Cellular Immune responses to human betaretrovirus in patients with primary biliary cholangitis

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INTRODUCTION

Our lab has characterized a human betaretrovirus (HBRV) in patients with primary biliary cholangitis (PBC) that triggers the presentation of mitochondrial autoantigens *in vitro*.[1] We have found proviral HBRV integrations in the majority of patients with PBC [2] and shown histological and biochemical responses in PBC patients treated with combination antiretroviral therapy.[3] Previous cellular immune studies suggested that PBC patients make robust immune responses to HBRV Gag and Env peptides [4] but limited serological responses to HBRV Env. [5] Accordingly, our goal was to develop an interferon (IFN)-γ release assay to detect cellular immune responses to HBRV. These "QuantiFERON" assays are useful for diagnosis and management of infectious diseases where serological diagnostics do not provide sufficient data concerning either diagnosis or disease activity.

AIM

(i) Map individual HBRV Gag and Env peptides that trigger robust cellular immune responses in intrahepatic lymphocytes.

(ii) Create a peptide pool sufficient to construct an IFN-γ release assay.

(iii) Conduct a survey for the prevalence of HBRV infection in patients with PBC, other liver diseases and healthy subjects.

METHOD

- Intrahepatic lymphocytes were isolated from transplant recipients' liver by flushing the portal vein (PBC n = 8, other hepatic disease n = 9) **Fig. 1-A**.
- PBMC were isolated from PBC patients (n = 32), other liver diseases (n = 30) and healthy controls (n=15)
- Lymphocytes were stimulated with individual HBRV peptides **Fig.1-B** or a pool of 22 HBRV peptides
- IFN-γ production was assessed by either ELISpot to measure IFN-γ spot forming colonies **Fig.1-C** or Mesoscale V-Plex ELISA to quantify IFN-γ release **Fig.1-D**. 8μg Nivolumab anti-PD1 checkpoint inhibitor was added to the stimulation assay to improve sensitivity.

Fig.1-A

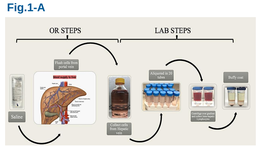


Fig.1-B

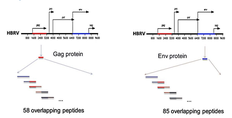
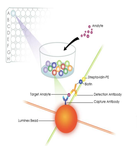
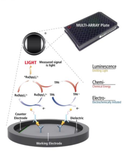


Fig.1-C



1-D



RESULTS

Fig. 2-A

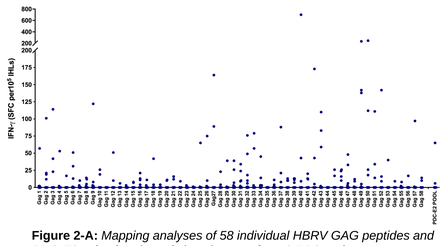


Figure 2-A: *Mapping analyses of 58 individual HBRV GAG peptides and PDC-E2 using intrahepatic lymphocytes from 8 PBC patients.*

Fig. 2-B

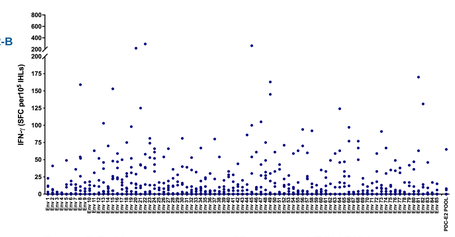


Figure 2-B: *Mapping analyses of 85 individual HBRV ENV peptides and PDC-E2 using intrahepatic lymphocytes from 8 PBC patients*

Fig. 3-A

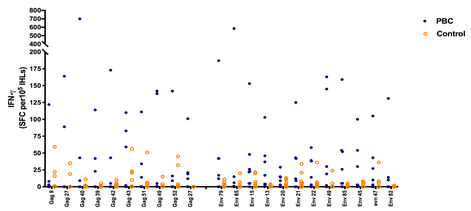


Figure 3-A: *Using a cut off level of 100 SFC, 22 individual HBRV peptides were identified that stimulated intrahepatic lymphocytes with 100% sensitivity and specificity in 8 PBC vs. 9 liver disease controls.*

Fig. 3-B

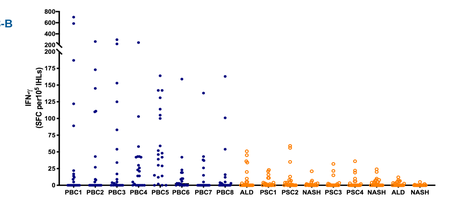
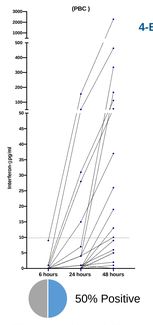
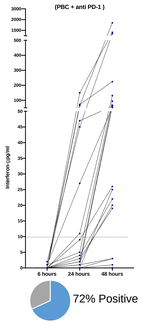


Figure 3-B: *SFC for each individual peptide shown for 8 PBC patients and 9 disease controls.*

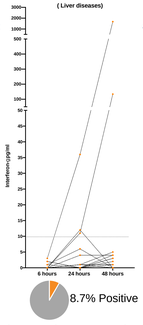
Fig. 4-A



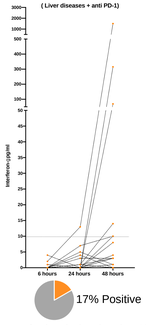
4-B



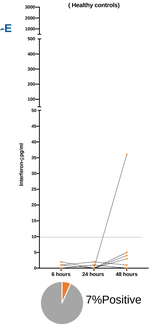
4-C



4-D



4-E



4-F

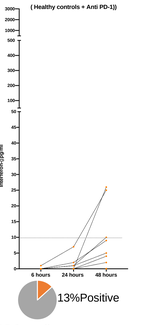


Figure 4: *(A and C) show PBC patients rarely make adequate (memory) responses by 6 hours of stimulation but by 48 hours, half of the PBC patients are positive (PBC vs. liver disease: 50% vs. 8.7%, p<0.03). (B and D) With the addition of the anti-PD1 check point inhibitor, the positivity increased to 72% vs. 17%, p=0.0003*

RESULTS: ELISPOT using Intrahepatic lymphocytes

- 10 HBRV Gag and 12 HBRV Env peptides were found to stimulate IHL with > 100 spot forming colonies (SFC). **Fig. 2- A & B**
- SFC production was higher in PBC vs. control patients (Mean HBRV Gag: 280 vs. 60, P=0.004; HBRV Env: 320 vs.40, P<0.0001). Notably, only one PBC patient had detectable IFN-γ producing IHL > 100 SFC following stimulation with the characterized mitochondrial PDC-E2 peptide. **Fig. 3-A**
- SFC for each individual peptide shown for 8 PBC patients and 9 disease controls**. Fig. 3-B**

RESULTS: Interferon-γ release assay using PBMC

- The 22 HBRV peptide pool stimulation assay was optimized for stimulating PBMC by modulating the amount of peptide, increased timing of stimulation, and use of the anti-PD1 checkpoint inhibitor (8 μg Nivolumab). **Fig. 4**
- Using a cut off > 10 pg/ml IFN-γ, half of the PBC patients showed reactivity to the 22 HBRV peptide pool (PBC vs. liver disease: 50% vs. 8.7%, p<0.03). **Fig. 4-A&C**
- With the addition of the anti-PD1 check point inhibitor, the positivity increased to 72% vs. 17%, p=0.0003 **Fig. 4-B&D**

CONCLUSIONS

In our peptide mapping studies, we show that all PBC patients' intrahepatic lymphocytes make IFN-γ when stimulated by individual HBRV peptides. The proportion of HBRV reactive lymphocytes in PBC patients was ~ $1:10^2$ - 10^3 whereas the PDC-E2 reactive lymphocytes was $1:10^3$-10^5 consistent with prior studies.[6] The characterized 22 HBRV Gag and Env peptides were combined to create an IFN-γ release assay and optimized for use with PBMC. We found that 50% of PBC patients produced > 10pg IFN-γ following stimulation with the 22 HBRV peptide pool and the use of anti-PD1 increased the sensitivity to 72 % but reduced the specificity. This study provides further support for our hypothesis that HBRV infection plays a role in the pathogenesis of PBC. Further studies will be required to refine the IFN-γ release assay as a diagnostic tool using whole blood. This assay can then be used to determine the prevalence of HBRV in patients with liver disease and other disorders.

ACKNOWLEDGEMENTS

liver.ca Canadian Liver Foundation Fondation canadienne du foie
Li Ka Shing Applied Virology Institute
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

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