of a Live/Dead discriminator dye. Cells were thawed and
stained individually with DNA-intercalating cell surface
Abs. The cells were then permeabilized using the BD Biosciences
Transcription Factor Phospho Buffer Set according to the
manufacturer’s protocol. Following permeabilization, the cells
were stained with the intracellular Abs, followed by DNA in-
tercalator to identify cellular events.

**Mass cytometry run and sample normalization/debarcoding**

Samples were collected on a Helios mass cytometer (Fluidigm).
Samples were resuspended with equilibration beads to allow
for signal normalization using the normalization software down-
loaded from the Nolan laboratory GitHub page (https://github.com/
nolanlab) (23). Debarcoding software was used following normal-
ization (https://github.com/nolanlab/single-cell-debarcoder). Nor-
malized, debarcoded data were subjected to traditional Boolean
gating in FlowJo, identifying nucleated events (191Ir+ 193Ir+). These
events were then gated and exported for downstream analysis.
Additional Boolean gating was later performed for CD45+ events
(Fig. 3), CD4+CD3+CD19+ (T cells, Fig. 4) events, or CD45+CD3–
CD19– (myeloid cells, Fig. 5) events, with gating criteria identified
within each figure.

**PhenoGraph software downloads.** Version 1.1.453 of R Studio was
downloaded from the official R Web site (http://www.r-project.
.org/). XQuartz v2.7.11 was downloaded (https://www.xquartz.
.org/). Release 3.7 of the Cytofkit package was downloaded from
Bioconductor and opened using R Studio and XQuartz (http://
Excel 16.15, FlowJo 10.5.3, GraphPad Prism 8.1.2, and Adobe
Illustrator CC 22.1 were used to generate figures and graphs.

Manually gated nucleated events (191Ir+ 193Ir+) and further
gated populations were selected, and relevant markers for
clustering were chosen (note that markers used for gating
imported populations were not used for clustering). PhenoGraph
was run with the following settings: files were 1) merged using
the merge method “min,” 2) transformed using the transformation
method “cytofAsinh,” and 3) clustered using the “Rphenograph”
method, coupled with the t-distributed stochastic neighbor
embedding–visualization method. All other settings were au-
tomatically chosen, using default PhenoGraph settings.

PhenoGraph–defined clusters were displayed on t-distributed
stochastic neighbor embedding plots within the R package “Shiny.”
Within the Shiny application, cluster color was altered or colored
according to protein expression. Multiple .csv files were produced
by the PhenoGraph analysis, including “cluster median data” and
“cluster cell percentage,” which were used to determine
cluster phenotype, distribution between conditions, and statistical
significance between groups. These .csv files included mean metal
intensity values for each cluster. The mean metal intensity was
quantified in a similar manner to mean fluorescence intensity
used in conventional flow cytometry to calculate the relative
expression of each marker.

**Statistical analysis**

A two-tailed t test was used to compare patient characteristics
between the two groups. An ordinary two-way ANOVA was used
to assess the statistical significance between two groups across
multiple time points. We corrected for multiple comparisons using
the Šidák method for the clinical data and Tukey method for CyTOF
analysis. One-way ANOVA was used to assess the statistical signifi-
cance between each time point and the preoperative value. We
corrected for multiple comparisons using the Bonferroni method.

Statistical analysis was performed using GraphPad Prism
version 8.1.2 for Mac (GraphPad) and IBM SPSS Statistics version
25.0.0.1. A p value <0.05 was considered to be statistically significant.

**RESULTS**

To compare circulating immune cell populations before and after
splenectomy, we compared clinical blood draws from 95 patients
before and after a non–trauma-related, elective splenectomy across
a broad range of time points: preoperation; 1–3 and 7–30 d post-
operation; 1–2, 2–6, and 6–12 mo postoperation; and 1–2, 2–5,
and >5 y postoperation. To control for the effect of a major
abdominal surgery on circulating immune cells, we included a
total of 91 patients who underwent a Whipple procedure
(pancreaticoduodenectomy) at the same institution, during the
same time period. We retrospectively obtained patient charac-
teristics (Table I), overall survival, and the absolute number
and percentage of the major populations of blood cells using
standard clinical laboratory complete blood counts.

**Patient characteristics**

The median age was 58 y for the splenectomy group and 66 y for
the nonsplenectomy group. Sixty-nine percent of the splenectomy
group and 47% of the nonsplenectomy group were female. Median
body mass index was 25.2 and 25.5, respectively. Median follow-up
was 2.67 y for the splenectomy group, compared with 4.68 y for the
nonsplenectomy group. Of the patients in the splenectomy group,
63% were diagnosed with a malignancy, and in the comparator
nonsplenectomy group, 67% had a malignancy. At the time of
surgery, 34% of the splenectomized patients had lymph node
metastases, compared with 36% in the nonsplenectomized pa-
tients. During the follow-up period, 20% of the splenectomized
patients and 10% of the nonsplenectomized patients developed
distant metastatic disease (Table I).

**Survival**

Although there was not a statistically significant difference in
survival, there was a trend toward improved overall survival in the
splenectomy group compared with controls (p = 0.1846, Fig. 1A).

https://doi.org/10.4049/immunohorizons.1900089
TABLE I. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Splenectomy (n = 95)</th>
<th>Nonsplenectomy (n = 91)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at surgery, y, median (range)</td>
<td>58 (25–85)</td>
<td>66 (19–88)</td>
</tr>
<tr>
<td>Sex, percent female (n)</td>
<td>69% (66)</td>
<td>47% (43)</td>
</tr>
<tr>
<td>BMI, median (range)</td>
<td>25.2 (17.4–51.6)</td>
<td>25.5 (16.7–38.0)</td>
</tr>
<tr>
<td>Follow up, y (range)</td>
<td>2.67 (0.088–7.6)</td>
<td>4.68 (0.16–7.10)</td>
</tr>
<tr>
<td>Deceased, percent (n)</td>
<td>26% (25)</td>
<td>45% (41)</td>
</tr>
</tbody>
</table>

Cancer, n
- Adenocarcinoma: head*
  0
- Adenocarcinoma: tail
  22
- Neuroendocrine tumor: head
  1
- Neuroendocrine tumor: tail
  20
- Other tumors*
  17
- Noncancer, n
  16
- Pancreatic cysts
  14
- Intraductal papillary mucinous neoplasm
  5

Other pathologies
- Node positive, percent (n) | 34% (32) | 36% (34) |
- Tumor, percent (n) | 19% (18) | 37% (34) |
- Locally advanced*g | 20% (19) | 10% (9) |
- Distant metastasis* | | |

*Including adenocarcinomas of the extrahepatic bile duct, common bile duct, intrapancreatic bile duct, papilla of Vater, and duodenum.
*Including melanoma, ovarian cancer, endometrium cancer, colon cancer, gastrointestinal stromal tumor, and solid pseudopapillary neoplasm.
**Including immune thrombocytopenia, pancreatitis, pancreatolithiasis, benign vascular lesions, primary sclerosing cholangitis, and necrosis.
At the time of surgery.
*Post surgery.

Changes in major blood cell populations

The blood values revealed broad, large-scale, and long-lasting alterations in several populations of circulating blood cells following both splenectomy and comparator Whipple procedures. These changes were significantly more pronounced and longer lasting in the splenectomized patients compared with controls. As previously observed, platelet count increased and remained elevated after splenectomy compared with control surgery, peaking 7–30 d postoperation (Fig. 1B) (24). RBC counts were significantly decreased up until 2–6 mo postoperation in both groups (Fig. 1C). Finally, we observed a significant increase in the absolute number of total white cells in patients immediately following splenectomy compared with control surgery (Fig. 1D).

Changes in individual WBC populations

We further analyzed both the percentage and absolute numbers of the individual populations of WBCs to determine which specific populations contribute to the increased WBC counts in splenectomized patients. After an initial increase in the percentage of neutrophils in both groups, we noted a decreased percentage in the splenectomy patients. In contrast with the comparator surgery patients who returned to baseline 2–6 mo postoperation, the percentage of neutrophils in the splenectomized patients dropped below baseline between 2–6 and 6–12 mo postoperation before returning to baseline after 1–2 y (Fig. 2A). When looking at the absolute neutrophil count, the splenectomized patients showed a higher number at 1–3 and 7–30 d postoperation; this was not statistically significantly different at later time points (Fig. 2B). As expected, the percentage of lymphocytes was significantly decreased immediately following surgery in both groups because of the major surgical stress (25). However, the percentage of lymphocytes was significantly elevated compared with baseline at 6–12 mo postoperation and remained higher than the control group until 2–5 y postoperation (Fig. 2C). Similarly, the absolute number of lymphocytes was significantly increased compared with the control group throughout the study (Fig. 2D). Although there were no differences in the percentages of monocytes between the two groups (Fig. 2E), the absolute number was significantly increased in the splenectomy group, both compared with baseline and the control group (Fig. 2F). We further analyzed the differences between the two groups at 2–5 y postoperation (the longest follow-up time with sufficient patients for analysis) to compare the major populations of total white cells in each patient at this time point to visualize the differences in cellular subsets (Fig. 2G).

Both groups demonstrated a steep decrease in percentage and absolute number of circulating eosinophils shortly after surgery, returning to baseline after 7–30 d postoperation (Supplemental Fig. 1A, 1B). Similarly, the percentage of basophils did not change between the two groups; however, the splenectomized patients exhibited increased absolute numbers of basophils compared with the control group starting at 1–2 mo postoperation (Supplemental Fig. 1C, 1D). Finally, we observed small differences in both the percentages and absolute numbers of the immature granulocytes (Supplemental Fig. 1E, 1F).

High-dimensional analysis of pre- and postsplenectomy blood samples

We have developed a 38-metal isotope-tagged mAb panel (Supplemental Table II) to identify the major circulating immune cell subsets and characterize their function. With this panel, we analyzed lysed whole-blood samples from five patients before and after splenectomy. We sought to visualize the potential relationships between cell populations without prior designation using the PhenoGraph clustering algorithm in Cytokit (Fig. 3A) (26). Using this software, we identified the major populations of circulating immune cells: B cells, T cells, myeloid cells, and NK and other cell types (Fig. 3A–C, Supplemental Table III). Although we did not observe statistically significant changes in the frequency of any immune cell populations using the markers described, we did observe significant changes in the expression of several immunologically relevant markers across the various clusters.

CCR6 and CCR4 expression are increased after splenectomy

We observed that both C-C chemokine receptors type 6 and 4 (CCR6 and CCR4) were increased on CD45+ cells postsplenectomy across a broad variety of immune cell populations (Fig. 3D, 3E). We further noted that both CCR6 and CCR4 were specifically upregulated on T cells (CD45+CD3+CD19-) and myeloid cells (CD45+CD3-CD19+CD14/CD15/CD66b+) (Fig. 3E). Although B cells represent a significant proportion of the lymphocytes, they exhibit CD19-CD45+CD3+ surface expression, and therefore, this analysis may not fully capture all possible inflammatory effects of splenectomy on B cells.
residing in the spleen (27), we did not note significant changes in this population in the peripheral blood, in either frequency or the expression of immunologically relevant cell surface marker expression following splenectomy.

**Circulating T cells are more activated following splenectomy**

To further characterize the differences in the T cell populations before and after splenectomy, we clustered and analyzed the CD45+CD3–CD19– T cells alone (Fig. 4A–C, Supplemental Table III). Although there were no statistically significant changes in the frequencies of any of these major groups of T cells, we did find increased naive CD4+ T cells and decreased terminally differentiated CD4+ T cells in four of the five patients following splenectomy (Fig. 4B, 4C) (28). Furthermore, we observed changes in two of the memory and effector memory CD4+ T cell populations in four of the five patients after splenectomy: a decrease in cluster 21 (more immature or less activated) and an increase in cluster 22 (more mature or activated) (Fig. 4D, 4E). This change in frequency was associated with an increased frequency of cells expressing higher levels of CCR6, CCR4, CD25, CD127, programmed death-ligand 1 (PD-L1), and CD56, as well as decreased expression of programmed death receptor 1 (PD1) (Fig. 4F, 4G). Additionally, we noted that the expression of CCR4 and CD25 was increased on the cells in cluster 22 after splenectomy (Fig. 4G).

**Circulating monocytes, but not neutrophils, are activated following splenectomy**

Similar to the T cells, we further analyzed the myeloid and NK cell populations by focusing on CD45+CD3–CD19– cells (Fig. 5A). In line with our findings in the larger clinical data set (Fig. 2A, 2F), we observed a decreased frequency of neutrophils (CD45+CD3–CD19–CD14–CD15–CD66b) and increased frequency of monocytes (CD45+CD3–CD19–CD14+) in the majority of patients following splenectomy (Fig. 5B, 5C). We noted heterogeneous changes in frequency within the neutrophil clusters (clusters 2 and 14) (Fig. 5D, 5E). However, we did observe a decreased frequency of activated neutrophils (cluster 2, expressing higher levels of CCR7, CD123, CD127, HLA-DR, CD33, and CD16) in four of the five patients (Fig. 5D–G). In contrast with the majority of other circulating immune cell populations, we did not observe any changes in the expression of CCR6 or CCR4 in either population of neutrophils (Fig. 5F, 5G).

Within the monocyte clusters, we noted a trend toward a decreased frequency of immature monocytes (cluster 24) and increased frequency of mature monocytes (cluster 15) (Fig. 5D, 5E). The mature monocytes (cluster 15) expressed higher levels of CD14, CD11c, CD33, PD-L1, HLA-DR, and CD56 (Fig. 5F, 5G). There was a trend toward increased CCR6 but not CCR4 expression among both populations of monocytes following splenectomy, with higher CCR4 expression on the immature monocytes (cluster 24) (Fig. 5F, 5G).

**DISCUSSION**

Previous studies on the immunologic consequences of splenectomy have focused on changes shortly after splenectomy or have compared asplenic patients to a healthy control population (9, 14, 17, 20). Despite these studies, the human spleen, and its contributions to populations of circulating immune cells, remains understudied. In this study, we performed longitudinal clinical and mass cytometric analysis of circulating immune cells to address questions concerning alterations in human immune responses before and after splenectomy. In this study, to control for the effects of major abdominal surgery, we compared clinical blood counts and other patient characteristics between patients undergoing elective splenectomy (most often with a distal pancreatectomy or other concomitant organ removal) and those undergoing a Whipple procedure.

As has been previously reported (24), we observed a strikingly increased platelet count following splenectomy, which lasted up until 1–2 y postsurgery. This observation may be linked to the increased risk of thromboembolic and various other cardiovascular events in asplenic patients (29–31). Despite this, we did not observe an increased mortality in the splenectomized patients compared with those undergoing a Whipple procedure. Whereas previous studies have compared splenectomized patients to healthy controls, our control group mainly consisted of patients with a similar diagnosis to those undergoing splenectomy, in this case, pancreatic cancer (14, 20). Notably, cancer-specific
survival may be the limiting factor determining the overall survival of both patient groups, considering that the majority of enrolled patients were diagnosed with a malignancy. However, differences in overall survival may become more evident during a longer follow-up.

In this study we found that, compared with control patients, patients undergoing splenectomy had increases in the absolute numbers of circulating lymphocytes, monocytes, and basophils. In contrast to these cell populations, we observed a decreased percentage of circulating neutrophils in splenectomized patients. This observation is likely the result of increased absolute numbers of monocytes and lymphocytes, as the absolute number of neutrophils remained unchanged. The increases in circulating lymphocytes, monocytes, and basophils were significant and long-lasting. We observed that the absolute number of lymphocytes and basophils was significantly elevated >5 y after splenectomy, whereas these cells rapidly returned to baseline levels in the control group. These results were mirrored in the monocyte population that was...
We observed several changes in the frequency and level of activation and maturation of both T cells and myeloid cells following splenectomy. We found increased frequencies of activated mature CD4+ T cells and monocytes. For the CD4+ T cells, we noted increased expression of CD25 and an increased frequency of cells expressing the NK cell marker CD56. CD4+ T cells expressing CD69 are increased in frequency in patients infected with CMV and noted to be highly cytolytic (32, 33). Expansion of CD56+ CD4+ T cells may be indicative of reactivation of latent CMV associated with diminished IgM following splenectomy (32, 34, 35). CMV may be reactivated following splenectomy by the loss of IgM-producing memory B cells that normally reside in the spleen (35–37).

Additionally, we observed increased expression of several cell surface markers, including HLA-DR, CD14, CD33, and CD56, on monocytes that are associated with monocyte maturation and activation (38). These results, along with a shift in the frequency of immature populations toward mature populations, suggest that the monocytic populations that were increased in the clinical blood draws are more differentiated and mature than those from presplenectomy blood draws. We found smaller changes in the frequency and activation of B cells, CD8+ T cells, and neutrophils.

The most common statistically significant change observed across many of the cellular populations was increased expression of CCR6 and, to a lesser extent, CCR4. This change was more emphasized in the T cell and monocyte populations than in the neutrophils or B cells and may be a possible mechanism accounting for the differences in cellular frequencies observed in our clinical blood analysis. CCR4 and CCR6 expression are associated with Th2 and Th17 cell polarization (39–41). These cells produce cytokines and perform functions that are often associated with unfavorable outcomes in multiple types of cancer (39, 42, 43). In a large study of American military veterans, splenectomy was associated with an increased risk of several types of malignancies (20). While taking into account that the patients in our study have been previously diagnosed with a malignancy, one preliminary hypothesis that stems from this observation is that increased frequencies of CD4+ cells expressing CCR4 and CCR6 may prevent successful antitumor immunity in those not previously diagnosed with cancer. This could possibly account for the increased risk of postsplenectomy malignancies. However, the mechanism(s) that accounts for the increased expression of CCR6 and CCR4 was not analyzed as part of this study.

Patients undergoing elective splenectomy receive four prophylactic vaccines to protect against infections often observed in asplenic patients (44). These vaccines may play a role in the global immune perturbations we observed following splenectomy. Furthermore, these vaccines have been shown to increase the expression of CCR4 and CCR6 (32). However, these vaccines are generally given at least 2 wk prior to surgery (44), and with the presplenectomy blood draws occurring on the day of surgery, it is unlikely that the presplenectomy and postsplenectomy blood draws would differ drastically because of responses to these vaccines.

The role of the spleen has been extensively studied in rodent models of cancer (45). Mouse models have shown that the spleen...
provides a specialized environment for the generation of immune tolerance to tumor Ags (46). Additionally, in mice, the timing of splenectomy can result in significantly altered outcomes (47). Significant differences exist between the structure and function of human and mouse spleen (48). Much less is known about the effects of splenectomy in human cancer patients (49). Although some studies have shown decreased overall survival in pancreatic and gastric cancer patients who undergo splenectomy versus those who do not, the mechanisms underlying the decreased overall survival have not been elucidated (50) and may be more related to

FIGURE 4. Splenectomy increases the frequency of naive CD4+ T cells, activates memory or effector memory CD4+ T cells, and increases the expression of CCR6 and CCR4 on these cells.

Mass cytometric analysis of lysed whole blood from patients before and after splenectomy. (C) The timing of the post-splenectomy blood draws from each patient. Files were normalized and gated on single CD45+CD3+CD19− cells prior to analysis. (A) PhenoGraph analysis of CD45+CD3+CD19− cells comparing pre- and post-splenectomy samples from all five patients, colored by cellular phenotype. (B) The frequencies of PhenoGraph-defined clusters comparing the values of each cell type between pre- and post-splenectomy samples from each patient (cell populations were identified based on the markers in Supplemental Table III). (C) Comparison of the frequencies of PhenoGraph-defined clusters between pre- and post-splenectomy samples from each patient, including the number of days postsplenectomy the blood draw occurred. (D and E) Identification of the two populations with the greatest degree of change between pre- and postsplenectomy. (F) Dendrogram heatmap generated in Cytofkit depicting the differences in the expression of the designated proteins between clusters 21 and 22. (G) Quantification of the mean metal intensity (relative expression level) of the designated proteins compared between clusters 21 and 22 from pre- and post-splenectomy samples. *p < 0.05.

https://doi.org/10.4049/immunohorizons.1900089
FIGURE 5. Splenectomy alters the frequency and activation of monocytes and neutrophils.

Mass cytometric analysis of lysed whole blood from patients before and after splenectomy. (C) The timing of the postsplenectomy blood draws from each patient. Files were normalized and gated on single CD45⁻CD3⁻CD19⁻ cells prior to analysis. (A) PhenoGraph analysis focusing on CD45⁺CD3⁺CD19⁻ myeloid cells, comparing pre- and postsplenectomy samples from all five patients, colored by cellular phenotype. (B) The frequencies of PhenoGraph-defined clusters comparing the values of each cell type between pre- and postsplenectomy samples from each patient. (C) Comparison of the frequencies of PhenoGraph-defined clusters between pre- and postsplenectomy samples from each patient, including the number of days postsplenectomy the blood draw occurred. (D and E) Identification of the populations with the greatest degree of change between pre- and postsplenectomy (cell populations were identified based on the markers in Supplemental Table III). (F) Dendrogram heatmap generated in CytoKit depicting the differences in the expression of the designated proteins between clusters 2 and 14 or 15 and 24. (G) Quantification of the mean metal intensity (relative expression level) of the designated proteins compared between clusters 2 and 14 and between 15 and 24 from pre- and postsplenectomy samples. *p < 0.05.

https://doi.org/10.4049/immunohorizons.1900089
the anatomic relationship of the tumor to the spleen than to splenic function. Analogous to the known increased risk of overwhelming postsplenectomy sepsis (51), splenectomy may perturb the antitumor immune responses required for the control or clearance of cancer cells in humans.

This study should be interpreted in light of its limitations. First, to control for the effects of surgery, we compared patients undergoing splenectomy to those undergoing a Whipple procedure. The vast majority of patients in both groups had been diagnosed with cancer, which may have altered the circulating immune cell populations reported in this study. Second, because of the heterogeneity of the patients selected for the CyTOF portion of this study, only broad postsplenectomy immunologic changes were observed. Furthermore, one of the patients in the CyTOF analysis portion presented with poor appetite and drainage from their surgical wound on the day of the postsurgical blood draw. This patient was subsequently diagnosed with pneumonia, a peri gastric abscess, and a suspected Clostridium difficile infection 15 d after their postsplenectomy blood draw. This was evident in both the clinical blood draw and CyTOF analysis performed at this time point. Heterogeneity of malignancy and postsplenectomy infections are common clinical presentations for this patient population, and comparisons across these patients are representative of this complex population. Despite the variability in timing and heterogeneity of diagnoses, we observed consistent and statistically significant changes when comparing pre- and postsplenectomy blood draws across these patients. These data significantly expand our knowledge of how the human immune system is altered by splenectomy and may point to new avenues of research into why patients undergoing splenectomy for nonmalignant diagnoses are at a higher risk of death.

DISCLOSURES

The authors have no financial conflicts of interest.

ACKNOWLEDGMENTS

We thank the Human Immune Monitoring Shared Resource within the University of Colorado Human Immunology and Immunotherapy Initiative for expert assistance in analysis of the peripheral blood samples required to complete this study.

REFERENCES


