PERSISTENT HEPATITIS C VIRUS (HCV) INFECTION IMPAIRS HCV-SPECIFIC CYTOTOXIC T CELL REACTIVITY THROUGH MCL-1/BIM IMBALANCE DUE TO CD127 DOWN-REGULATION.

Juan R Larrubia, Megha U Lokhande, Joaquín Miquel, Silvia García-Garzón, Alejandro González-Praetorius y Eduardo Sanz-de-Villalobos.
Translational Hepatology Unit, Guadalajara University Hospital. University of Alcalá, Guadalajara, Spain

BACKGROUND AND AIMS

In persistent hepatitis C virus (HCV) infection, HCV-specific cytotoxic T lymphocyte (CTL) reactivity is impaired and this affects HCV control. Interleukin-7 receptor (CD127) expression on these cells could regulate CTL reactivity through Mcl-1/Bim balance modulation. Bim is a pro-apoptotic molecule blocked by the action of Mcl-1.

METHODS

Mcl-1/Bim expression and T cell reactivity on HCV-specific CTLs were compared according to CD127 phenotype. Peripheral blood lymphocytes (PBL) from HLA-A2+ HCV+ patients were obtained. HCV-specific CTLs were visualized by staining PBL with anti-CD8 and HLA-A2/peptide pentameric complexes (pentamer). Mcl-1/Bim/CD127 phenotype of HCV-specific CTLs was tested by staining detectable CD8+/pentamer+ cells with anti-Mcl-1/Bim/CD127 antibodies. HCV-specific CTL proliferation ability after specific in vitro challenge was tested in the presence and absence of pancaspase inhibitor z-VAD-fmk. All stained cells were analysed by flow cytometry.

RESULTS

CD127low-expressing HCV-specific CTLs associated with high HCV viraemia, while CD127high correlated with undetectable viral loads (p<0.001) (Fig.1). Directly ex vivo, pentamer+ cell frequency was similar according to CD127 expression level. Nevertheless, CD127low pentamer+ cell proliferation after specific in vitro challenge was impaired (p<0.05), although this was corrected by z-VAD-fmk treatment (p<0.05) (Fig.2). Mcl-1 expression was low directly ex vivo (p<0.01), and Bim was up-regulated after antigen encounter (p<0.05) of CD127low pentamer+ cells (Fig.3). The ex-vivo difference between Mcl-1 and Bim expression on pentamer+ cells correlated positively with CD127 expression level (p<0.001) and with pentamer+ cell reactivity (p<0.05) (Fig.4).

CONCLUSIONS

A low ex vivo Mcl-1 expression and Bim up regulation after antigen encounter are involved in CD127low HCV-specific CTL hyporeactivity during chronic infection, but it can be overcome by apoptosis blockade.

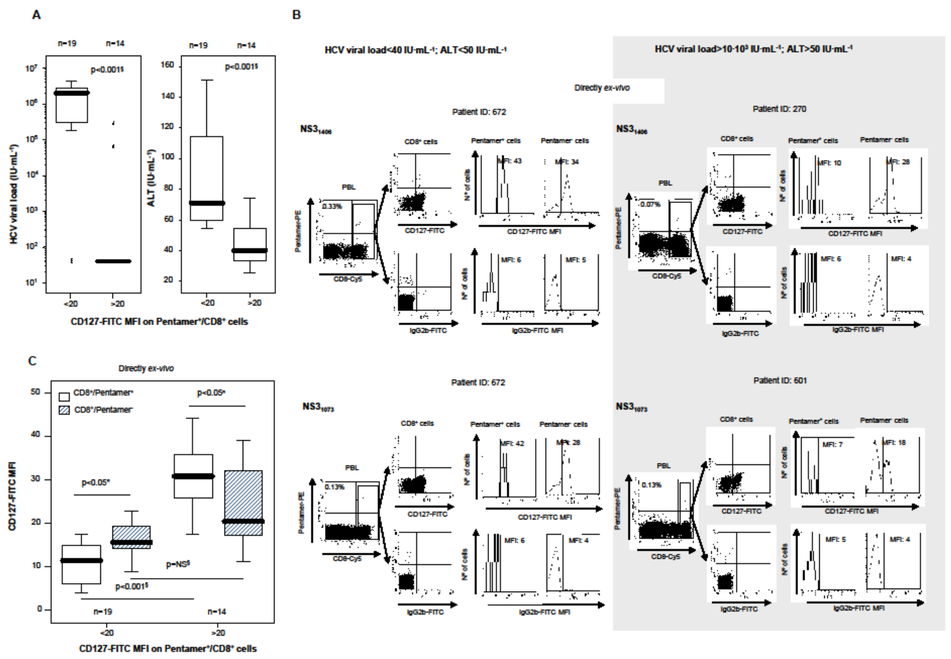

Fig.1 A. Liver damage and viral control according to CD127 expression on hepatitis C virus (HCV)-specific cytotoxic T cells. (a) Box plots showing serum ALT (IU/mL) and viral load (IU/mL) levels according to the CD127 expression on HCV-specific cytotoxic T cells (pentamer+/CD8+ cells). (B) Representative FACSÒ dot plots and MFI histograms of peripheral T cells stained with labelled mAb against CD8 and CD127 and with pentameric HLA-A2/peptide PE-labelled complexes against NS31406 and NS31073 HCV epitopes from cases with different degrees of liver damage and viral control. The figure on the upper left corner of the FACSÒ dot plots for pentamer/CD8 staining shows the frequency of pentamer+ cells out of the total CD8+ cells. FACSÒ histograms are gated on pentamer+ and pentamer- CD8+ cells. The dashed line in the histograms represents the upper limit of the staining with the isotypic control. (c) Box plots showing the directly ex vivo CD127 MFI on total CD8+ and pentamer+ cells in the two groups of the study. §Mann–Whitney U test. ¤Wilconxon test. ID, patient identification; MFI, mean fluorescence intensity; n, number of cases; PBL, peripheral blood lymphocytes. *Outlier values.

Fig.2. Bim and Mcl-1 expression directly ex vivo and after specific stimulation on peripheral CD8+/pentameter+ cells according to CD127 level. Representative FACSÒ dot plots and mean fluorescence intensity (MFI) histograms showing the Mcl-1 and Bim expression on peripheral CD8+/pentamer+ cells, directly ex vivo and after specific in vitro challenge, with regard to the CD127 expression level on those cells. Peripheral blood lymphocytes were subjected to specific in vitro challenge in the presence of z-VAD-fmk and β-galactosidase as control. The figure on the top of the dot plots represents the frequency of pentamer+ cells out of the total CD8+ cells. The figure in the upper right corner in the histogram plot represents the MFI for Bim and Mcl-1 staining. The continuous and dashed line in the dot plots and histograms represent the cut-off point for positive staining according to the negative control. IC, isotypic control; ID, patient identification; N.D., not done because of lack of pentamer+/CD8+ proliferation after specific stimulation.

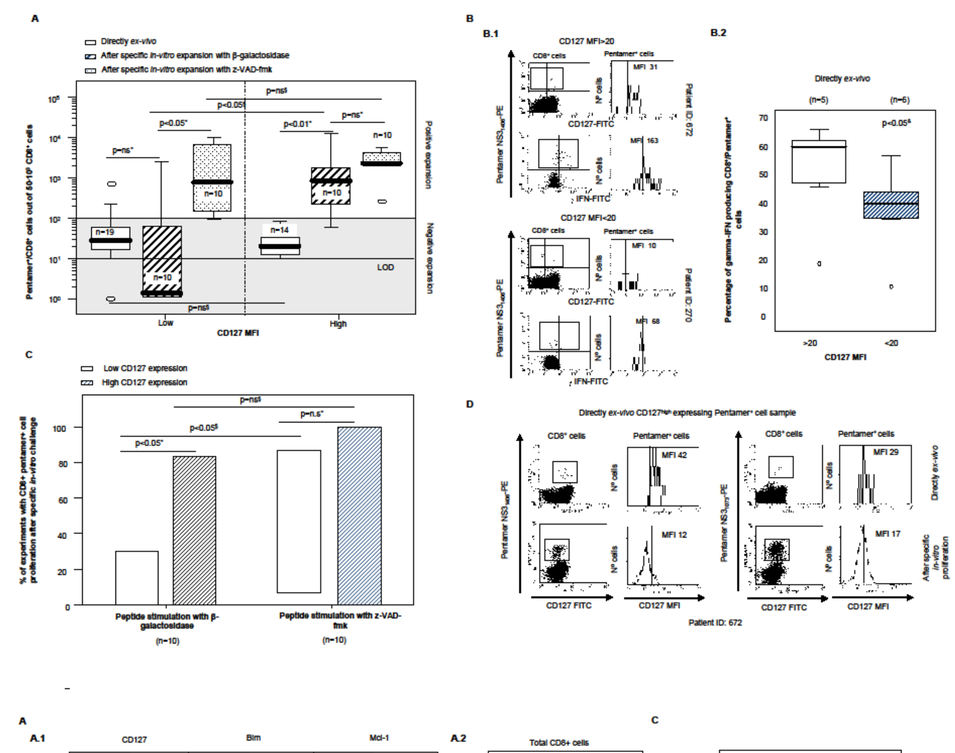

Fig.3. Reactivity of CD8+/pentamer+ cells according to CD127 expression level. (a) Box plots showing the frequency of CD8+/ pentamer+ cells directly ex vivo and after specific in vitro challenge in the presence of either z-VAD-fmk or b-galactosidase as control in high and low CD127-expressing pentamer+ cells. (b) (b.1) Representative FACS dot plots and mean fluorescence intensity (MFI) histograms showing directly ex vivo c-interferon (IFN) production on pentamer+ cells according to CD127 expression. (b.2) Box plots showing the percentage of IFN-producing pentamer+ cells, in relation to CD127 expression level. The figure in the upper right corner in the histogram plot represents the MFI for IFN staining. (c) Bar plots showing the percentage of experiments with CD8+/pentamer+ cell proliferation after specific in vitro challenge in the presence of either z-VAD-fmk or b-galactosidase as control, with regard to CD127 expression level. Positive proliferation was considered when more than 0.2% of CD8+/pentamer+ cells in a clear cluster were observed after specific stimulation. (d) Representative FACSÒ dot plots and MFI histograms from CD127high-expressing pentamer+ cells, with CD127 expression on CD8+/pentamer+ cells directly ex vivo and after specific in vitro challenge. *Wilconxon test. §Mann–Whitney U test. LOD, limit of detection; MFI, mean fluorescence intensity; n, number of cases; n.s., nonsignificant; O, outliers.

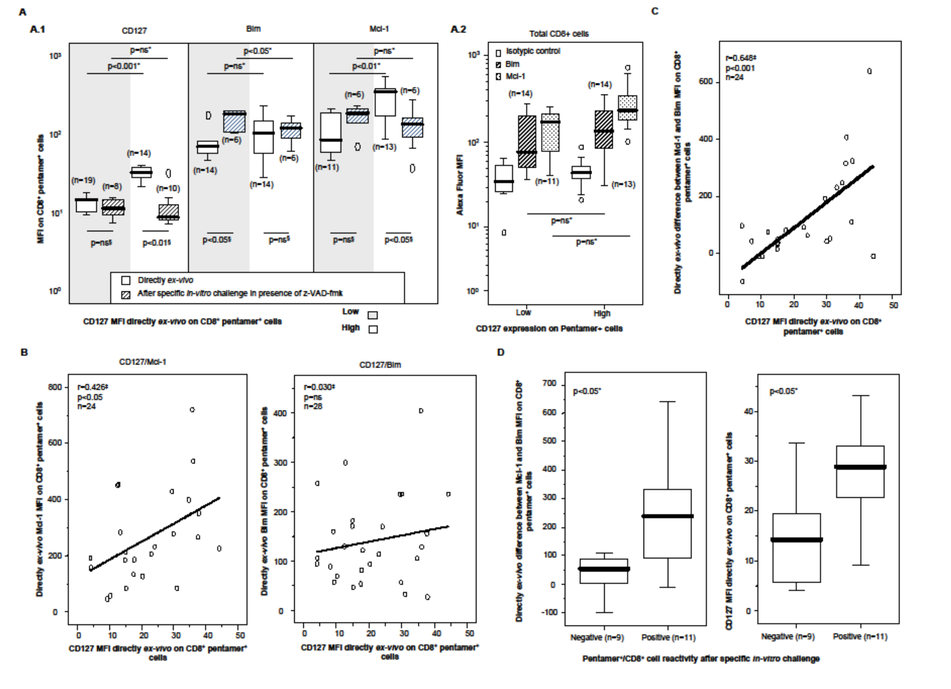

Fig.4. Mcl-1 and Bim expression on CD8+/pentamer+ cells according to CD127 expression level and their relationship with CD8+/pentamer+ cell reactivity after antigen encounter. (a) (a.1) Box plots showing the MFI for CD127, Mcl-1 and Bim staining on peripheral CD8+/pentamer+ cells directly ex vivo and after specific in vitro challenge, blocking apoptosis, according to CD127 expression level. (a.2) Box plots describing the directly ex vivo Mcl-1/Bim expression on total CD8+ cells in the two groups of the study. (b) Scatter plots showing the correlation between CD127 expression on CD8+/pentamer+ cells and Mcl-1/ Bim expression on peripheral CD8+/pentamer+ cells. (c) Scatter plot showing the positive correlation between the Mcl-1/Bim expression subtraction and CD127 expression level on peripheral CD8+/pentamer+ cells. (d) Box plots describing the CD127 expression level and Mcl-1/Bim expression difference on peripheral CD8+/pentamer+ cells with regard to their reactivity after antigen encounter. §Wilconxon test. *Mann–Whitney U test. àSpearmanÕs correlation coefficient. MFI, mean fluorescence intensity; n, number of cases; n.s., nonsignificant; O, outliers.