

EXPANSION OF CD4+CD25+CD45RO+IL-7R+ ACTIVATED T CELLS IN LIVER TRANSPLANT RECIPIENTS RECEIVING CNI- BASED THERAPY: DOWNMODULATION BY HCV

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INTRODUCTION

Among CD4+CD25+ T cells there are T regulatory (T regs) cells and activated CD4 T cells.

T regulatory cells are recognized to have a crucial role in suppressing immune responses to self antigens, in preventing autoimmune diseases and in controlling immune responses to pathogens. In the last years there is emerging evidence of a critical role of Tregs in the context of transplantation and on the role of T regs to induce transplantation tolerance.

CD4+CD25+ T cells can also be a population of activated T cells that exert effector functions with production of cytokines and can be responsible, in the context of transplantation, for donor specific responses that induce to the rejection of the graft.

Both CD4+CD25+ T regs cells and activated CD4 T cells can have a crucial effect in the context of transplantation, the first with the possible role of inducing tolerance to the graft and the second in its rejection.

Some authors have observed a higher number of CD4+CD25+ T regulatory cells in individuals chronically infected with HCV. This observation could be relevant in the context of liver transplant recipients infected with HCV. A higher number of T regs in liver transplant recipients infected with HCV, even if detrimental for the persistence of infection, could be positive towards the tolerance of the graft.

Different Populations of Activated CD4+ T Cells

Activated CD4 T cells

- transient expression of CD25
- CD45RO positive
- CD127(IL7-R) positive
- no constitutive expression of Fox p-3
- effector functions

T regulatory cells

- sustained expression of higher level of CD25
- CD45RO positive
- CD127(IL7-R) negative
- constitutive expression of Foxp3
- suppressor functions

AIM OF THE STUDY

The aim of our study was to define the different sub-populations of CD4 T cells distinguishing between T regs and activated CD4 T cells in different cohorts of liver transplant recipients and to evaluate the role of HCV on the different subsets of CD4 T cells.

PATIENTS AND METHODS

We enrolled for this study 21 liver transplant recipients infected with HCV (end stage liver disease HCV related) and 22 liver transplant recipients non HCV infected. As controls we studied 25 healthy individuals.

All patients gave informed written consent to participate to the study approved by local IRB.

Cell surface analysis and sorting were performed using a combination of surface markers for the expression of: CD4, CD25, CD45RO and IL7-R. The different sub-populations of CD4T cells were sorted using FACS vantage.

To evaluate the suppression function of T regs cells we used mixed lymphocyte reaction (MLR) using the recipient's cells as responders.

The expression of Foxp-3 were evaluated through RT-PCR.

RESULTS

Selective Expansion of CD4+CD25+CD45RO+IL-7R+ T Cells in HCV Negative Liver Transplanted Patients

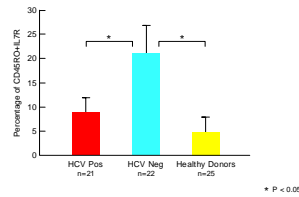


Figure 1
The CD45RO+IL-7R+ T cells were significantly expanded (21%+/- 7% of CD4+CD25+ cells) in HCV negative transplant recipients compared to HCV positive transplant recipients (9%+/-5%) and to healthy individuals (5%+/-3%)(P<0.05).

Suppression Capacity of CD4+CD25+CD127+ and CD4+CD25+CD127- T Cells Populations in MLR

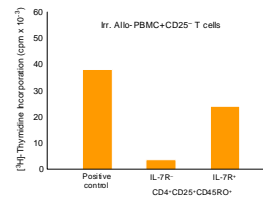


Figure 2
To better characterised the different sub-populations of CD4 T cells we sorted the population expressing CD25, CD45RO and IL-7R and the population expressing CD25, CD45RO but negative for IL-7R to perform functional analysis. The population expressing IL-7R had no suppressor functions compared to population negative for IL-7R that had suppressor functions.

Expression of Fox-p3 in CD4+CD25+IL-7R+ and CD4+CD25+CD127- T Cells Populations

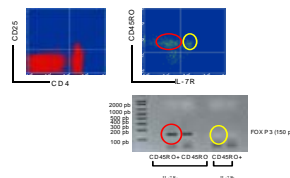


Figure 3
The CD4+CD25+CD45RO+IL-7R- cells do not express Foxp-3 while CD4+CD25+CD45RO+IL-7R+ express Foxp-3 as typical T regs cells.

Changes in CD4+CD25+CD45RO+IL-7R+ T Cells after Viral Clearance Following Antiviral Therapy (PEGIFN+RBV)

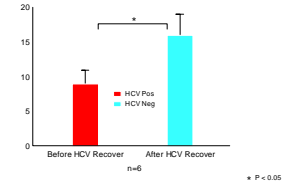


Figure 4
To analyse the impact of HCV infection we evaluated the percentage of CD4+CD25+CD45RO+IL-7R+ before and after clearance of the virus through the treatment with ribavirin and PEGIFN. The patients treated with antiviral therapy with clearance of the virus present a higher expression of CD4+CD25+CD45RO+IL-7R+.

No Changes in CD4+CD25+CD45RO+IL-7R+ T Cells in Absence of Clearance Following Antiviral Therapy (PEGIFN+RBV)

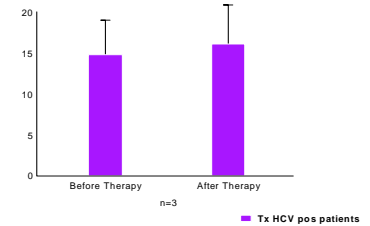


Figure 5
We analysed three HCV positive transplant recipients treated with ribavirin and PEGIFN who did not respond to the antiviral treatment and remained always positive for HCV. In this patients the percentage of CD4+CD25+CD45RO+IL-7R+ T cells remained unchanged.

CONCLUSIONS

We have recently identified IL-7 receptor (IL-7R) as a useful marker to distinguish different populations of CD4 T cells; regulatory T cells (defined by the expression of Foxp3) are mainly CD45RO+IL-7R- while activated T cells (which are Foxp3 negative) are mainly CD45RO+IL-7R+.

In liver transplant recipients we found that the CD45RO+IL-7R+ subset is significantly expanded. This expanded population is Foxp3 negative and does not have suppressor functions in MLR as typical activated antigen specific CD4+CD25+ T cells.

This population seems to be modulated by HCV: in HCV negative transplant recipients it is significantly expanded compared to HCV positive transplant recipients.

Furthermore in HCV positive transplant recipients treated successfully with antiviral therapy we observed a significant increase of this population reaching values similar to HCV negative transplant recipients.

Changes in this population are due to the clearance of the virus and not to the presence of antiviral treatment (PEGIFN) since we do not observe any changes of this subset in HCV patients on treatment but without virological response.

Further phenotypical and functional analyses are ongoing to determine whether this T cell population is donor specific.