SUPPLEMENTAL MATERIAL

Kuang et al., http://www.jem.org/cgi/content/full/jem.20082173/DC1

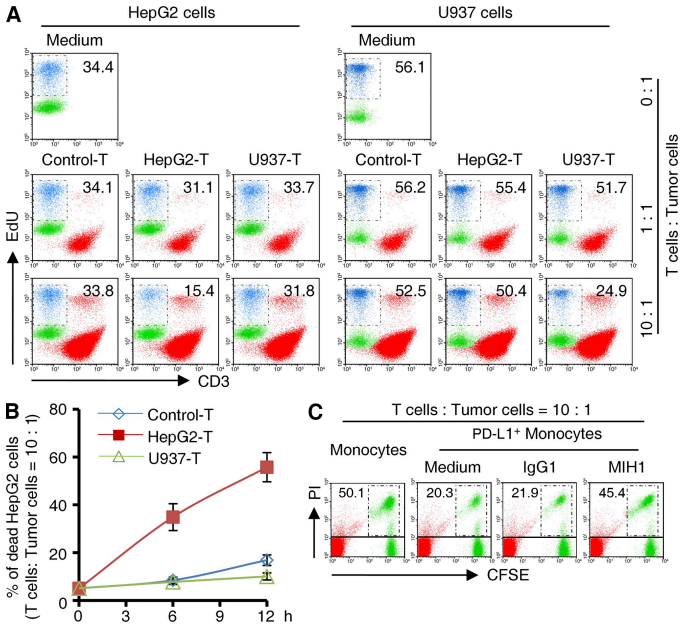

Figure S1. TSN-exposed PD-L1⁺ monocytes regulated T cell cytotoxicity. (A) HepG2 or U937 cells were cultured with control, HepG2-, or U937-associated T cell lines at the indicated ratios for 4 h, with EdU (Invitrogen) present during the final 1 h. Thereafter, cells were collected for EdU staining according to the manufacturer's instructions. The numbers within the dot plots show the percentage of proliferated tumor cells (blue population within $CD3^-$ cells). (B) CFSE-labeled HepG2 cells were cultured with control, HepG2-, or U937-associated T cell lines at a ratio of 1:10 for the indicated times. Thereafter, cells were collected for propidium iodide (PI) staining, and the death of $CSFE^+$ HepG2 cells was determined by FACS. Results are expressed as means ± SEM. (C) HepG2-associated T cells were co-cultured with normal or TSN-exposed PD-L1⁺ monocytes (10:1) in the presence or absence of anti-PD-L1 antibody (MIH1) or control antibody for 24 h. Thereafter, CFSE-labeled HepG2 cells were incubated with these T cells for 6 h, and the death of HepG2 cells was analyzed by FACS. The numbers within the dot plots show the percentage of dead tumor cells (PI^+ in $CFSE^+$ cells). The results shown in A and B suggest that HepG2- or U937-associated T cells can specifically inhibit the proliferation and exert their cytotoxicity against corresponding tumor cells. All FACS data shown are representative of three separate experiments.

Table S1. Blocking effects of IL-10, TNF-α and IL-6 in the co-culture system by specific mAbs

Treatment	IL-10	TNF-α	IL-6
	pg/ml	*pg/ml*	*pg/ml*
Untreated	1,307 ± 138	1,903 ± 242	5,676 ± 691
IL-10 antibody	241 ± 58**	2,056 ± 473	5,184 ± 712
TNF-α antibody	1,136 ± 287	31 ± 7**	4,235 ± 431
IL-6 antibody	1,227 ± 385	2,214 ± 402	508 ± 41**

Monocytes were left untreated or were pretreated with an antibody specific for IL-10, TNF-α, or IL-6 for 1 h, and were then cultured with TSN from SK-Hep-1 cells for 24 h. The concentrations of cytokines in culture supernatants were determined by ELISA. Each value represents the mean ± SD of results from at least four separate experiments. Significant differences from untreated monocytes are indicated (*, $P < 0.05$; and **, $P < 0.01$).

Table S2. Association of peritumoral CD68 cells with clinicopathological characteristics

		Peritumoral stroma CD68 cells		
Variable		Low (cases)	High (cases)	p-value
Age (yr)	≤48	70	61	0.622
	>48	66	65	
Gender	Male	127	108	0.71
	Female	13	14	
HbsAg	Negative	76	85	0.054
	Positive	60	41	
Cirrhosis	Absent	29	20	0.441
	Present	107	106	
ALT (U/liter)	≤42	68	64	0.898
	>42	68	62	
α-Fetoprotein (ng/ml)	≤25	45	32	0.142
	>25	91	94	
Tumor size (cm)	≤5	61	41	<u>0.032</u>
	>5	75	85	
Tumor multiplicity	Solitary	118	90	<u>0.003</u>
	Multiple	18	36	
Vascular invasion	Absent	128	111	0.058
	Present	8	15	
Intrahepatic metastasis	No	124	108	0.151
	Yes	12	18	
TNM stage	I+II	111	82	<u>0.002</u>
	III+IV	25	44	
Tumor differentiation	I+II	112	96	0.265
	III+IV	24	30	
Fibrous capsule	Absent	22	31	0.081
	Present	114	95	

The underlined terms represent statistical significance. ALT, alanine aminotransferase; HbsAg, hepatitis B surface antigen; TNM, tumor node metastasis.

Table S3. Clinical characteristics of the 312 HCC patients

	Group 1	Group 2
Patient characteristics	**Paraffin samples**	**(Blood + tissues)**
Number of patients	262	50
Age (yr; median, range)	48, 17–78	49, 16–78
Gender (male/female)	235/27	44/6
HbsAg (negative/positive)	161/101	31/19
Cirrhosis (absent/present)	49/213	20/30
ALT (U/liter; $\leq$42/>42)	132/130	21/29
AFP (ng/ml; $\leq$25/>25)	77/185	22/28
Tumor size (cm; $\leq$5/>5)	102/160	27/23
Tumor multiplicity (solitary/multiple)	208/54	35/15
Vascular invasion (absent/present)	239/23	45/5
Fibrous capsule (absent/present/ND)	53/209	14/27/9
Intrahepatic metastasis (No/Yes)	232/30	38/12
Tumor differentiation (I+II/III+IV)	208/54	34/16
TNM stage (I+II/III+IV)	193/69	32/18
Peritumoral stroma Mφ (median, range)	118, 22–291	ND

AFP, α-fetoprotein; ALT, alanine aminotransferase; HbsAg, hepatitis B surface antigen; ND, not determined; TNM, tumor node metastasis.