# Increased T and B Regulatory Cell Function Contributes to the Persistence of HCV and Other Viral Infections

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Viral infections are eradicated by efficient T and natural killer (NK) cell responses. When these responses fail, viruses escape and persist in relevant cells causing continuous inflammation and chronic organ damage. In this review, we will focus on the mechanisms by which hepatitis C virus (HCV) infection escapes efficient peripheral clearance, and on the role of T regulatory (Treg) cells in the persistence of HCV infection. We will also review the issue of B regulatory (Breg) cells and their role in viral evasion and failure of cellular immune responses to eliminate these infections.

Peripheral immune responses against HCV antigens, namely effector T cells, and natural killer (NK) cell activity act as a first-line defense aiming to clear HCV infection and inhibit its escape to the liver. The failure of the above immune responses to clear HCV from peripheral blood was considered to be the result of one of two mechanisms. The first is ligation of CD81 (HCV receptor on many immune cells) by binding major envelope protein HCV (HCV-E2). Engagement of CD81 on NK cells inhibits their cytotoxic activity through a mechanism that is distinct from the negative signaling pathways associated with NK cell inhibitory receptors for major histocompatibility complex class I. Thus, NK cell inhibition allows HCV evasion strategy and the development of chronic infection [1]. The second is enhanced peripheral T cell apoptosis, reported by us to be another mechanism by which HCV could possibly escape efficient peripheral cellular immune responses. Increased Th1 apoptosis was found to be in association with decreased NF-kB (but not bcl-2) expression. Of the other possible mechanisms by which HCV infection increases the sensitivity of T cells to undergo apoptosis, one could mention: (i) down-regulation of MHC class II and B7 molecules on HCV-infected dendritic cells, thus attenuating the delivery of co-stimulation; (ii) inhibition of the production of interferon-gamma (IFNγ) and interleukin (IL)-12, leading to enhanced responsiveness to IL-10 modulating effects and down-regulation of Th1 responses; (iii) induction of an autoimmune responses due to cross-reactions between HCV core proteins and cryptic epitopes on activated T cells; and (iv) enhancement of T cell apoptosis by causing G1/S arrest and c-myc up-regulation [2].

## T REGULATORY CELLS AND HCV INFECTION

T regulatory cells (Tregs) (CD4+CD25high) are highly important in maintaining self-tolerance and therefore very important in defining the course of many autoimmune diseases, malignancy and infections (mainly viral) [3]. In this case the balance between Treg cell function and the efficient antiviral effector Th1 cell function was discussed with respect to HCV chronic infection. In a recent study, Treg cell function was found to be enhanced in patients suffering from chronic HCV infection. Here, the expression of granzyme B on CD4+CD25high T cells of HCV patients was significantly higher than that on Treg cells of normal individuals (in which granzyme B is almost absent). Treg cells from chronic HCV patients produced higher amounts of granzyme B, thereby increasing Th1 apoptosis and suppressing autologous CD4+CD25low effector T cell activity and reducing CD4+ T cell response against HCV [4]. In another study, CD4+CD25highFoxP3+ Treg cells were increased in patients with chronic hepatitis C and were positively correlated with HCV RNA and HCV core protein levels in the blood. In addition, HCV core protein induced the secretion of IL-10 and transforming growth factor-beta (TGFβ) by CD4+CD25high Treg cells, thereby decreasing CD4+ T cell proliferation and INFγ production [5]. In agreement with the above, T regulatory cells (CD4+CD25high) were found to be activated early in patients with acute and chronic HCV, probably causing low activity of effector T cells which allows the survival of hepatitis C virus [6]. The eradication of HCV by ribavirin was shown to be through polarization of Th cell balance into Th1 dominance. In this respect, ribavirin inhibited the differentiation of naïve Th cells into adaptive Treg cells by down-modulating FoxP3. This results in the maintenance of Th1 activity, pointing to the role of increased Treg function in HCV infection [7].

## B REGULATORY CELLS

B lymphocytes play a key role in both the innate and adaptive immune systems. Apart from being the source of antibodies,

they are efficient antigen-presenting cells (APCs) and producers of pro-inflammatory cytokines. On the other hand, B cells are a source of inhibitory cytokines such as IL-10 and TGFβ. Depending on the signals B cells receive, pro- or anti-inflammatory cytokines can be produced, and the shift towards an inflammatory or a protective/regulatory response will be induced.

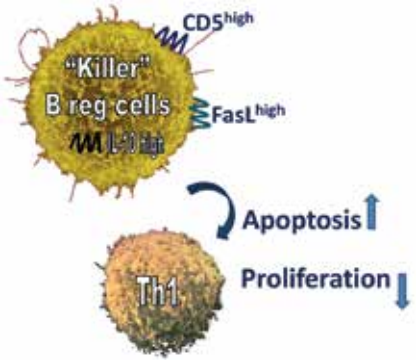
B regulatory (Breg) cells were intensively studied in the last decade and were shown to be of many phenotypic subsets. CD19+CD38highCD24highCD1dhigh were reported to be IL-10 producers, able to suppress the differentiation of Th1effector cells. When assessed in patients with systemic lupus erythematosus (SLE), they failed to suppress tumor necrosis factor (TNF) and IFN production by Th1 cells compared to their ability in healthy individuals [8]. In our laboratory, we characterized Breg cells being CD19+CD25highCD86highIL-10high. Here also, these cells were able to suppress Th1 cell proliferation and also to enhance Treg cell phenotypic characteristics when co-cultured together. These results suggested that Breg cells may induce their suppressive function by increasing Treg cell function [9]. Similar to T regulatory cells, the function of B regulatory cells was also shown to be enhanced in malignancy and during long-lasting infections, thus delaying proper T cell responses and control of malignancy spread [10]. This concept was recently strengthened by a study demonstrating that B regulatory cells were increased in HIV patients. In this study, it was suggested that in an IL-10 and PD-L 1 synergistic mechanism, regulatory B cells may inhibit APC function and autologous CD4+ T cell proliferation, leading to anti-HIV CTL attenuation, thereby hindering viral eradication [11]. B regulatory cells are expanded by many well-established stimulatory signals such as CD40L and CpG. In a recent study it was also elegantly shown that the addition of IL-21 and IL-35 increased regulatory activity and IL-10-producing B cells, resulting in the modulation of inflammatory immune responses and limitation of autoimmune diseases [12,13].

#### "KILLER" B REGULATORY CELLS IN VIRAL INFECTIONS

Herpes simplex virus type I (HSV) was reported to induce a population of B cells in spleens of mice, which can inhibit lymphocyte proliferation upon stimulation with lectins and alloantigens. In this early study, B cell suppression was suggested to occur by mechanisms such as interference with antigen-presenting cells, the neutralization of cytokines/lymphokines, and the release of mediators that inhibit T cell activation. Such suppression may allow the escape of HSV from efficient immune responses [14]. The FasLigand/Fas receptor pathway was studied extensively as a mechanism of killing over-active CD4+ T cells and other immune cells, thereby preventing autoimmunity. Evidence has emerged that in addition to activated cytotoxic T cells (CTL) and natural killer (NK) cells, B lymphocytes were also found to express Fas ligand, thus mediating cell death of many over-activated immune cells. The expression of Fas ligand and IL-10 and other death-inducing ligands such as TRAIL, PD-L1 and PD-L2 was highest on CD5+ B cells, suggesting this subset to be unique in the field of human Breg cells. In this case CD19+CD5highFaslhigh were defined as "killer" Breg cells and considered to play a role in preventing autoimmunity but allowing cancer spread and persistence of infections [Figure 1] [15]. Killer B cells were initially reported in a murine model of *Schistosoma mansoni* infection. In this model, female worms secrete soluble antigens leading to T cell-mediated granulomatous inflammation in the host liver. The study showed that splenic B cells defined as CD5+FasLhigh mediate apoptosis of CD4+ T lymphocytes during the early stage of *Schistosoma* infection [16]. Furthermore, it was shown that the lacto-N-fucopentaose (LNFP-III), a component of *Schistosoma* eggs, is able to stimulate IL-10 production from B cells and induce Fas ligand expression on CD5+ B cells [17]. Viral infections have been reported to induce Fas ligand expression in CD5+ B cells in human peripheral blood. In a study of patients with acute infectious mononucleosis (caused by Epstein-Barr virus), it was shown that EBV increases Fas receptor expression on CD4+ T cells as well as Fas ligand on B cells and macrophages, leading together to increased CD4+T cell apoptosis. In addition, the study showed that EBV glycoprotein gp350, which initiates virion attachment to CD21 receptor on host B cell leading to virus entry, can function as an immune modulator and leads to increased CD95 expression in CD4+ T cells [18]. A similar study on HIV-infected individuals has shown over-expression of Fas ligand on CD5+ B cells and increased levels of Fas receptor on T cells stimulated by HIV gp120 which initiates virion attachment to the host cell leading to virus entry [19].

The expansion of CD5+FasLhigh B cells allows persistence of viruses and their escape from efficient T cell responses. Future studies should assess the possible role of this subset of cells in the development of chronic HCV and other viral infections. Targeting "killer" Breg cells may become one of the therapeutic tools in viral infections.

**Figure 1.** "Killer" B regulatory cells are characterized by being CD5(high)Fasligand(high) and expressing/producing high amounts of IL-10. They decrease their proliferation and increase Th1 apoptosis



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