IL-21 Attenuates Liver Inflammation By Enhancing Myeloid-Derived Suppressor Cells Immunosuppressive Function In Chronic HBV Infection

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Fig. S1

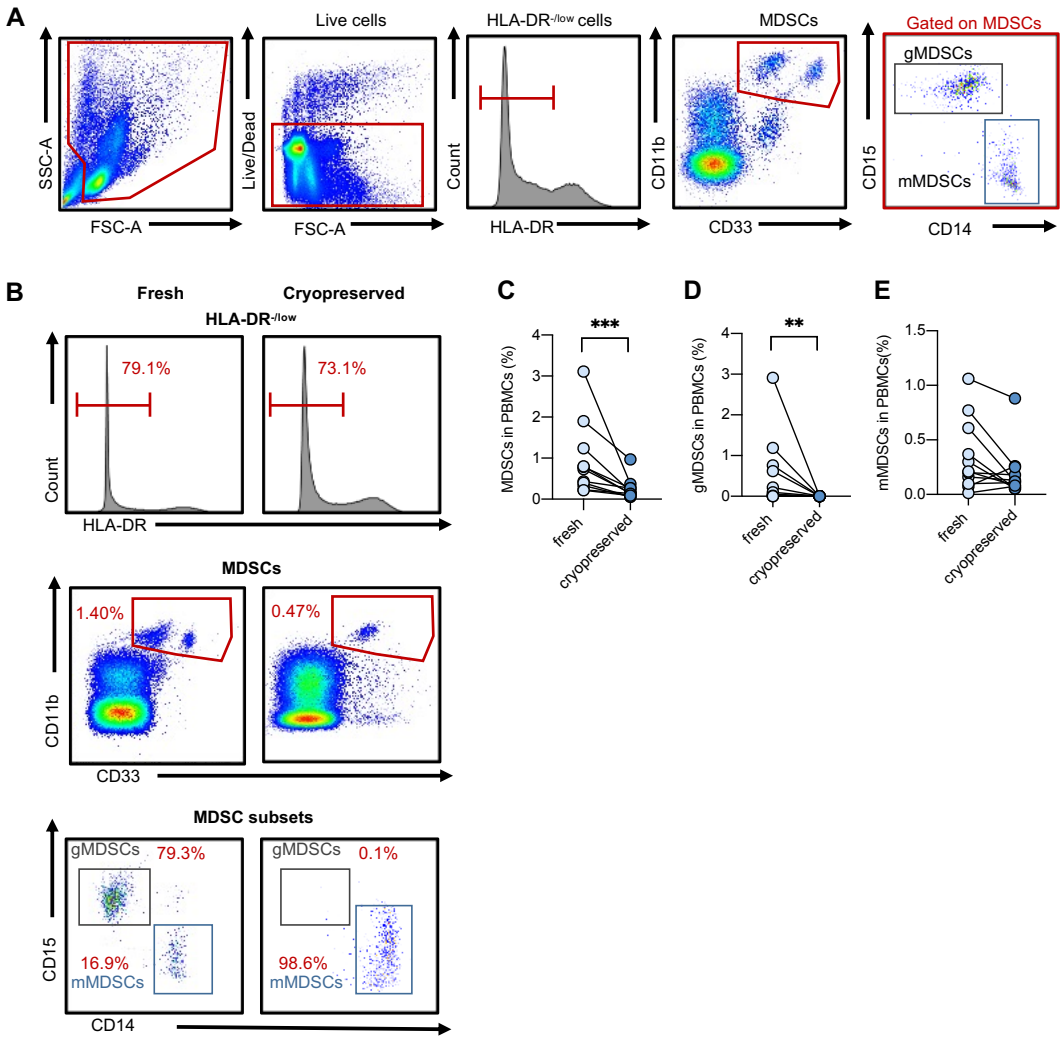

Fig. S1. MDSCs were fragile to freezing and thawing. (A) Flow cytometric gating strategy for identifying MDSCs (HLA-DR$^{-/low}$CD11b^{+}CD33^{+}), using freshly isolated PBMCs from a patient with chronic HBV infection. **(B)** Representative FACS plots showing the gating strategy for MDSC population from paired fresh and cryopreserved blood samples. **(C-E)** Comparison of the frequencies of MDSC **(C)**, gMDSCs **(D)**, and mMDSCs **(E)** between paired fresh and cryopreserved blood samples.

Fig. S2

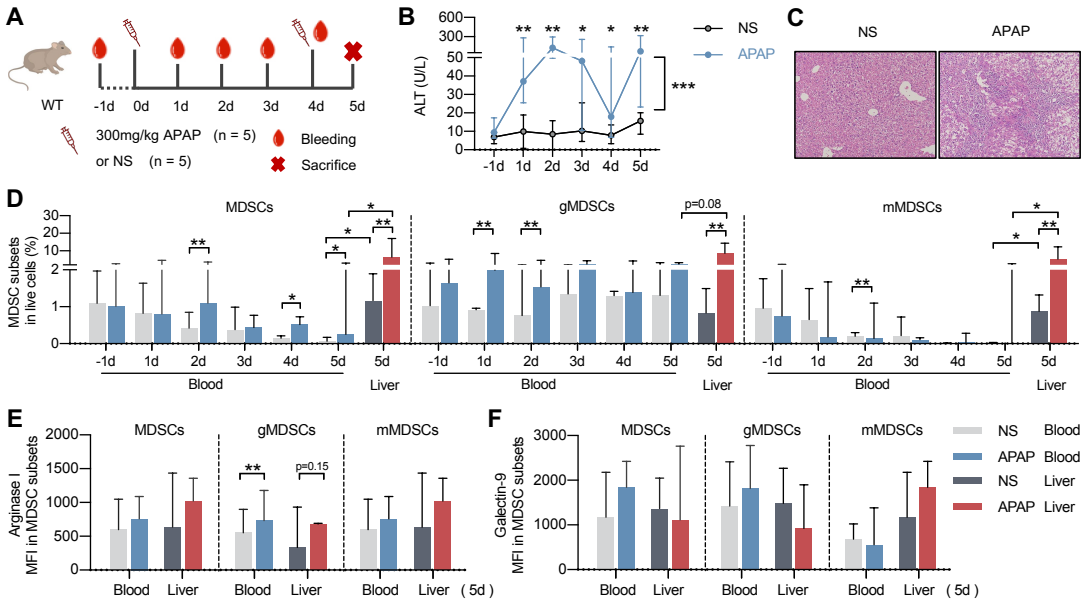

Fig. S2. MDSCs accumulate in the liver in a mice acute liver injury model induced by APAP. (A) Schematic representation of intraperitoneal injection of 300 mg/kg acetaminophen (APAP, n=5) or normal saline (NS, n=5). **(B)** Serum ALT levels at the indicated time points. **(C)** Representative hematoxylin and eosin staining of liver tissues at day 5. **(D)** Frequencies of circulating and intrahepatic MDSC subsets detected at the indicated time points post-injection of APAP or NS. **(E-F)** Mean fluorescence intensity (MFI) of intracellular Arginase I **(E)** and Galectin-9 **(F)** in circulating and intrahepatic MDSC subsets at day 5. *P < 0.05, **P < 0.01.

Fig. S3

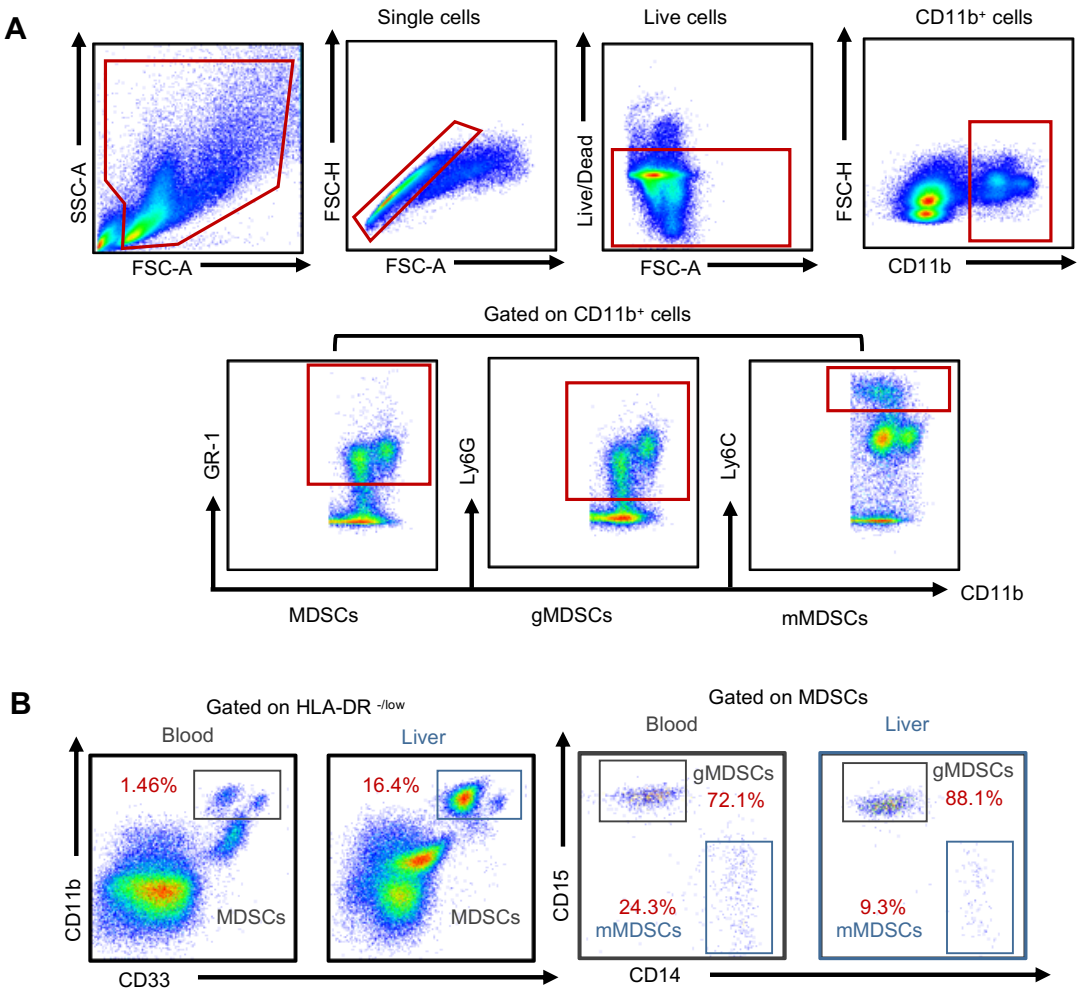

Fig. S3. (A) Flow cytometric gating strategy for the identification of mice MDSCs ($CD11b^+GR\text{-}1^+$), gMDSCs ($CD11b^+Ly6G^+$), and mMDSCs ($CD11b^+Ly6C^+$). **(B)** Representative FACS plots showing the MDSC population gated on the HLA-$DR^{-/low}$ population, and the gMDSC and mMDSC populations gated on the MDSC population from paired blood and liver samples.

Fig. S4

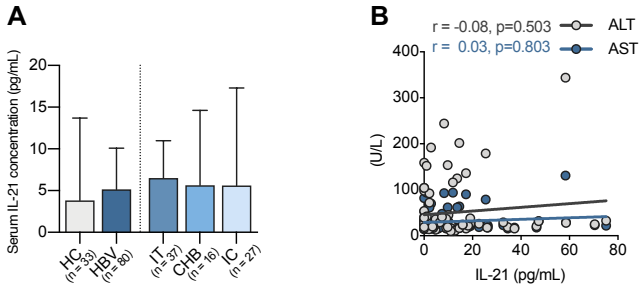

Fig. S4. (A) Comparison of serum IL-21 concentrantion between HC (n=33) and patients with chronic HBV infection (n=80). **(B)** Correlations between IL-21 concentration and serum levels of ALT and AST in chronic HBV infected patients (n=80).

Fig. S5

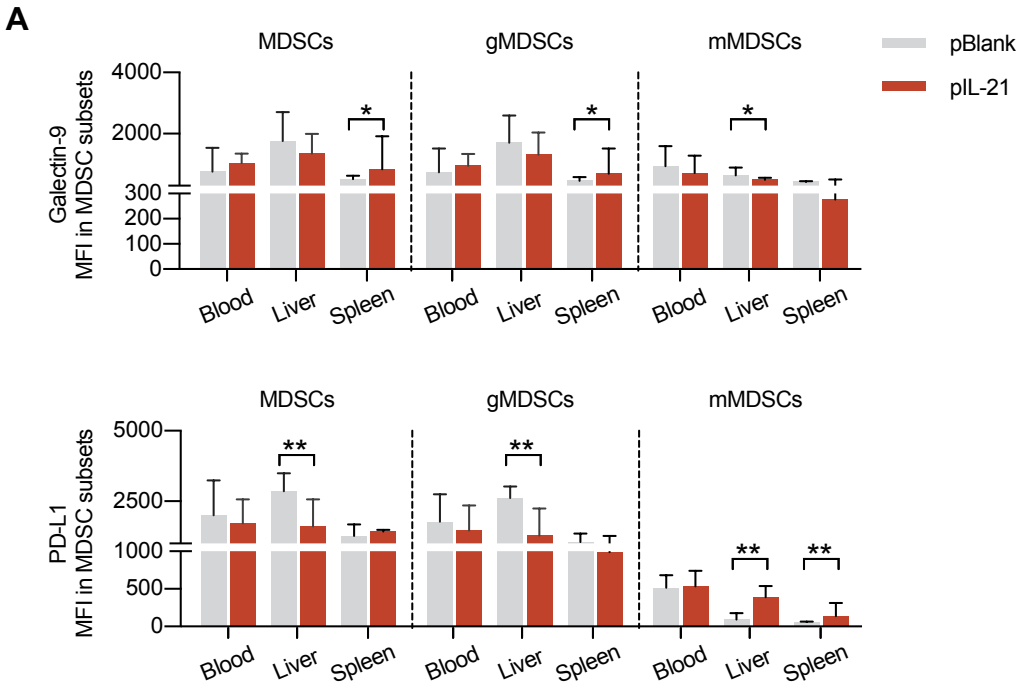

Fig. S5. (A) The expression of Galectin-9 and PD-L1 in circulating, intrahepatic, and intrasplennic MDSC subsets in WT mice administrated with pAAV-HBV1.2 in combination with pIL-21 at day 7 post hydrodynamic injection. *P < 0.05, **P < 0.01. MFI, mean fluorescence intensity.

Table S1. **Clinical characteristics of healthy controls (HCs) and chronically HBV-infected patients classified as IT, hepatitis B e antigen (HBeAg)-positive CHB, and IC.**

	HCs	IT	CHB	IC
Number	33	37	16	27
Gender (male/female)	14/19	23/14	11/5	18/9
Age (years)*	24 (20-32)	26 (19-44)	30 (23-44)	34 (23-58)
ALT (IU/L)*	NA	26.5 (16-45)	144 (102-159)	19.5 (15-35)
HBV DNA (log_{10} IU/mL)*	NA	8.31 (7.16-8.91)	7.92 (6.43-8.93)	2.15 (1.98-3.85)
HBsAg (IU/mL)*	NA	40549.5 (784-52000)	18449 (1017-52000)	1250 (47-11542)
HBeAg (positive/negative)	NA	37/0	16/0	0/27

*Data are shown as median (range). ALT, alanine aminotransferase; HCs, healthy controls; IT, immune tolerant carrier; CHB, hepatitis B e antigen (HBeAg)-positive chronic hepatitis B; IC, inactive carrier; NA, not applicable.

Table S2. **Clinical characteristic of patients with HBV-related hepatocellular carcinoma (HCC) who underwent curative hepatectomy and non-HBV-infected patients with hepatic hemangioma.**

	HBV-related HCC	hepatic hemangioma
Number	9	4
Gender (male/female)	8/1	2/2
Age (years)*	37 (31-56)	42 (38-46)
ALT (IU/L) *	30 (12-65)	11 (5-15)
HBV DNA (log_{10} IU/mL) *	1.98 (1.98-4.65)	NA
HBsAg (IU/mL) *	250 (63-250)	NA
HBeAg (positive/negative)	2/7	NA

*Data are shown as median (range). ALT, alanine aminotransferase; HBV-related HCC, HBV-related hepatocellular carcinoma.

Table S3. **Antibodies used for flow cytometry staining of human and mice cells**

Name	Reactivity	Fluorescence	Supplier	cat no.
Arginase I	Mouse, Human	PE	R&D	IC5868P
CD11b	Human	PE	BD	555388
CD11b	Mouse, Human	FITC	Biolegend	101206
CD11b	Mouse, Human	PE	Biolegend	101208
CD14	Human	APC-H7	BD	560180
CD14	Human	Brilliant Violet 421	BD	563743
CD14	Human	PE/Cyanine7	Biolegend	301814
CD15	Human	APC	BD	551376
CD15	Human	Brilliant Violet 421	Biolegend	323040
CD163	Human	PE/Cyanine7	Biolegend	326514
CD181/CXCR1	Human	Brilliant Violet 510	BD	743420
CD182/CXCR2	Human	Brilliant Violet 510	BD	744196
CD183/CXCR3	Human	Brilliant Violet 421	BD	562558
CD183/CXCR3	Human	PE	Biolegend	353706
CD184/CXCR4	Human	Brilliant Violet 421	BD	562448
CD206	Human	APC	Biolegend	321110
CD33	Human	Brilliant Violet 510	Biolegend	303422
CD33	Human	PE/Dazzle™ 594	Biolegend	303432
CD33	Human	PerCP/Cyanine5.5	Biolegend	303414
CD39	Human	Brilliant Violet 510	Biolegend	328219
CD68	Human	FITC	Biolegend	333806
CD73	Human	Brilliant Violet 421	Biolegend	344008
CD80	Human	PE/Cyanine7	Biolegend	305218
CD86	Human	APC	Biolegend	305411
Galectin-9	Mouse	Brilliant Violet 421	BD	566028
Galectin-9	Human	PE	Biolegend	348906
GR1	Mouse	PerCP/Cyanine5.5	Biolegend	108428
HLA-DR	Human	PE/Cyanine7	BD	560651
HLA-DR	Human	PE	Biolegend	327008
HLA-DR	Human	PE/Cyanine7	Biolegend	307016
HLA-DR	Human	PerCP/Cyanine5.5	Biolegend	307630
IDO	Human	PE	R&D	IC6030P
IL-10R	Human	PE	Biolegend	308804
IL1-RII	Human	PE	R&D	FAB663P
IL-21R	Human	PE	Biolegend	347806
IL-4Rα	Human	PE	Biolegend	355004
IL-6R	Human	Brilliant Violet 480	BD	752530
IL-6Rα	Human	PE	Biolegend	352804

Table S3. **Antibodies used for flow cytometry staining of human and mice cells**

(continuted)

L/D	Mouse, Human	APC-Cyanine7	Invitrogen	L34976
Ly6C	Mouse	APC	Biolegend	128016
Ly6G	Mouse	PE/Cyanine7	Biolegend	127618
NF-κB (pS529)	Human	Brilliant Violet 421	BD	565446
PD1	Human	Brilliant Violet 421	Biolegend	367422
PD-L1	Human	Brilliant Violet 510	Biolegend	329734
PD-L1	Human	PE	Biolegend	329706
PD-L1	Mouse	PE/Dazzle™ 594	Biolegend	124323
STAT1 (pY701)	Human	PerCP/Cyanine5.5	BD	560113
STAT3 (pS727)	Human	Alexa Fluor® 647	BD	558099
STAT5 (pY694)	Human	PE	BD	612599