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Major Hepatectomy Induces Phenotypic Changes in Circulating Dendritic Cells and Monocytes

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Abstract

Patients undergoing major hepatectomy are at increased risk for post-operative morbidity and mortality, and changes in the phenotype of effector cells may predispose these patients to infectious sequelae. To better understand post-hepatectomy immune responses, peripheral blood from fifteen hepatectomy patients was drawn immediately before and after liver resection and on post-operative days one, three and five. Circulating monocytes and dendritic cells were analyzed by flow cytometry for quantity, phenotype, activation status, HLA-DR expression, and toll-like receptor-2 and 4 expression. Major hepatectomy increased the numbers of activated CD16^{bright} blood monocytes and the percentage of activated dendritic cells, although monocyte HLA-DR expression was reduced. These results may represent both dysfunctional antigen presentation and pending anergy, as well as cellular priming of immune effector cells. Better understanding of the alterations in innate immunity induced by hepatectomy may identify strategies to reduce infectious outcomes.

Keywords

inflammation; dendritic cell maturation; monocyte activation; hepatectomy; innate immunity

Introduction

Liver resection is an effective treatment for many benign and malignant diseases, and in the past decade significant advances have been made in reducing postoperative mortality of hepatectomy patients (16). Infections and sepsis are among the most common post-hepatectomy complications, with post-operative infectious complications occurring in 4–20% of these patients (6, 12, 13, 16, 22). Even in institutions with improved morbidity and mortality rates after hepatectomy, post-operative infections and sepsis still have an increased association with mortality, and can account for up to 40% of post-operative deaths (6, 12, 13, 16, 22). The mechanism(s) behind this increased risk of infection and subsequent mortality in patients after liver resection remains unclear. One possible mechanism could be surgery and/or hepatic injury-induced changes in innate immunity.

Two of the key cells of innate immunity include monocytes and dendritic cells (DCs). Circulating monocytes have been demonstrated to play a central role in the innate immune response, and among this cell population, it is thought that the CD16^{bright} (Fcγ receptor III expressing) subpopulation is of critical importance (5, 50). CD16^{bright} monocytes are more

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mature, have an increased activation status, play a greater role in cytokine secretion, and are more responsible for monocyte/T-cell interactions than their CD16^{dim} counterparts (5, 33, 41). The CD16^{bright} monocyte subpopulation is known to expand during sepsis (14).

Dendritic cells (DCs) are the most potent antigen presenting cell and serve as a critical link between the innate and acquired immune systems (10). Peripheral DCs contain several subsets, which include DC1, DC2 and less differentiated subtypes. In humans, DC1s are predominantly myeloid DCs that express CD11c (p150, integrin α chain) and are thought to be the more immunogenic of the DC subtypes (4, 17, 36, 37). DC2s are plasmacytoid DCs, so called due to their morphology, and they express CD123 (IL-3 receptor α chain) (34). The DC2 subtype is thought to be the more tolerogenic subset, with the capacity to generate either a Th₂ response, anergy or antigen-specific non-responsiveness (17, 23, 25, 37). Finally, an additional DC subtype has been characterized as a less differentiated DC (IdDC). These cells have been described predominantly in fetal cord and pediatric blood samples (18, 29, 45). The number of these cells in the systemic circulation decreases with age, similar to other immature cell types. However, the origin, function, and migration pattern of these cells have yet to be fully elucidated (18, 29, 45).

With the key roles that monocytes and DCs play in innate immunity and their link to the acquired immune system, changes in these cell numbers and phenotype may explain some of the immune dysfunction that has been seen in patients undergoing hepatic resection. Our goal was to analyze circulating monocyte and DC numbers and phenotype in the immediate pre-operative and post-operative period in patients undergoing hepatectomy. We hypothesized that the phenotypic changes in blood monocytes and DCs following hepatectomy would be consistent with a reduced capacity for immune surveillance.

Material and Methods

The University of Florida Institutional Review Board approved this study. Informed signed consent was obtained from all patients prior to inclusion in the study.

Patients

Fifteen adult patients (< 21 years) undergoing major hepatic resection were prospectively analyzed (Table I). Exclusion criteria included: evidence of active systemic or localized infection at the time of surgery; infection with HIV; previous organ transplant; current immunosuppressive treatments; current use of steroids or non-steroidal anti-inflammatory medications; previous hepatic resection, or arterial chemoembolization or radiofrequency ablation less than three months prior to surgery. Patients with planned complex combined hepatic resection with vascular reconstruction that might lead to increased liver ischemic injury were also excluded.

Surgical technique

All surgery was performed by a single surgeon (AWH) and was standardized. Briefly, the abdomen was opened and assessed for evidence of extra hepatic disease or unresectability. Intraoperative ultrasound was performed to assess the extent of intrahepatic disease. The liver was mobilized by dividing the triangular ligaments and by mobilizing the liver off of the inferior vena cava. Extra-hepatic control of the hepatic veins was achieved prior to parenchymal transection of the liver. In order to standardize the ischemic injury sustained by the liver, 30 minutes of hepatic arterial and portal venous inflow occlusion (Pringle maneuver) was used in all cases during the parenchymal transaction phase of the procedure. In the two cases that required portal venous reconstruction, the reconstruction was performed during the 30 minutes of inflow occlusion; however, additional dissection of the

portal vein was required in order to obtain distal control. Parenchymal transection was performed using low central venous pressure conditions and the liver divided with an ultrasonic aspirator.

Samples

Blood samples were obtained from central or peripheral venous access at the following time points: after laparotomy prior to mobilization of the liver, 30 minutes post resection of the liver prior to closure of the abdomen, and post operative days (POD) one, three and five. Initially, 2 ml of blood were withdrawn into a separate container and discarded. Next, blood was withdrawn into a 3 ml Becton Dickinson Vacutainer™ K₃ EDTA tube (BD, Franklin Lakes, NJ), placed on ice, and immediately processed. In addition, an extra aliquot of blood was collected into another 3 ml Vacutainer™ K₃ EDTA tube for determination of a complete and differential blood count.

Flow cytometry

Blood from the first Vacutainer™ K₃ EDTA tube was transferred into six polystyrene 12 × 75 mm test tubes (Fischer Scientific, Pittsburg, PA) for fluorescent antibody staining, three for DCs (200 µl each) and three for monocytes (100 µl each). DC staining was performed as previously described with some minor modifications (48). The following antibodies were utilized to phenotype DCs: fluorescein conjugated (FITC)-Lineage Cocktail (CD3, 14, 16, 19, 20, 56), R-phycoerythrin conjugated (PE)-CD123, peridinin chlorophyll protein conjugated (PerCP)-DR, allophycocyanin conjugated (APC)-CD11c, PE-CD86 (BD Biosciences, San Jose, CA), PE-TLR2, and APC-TLR4 (eBioscience, San Diego, CA). The following antibodies were used to phenotype blood monocytes: FITC-CD16, PE-CD11b, PerCP-CD14, APC-CD18, FITC-CD69, PE-CD16, PerCP-DR, APC-CD14 (BD Biosciences), PE-TLR2, and APC-TLR4 (eBioscience). Appropriate isotype controls were run with blood obtained from healthy controls and used for compensation and gating blood samples from the hepatectomy patients. In addition, gating was performed on blood from healthy subjects after being incubated for one hour at 37° C with and without 1 µg/ml lipopolysaccharide (LPS (Sigma, St. Louis, MO)) to guarantee appropriate detection of markers of DC and monocyte activation by flow cytometry. After addition of the antibody cocktail, all tubes were gently mixed and left for 20 minutes in the dark. During this time period, the samples were gently mixed an additional two times. Subsequently, leukocyte fixation and red cell lysis were performed using BD FACS™ Lysing Solution (BD Biosciences) following the manufacturer's instructions. Cells were then washed twice with 1% flow buffer (HBSS containing 1% BSA, 1 mM EDTA (Fisher Scientific, Pittsburgh, PA) and 0.1% sodium azide (NaN₃, Sigma)). The fixed leukocytes were then resuspended in 400 µl of 1% flow buffer. Samples were acquired and analyzed on a six-parameter FACSCalibur™ machine with Cellquest™ Software (Becton Dickinson Systems, San Jose, CA) at the University of Florida Flow Cytometry Core Laboratory. For DCs, debris was excluded and flow cytometry acquisition was continued until the tube was empty or until 1,500 Lineage Cocktail⁻ and HLA-DR⁺ events were collected. For monocytes, debris was excluded and 10,000 CD14⁺ events were collected per sample.

Flow cytometry analysis

Compensation and isotype gating were performed prior to hepatectomy sample acquisition using blood from healthy non-hepatectomy control subjects. For DC analysis, debris (events too small to be considered cells) was eliminated using a forward versus side scatter density plot. Subsequently, the remaining events were gated to a Lineage Cocktail vs HLA-DR density plot, on which DCs were identified as being Lineage Cocktail⁻ and HLA-DR⁺ events (Figure 1a). These cells were analyzed for their expression of CD123 and CD11c to determine their phenotype (DC1 = CD11c⁺, DC2 = CD123⁺, IdDC CD11c⁻ CD123⁻)

(Figure 1b). Activation status was determined by expression of CD86. TLR-2 and TLR-4 expression were also identified. Monocyte analysis used a mixture of forward and side scatter characteristics as well as the CD14 marker in order to isolate CD16^{bright/dim} monocytes for analysis. Like the DC analysis, monocyte analysis used a forward versus side scatter density plot to eliminate debris. However, lymphocytes were also eliminated on this plot. Non-debris, non-lymphocyte events were then gated to a CD14 histogram. CD14⁺ events were then back gated to a forward versus side scatter plot and a gate was drawn around the apparent cells. Next, the gate only (not the cells) was copied back into the original non-debris non-lymphocyte forward versus side scatter density plot (which had no gating based on CD14). The events in the forward versus side scatter plot that were contained in this newly copied region were then gated to a CD14 versus CD16 plot, which allowed determination of CD16^{bright/dim} monocytes (Figure 1c). This form of monocyte analysis was necessary for the following reasons: gating on a CD14 histogram alone would have excluded some CD16^{bright} monocytes which are CD14^{low}; and gating on forward and side scatter characteristics alone would not always allow complete differentiation between monocytes or granulocytes, causing either the exclusion of some monocytes or the inclusion of some granulocytes in the monocyte analysis. These monocytes were analyzed for their expression of activation markers (CD11b, CD18, and CD69), human MHCII (HLA-DR) and TLR-2 and TLR-4. For both DC and monocyte analysis, events were evaluated for both their proportion of positive cells as well as for their mean fluorescence intensity (MFI) for each antibody to determine the number of cells positive for a cell surface marker and the expression quantity of that marker on the cell surface membrane, respectively.

Statistical analysis

Data are reported as the mean $\pm$ SEM. An $n = 9-15$ was utilized for the data analysis for each staining type for each time period. An $n < 15$ was used when data points were not available due to limitations in the staining procedure and the blood collection volumes. Data were analyzed using the statistical software program SigmaStat[®] v.2.03 (SPSS Inc, Chicago, IL). For multivariate comparison among groups, a one-way ANOVA was used with an all pairwise multiple comparison procedure being performed using the Fisher Least Significant Difference method. The Kruskal-Wallis ANOVA on ranks and the Dunn's Method were utilized when tests for normality or equal variance failed. Differences were considered significant at $p < 0.05$.

Results

In this group of 15 patients undergoing hepatic resection, there were seven male and eight female subjects. The patients' mean age was 61 ± 2 years, with 12 of the 15 having a final diagnosis of malignancy (Table I). The length of hospital stay was 15 ± 5 days, with a median of nine days. Eleven patients had uneventful post-operative courses, with nine patients having no complications and two having minor complications. This included one patient who developed a superficial wound infection and one who exhibited minor symptoms from a possible withdrawal from alcohol. Two patients required readmission within 30 days of surgery for operation-related complications (one subphrenic abscess, one sympathetic pleural effusion). Finally, patient number eight required an in-hospital intravenous antibiotic treatment course for bacterial colonization of a pre-operatively placed biliary stent and a possible postoperative pneumonia, while patient number two developed an ileocolic anastomotic leak at 8 days after the initial operative procedure. There were no deaths within 30 days of surgery. The infectious complication rate was three out of fifteen (20%). Four patients received blood during the study (<3 units).

Relative and absolute numbers of circulating monocyte and DC populations and subpopulations

Patients undergoing partial hepatectomy manifested a significant leukocytosis on post-operative day one, which subsequently normalized (Figure 2). The relative and absolute monocyte population significantly increased in the post-operative period (Figures 3a and b). Although there was an increase in both the CD16^{bright} and CD16^{dim} populations postoperatively, there was a relative decrease in the CD16^{bright} population as compared to CD16^{dim} monocytes initially post-resection. This was followed by an increase in CD16^{bright} monocytes as compared to the CD16^{dim} population on post-operative day one, which subsequently normalized (Figure 3a and b).

The relative and absolute numbers of DCs declined post-resection and did not significantly recover within five days (Figures 4a and 4b). There was a relative and absolute increase in the plasmacytoid DC2 population, as compared to the DC1 population in the immediate post-hepatectomy period. This was followed by a reversal to a predominantly DC1 phenotype on post-operative day one, although total DC numbers were still decreased. By post-operative day five, IdDCs were the primary circulating DC phenotype (Figures 4a and 4b).

Relative and absolute changes in the activation status of circulating monocyte and DC populations and subpopulations

In general, there was no change in the monocyte expression of either CD18 or CD11b, although the latter was expected, as most monocytes were already expressing CD11b. However, there was a significant increase in the cell-surface expression of CD11b in the CD16^{bright} subpopulation starting on post-operative day one, as demonstrated by a significant increase in the MFI of these cells (Figure 5). The absolute number of CD69⁺ monocytes also increased after the hepatectomy, but this appeared to be related to the overall increase in the numbers of circulating monocytes, rather than to any proportional increase in monocyte CD69 expression (data not shown).

CD86 expression was used to examine DC maturation or activation status. The DC population was subdivided into three phenotypes based upon their level of CD86 expression: CD86⁻, CD86^{low}, and CD86^{high} (Figure 6a). CD86 expression by DCs significantly decreased in the immediate post-operative period with CD86⁻ expression becoming the predominant phenotype (Figure 6b). This was followed by CD86^{low} DCs becoming the predominant phenotype on post-operative day one. By post-operative day five, however, the relative population of CD86^{high} DCs had significantly increased, although an increase in their absolute concentration did not reach statistical significance (Figure 6).

Changes in monocyte HLA-DR expression

There was a significant relative decrease in the HLA-DR⁺ monocyte population post-hepatectomy (Figure 7A). Cell surface expression (MFI) of HLA-DR significantly declined post-operatively and then began to increase by post-operative day three (Figure 8). HLA-DR⁺ expression on the CD16^{dim} monocyte population declined, but absolute reductions in the number of circulating CD16^{dim} HLA-DR⁺ monocytes, as well as changes in their HLA-DR cell surface expression (based on MFI), were not seen (Figure 7A).

In contrast, the percentage of CD16^{bright} monocytes expressing HLA-DR significantly declined until post-operative day three, when there was a significant restoration in their relative population (Figure 7A). This was followed by a significant decline again on post-operative day five. The absolute numbers of CD16^{bright} HLA-DR⁺ monocytes declined post-resection, but subsequently normalized, most likely due to the post-operative increase in

total circulating monocytes (Figure 7B). More interestingly, the amount HLA-DR cell surface expression (as based on MFI) in CD16^{bright} monocytes significantly decreased post-resection without significant recovery (Figure 8).

Relative and absolute TLR-2 and -4 expression in circulating monocytes and DCs

Significant differences were also found in the total number of blood monocytes expressing TLR-2 in the post-operative period, but these were generally a reflection of total monocyte numbers, as most monocytes were TLR-2⁺, and there were no significant changes in the relative percentage of this population (data not shown). However, there was a significant upregulation of TLR-2 cell surface expression (MFI) beginning on post-operative day one in both the total and CD16^{bright} monocytes (Figure 9), an occurrence not seen in the CD16^{dim} subpopulation (data not shown). In addition, there was a relative increase in the percentage of TLR-4⁺ monocytes and TLR-4⁺ CD16^{bright} monocytes, as well as an absolute increase in the numbers of TLR-4⁺ monocytes and TLR-4⁺ CD16^{bright} monocytes by post-operative day one (Figure 10).

There was a significant increase in the percentage of TLR-2⁺ DCs on postoperative day one (Figure 9). However, the total numbers were decreased due to the overall loss of DCs from the circulation during this period, and there was no difference in the cell surface of expression (MFI) of TLR-2 on these DCs (data not shown).

With the relatively small sample size, no temporal differences in cell phenotype could be identified between patients who developed infectious complications and those who did not, or in patients who received red blood cell transfusions versus those who did not. In addition, no differences were identified in those patients that underwent surgery additional to a liver resection. However, those patients with infectious complications essentially displayed exacerbations of the data presented below (data not shown).

Discussion

Changes in the numbers and phenotype of circulating cells of the innate immune system may contribute to the increased post-operative risk for infectious complications seen in patients undergoing major hepatectomy. In the present report, we examined the changes in the numbers and phenotypes of both monocytes and DCs (Table II). By post-operative day one, there was an increase in the total number of circulating monocytes and the percentage of monocytes expressing CD16, and these cells were activated based on their increased CD11b expression. There was also upregulation of TLR-2 and TLR-4 expression, while there was a simultaneous decline in their HLA-DR expression. Unlike circulating monocytes, DC numbers significantly declined after surgery, shifting from a pre-operative DC1 phenotype (myeloid) to a post-resection DC2 phenotype (plasmacytoid) and back to a DC1 phenotype on post-operative day one. As the post-operative period progressed, however, the phenotype of the circulating DCs shifted to a more IdDC phenotype by post-operative day five. These cells initially appeared to have a reduced activation status, but cell surface CD86 expression increased by day five. In addition, there was an increased percentage of circulating DCs expressing the TLR2 receptor (Table II). Altogether, these data confirm that the innate immune system was significantly altered in the immediate post-resection and post-operative period.

Changes in the monocyte phenotype, specifically the CD16^{bright} population, in the post-operative period were somewhat unexpected for patients undergoing hepatic resection. Although the leukocytosis on post-operative day one and the increased relative and absolute levels of monocytes in the post-operative period were not surprising, the specific loss of HLA-DR expression on CD16^{bright} monocytes was unanticipated. It is possible that the loss

of HLA-DR expression on monocytes represented an efflux of less mature cells from other compartments in response to surgical stress, but an increase in immature monocytes should have been associated with a decrease in monocyte CD16 expression. This was not observed; thus, we believe our data demonstrates a specific downregulation of HLA-DR on circulating monocytes. HLA-DR downregulation is thought to contribute to reduced antigen presentation capability and immune paralysis (47). Monocyte HLA-DR downregulation has been reported in cardiopulmonary bypass operations and can predict patients at elevated risk for post-operative infections (15, 35, 39, 43). Although data from our laboratory have demonstrated that both sepsis and gastrointestinal surgery decrease monocyte HLA-DR expression, only sepsis decreases HLA-DR expression on CD16^{bright} monocytes (27, 47).

Abdominal surgery has been generally characterized by an increased monocyte responsiveness, consistent with activation and/or priming. For example, LPS-induced monocyte tumor necrosis factor-alpha (TNF- α) production has been shown to increase after gastrointestinal surgery (2, 11, 30). Since our post-hepatectomy patients have a relative and absolute increase in circulating monocytes that shifts to a CD16^{bright} phenotype, as well as having an increased activation status, it would appear that the innate immune system was activated by the surgical resection of the liver. However, the observation that HLA-DR expression, a marker of the cell's capacity to present antigens and to drive an acquired immune response, was reduced complicates that earlier observation. In this regard, the monocytic phenotype observed after major liver resection is more similar to that seen in sepsis or with cardiopulmonary bypass than standard general surgery operations. These multiple disparate findings would imply that these patients may be manifesting complex phenotypic patterns consistent not only with monocyte activation, but also with anergy and suppression of acquired immunity.

Similarly, hepatectomy patients demonstrated a significant decline in their circulating DC population immediately following resection. Specific analysis of DC kinetics and phenotypic changes in the immediate post-operative period with other types of surgery is limited, but similar decreases have been seen in patients following gastrointestinal tract surgery (7). The pattern seen in hepatectomy patients is notably different than that seen in laparoscopic cholecystectomy, open cholecystectomy, and elective hysterectomy patients. These patients actually have an increase in circulating DC numbers intraoperatively, with the restoration of normal DC concentrations not occurring until post-operative day two or three, and with DC concentrations not declining to the levels displayed by the hepatectomy patients (20). In addition, there is an eventual phenotypic shift to CD11c⁻, CD123⁻ DCs. We have labeled these cells ldDCs, a term fashioned by Hagendorens, *et al.* as our CD11c⁻ CD123⁻ DCs correlate with their described CD11c⁻ CD123^{low} ldDCs (18). Although the function of these cells is presently unknown, they are the predominant DC phenotype found in cord blood, and they are found to be in higher numbers in pediatric patients with atopic dermatitis as compared to healthy and asthmatic children (18). The shift to ldDCs in the hepatectomy patient is most likely due to combination of DC migration out of the blood stream as well as an effort to replenish the circulating DC population from the patient's progenitor cells. Regardless, the appearance of these ldDCs represents a shift away from a classical Th1 response. These shifts are not seen in patients who are undergoing less invasive operations (20).

Most circulating DCs are not activated, but rather are usually in a precursor (CD86⁻) or immature (CD86^{low}) state. The function of these circulating DC1s is to migrate to areas of inflammation prior to complete maturation. After encountering a stimulus or antigen, these DCs will undergo a number of functional and phenotypic changes, including decreased endocytosis, upregulation of membrane MHCII and co-stimulatory molecules, and increased specific cytokine expression. These phenotypic changes result in the DC becoming a potent

stimulator of T lymphocytes, and mature DCs also have an increased ability to migrate to lymphoid tissue, usually through the lymph system (10). The loss of precursor CD86⁻ and immature CD86^{low} DCs in the circulation could reflect migration of these immature cells out of blood stream to areas of infection or inflammation. Interestingly, we observed a significant decrease in the percentage of CD86⁻ and CD86^{low} DCs along with a significant increase in the percentage of CD86⁺ DCs by post-operative day five. Although this may represent an increase in the activation status of the circulating DCs, IdDCs make up the primary DC phenotype at this time point. Data from our laboratory as well as others have demonstrated that DCs which are phenotypically mature (*i.e.* express high levels of costimulatory molecules) but are functionally immature (in terms of cytokine expression and ability to induce Th1 or Th2 responses), can induce the expansion of T regulatory cell populations (unpublished data) (1, 10). Like activated monocytes lacking HLA-DR, these DCs may inhibit an appropriate response to infection post-hepatectomy.

TLRs are a family of ancient and conserved receptors among organisms that recognize pathogen-associated molecular patterns (PAMPs), which are conserved structural components found in many microorganisms (28, 44, 49). TLR-2 has been shown to recognize Gram-positive bacterial, mycobacterial, fungal, and spirochetal cell wall. TLR-4 recognizes the Gram-negative PAMP LPS as well as other endogenous ligands such as fibrinogen, neutrophil elastase, and high mobility group box 1 (HMGB-1) (8, 32, 38, 40, 42, 52). Other studies have demonstrated a significant suppression of TLR-2 and TLR-4 surface expression after alimentary tract operations (21). Cardiopulmonary bypass produces an initial downregulation of TLR-2 and TLR-4 expression in the immediate post-operative period followed by an upregulation of both TLRs on post-operative day one (9). We and others have demonstrated during sepsis that there is an increase in monocyte TLR-2 and TLR-4 expression (3, 19, 46, 48). In this report, we again have chosen to analyze TLR-2 and TLR-4 for the reason that this would most likely include the TLRs for the most commonly encountered organisms in post-operative infection (Gram positive and negative bacteria), as well as giving us a reference point from which to compare our data. Our data displays that there is also increased expression of TLR-2 on monocytes as well as an increase in the percentage of TLR-4⁺ monocytes in the circulation consistent with an increased immune surveillance during the post-operative period. It should be noted that responses in TLR-2 and TLR-4 expression do not represent changes in all TLR expression, such as TLR-3, 5, 7, 8, and 9, which bind to other specific PAMPs.

The changes seen in the numbers and phenotype of monocytes and DCs in post-hepatectomy patients are most likely multifactorial. The liver is the principal source of complement and acute phase reactants, such as IL-6, both of which are essential components of innate immunity. In addition, some may be related to the inflammatory response previously noted to occur following liver resection. In patients undergoing liver resection, increased portal IL-6 and systemic IL-8 concentrations correlated with hepatocyte injury (24). Patients who developed SIRS after hepatectomy exhibited elevated serum post-operative IL-6 levels (31). However, some studies have demonstrated no difference in the magnitude of the inflammatory response, as determined by plasma IL-6, IL-8, secretory phospholipase A2 (sPLA2) and elastase levels, in hepatectomy patients as compared to other major abdominal operations. These data suggest that the inflammatory response may not be responsible for the increased complication rate observed in these patients (51). Other factors may play a role, including bacterial translocation. In a porcine model of liver ischemia, partial liver resection and liver reperfusion, bacterial translocation to the thoracic duct contributed to any subsequent bacteremia and SIRS response (26). All patients in this study underwent a standardized 30 minutes of hepatic vascular inflow occlusion. The normal human liver tolerates up to an hour of warm ischemia during hepatectomy without major sequelae, however this short period of ischemia and subsequent reperfusion may play a role in the

observed response. Finally, we cannot rule out the possibility that the observed response is specifically related to liver malignancy and its operative management, as most of our patients had a final diagnosis of cancer.

Conclusion

Our data demonstrate that hepatectomy is associated with a complex monocytic phenotype, consistent with activation of monocyte populations, increased immune surveillance and simultaneous reduced antigen presentation capacity. DC populations are equally affected, with the ultimate generation of a novel IdDC population expressing increased levels of T cell costimulatory molecules. These overall changes in the phenotype of effector cell populations would theoretically place the post-hepatectomy patient at increased risk of an exaggerated secondary inflammatory response to a subsequent infectious challenge, with a reduced capacity to stimulate a Th1 acquired immune response. These changes may contribute to the increased risk of infectious complications in this patient population.

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Abbreviations

APC	allophycocyanin conjugated
BSA	bovine serum albumin
CC	cholangiocarcinoma
DC1	myeloid dendritic cells
DC2	plasmacytoid dendritic cells
DCs	dendritic cells
EDTA	ethylenediaminetetraacetic acid
FITC	fluorescein
FNH	focal nodular hyperplasia
HBSS	Hanks balanced salt solution
HCC	hepatocellular carcinoma
HD	hospital days
HLA	human leukocyte antigen
HMGB-1	high mobility group box 1
IFN	interferon
IL	interleukin
Id	less differentiated
LHL	left hepatic lobectomy
LPS	lipopolysaccharide
LTSG	left trisegmentectomy

MCC	metastatic colon cancer
MFI	mean fluorescence intensity
MHC	major histocompatibility complex
NaN₃	sodium azide
PAMPs	pathogen-associated molecular patterns
PE	phycoerythrin
PerCP	peridinin chlorophyll protein
POD	post operative day
RHL	right hepatic lobectomy
RTSG	right trisegmentectomy
SIRS	systemic inflammatory response syndrome
sPLA2	secretory phospholipase A2
Th1	type one T helper cell
Th2	type two T helper cell
TLR	toll-like receptor
TNF-α	tumor necrosis factor-alpha

References

1. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol.* 2001; 2:725–731. [PubMed: 11477409]
2. Aosasa S, Ono S, Mochizuki H, Tsujimoto H, Osada S, Takayama E, Seki S, Hiraide H. Activation of monocytes and endothelial cells depends on the severity of surgical stress. *World J Surg.* 2000; 24:10–16. [PubMed: 10594196]
3. Armstrong L, Medford AR, Hunter KJ, Uppington KM, Millar AB. Differential expression of Toll-like receptor (TLR)-2 and TLR-4 on monocytes in human sepsis. *Clin Exp Immunol.* 2004; 136:312–319. [PubMed: 15086396]
4. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, Pulendran B, Palucka K. Immunobiology of dendritic cells. *Annu Rev Immunol.* 2000; 18:767–811. [PubMed: 10837075]
5. Belge KU, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, Espevik T, Ziegler-Heitbrock L. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. *J Immunol.* 2002; 168:3536–3542. [PubMed: 11907116]
6. Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg.* 2000; 191:38–46. [PubMed: 10898182]
7. Brivio F, Gilardi R, Bucoccev R, Ferrante R, Rescaldani R, Vigore L, Fumagalli L, Nespoli A, Lissoni P. Surgery-induced decline in circulating dendritic cells in operable cancer patients: a possible explanation of postoperative immunosuppression. *Hepatogastroenterology.* 2000; 47:1337–1339. [PubMed: 11100346]
8. Devaney JM, Greene CM, Taggart CC, Carroll TP, O'Neill SJ, McElvaney NG. Neutrophil elastase up-regulates interleukin-8 via toll-like receptor 4. *FEBS Lett.* 2003; 544:129–132. [PubMed: 12782302]
9. Dybdahl B, Wahba A, Lien E, Flo TH, Waage A, Qureshi N, Sellevold OF, Espevik T, Sundan A. Inflammatory response after open heart surgery: release of heat-shock protein 70 and signaling through toll-like receptor-4. *Circulation.* 2002; 105:685–690. [PubMed: 11839622]

10. Efron P, Moldawer LL. Sepsis and the dendritic cell. *Shock*. 2003; 20:386–401. [PubMed: 14560102]
11. Ertel W, Kremer JP, Kenney J, Steckholzer U, Jarrar D, Trentz O, Schildberg FW. Downregulation of proinflammatory cytokine release in whole blood from septic patients. *Blood*. 1995; 85:1341–1347. [PubMed: 7858264]
12. Fan ST, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg*. 1999; 229:322–330. [PubMed: 10077043]
13. Finch MD, Crosbie JL, Currie E, Garden OJ. An 8-year experience of hepatic resection: indications and outcome. *Br J Surg*. 1998; 85:315–319. [PubMed: 9529482]
14. Fingerle G, Pforte A, Passlick B, Blumenstein M, Strobel M, Ziegler-Heitbrock HW. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood*. 1993; 82:3170–3176. [PubMed: 7693040]
15. Fink R, Al-Obaidi M, Grewal S, Winter M, Pepper J. Monocyte activation markers during cardiopulmonary bypass. *Perfusion*. 2003; 18:83–86. [PubMed: 12868785]
16. Garwood RA, Sawyer RG, Thompson L, Adams RB. Infectious complications after hepatic resection. *Am Surg*. 2004; 70:787–792. [PubMed: 15481295]
17. Gomez J, Borrás FE, Singh R, Rajanathanan P, English N, Knight SC, Navarrete CV. Differential up-regulation of HLA-DM, invariant chain, and CD83 on myeloid and plasmacytoid dendritic cells from peripheral blood. *Tissue Antigens*. 2004; 63:149–157. [PubMed: 14705986]
18. Hagendorens MM, Ebo DG, Schuerwegh AJ, Huybrechts A, Van Bever HP, Bridts CH, De Clerck LS, Stevens WJ. Differences in circulating dendritic cell subtypes in cord blood and peripheral blood of healthy and allergic children. *Clin Exp Allergy*. 2003; 33:633–639. [PubMed: 12752592]
19. Harter L, Mica L, Stocker R, Trentz O, Keel M. Increased expression of toll-like receptor-2 and -4 on leukocytes from patients with sepsis. *Shock*. 2004; 22:403–409. [PubMed: 15489631]
20. Ho CS, Lopez JA, Vuckovic S, Pyke CM, Hockey RL, Hart DN. Surgical and physical stress increases circulating blood dendritic cell counts independently of monocyte counts. *Blood*. 2001; 98:140–145. [PubMed: 11418473]
21. Ikushima H, Nishida T, Takeda K, Ito T, Yasuda T, Yano M, Akira S, Matsuda H. Expression of Toll-like receptors 2 and 4 is downregulated after operation. *Surgery*. 2004; 135:376–385. [PubMed: 15041961]
22. Jarnagin WR, Gonen M, Fong Y, DeMatteo RP, Ben-Porat L, Little S, Corvera C, Weber S, Blumgart LH. Improvement in perioperative outcome after hepatic resection: analysis of 1,803 consecutive cases over the past decade. *Ann Surg*. 2002; 236:397–406. discussion 406–397. [PubMed: 12368667]
23. Kiertscher SM, Gitlitz BJ, Figlin RA, Roth MD. Granulocyte/macrophage-colony stimulating factor and interleukin-4 expand and activate type-1 dendritic cells (DC1) when administered in vivo to cancer patients. *Int J Cancer*. 2003; 107:256–261. [PubMed: 12949803]
24. Kim YI, Song KE, Ryeon HK, Hwang YJ, Yun YK, Lee JW, Chun BY. Enhanced inflammatory cytokine production at ischemia/reperfusion in human liver resection. *Hepatogastroenterology*. 2002; 49:1077–1082. [PubMed: 12143206]
25. Kuwana M, Kaburaki J, Wright TM, Kawakami Y, Ikeda Y. Induction of antigen-specific human CD4(+) T cell anergy by peripheral blood DC2 precursors. *Eur J Immunol*. 2001; 31:2547–2557. [PubMed: 11536152]
26. Lemaire LC, van Wagensveld BA, van Gulik TM, Dankert J, van Lanschot JJ, Gouma DJ. Bacterial translocation to the thoracic duct in a setting of ischemia, partial resection and reperfusion of the porcine liver. *Dig Surg*. 1999; 16:222–228. [PubMed: 10436371]
27. McAuliffe PF, Efron PA, Scumpia PO, Uchida T, Mutschlecner SC, Rout WR, Moldawer LL, Cendan JC. Varying blood monocyte and dendritic cell responses after laparoscopic versus open gastric bypass surgery. *Obesity surgery*. 2005; 15:1424–1431. [PubMed: 16354522]
28. Medzhitov R, Janeway CA Jr. Innate immunity: the virtues of a nonclonal system of recognition. *Cell*. 1997; 91:295–298. [PubMed: 9363937]
29. Motley D, Meyer MP, King RA, Naus GJ. Determination of lymphocyte immunophenotypic values for normal full-term cord blood. *Am J Clin Pathol*. 1996; 105:38–43. [PubMed: 8561086]

30. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Invest.* 1991; 88:1747–1754. [PubMed: 1939659]
31. Namekata K, Takamori S, Kojima K, Beppu T, Futagawa S. Significant changes in the serum levels of IL-6, h-HGF, and type IV collagen 7S during the perioperative period of a hepatectomy: relevance to SIRS. *Surg Today.* 2000; 30:403–409. [PubMed: 10819474]
32. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E. Involvement of toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. *J Biol Chem.* 2004; 279:7370–7377. [PubMed: 14660645]
33. Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood.* 1989; 74:2527–2534. [PubMed: 2478233]
34. Peng R, Li Y, Brezner K, Litherland S, Clare-Salzler MJ. Abnormal peripheral blood dendritic cell populations in type 1 diabetes. *Ann N Y Acad Sci.* 2003; 1005:222–225. [PubMed: 14679064]
35. Rinder CS, Mathew JP, Rinder HM, Tracey JB, Davis E, Smith BR. Lymphocyte and monocyte subset changes during cardiopulmonary bypass: effects of aging and gender. *J Lab Clin Med.* 1997; 129:592–602. [PubMed: 9178725]
36. Rissoan MC, Soumelis V, Kadowaki N, Grouard G, Briere F, de Waal Malefyt R, Liu YJ. Reciprocal control of T helper cell and dendritic cell differentiation. *Science.* 1999; 283:1183–1186. [PubMed: 10024247]
37. Robinson SP, Patterson S, English N, Davies D, Knight SC, Reid CD. Human peripheral blood contains two distinct lineages of dendritic cells. *Eur J Immunol.* 1999; 29:2769–2778. [PubMed: 10508251]
38. Sasu S, LaVerda D, Qureshi N, Golenbock DT, Beasley D. Chlamydia pneumoniae and chlamydial heat shock protein 60 stimulate proliferation of human vascular smooth muscle cells via toll-like receptor 4 and p44/p42 mitogen-activated protein kinase activation. *Circ Res.* 2001; 89:244–250. [PubMed: 11485974]
39. Sbrana S, Parri MS, De Filippis R, Gianetti J, Clerico A. Monitoring of monocyte functional state after extracorporeal circulation: a flow cytometry study. *Cytometry B Clin Cytom.* 2004; 58:17–24. [PubMed: 14994371]
40. Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem.* 1999; 274:17406–17409. [PubMed: 10364168]
41. Skrzeczynska J, Kobylarz K, Hartwich Z, Zembala M, Pryjma J. CD14+CD16+ monocytes in the course of sepsis in neonates and small children: monitoring and functional studies. *Scand J Immunol.* 2002; 55:629–638. [PubMed: 12028567]
42. Smiley ST, King JA, Hancock WW. Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol.* 2001; 167:2887–2894. [PubMed: 11509636]
43. Strohmeyer JC, Blume C, Meisel C, Doecke WD, Hummel M, Hoeflich C, Thiele K, Unbehauen A, Hetzer R, Volk HD. Standardized immune monitoring for the prediction of infections after cardiopulmonary bypass surgery in risk patients. *Cytometry B Clin Cytom.* 2003; 53:54–62. [PubMed: 12717692]
44. Takeshita F, Leifer CA, Gursel I, Ishii KJ, Takeshita S, Gursel M, Klinman DM. Cutting edge: Role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. *J Immunol.* 2001; 167:3555–3558. [PubMed: 11564765]
45. Teig N, Moses D, Gieseler S, Schauer U. Age-related changes in human blood dendritic cell subpopulations. *Scand J Immunol.* 2002; 55:453–457. [PubMed: 11975756]
46. Tsujimoto H, Ono S, Hiraki S, Majima T, Kawarabayashi N, Sugawara H, Kinoshita M, Hiraide H, Mochizuki H. Hemoperfusion with polymyxin B-immobilized fibers reduced the number of CD16+ CD14+ monocytes in patients with septic shock. *J Endotoxin Res.* 2004; 10:229–237. [PubMed: 15373966]
47. Tsujimoto H, Ono S, Majima T, Efron PA, Kinoshita M, Hiraide H, Moldawer LL, Mochizuki H. Differential toll-like receptor expression after ex vivo lipopolysaccharide exposure in patients with sepsis and following surgical stress. *Clinical immunology (Orlando, Fla.)* 2006; 119:180–187.

48. Tsujimoto H, Ono S, Majima T, Kawarabayashi N, Takayama E, Kinoshita M, Seki S, Hiraide H, Moldawer LL, Mochizuki H. Neutrophil Elastase, Mip-2, and Tlr-4 Expression During Human and Experimental Sepsis. *Shock*. 2005; 23:39–44. [PubMed: 15614130]
49. Ulevitch RJ, Tobias PS. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol*. 1995; 13:437–457. [PubMed: 7542010]
50. Wang SY, Mak KL, Chen LY, Chou MP, Ho CK. Heterogeneity of human blood monocyte: two subpopulations with different sizes, phenotypes and functions. *Immunology*. 1992; 77:298–303. [PubMed: 1427982]
51. Wiezer MJ, Meijer C, Wallast-Groenewoud HP, Tool AT, Prins HA, Houdijk AP, Beelen RH, Meijer S, Hack CE, van Leeuwen PA. Impaired leukocyte phagocytosis in patients undergoing hemihepatectomy for liver metastases. *Liver Transpl Surg*. 1999; 5:238–245. [PubMed: 10226116]
52. Yang H, Young DW, Gusovsky F, Chow JC. Cellular events mediated by lipopolysaccharide-stimulated toll-like receptor 4. MD-2 is required for activation of mitogen-activated protein kinases and Elk-1. *J Biol Chem*. 2000; 275:20861–20866. [PubMed: 10877845]

Figure 1. Examples of flow cytometry analysis

(A). Identification of circulating DCs. After debris was gated out on a forward versus side scatter density plot, DCs were identified on a Lineage Cocktail versus HLA-DR density plot as Lineage Cocktail⁻ HLA-DR⁺ events. Lineage Cocktail⁻ HLA-DR⁻ basophils were usually identifiable as well. (B). Phenotyping DCs. Lineage Cocktail⁻ HLA-DR⁺ events were gated to a CD11c versus CD123 density plot in order to determine DC1 (CD11c⁺CD123⁻), DC2 (CD11c⁻CD123⁺), and IdDC (CD11c⁻CD123⁻) populations. (C). Phenotyping circulating monocytes. Events representing circulating monocytes were identified through a sequential analysis of forward scatter, side scatter, and CD14 expression. This analysis allowed CD16^{bright/dim} monocytes to be identified on a CD14 versus CD16 density plot.

Figure 2. WBC counts of patients undergoing hepatectomy

Hepatectomy patients have a significant leukocytosis on post-operative day (POD) one as compared to earlier ($p < 0.001$ versus pre and post-hepatectomy) and later time points ($p < 0.05$ versus POD3 and 5).

Figure 3. Relative (A) and absolute (B) changes in blood monocyte levels and their CD16 phenotype

Although the relative decline in monocyte population immediately post-resection did not reach statistical significance, there was a significant recovery and increase in the circulating monocyte population on POD3 and 5 ($p < 0.05$) (Figure 3A). This was reflected in the absolute numbers of circulating monocyte as there was a significant increase in monocytes in the post-operative period as compared to pre-hepatectomy ($p < 0.05$ versus POD3 and 5, $p < 0.001$ versus POD1) and post-hepatectomy period ($p < 0.001$ versus POD1,3, and 5) (Figure 3B). The percentage of CD16^{dim} monocytes significantly increased post-hepatectomy and then decreased on POD1 ($p < 0.05$), with CD16^{bright} monocytes expectedly demonstrating an inverse effect ($p < 0.05$) (Figure 3A). This relative change was not necessarily reflected in the absolute number of circulating CD16^{dim} and CD16^{bright} monocytes (Figure 3B). There was an absolute increase in the CD16^{dim} monocyte number from pre-hepatectomy ($p < 0.05$ versus POD1 and 5) and post-hepatectomy levels ($p < 0.001$ versus POD1 and 5, $p < 0.05$ versus POD3). There was a significant drop in the CD16^{bright} monocyte population post-hepatectomy ($p < 0.05$ versus pre-hepatectomy), which was followed by a significant increase in their numbers ($p < 0.05$ versus POD1 and 3).

Figure 4. Relative (A) and absolute (B) alterations in circulating DC levels and phenotypes in hepatectomy patients

The percentage of circulating DCs significantly decreased after liver resection ($p < 0.05$ versus post-hepatectomy, POD1, 3, and 5), which was reflected in their absolute circulating numbers ($p < 0.05$ versus POD3). CD11c⁺ DCs exhibited a relative loss post-hepatectomy followed by a relative increase on POD1 followed by another relative loss in their circulating levels (all $p < 0.001$ except pre-hepatectomy vs POD1, $p < 0.05$). Absolute CD11c⁺ DC counts displayed pre-hepatectomy and POD1 numbers to be different than other time points ($p < 0.05$ versus post-hepatectomy, POD3, POD5 and $p < 0.05$ versus pre-hepatectomy and POD3, respectively). There was a significant increase in the percentage of CD123⁺ DCs post-hepatectomy ($p < 0.001$), which was followed by significant decline on POD1 and 5 ($p < 0.001$ and $p < 0.05$ as compared to pre-hepatectomy, respectively). An absolute loss of CD123⁺ DCs was also observed in the post-operative period ($p < 0.05$ pre-hepatectomy versus POD1, 3 and 5; $p < 0.05$ post-hepatectomy versus POD1 and 3). By POD5, the percentage of circulating IdDCs increased ($p < 0.05$ versus pre-hepatectomy, post-hepatectomy, and POD1) and became the predominant DC phenotype, although changes in the absolute numbers of these cells never reached significance.

Figure 5. CD11b expression on circulating CD16^{bright} monocytes
 CD16^{bright} monocytes significantly upregulated their expression of CD11b in the post-operative period ($p < 0.05$ pre-hepatectomy versus POD1, 3 and 5).

Figure 6. CD86 expression on circulating DCs

(A). Examples of CD86 flow cytometry analysis in healthy non-hepatectomy volunteers. (i). CD86⁻ cells were determined by the appropriate isotype control. (ii). CD86^{low} and CD86^{high} DC populations were easily identified after activating DCs in whole blood by incubating them with 1 μ g/ml of LPS for one hour. (B) Alterations in DC CD86 expression. There was a significant increase in the relative CD86⁻ DC population post-hepatectomy as compared to all other time points (p<0.05). However, absolute numbers of these cells declined (p<0.05 pre-hepatectomy versus POD1) due to the overall loss of circulating DCs. The relative number of CD86^{low} DCs was greater pre-hepatectomy and on POD 1 as compared to post-hepatectomy, POD3 and POD5 (p<0.05). This was also reflected in the absolute numbers, with a significant increase on POD1 as compared to post-hepatectomy levels (p<0.05). The proportion CD86^{high} increased later in the post-operative period (p<0.05 POD3 versus pre-hepatectomy; POD5 versus pre-hepatectomy, post-hepatectomy and POD1), although change in the absolute number of mature CD86^{high} DCs did not reach significance.

Figure 7. Changes in relative and absolute populations of HLA-DR⁺ total, CD16⁻, and CD16⁺ monocytes

A. Percentage of HLA-DR⁺ monocytes. There was a significant decline in the relative HLA-DR⁺ population post liver resection ($p < 0.05$ pre-hepatectomy versus post-hepatectomy; $p < 0.001$ pre-hepatectomy and post-hepatectomy versus POD1, 3, and 5). The percentage of circulating CD16^{dim} HLA-DR⁺ monocytes decreased ($p < 0.001$ pre-hepatectomy and post-hepatectomy versus POD1, 3 and 5) while the CD16^{bright} HLA-DR⁺ monocytes exhibited a relative decrease in their population ($p < 0.001$ pre-hepatectomy versus post-hepatectomy and POD5; $p < 0.05$ pre-hepatectomy versus POD1), which normalized on POD3 ($p < 0.05$ post-hepatectomy versus POD3; no significant difference pre-hepatectomy versus POD3) before declining again. B. Total number of circulating HLA-DR⁺ monocytes. There were no significant changes in the number of all HLA-DR⁺ monocytes (data not shown) and CD16^{dim} HLA-DR⁺ monocytes. There was, however, a significant decrease in the number of CD16^{bright} HLA-DR⁺ monocytes post-hepatectomy ($p < 0.001$ post-hepatectomy versus POD1, 3, and 5; $p < 0.05$ post-hepatectomy versus pre-hepatectomy), which subsequently normalized.

Figure 8. HLA-DR MFI of total, CD16^{dim}, and CD16^{bright} monocytes

HLA-DR was significantly downregulated on all monocytes ($p < 0.001$ pre-hepatectomy versus post-hepatectomy; $p < 0.05$ pre-hepatectomy versus POD1), which then increased, later in the post-operative period ($p < 0.05$ post-hepatectomy versus POD3 and 5). CD16^{dim} monocytes never significantly altered their amount of HLA-DR cell surface expression. However, CD16^{bright} monocytes significantly downregulated their expression of HLA-DR post-operatively and never recovered to pre-resection levels ($p < 0.001$ pre-hepatectomy versus POD1; $p < 0.05$ pre-hepatectomy versus post-hepatectomy, POD 3 and 5).

Figure 9. TLR-2 expression on circulating monocytes and DCs

Monocytes significantly upregulated their cell surface expression of TLR-2 post-resection ($p < 0.05$ post-hepatectomy versus POD1, 3 and 5; pre-hepatectomy versus POD3) which normalized by POD5. CD16^{bright} monocytes TLR-2 cellular membrane expression increased post-resection and remained elevated ($p < 0.05$ pre-hepatectomy and post-hepatectomy versus POD1, 3 and 5). The percentage of TLR-2⁺ DCs significantly increased on POD1 ($p < 0.05$ pre-hepatectomy versus POD1).

Figure 10. Relative and absolute circulating TLR4⁺ monocytes

As a group, the percentage of TLR4⁺ monocytes and TLR4⁺ CD16^{bright} monocytes differed with liver resection. Absolute numbers of circulating TLR4⁺ monocytes and TLR4⁺ CD16^{bright} monocytes significantly increase on POD1 ($p < 0.05$ pre-hepatectomy versus POD1 and POD3).

Table 1

Characteristics of patients undergoing hepatectomy. In addition to the operation listed below, all patients had an intraoperative ultrasound and cholecystectomy.

Patient	Gender	Age	Operation	Diagnosis	HD	Post-Operative Complications
1	male	55	LHL; radiofrequency ablation of right lobe liver lesion	MCC	8	none
2	male	65	RHL; resection of small bowel/colon with ileocolic anastomosis	MCC	86	ileocolonic anastomotic leak with subsequent resection and ileostomy; VRE from abdominal cultures
3	female	49	RHL; wedge resection of left lateral segment of the liver	MCC	8	none
4	female	61	RHL	MCC	9	pleural effusion within 30 days of operation
5	male	73	LHL	MCC	9	possible ethanol withdrawal
6	female	63	RHL	Polycystic liver	8	none
7	female	70	LHL	HCC	9	none
8	male	52	RTSG; extrahepatic bile duct resection; resection of portal vein with primary anastomosis; hepaticojejunostomy	CC	17	pleural effusion; leukocytosis; possible pneumonia; bacterial colonization of pre-operatively placed biliary stent
9	female	54	LTSG; extrahepatic bile duct resection; pancreaticoduodenectomy; hepaticojejunostomy	CC	13	subphrenic abscess
10	female	59	RHL + segment 4B resection; extrahepatic bile duct resection; hepaticojejunostomy	Gall-bladder adeno-carcinoma	9	none
11	male	74	RHL; wedge resection of segment 4	HCC	8	none
12	male	53	RTSG	HCC	8	none
13	male	65	RTSG; extrahepatic bile duct resection; resection of portal vein bifurcation with primary anastomosis; hepaticojejunostomy	CC	13	superficial wound infection
14	female	51	LHL + caudate & segment 8 resection; wedge resection of segment 6	FNH & hepatic adenoma	9	none
15	female	71	RHL	cystadenoma	9	none

HD = hospital days; LHL = left hepatic lobectomy; RHL = right hepatic lobectomy; RTSG = right trisegmentectomy; LTSG = left trisegmentectomy; MCC = metastatic colon cancer; HCC = hepatocellular carcinoma; CC = cholangiocarcinoma; FNH = focal nodular hyperplasia

Summary of alterations in the innate immune system after hepatectomy (in comparison to its preoperative state).

Table II

Cell Type	Parameter	Timepoint			
		Post-Hepatectomy	POD1	POD3	POD5
Leukocytes	WBC		↑		
Monocytes	Numbers			↑	↑
	Phenotype	↑ CD16 ^{dim}	↑ CD16 ^{bright}	↑ CD16 ^{bright}	
	Activation Markers (on CD16 ^{bright})		↑	↑	↑
	APC Capacity Marker (on CD16 ^{bright})	↓ (CD16 ^{bright})	↓ CD16 ^{bright}	↓ CD16 ^{bright}	↓ CD16 ^{bright}
	TLR expression (on CD16 ^{bright})		↑ TLR2 & TLR4	↑ TLR2 & TLR4	↑ TLR2
DCs	Numbers	↓	↓	↓	↓
	Phenotype	↑ DC2	↑ DC1		↑ IdDC
	Activation Marker			↑	↑
	TLR expression		↑ TLR2		