World Vaccine Congress Europe 2021

Detection and Deep Profiling of Immune Cells in HBV-infected Liver Biopsies

Anna Juncker-Jensen • Mate Levente Nagy • Erinn A. Parnell • Nickolas Stavrou • Qingyan Au • Judy Kuo • Eric Leones • Flora Sahafi • Kathy Pham • Julie Sakaki • Jessica Lin • Josette William Ragheb

NeoGenomics Laboratories, Aliso Viejo, CA

Background: Hepatitis B virus (HBV) infection is the main cause of cirrhosis and hepatocellular carcinoma (HCC), and with more than 250 million people worldwide living with chronic HBV presents a major global health problem. However, a detailed analysis of HBV pathogenesis and host immune responses are often hindered by access to sufficient amounts of liver tissues. Here we present a multiplexed immunofluorescence approach for deep profiling of host immune cells and HBV-infected hepatocytes with the goal of providing novel insights into HBV immunopathogenesis.

Methods: We used the mIF assay MultiOmyx™ which utilizes a pair of directly conjugated Cyanine dye-labeled (Cy3, Cy5) antibodies per round of staining followed by a dye inactivation step, illustrated in figure 1. Using a 19-marker panel and proprietary cell segmentation and classification algorithms developed at NeoGenomics, we have analyzed the presence of HBV-infected hepatocytes and key immunophenotypes in 8 FFPE core needle biopsies from HBV-infected patients with chronic hepatitis.

Results: We used antibodies against HBcAg (4.7%) and HBsAg (13.1%) to quantitate the proportion of cells infected with HBV. Key immune cell types were quantitated using co-expressions outlined in table 2, with the major immune cell type present being T cells (7.2%), followed by Kupffer cells (6.6%), MDSCs (5.6%), NK cells (5.5%), dendritic cells (2.7%), and B cells (0.9%). Further subtyping of these immune cells can be seen in figure 2.
Overall we found a high density of immune cell types clustering indicating a high degree of immune interactions within the hepatic microenvironment.

Conclusions: With this study we provide proof of concept for the use of highly multiplexed IF analysis of liver biopsies, allowing for a detailed analysis of HBV pathology with potential implications for future therapies aimed at treating viral immune pathogenesis of the liver.

MultiOmyx Workflow and Biomarker Panel

A.

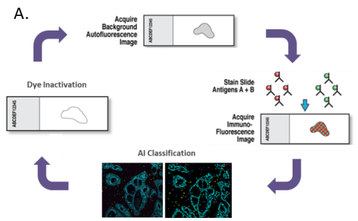


Table 1

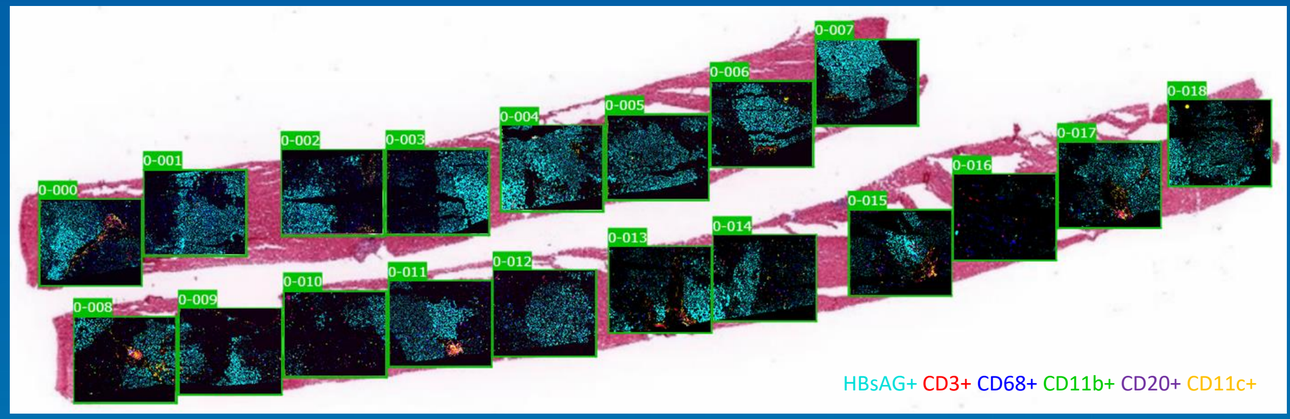
19-marker panel			
HBcAg	HBsAg	CD3	CD4
CD8	FoxP3	CD68	CD163
CD11b	MHC-II	CD20	CD56
CD11c	PD-1	PD-L1	DC-SIGN
IDO-1	Ki67	Na,K-ATPase	

Table 2

Co-Expression	Immune Phenotypes
HBcAg+/HBsAG+	HBV-infected cell
CD3+CD4+	T helper
CD3+CD4+FoxP3+	T regulatory
CD3+CD4+PD1+	T helper PD-1 positive
CD3+CD8+	CTL
CD3+CD8+PD1+	CTL PD-1 positive
CD68+CD163+	Kupffer cells
CD68+CD163+PDL1+	Kupffer cells PD-L1+
CD68+CD163+DCSIGN+	Kupffer cells DC-SIGN+
CD11b+MHCII-	MDSC
CD11c+MHCII+	Dendritic cells
CD11c+MHCII+DCSIGN+	Immature dendritic cells
CD56+CD3-	NK cells
CD56+CD3-MHCII+	Activated NK cells

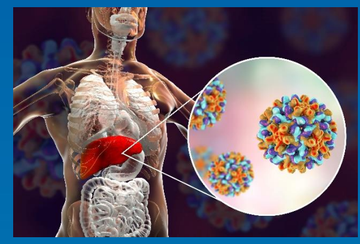
Figure 1. A) MultiOmyx staining protocol (described in Gerdes et al. 2013. PNAS USA. 110:11982-7). **Table 1)** Protein Panel Composition. **Table 2)** Phenotyping of human immune cells. HBcAg: Hepatitis B core antigen, HBsAg: Hepatitis B surface antigen, CTL: cytotoxic T lymphocyte, MDSC: myeloid derived depressor cell, NK: natural killer.

Using MultiOmyx™ mIF Technology to Characterize HBV Biopsies



Key Study Highlights

- We provide an extensive visualization and quantitation of immune subsets in patients with chronic HBV.
- Performing high-plex spatial immuno-histochemical analysis can lead to valuable insights into HBV-pathology.



MultiOmyx Overlay Images & Quantitation of Immune Cell Populations in HBV-infected Liver Biopsies

1.

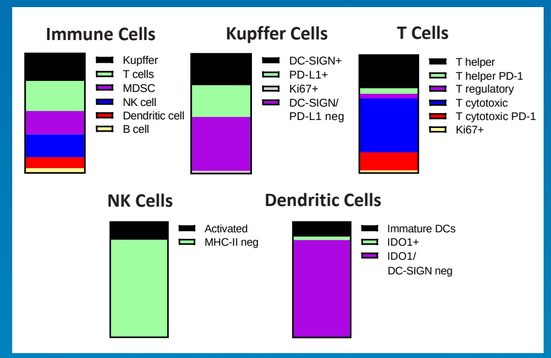


Figure 2. Parts of whole graphs displaying the proportions of the main immune phenotypes. Kupffer and T cells constitute the main immune phenotypes detected, while the main T cell subset detected is T cytotoxic cells.

2.

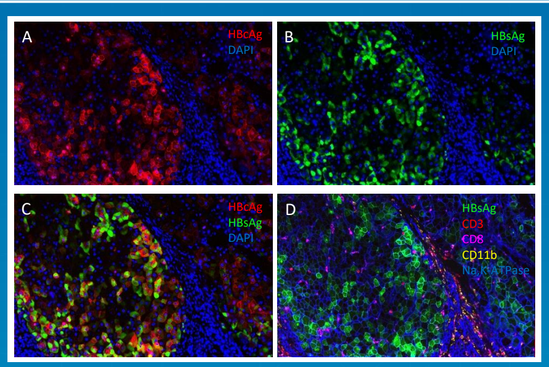


Figure 3. HBV-infected hepatocytes. Multiplexed overlaid images of **A)** HBcAg+ cells. **B)** HBsAG+ cells. **C)** co-expression of HBcAG and HBsAG, and **D)** HBsAG+ cells, T cells (CD3+), CTLs (CD3+CD8+), myeloid cells (CD11b+). Na,K-ATPase is used as a general tissue segmentation marker in figure 3-5.

3.

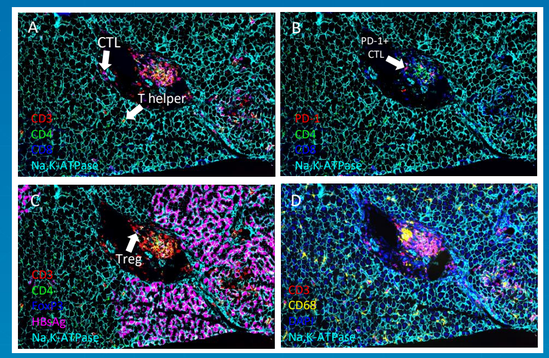


Figure 4. T cells. Multiplexed overlaid images of **A)** T helper cells (CD3+CD4+) and CTLs (CD3+CD8+). **B)** PD-1+ T helper cells (CD4+PD1+) and PD-1+ CTLs (CD8+PD1+), **C)** HBsAG+ cells, and T regulatory cells (CD3+CD4+FoxP3+), and **D)** T cells (CD3+), and Kupffer cells (CD68+).

4.

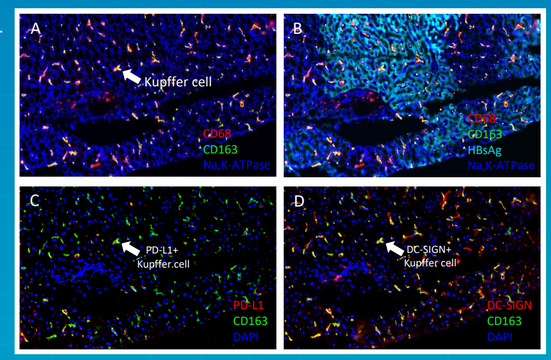


Figure 5. Kupffer cells. Multiplexed overlaid images of **A)** Kupffer cells (CD68+CD163+). **B)** HBsAG+ and Kupffer cells (CD68+CD163+). **C)** PD-L1+ Kupffer cells (PDL1+CD163+), and **D)** DC-SIGN+ Kupffer cells (DCSIGN+CD163+).

5.

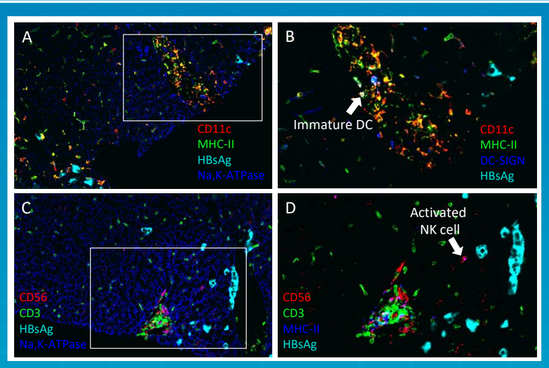
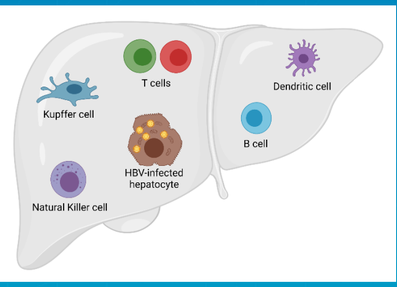


Figure 6. NK & dendritic cells. Multiplexed overlaid images of **A)** HBsAG+ and dendritic cells (CD11c+MHCII+). **B)** Immature dendritic cells (CD11c+MHCII+DCSIGN+). **C)** HBsAG+ and NK cells (CD56+CD3-), and **D)** Activated NK cells (CD56+CD3-MHCII+).



Created with BioRender.com

anna.juncker-jensen@neogenomics.com

NEO GENOMICS