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The portal vein as a distinct immunological compartment – A comprehensive immune phenotyping study

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ABSTRACT

Advanced liver diseases are associated with impaired intestinal barrier function, which results in bacterial influx *via* the portal vein to the liver, causing hepatic and systemic inflammation. Little is known about possible concomitant trafficking of immune cells from the intestines to the liver. We therefore performed a comprehensive immunophenotyping study of the portal venous versus peripheral blood compartment in patients with liver cirrhosis who received a transjugular intrahepatic portosystemic stent shunt (TIPS). Our analysis suggests that the portal vein constitutes a distinct immunological compartment resembling that of the intestines, at least in patients with advanced liver cirrhosis. In detail, significantly lower frequencies of naïve $CD4^+$ T cells, monocytes, dendritic cells and Vδ2 T cells were observed in the portal vein, whereas frequencies of activated $CD4^+$ and $CD8^+$ T cells, as well as of mucosa-associated Vδ1 T cells were significantly higher in portal venous compared to peripheral blood. In conclusion, our data raises interesting questions, e.g. whether liver cirrhosis-associated chronic inflammation of the intestines and portal hypertension promote an influx of activated intestinal immune cells like γδ T cells into the liver.

1. Introduction

The intestinal mucosa is harboring a unique immunologic compartment characterized by specialized populations of epithelial associated lymphocytes [1]. Intraepithelial lymphocytes (IEL) are predominantly T cells [2], which differ remarkably from peripheral T cells with respect to subpopulation frequencies and functions. For example, the intraepithelial T cell compartment contains higher frequencies of γδ T cells (10–14%), compared to the compartment of peripheral T cells (5%) [3]. Comparable frequencies of γδ T cells are present in the epithelial layer of the skin and the respiratory tract [4–6], suggesting a significant role of γδ T cells for epithelial barrier defense. γδ T cells are named for their different T cell receptor in contrast to conventional αβ T cells, including one γ- and one δ-chain. In contrast to αβ T cells, γδ T cells do not depend on MHC-restricted antigen-presentation but recognize non-classical antigens like phosphorylated non-peptide antigens or MHC molecules like CD1 or MICA [7,8]. Although the definitive role of γδ T cells is still under investigation, one of their likely important functions is the protection of epithelial barriers against microbial invasion by secretion of interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and Interleukin (IL)-17 [9]. γδ T cells can trigger inflammatory responses in reaction to infectious stimuli, which may – on the contrary – be mechanistically involved in the development of inflammatory bowel disease in rodents [10]. On the other hand, γδ T cell subpopulations which are characterized by production of anti-inflammatory cytokines TGF-β and IL-10 are also involved in

Abbreviations: ASH, alcoholic steatohepatitis; BSA, bovine serum albumin; CCR, chemokine receptor; CD, cluster of differentiation; CM, classical monocytes; DMSO, dimethyl sulfoxide; FACS, fluorescence-activated cell sorting; HIPC, Human Immunophenotyping Consortium; IEL, intraepithelial lymphocytes; MELD, model for end-stage liver disease; MHC, major histocompatibility complex; NASH, non-alcoholic steatohepatitis; NCM, non-classical monocytes; NK, natural killer cell; PAMPs, pathogen-associated molecular patterns; PBS, phosphate buffered saline; PV, portal vein; PVT, portal vein thrombosis; TCR, T cell receptor; TIPS, transjugular intrahepatic portosystemic stent shunt

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immunoregulation and tolerance of the intestinal epithelium [11–13].

In addition to their cytokine secretion profile, γδ T cells are classified as Vδ1 and Vδ2 T cells, depending on the δ-chain of the γδ T cell receptor complex [14,15]. Whereas Vδ2 T cells account for the majority of γδ T cells in the peripheral blood, Vδ1 T cells are predominantly found in the gut and other peripheral tissues [16,17]. These, tissue associated, Vδ1 T cells have cytolytic and antimicrobial properties via perforin-, granulysin- and Fas-mediated cytotoxicity, and produce T_{H1}-type cytokines [18,19].

Interestingly, the liver also comprises large numbers of γδ T cells [20,21]. In the liver, γδ T cells can either provide protection from or induce tissue damage, depending on their Vδ chain expression [22]. For example, infiltration of Vδ1 T cells is associated with higher degrees of inflammation and necrosis in the liver of patients with chronic hepatitis C virus infection [23], whereas Vδ2 T cells can exert anti-tumoral functions [24].

Already in steady state, the liver is exposed to continuous influx of antigens and bacterial products like lipopolysaccharide (LPS) from the gut *via* the portal vein [25]. In patients with advanced liver disease, a disturbed intestinal barrier results in a profound increase in bacterial influx from the intestines to the liver, causing hepatic and systemic inflammation [26,27]. As described above for γδ T cells, the immune cell composition of the intestines resembles to that of the liver. Yet, little is known about a possible trafficking of specific immune cells from the intestines to the liver in humans. Therefore, we aimed to perform a comprehensive immunophenotyping study including a characterization of immune cell frequencies in the blood of the portal vein, as a putative intermediate between gut and liver, in comparison to peripheral blood in patients with liver cirrhosis.

2. Patients, materials and methods

2.1. Patients

Consecutive patients with liver cirrhosis who were admitted to Department of Internal Medicine I, University Hospital Frankfurt, Germany, for the insertion of a transjugular intrahepatic portosystemic stent shunt (TIPS) between December 2016 and July 2017 were included in our study. Patients were excluded in case of age below 18 years, pregnancy or breastfeeding, presence of hepatocellular carcinoma (HCC) beyond Milan criteria, presence of bacterial or fungal infections, presence of infection with human immunodeficiency virus (HIV) or usage of immunosuppressive drugs. All patients provided written informed consent to the study protocol, and the study was approved by the local ethic committee of the University Hospital Frankfurt, Germany.

2.2. Immunophenotyping

Blood of the portal vein was sampled in EDTA-tubes directly at the first puncture of the portal vein during TIPS insertion. Corresponding peripheral blood was collected from decubital vein and equally sampled in EDTA-tubes. Flow cytometric analysis was performed on peripheral blood mononuclear cells (PBMCs) of patients. PBMCs were isolated by Ficoll density gradient separation (Biocoll Separating Solution, Merck Millipore). After isolation, the cells were resuspended (1 ml 90%FCS/10%DMSO) and stored in liquid nitrogen. After defrosting the cells (using a water bath with 37 °C – 98,6°F), centrifugation (500rcf, 5 min, 4 °C) and discarding the supernatant, cells were counted and washed. Cells were resuspended in 50 µl staining buffer (PBS/BSA 0.5%) and blocked with 2 µl human FcR Blocking Reagent (Miltenyi Biotec, Bergisch-Gladbach Germany) for ten minutes on ice. Then, cells were incubated for 25 min. on ice in the dark with a mastermix including 50 µl/sample of Brilliant stain buffer (BD Biosciences, San Jose, USA) and one of the two panels of antibodies as shown in SI Tables 1 and 2. Antibody panel 1, regarding the differentiation of γδ T cells, was adapted from the publication of Wistuba-Hamprecht [28]. Composition of antibody panel 2 was chosen according to the recommendations of the Human Immunophenotyping Consortium [29]. After another washing cycle, cells were resuspended in 300 µl staining buffer (PBS/BSA 0.5%) and acquired on BD LSR II Fortessa flow cytometer. Frequency of cell populations were analyzed by using FlowJo V10 software (Tree star). The complex gating strategy to define all different immune cell populations is shown in Figs. 5–7. Cellular viability was estimated by 7-AAD incorporation (BioLegend). For correct gating fluorescence minus one controls (FMOs), i.e. fully stained cells with the exception of one particular antibody-fluorochrome conjugate, were used to identify truly positive cells for a given marker, as shown exemplarily in SI Fig. 1.

2.3. Statistical analyses

The Wilcoxon matched-pairs test was used for paired intraindividual comparisons of portal versus peripheral blood immune cell subsets. Group differences were assessed by the Wilcoxon-Mann-Whitney-U-Test. P-values < 0.05 were considered statistically significant and marked with one star, < 0.01 with two stars and < 0.001 with three stars. Statistical analyses were performed using GraphPad Prism 5 for Windows (GraphPad Software, Inc.).

3. Results

3.1. General characteristics of patients

A total of 17 patients receiving a TIPS were included in the study. The medium age was 61.5 years. Two (12%) of all patients had a portal vein thrombosis. Indications for TIPS-insertion were refractory ascites in 12 (71%) patients, secondary prophylaxis for recurrent bleeding from esophageal varices in 3 (18%) patients and presence of both causes (history of esophageal bleedings and ascites) in another two (12%) patients. More demographic and clinical patient characteristics are shown in Table 1.

3.2. Frequencies of αβ T cells in portal versus peripheral blood

The frequency of all T cells was significantly higher in the portal vein compared to peripheral blood ($p = 0.02$). In detail, naïve $CD4^+$ T cells ($p = 0.0008$) and effector $CD4^+$ T cells ($p = 0.03$) were more

Table 1
Baseline characteristics of patients.

Character	All patients (n = 17)
Age, y (range)	61.5 (53–70)
Gender, male/female (%)	12/5 (71/29)
Etiology of liver cirrhosis	
BMI, kg/m^2	25 (11–48)
Portal vein thrombosis, n (%)	2 (12)
Hepatic encephalopathy, any time, n (%)	5 (29)
MELD-score, mean (range)	12.9 (6–24)
Child Pugh Class (A/B/C), n	1/14/2
Presence of ascites (none/mild/severe)	3/1/13
Presence of esophageal varices (Grade 0/1/2/3)	6/6/4/1
Hepatorenal syndrome (absent/present)	11/6
Indication for TIPS – insertion (ascites/bleeding/both)	12/3/2

PVT = portal vein thrombosis; BMI = body mass index; MELD = model of end stage liver disease; TIPS = transjugular intrahepatic portosystemic stent shunt.