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Evaluation of T cell response in chronic HCV patients treated with PegINF-free regimen compared to PegINF therapy

Hepatitis C virus (HCV) is a major cause of chronic hepatitis worldwide. Present estimates predict that between 185 million people worldwide are infected with HCV with the majority of all infections progressing to chronicity, ultimately leading to fibrosis, cirrhosis and hepatocellular carcinoma. Virus-specific $CD8^+$ T cells play a central role in the outcome of acute HCV infection, indeed, spontaneous viral clearance is associated with vigorous and functional $CD8^+$T cell responses (1).

In contrast, a hallmark of chronic HCV infection is the presence of functionally impaired virus-specific $CD8^+$ T cells that are characterized by their inability to secrete antiviral cytokines, such as IFN-gamma, or to proliferate (2). This state of T cell exhaustion is characterized by an up-regulation of PD1 and low expression of CD127 (3), and is mediated by the co-expression of several different inhibitory receptors. Indeed, CD127low HCV-specific $CD8^+$ T cells were shown to co-express the inhibitory receptors 2B4, KLRG1, and CD160 in addition to PD-1 in chronic HCV infection (4). It has also been shown that the composition of the memory $CD4^+$ and $CD8^+$ T lymphocyte compartment is altered during HCV infection (5). Furthermore natural killer (NK) cells, are important mediator of antiviral response at all stages of HCV infection (6), and have been demonstrated to have altered frequency in circulating and hepatic compartments during HCV infection (7). Additionally, emerging data suggest that rapid viral clearance resets the changes that occur in NK cell compartment due to chronic HCV infection (8).

Besides regulatory T cells (Tregs) has a pivotal role in the control of the balance between host damage and viral control produced by specific immune response. HCV infection increases the frequency of Treg cells and the exent of suppression irrespective of the outcome of the infection. (9). Early successful treatment with pegylated type I interferon (PegIFN) during acute infection may rescue functional HCV-specific $CD8^+$T cells and thus prevent the development of T cell exhaustion

caused by ongoing recognition of viral antigen (10). In contrast, PegIFN therapy mediated immune restoration has not been reported at later stages of infection, during chronic infection. The recent introduction of direct antiviral agents (DAAs) regimens that do not involve PegIFN, provides a unique opportunity to determine whether successful treatment-induced eradication of viral antigen leads to reconstitution of T-cell immunity.

Therefore the aim of this study is to analyse and compare the impact of inhibition of ongoing viral replication by IFN-free therapies with DAAs on the phenotype and function of the innate and adaptive immune responses as compared to PegIFN+Ribavirine therapy. The study is carried out in collaboration with the Infectious Diseases Center of Azienda Ospedaliera of Padova.

To this purpose, I will analyze groups of patients with HCV chronic infection i) untreated, ii) treated with PegIFN+Rib, iii) treated with the second generation of DAAs (that includes sofosbuvir, simeprevir, and fixed combination medicines Harvoni and Viekira Pak) vi) pharmacologically cured patients. After purification and stimulation of peripheral blood mononuclear cells, the phenotype of HCV-specific $CD8^+$ $CD4^+$ T cells (CD3, CD4, CD8) will be studied; by means of flow cytofluorimetry, T cell activation (IFN-gamma, CD45, CD38, CD57, CD27, CD31) and T cell exhaustion (CD127, 2B4, KLRG1, CD160 and PD1) phenotypes will be determined. In addition, quantification of T general population through proliferation assay and apoptotic assay (Annexin V), T-reg (CD4, CD25, Foxp3) and NK cell activation (CD16, CD56 and CD57), will be analyzed.

The expected results are the restoration of T cell response, therefore inhibition of T cell exhaustion. This study will give information about T cell response in patient pharmacologically cured in order to prevent a new reinfection with HCV. Besides, reversing T cell exhaustion could provide a promising therapeutic approach for restoring an effective natural immunological control over persistent viral infections.
It will be useful to determine how information about T cell response can be integrated into the development of therapeutic and prophylactic vaccination strategies for this disease.

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