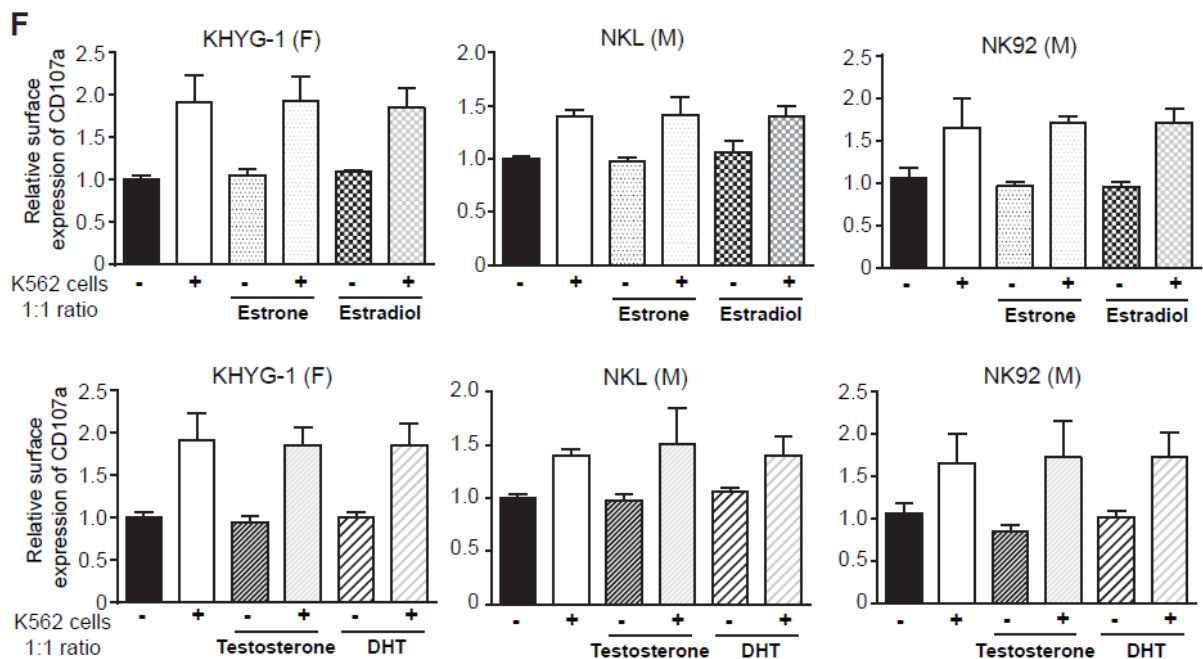
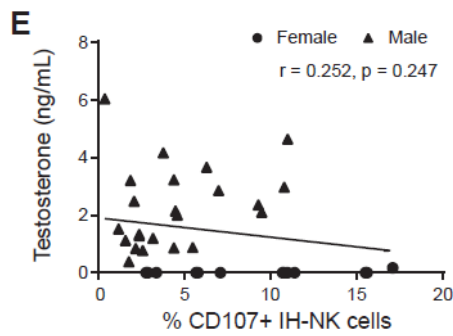
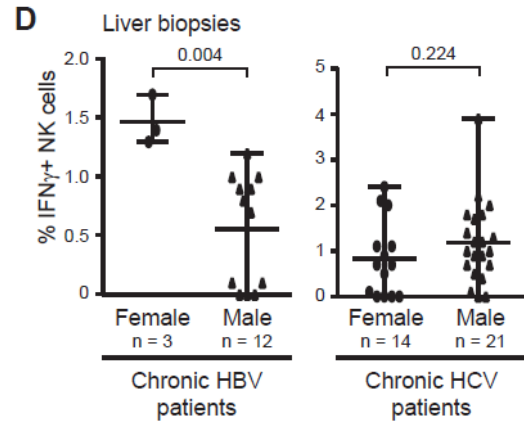
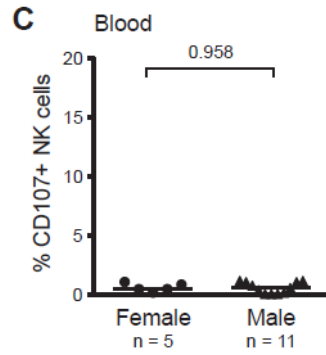
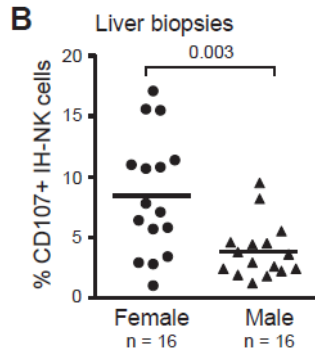
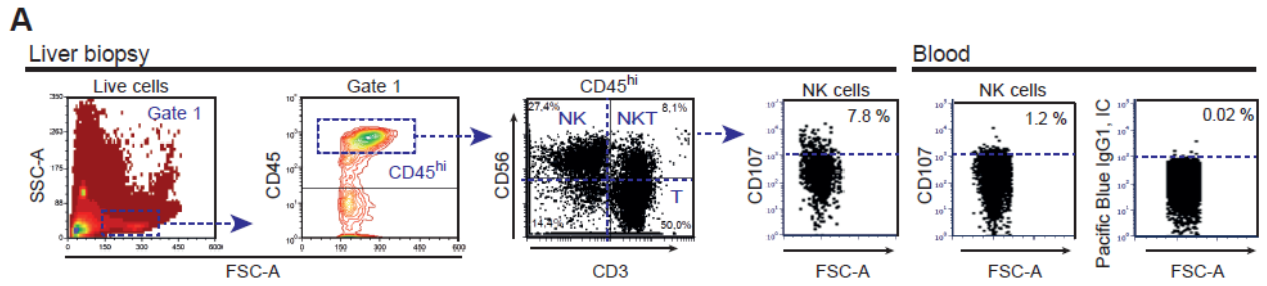


Supplementary Information

Sex differences in spontaneous degranulation activity of intrahepatic natural killer cells during chronic hepatitis B; association with estradiol levels

Zuzana Macek Jilkova^{1,2}, Thomas Decaens^{1,2,3}, Alice Marlu³, Hélène Marche^{1,2}, Evelyne
Jouvin-Marche^{1,2} and Patrice N Marche^{1,2}

¹ Université Grenoble-Alpes, IAB, F38000 Grenoble, France; ² INSERM U1209, F-38000
Grenoble, France; ³ CHU-Grenoble Alpes, Département d'Hépatogastro-entérologie, F30700
La Tronche.



Supplementary Figure 1:

Gating strategy and degranulation of NK cells

A) Gating strategy to investigate degranulation activity of NK cells. Dead cells were excluded and immune cells were identified according their FSC and SSC parameters (left panel). Cells were further gated based on their CD45^{high+} expression and NK cells (CD56⁺CD3⁻), NKT cells (CD56⁺CD3⁺) and T cells (CD56⁻CD3⁺) were selected. To study degranulation activity, surface expression of CD107 was analysed. **B)** Degranulation of NK cells in liver of selected chronic HBV-infected patient cohort. **C)** Degranulation of NK cells from blood of chronic HBV-infected patients. **D)** Frequency of IFN gamma⁺ NK cells in the liver of chronic HBV-infected patients. In this cohort, the liver cell suspension was stained by surface antibodies: CD45, CD3, CD56, then fixed, permeabilized, and stained for IFN γ (BV421, Clone 4S.B3) **E)** Correlation of testosterone serum levels with spontaneous degranulation capacity of intrahepatic NK cells of chronic HBV-infected patients. **F)** Frequencies of CD107⁺ human NK cell lines cultured in media +/- sex hormones for 24h and stimulated by +/- 3h of K562 target cells. Human NK cell lines: KHYG1 (originally from female (F)), NK92 and NKL (originally from male (M)). Cell line experiments were performed in duplicates and repeated at least three times. Gaussian distribution was tested by D'Agostino & Pearson omnibus normality test and non-parametric Mann-Whitney test was used to test HBV cohort.

	Chronic HBV infection			Chronic HCV infection		
	Females	Males	<i>p-value</i>	Females	Males	<i>p-value</i>
N	16	27		39	43	
Age (yr) [*]	44.5 ± 2.9	40 ± 2.6	0.279	53.8 ± 1.8	50.0 ± 1.4	0.116
ALT (IU/L) [*]	39.0 ± 4.2	58.4 ± 10.9	0.217	69.7 ± 10.5	107 ± 13.8	0.033
AST (IU/L) [*]	36.8 ± 4.1	39.9 ± 6.0	0.921	48 ± 6.2	68 ± 11.1	0.117
Viral load (IU/mL) [#]	7574	7435	0.188	646000	869850	0.185
Metavir activity						
A0	4	6		0	0	
A1	9	18		21	14	
A2	3	2		14	21	
A3	0	1		4	8	
Metavir fibrosis						
F0	1	5		0	0	
F0/1	3	1		0	0	
F1	11	15		22	19	
F2	1	2		13	15	
F3	0	3		3	5	
F4	0	1		1	4	

Supplementary Table 1

Demographic and clinical parameters of cohorts.

Demographic and clinical parameters of chronically HBV and HCV-infected patients. ^{*}Data are Mean ± SE, [#]Data are expressed as Median.

Frequency of CD107⁺ intrahepatic NK cells			
Etiology (n=F/M)	Females	Males	<i>p-value</i>
Chronic HBV (16/27)	8.4 ± 1.2	4.5 ± 0.6	<i>0.0061</i>
Chronic HCV (39/43)	4.7 ± 0.6	5.1 ± 0.4	<i>n.s.</i>
NASH (8/11)	4.6 ± 0.5	3.5 ± 0.3	<i>n.s.</i>
AIH (15/2)	8.2 ± 0.9	9.2 ± 0.8	<i>n.s.</i>

Supplementary Table 2

Degranulation of intrahepatic natural killer cells during liver diseases.

Spontaneous degranulation activity of intrahepatic natural killer cells during chronic hepatitis B (HBV), chronic hepatitis C (HCV), Nonalcoholic steatohepatitis (NASH) and Autoimmune hepatitis (AIH). Data are Mean ± SE.

	Females n = 16	Males n = 16	<i>p-value</i>
Age (yr)	44.5 ± 2.9	43.7 ± 2.4	0.82
ALT (IU/L)	39.0 ± 4.2	40.2 ± 3.9	0.83
AST (IU/L)	36.8 ± 4.1	30.8 ± 3.4	0.13
Metavir activity			
A0	4	5	
A1	9	10	
A2	3	1	
Metavir fibrosis			
F0	1	3	
F0/1	3	1	
F1	11	10	
F2	1	2	

Supplementary Table 3

Demographic and clinical parameters of HBV cohort.

Demographic and clinical parameters of chronically HBV-infected patients (n=16). Data are Mean ± SE.

	Non-stimulated			Stimulated		
	F	M	<i>p-value</i>	F	M	<i>p-value</i>
CD 107 ⁺ NK cells	7.8 [2.8-17.1]	4.5 [1.8-6.9]	0.033	12.6 [9.7-20.5]	14.8 [4.9-30.74]	0.965
CD107 ⁺ CD56 ^{Bright} NK cells	6.7 [0-38.9]	3.5 [0-11.1]	0.104	9.1 [0-44.4]	8.8 [7.7-40.0]	0.592
CD107 ⁺ CD56 ^{Dim} NK cells	8.0 [2.7-18.2]	4.7 [1.9-6.5]	0.046	12.2 [9.6-22.7]	13.3 [15.1-4.6]	0.872

Supplementary Table 4

Degranulation of intrahepatic CD56 Bright versus Dim natural killer cells.

Frequency of intrahepatic CD107a⁺ NK cells of chronically HBV-infected females (F, n=7) and males (M, n=7). Without (Non-stimulated) or with (Stimulation) K562 target cells. Data are Median [Min-Max].