ADOPTIVE T-CELL THERAPY FOR HERPESVIRUS-INDUCED PATHOLOGIES IN TRANSPLANT RECIPIENTS

Monica Miele[a,b], Marco Sciveres [c], Mariangela Di Bella[a,b], Giandomenico Amico[a,b], Silvia Riva[c], Rosa Liotta[d], Aurelio Sonzogni[e], Paolo Grossi[f], Pier Giulio Conaldi[a,b]

[a]Ri.MED Foundation, Palermo; [b]Regenerative Medicine and Biomedical Technologies Unit, [c] Pediatric Hepatology and Liver Transplantation, [d] Pathology, ISMETT, University of Pittsburgh Medical Center Italy, Palermo; [e] Pathology, Ospedali Riuniti, Bergamo; [f]Infectious Diseases, Università dell'Insubria, Varese

BACKGROUND

Human Herpesviruses (HHV) are human pathogenic agents able to establish latent infections, these viruses rarely cause significant diseases in immunocompetent host. However, in a regimen of immunosuppression as after solid organ transplantation (SOT), patients are highly susceptible to the risk of primary infection/reactivation by HHV. SOT patients are not able to mount an adequate virus-specific T-cell response and can develop systemic or organ specific pathologies with considerable morbidity and mortality. The post-transplantation lymphoproliferative disorder (PTLD) is associated with Epstein-Barr virus (EBV) infection that results in immortalization and transformation of infected B-cells. PTLD is highly frequent in pediatric transplanted patients because it is related to the primary infection in EBV seronegative recipients.
Now, conventional therapeutic approaches can lead to the development of heavy side effects and when these treatments fail the overall mortality rate is above 50%.

Aim: restoration of EBV specific T-cell immunity by adoptive immunotherapy to treat PTLD in solid organ transplant (SOT) recipients

PATIENTS AND METHODS

Thirty liver transplanted pediatric patients were enrolled at ISMETT hospital. They developed biopsy-proven EBV-positive PTLD showing signs of no response or progression during reduction or change of immunosuppression therapy. Five patients were treated with five or six monthly infusions of autologous EBV-specific T-cells. Clinical response was recorded about 5-6 weeks after the first and the second infusion blocks.

CTL production and characterization: EBV-transformed B lymphoblastoid cell lines (LCLs) and EBV-Cytotoxic T lymphocytes (CTL) were generated from peripheral–blood mononuclear cells (PBMC). Protocol is summarized in **Figure 1**.

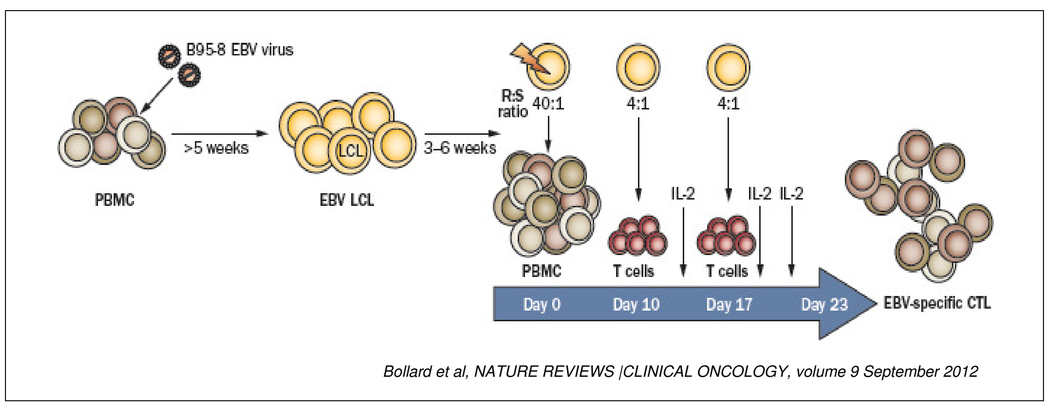

Figure 1: EBV-specific CTL production

RESULTS

CTLs phenotype (**Figure 2**) and *ex vivo* function (**Figure 3)** was characterized before infusion

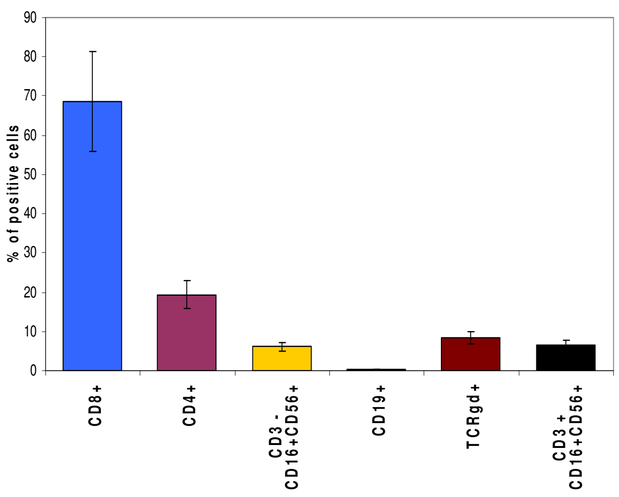

Figure 2: Immunophenotypic analisys by cytofluometry of CTL clones (n=30) expended *in vitro*; the majority of T-cells were CTL; data are shown as the mean ± SEM (Standard error mean)

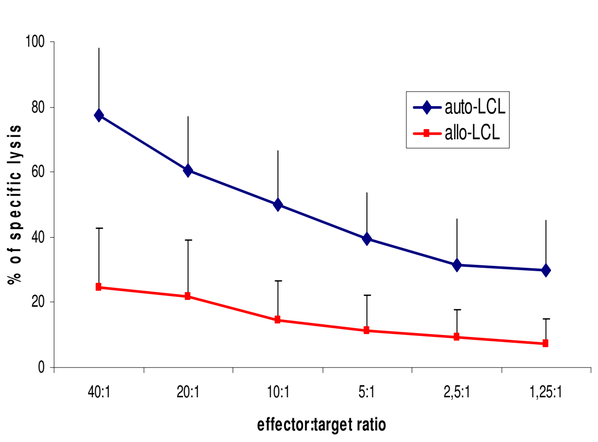

Figure 3: Cytotoxic activity of T-cells (n=29) measured by 4-hour Cr51 release assay; the EBV specifity of these CTLs was confirmed by their preferential lysis of autologous LCLs; data are shown as the mean ± SDV

We also evaluated the degranulation capacity of CTLs analyzing the surface expression of CD107a in response to autologous LCLs compared to allogenic LCLs. Stimulation with PMA-Ionomycin was used as positive control (**Fiaure 4**).

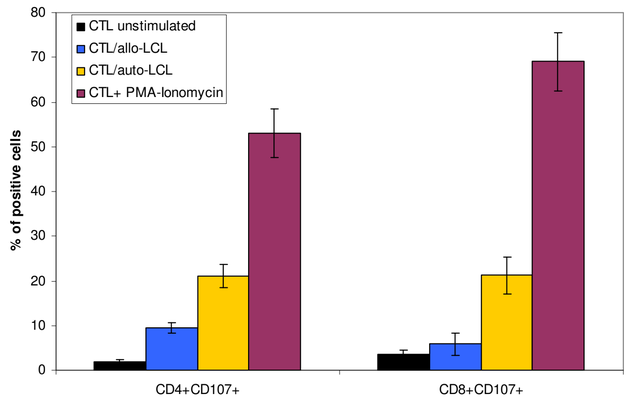

Figure 4: Flow cytometry analysis of CD107a surface expression of CTLs (n= 26), after 4 hour incubation, effector/target ratio 5:1); data are shown as the mean ± SEM.

The breadth of the response to EBV by CTL was investigated. Specifity to antigens, expressed in the latent and lytic phase, was measured by quantifying the frequency of IFN-γ–secreting cells upon stimulation with either autologous LCLs as well as EBV antigen mixtures (**Figure 5**). Furtheremore, the immunodominant EBV antigen were analyzed using a pool of overlapping peptides ranging 7 latent EBV proteins. The EBNA3 complex antigens resulted the strongest stimulus for IFN-γ secretion by T cells **(Figure 6**).

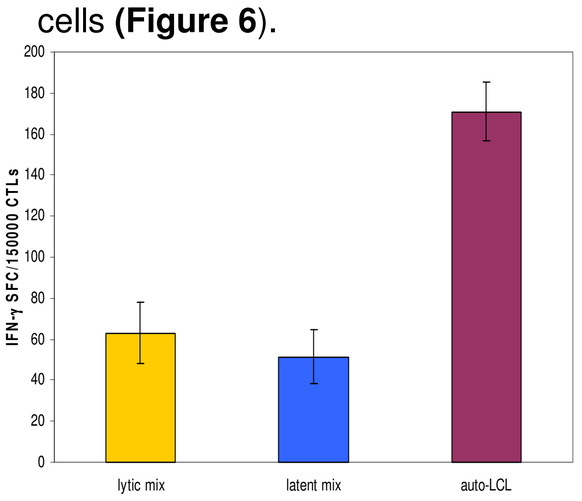

Figure 5: Analysis of EBV specifity of CTLs (n=30) by ELISPOT assay, SFC=spot-forming cells, data are shown as the mean ± SEM.

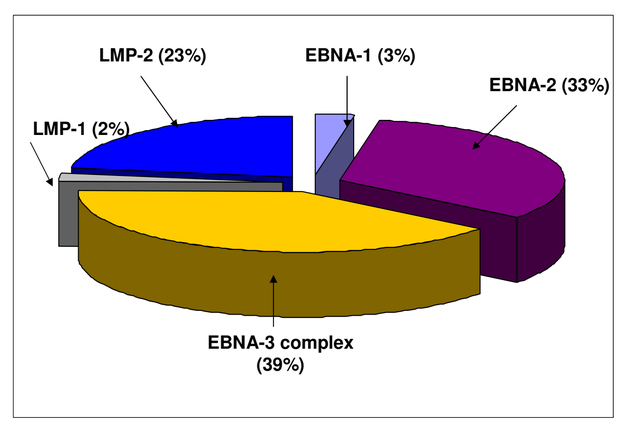

Figure 6: Panel of EBV latent antigen specifity of CTLs (n=10) measured by ELISPOT assay, data are shown as the mean ± SEM.

Five patients received EBV-CTLs as therapy. Infusions were well tolerated and no adverse reactions were recorded. Results of histological assessment (on tissue biopsy) before and after treatment are summarized in **table 1**.

Patient	(TIME 0)			(TIME 1)			(TIME 2)		
	Tonsils/ adenoids	Bowel	Stomach	Tonsils/ adenoids	Bowel	Stomach	Tonsils/ adenoids	Bowel	Stomach
005DM	PM (+)	PM (+)	PM (+)	NA	EL (+)	EL (+)	EL (+)	EL (+)	EL (-)
008MC	EL (+)	EL (+)	Neg	EL (-)	EL (+)	EL (+)	ND	Neg	Neg
009CV	EL (+)	EL (+)	EL (+)	Neg	EL (+)	EL (+)	Neg	EL (-)	Neg
010MG	PM (+)	EL (+)	Neg	EL (+)	EL (+)	EL (+)	EL (-)	EL (-)	EL (-)
011AR	EL(+)	EL (+)	Neg	PM (++)	EL (-)	EL (-)	Neg	EL (-)	Neg.

Table 1: Outcome of treatment after three infusions (1st block, time1) and after five or six infusions (2 nd block, time 2); PM:Polymorphic PTLD; EL: "Early Lesion"; Neg.: No PTLD; EBV–encoded small RNA evaluation (EBER), measured by FISH, is indicated in parenthesis

CONCLUSIONS

The use of cellular immunotherapy to prevent and treat Herpes virus-related complications is safe and feasible in liver transplanted children with EBV related B-cell PTLD. These results shown here confirm that CTLs, generated in our facility, are a reliable tool for cell therapy. We documented downgrading of polymorphic PTLD to "Early Lesions" or to normal tissue in 4/5 patients (one complete regression) and an overall decrease in EBER positive lymphocytes. These preliminary results are encouraging. Forthcoming experiments, include optimization of a novel culture conditions to generate different T-cell subsets characterized by stronger cytotoxic potential *in vivo,* in order to gain optimal therapeutic outcome.