Poster N° 705

Invariant Natural Killer T-Cells in HIV-HBV co-infection

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INTRODUCTION

Approximately 10% of 40 million HIV-infected individuals worldwide have chronic hepatitis B (CHB) (Alter et al. J. Hepatol. 2006). In the HAART era, deaths from AIDS-related causes have declined, yet liver disease has emerged as one of the leading causes of morbidity and mortality (Palella et al. JAIDS 2006). Indeed, HBV infection accelerates immunologic and clinical progression of HIV disease and increases the risk of hepatotoxicity when HAART is initiated, while HIV infection augments the risk of hepatitis events, cirrhosis, and end-stage liver disease related to CHB (Thio et al. Lancet 2002).

Invariant Natural Killer T (iNKT) cells are a unique subset of cells which bridge innate and adaptive immunity (Godfrey et al. JCI 2004) and mediate the pathogenesis of multiple clinical conditions, including HIV and HBV infections (Tupin et al. Nat. Rev. Microbiol. 2007).

In particular, iNKT cells are depleted and functionally impaired in the course of both infections and partially restored following specific treatment (Moll et al. EJI 2009, JIANG et al. PLoS One 2011). To date, however, the role of iNKT cells in the setting of HIV and HBV co-infection has yet to be investigated.

AIM OF THE STUDY

Assess iNKT frequency , phenotype and function in HIV+HBV+ co-infection.

PATIENTS AND METHODS

Cross sectional study on subjects belonging to the following groups:

I) HIV+HBV+ co-infected subjects (HIV Ab+ and HbsAg+); n=10 treated with a TDF/FTC-containing regimen; n=4 naïve
II) HIV+HBV- mono-infected subjects (HIV Ab+ and HBsAg-); n=10 HAART-treated; n=5 naïve
III) HIV-HBV+ mono-infected subjects (HIV Ab- and HbsAg+); n=9 treated; n=5 naïve
IV) HIV-HBV- uninfected healthy controls (HC) who had not received prior HBV vaccination; n=5

Cryopreserved peripheral blood mononuclear cells (PBMCS) were cultured with PMA (50 ng/ml) and Ionomicyn (500 ng/ml) or αGalactosylCeramide (αGalCer; 200ng/ml) along with Brefeldin A (10μg/ml) for 16 hours. Cells were then stained with the following antibodies: CD3-Pcy7, Vα24-biotin, Q-dot 655 strepdavidin. CD1d-PE, CD161 FITC, TNF-α/IFN-γ FITC to measure iNKT frequency (CD3+Vα24+CD1d+), CD161 and TNF-α/IFN-γ expression by flow cytometry. Data were analyzed with GraphPad Prism 5.01 software. Kruskall-Wallis and Wilcoxon tests were used for statistics.

RESULTS

Demographic and viro-immunologic parameters are show in the table below. Both treated and naïve HIV-HBV+ monoinfected pts displayed higher age compared to other groups. HBV viral load of HIV-HBV+ monoinfected pts was significantly lower compared to HBV viral load of HIV+HBV+ co-infected pts.

Table 1. Clinical characteristics of subjects enrolled in the study

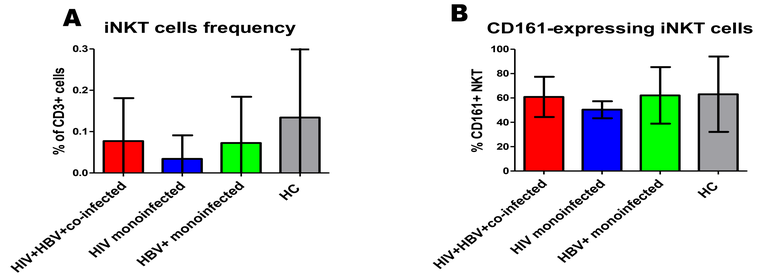
	NAÏVE			TREATED		
CHARACTERISTIC	**HIV+HBV+ co-infected n=4**	**HIV+HBV- monoinfected n=5**	**HIV-HBV+ monoinfected n=5**	**HIV+HBV+ co-infected n=10**	**HIV+HBV- monoinfected n=10**	**HIV-HBV+ monoinfected n=9**
Age, years (IQR)	39 (34-44)	33 (30-39)	57[a] (48-67)	46 (40-55)	43 (42-48)	60[ab] (54-67)
Sex, F (%)	0 (0%)	1 (20%)	3 (60%)	0 (0%)	3 (30%)	3 (33%)
ALT (u/l) (IQR)	82 (35-200)	26 (21-44)	26 (19-35)	33 (25-44)	29 (20-46)	33 (18-52)
AST (u/l) (IQR)	79 (34-87)	24 (22-34)	26 (22-33)	27 (18-47)	34 (22-35)	28 (23-35)
Current CD4 /mmc (IQR)	457 (170-601)	588 (548-649)	N.A.	653 (366-796)	597 (301-777)	N.A.
Current CD4 % (IQR)	33 (22-38)	26 (25-32)	N.A.	30 (27-36)	22 (11-28)	N.A.
Current HIV RNA log10 cp/ml (IQR)	3.26 (2.48-5.40)	3.61 (3.35-4.68)	N.A.	1.59	1.59	N.A.
Current HBV DNA Log10 (IU/ml) (IQR)	9,49 (4,51 – 11,50)	N.A.	2,71[c] (2,34 -3,35)	<10	N.A.	<10
Zenith HIV RNA log10 cp/ml (IQR)	4.92 (4.51-5.43)	4.61 (3.56-5.11)	N.A.	4.82 (4.22-5.34)	5.52 (2.41-5.60)	N.A.
Epidemiology, (%) Homo/bisex Eterosex exTD	2 (50%) 2 (50%) 0 (0%)	2 (40%) 3 (60%) 0 (0%)	N.A.	5 (50%) 3 (30%) 2 (20%)	3 (30%) 7 (70%) 0 (0%)	N.A

Note. Data were shown as median (IQR). ALT, alanina aminotransferase; AST, aspartate transaminase; Mann Whitney, Chi-Square and Kruskall Wallis tests used for statistics. p<0.05 : [a] naive HIV-/HBV+ vs naive HIV+/HBV+ and naive HIV+/HBV-; p<0.05 [b] treated HIV-/HBV+ vs treated HIV+/HBV+ and treated HIV+/HBV-; p<0.05 [c] naive HIV-/HBV+ vs naive HIV+/HBV+ . Both treated and naïve HIV-HBV+ monoinfected pts displayed higher age compared to other groups. HBV viral load of HIV-HBV+ monoinfected pts was significantly lower compared to HBV viral load of HIV+HBV+ co-infected pts, ad widely describe in literature.

We conducted a comparative analysis of iNKT cell frequency, phenotype and function among study groups according to their treatment status.

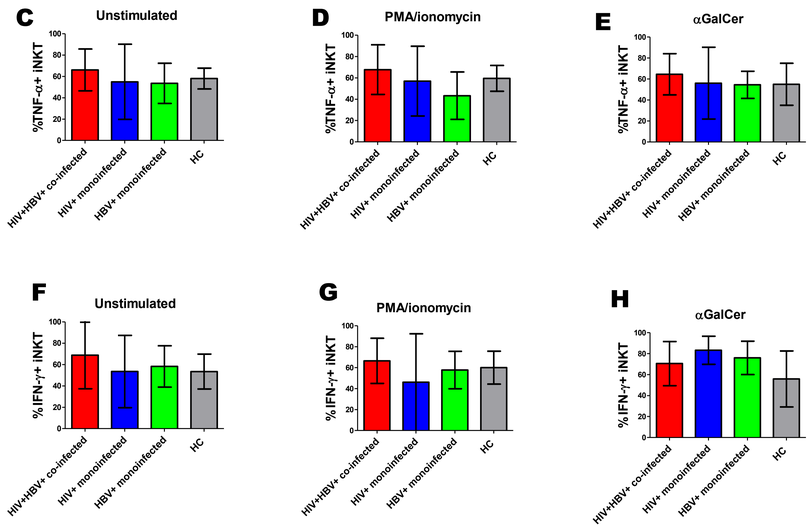
Therapy-naïve (mono-infected HBV or HIV and co-infected) and healthy controls showed similar circulating iNKT cells, despite lower frequencies in the former (HIV+HBV+: 0.028% IQR: 0.022-0,182; HIV+HBV-: 0.011% IQR: 0.007-0.074; HIV-HBV+: 0.02% IQR:0.014-0.158; HC: 0.048% IQR:0.006-0.306; p=0.46; figure 1.A). CD161 expression was also comparable among groups (HIV+HBV+: 67.74% IQR: 46.36-76.76; HIV+HBV-: 54.55% IQR: 43.05-55.56; HIV-HBV+: 67.74 IQR: 43.64-77.71; HC: 62.50% IQR:35.82-90.63; p=0.30; figure 1.B).

Figure 1. iNKT frequency (A) and CD161 expression (B) in HIV+HBV+ co-infected, HIV+ monoinfected, HBV+ monoinfected naïve patients and healthy controls



The investigation of functional capacity (TNF-α/IFN- γ) of iNKT cells in naive patients did not show any differences between the 4 study groups. Indeed, similar values of TNF-α were registered in all experimental conditions (unstimulated: p=0.09; figure 1.C; PMA/Iono: p=0.36; figure 1.D; αGalCer: p=0.89; figure 1.E). A similar trend was noted for IFN-γ production (unstimulated: figure 2.F p=0.58; PMA/Iono p=0.81; figure 1.G; αGalCer p=0.074; figure 1.H) .

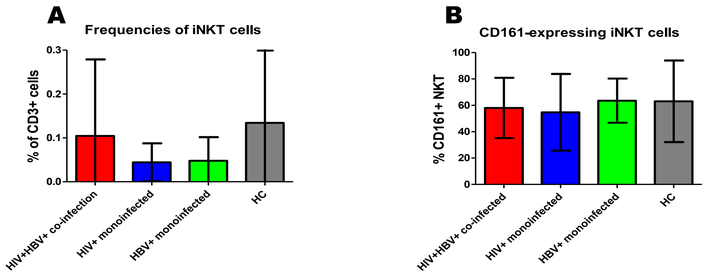
Figure 1. TNF-α (top)/IFN-γ (bottom) iNKT expressing cells prior to (C-F) and after PMA/Iono (D-G) or αGalCer (E-H) stimulation in HIV+HBV+ co-infected, HIV+ monoinfected, HBV+ monoinfected naïve patients and healthy controls



We then studied iNKT cell frequency, phenotype and intracellular cytokine expression in treated subjects enrolled in our study.

No significant differences were found among groups in terms of iNKT frequency (HIV+HBV+: 0.32 IQR: 0.021-0.12; HIV+HBV-: 0.028% IQR: 0.05-0.095; HIV-HBV+: 0.033 IQR: 0.012-0.071 ; HC: see above; p=0.46; figure 2.A) and CD161 expression (HIV+HBV+: 69.79% IQR: 23.26-76.62; HIV+HBV-: 56.04% IQR: 48.37-79.27; HIV-HBV+: 68.18% IQR: 46.91-80.44; HC see above; p=0.87; figure 2.B).

Figure 2. iNKT frequency (A) and CD161 expression (B) in HIV+HBV+ co-infected, HIV+ monoinfected, HBV+ monoinfected treated patients and healthy controls



Interestingly, HIV-infected individuals showed a peculiar functional capacity of iNKT cells. Indeed, mono-infected HIV+ showed the highest constitutive release of TNF-α, reaching statistical significance when compared to co-infected and HBV+ mono-infected subjects (77.90% IQR; 69.43-86.01 vs 54.47% IQR: 46.20-76.83 and 58.93% IQR: 39.05-66.45 respectively; p=0.011; figure 2.C). As similar hierarchy was noted also following PMA/ionomycin (co-infected: 72.22% IQR: 37.57-81.04; HIV monoinfected: 74.89% IQR: 70.25-82.73; HBV monoinfected: 59.18% IQR: 45.84-71.98; HC: 61.77% IQR: 48.29-69.65; p=.0.79 Fig 2D) and iNKT-specific stimulation (co-infected: 65.34% IQR: 51.25-76.79; HIV monoinfected: 77.84% IQR: 68.45-85.91; HBV monoinfected: 66.67% IQR: 43.87-71.04; HC: 58.43% IQR: 36.25-71.97; p=0.063 Fig 2E).

In accordance with these findings, also IFN-γ levels were higher in coinfected, subjects, reaching statistical significance when compared to healthy controls following PMA/ionomycin (88.35% IQR: 80.68-93.26 vs 62.5% IQR: 44.61-74.15; p=0.0087; figure 2.G).

When examining cytokine production within each patient group, only iNKT cells from co-infected HIV+HBV+ significantly increased TNF-α release upon αGalCer stimulation (us: 54.47% IQR: 46.20-76.83; αGalCer 65.34% IQR: 51.25-76.79; p=0.039; figure 3.A).

Figure 2. TNF-α (top)/IFN-γ (bottom) iNKT expressing cells prior to (C-F) and after PMA/Iono (D-G) or αGalCer (E-H) stimulation in HIV+HBV+ co-infected, HIV+ monoinfected, HBV+ monoinfected treated patients and healthy controls

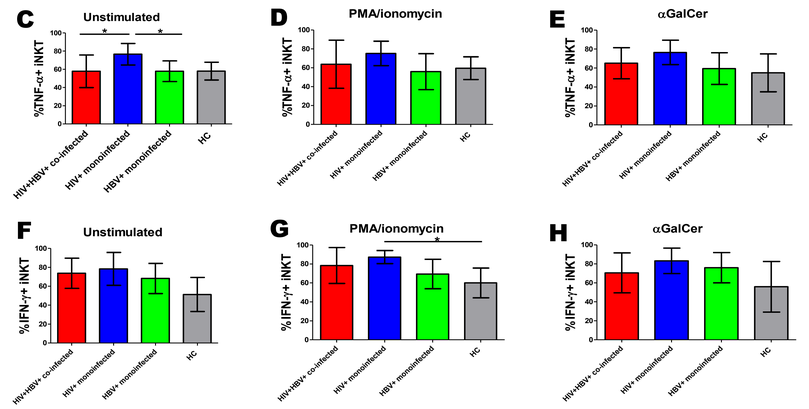
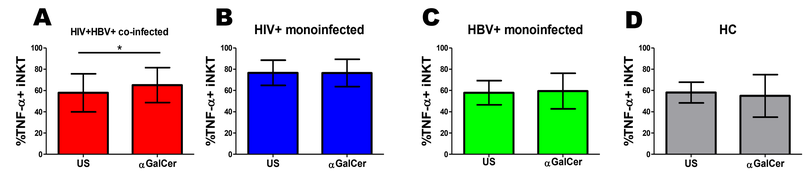
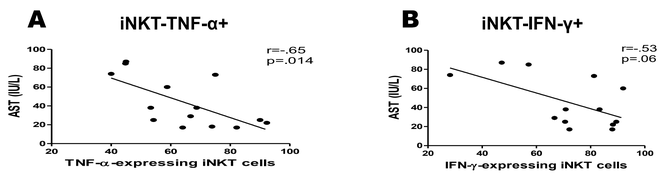


Figure 3. TNF-α production prior to and following αGalCer stimulation in treated patients and healthy controls



Interestingly, an inverse correlation was found between iNKT-specific TNF-α production and AST levels in the setting of HIV+HBV+ co-infection (p=0.014, r=-0.65; figure 4.A) as well as a trend to a negative correlation between IFN-γ and AST levels (p=0.06, r=-0.53; figure 4.B).

Figure 4. Inverse correlation between iNKT producing TNF-α (A) or IFN-γ (B) after αGalCer stimulation and AST levels in HIV+HBV+ co-infected treated and naïve patients



CONCLUSIONS

The inverse correlation between cytokine-expressing iNKT and liver function in HIV+HBV+ co-infected patients suggests the impairment of circulating iNKT cells in response to increased liver inflammation Despite no differences following αGalCer stimulation among all groups, treatment may restore iNKT specific function in the setting of HIV+HBV+ co-infection.

The role of iNKT cells in promoting liver inflammation requires further investigation to understand whether treatment of HIV+HBV+ ameliorates iNKT function and improves clinical outcome.

REFERENCES

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