

Role of IL-10 and IL28B in HCV Infected Patients: A Short Review

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ABSTRACT

The hepatitis C virus (HCV) infection is pandemic and has been systematically studied, characterized in North America and Europe, but this important public health problem has not received tantamount attention in other regions. Hepatitis C virus (HCV) was detected from the serum of infected animal by Choo *et al* (1989), it is now well accomplished that HCV infection pretends all countries, leading to a major global health problem that requires far-flung active interventions for its prevention and control. Subsequently HCV infection, the innate immune response is initially important for controlling viral replication with the adaptive immune reaction topping out at 8-weeks after infection. Finally, a coordinated attempt between the innate and adaptive immune responses is necessary to eliminate HCV from the liver. Cytokines play an important role in the regulation of immune response may influence the outcome of acute HCV infection. Interleukin-10 (IL-10) is having multiple function anti-inflammatory cytokine mainly produced by immune cells, such as T cells, monocytes, appropriately stimulated macrophages, some subsets of dendritic cells (DCs), and B cells. IL28B gene encodes a cytokine distantly related to type I interferon and the IL-10 family. IL-10, interleukin 28B (IL28B), and interleukin 29 (IL29) are three closely related cytokine genes that form a cytokine gene cluster on a chromosomal region mapped to 19q13. Expression of the cytokines encoded by the three genes can be induced by viral infection.

Keywords: Hepatitis C virus (HCV), Untranslated regions (UTRs), Interleukin, Cirrhosis.

INTRODUCTION

Hepatitis C virus (HCV) was detected from the serum of infected animal by Choo *et al* (1989). It is a single stranded RNA virus (family-Flaviviridae and genus-Hepacivirus) (Flamm *et al* 2003), 9.8 kb in length encoding for a single polyprotein precursor of approximately 3000 residues flanked by untranslated regions (UTRs) at both ends. Hepatitis C is an infection that leads to liver inflammation. Most people are symptom-free and remain unaware of the infection for a long time;

therefore it is known as a “silent killer”. Near about 15 to 20 percent of people infected with hepatitis C virus fight it off without antiviral treatment and suffer no long-term damage to their health. The first six months following infection is the acute phase of the disease. Most people who have been infected will progress to the chronic phase. However, even in the chronic phase, it may take years for symptoms to appear. Progression begins with inflammation of the liver, followed by the death of liver cells. This causes replacement of healthy liver cells by permanent scar tissue thereby causing scarring and hardening (cirrhosis) of liver tissue. In HCV infection, the production of inappropriate cytokine levels seems contribute to viral persistence and to affect response to therapy. Cytokine genes are polymorphic at particular sites and certain mutations located within coding regions and have been shown to affect the overall expression and secretion of cytokines.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine, produced by immune cells, such as T cells, monocytes, appropriately stimulated macrophages, some subsets of dendritic cells (DCs), and B cells. IL28B gene encodes a cytokine distantly related to type I interferon and the IL-10 family. Expression of the cytokines encoded by the three genes can be induced by viral infection. However the exact role of the immune response in patients with HCV infection is still unclear.

Hepatitis C Virus:

Following 2nd World War (1939-1945), blood transfusion-associated Hepatitis became one of the major hazards. After the identification of Hepatitis B Virus (HBV) as an infectious agent causing hepatitis and multiple screening assays for HBV antigen were introduced in the 1970's. Although these interventions dramatically decreased rates of transfusion-associated hepatitis, still many of the hepatitis patients were infected by unknown agents. A retrospective study showed that only 25% of transfusion-associated hepatitis was caused by HBV, indicating that 75% of cases were tentatively classified as Non-B Hepatitis (Alter and Klein, 2008; Alter *et al* 1972). At that time, the only other identified hepatitis virus was Hepatitis A Virus (HAV). However, surprisingly, there was not a single transfusion-associated hepatitis case caused by HAV. Therefore, the Non-A, Non-B Hepatitis (NANBH) was designated to describe the new form of blood transfusion-

associated Hepatitis caused by the unknown infectious agent (Alter *et al* 1979). The search for the causative agent of NANBH finally led to the identification of HCV. The only known natural host of HCV is humans. HCV is a prototype member of the *Hepacivirus* genus (from the Greek hepar, hepato, and liver) and is further classified into at least seven major genotypes (1, 2, 3, 4, 5, 6 & 7) which differ by about 30 per cent in their nucleotide sequence (Gottwein *et al.*, 2009, Kuiken *et al.*, 2009).

HCV possesses an approximately 9.6 kb positive-sense RNA genome, translated as a single polypeptide approx. 3000 amino acids in length (Baron, 1996; Lindenbach, *et al.* 2005). HCV is proteolytically cleaved into 10 viral proteins including the structural proteins core, E1, and E2 as well as the non-structural (NS) proteins p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Wang *et al.*, 1993).

HCV Life Cycle:

The HCV lifecycle constitutes viral entry, uncoating and release of the viral genome into the cytoplasm, translation and replication of the RNA, cleavage of the polyprotein, fabrication into new particles and emerge of new HCV particles. The major host cell supporting HCV infection and replication *in vivo* is the human hepatocyte. HCV mainly infect liver *in vivo*.

There are six step of viral life cycle:

1: Binding and Internalization: HSC70 is part of the viral particle and may play a role in viral entry. Also HCV internalization occurs at least in part through clathrin-mediated endocytosis which involves HSC70.
2: Cytoplasmic release and uncoating: The chaperone activity of E1 and E2 may be involved in membrane fusion that releases the core-encapsidated viral genome into the cytosol.

3: Protein translation and processing: HSP70, together with the DNAJA2 member of HSP40 co-chaperones, is the main chaperone involved in IRES-mediated translation of the viral genome, while HSP90 may play some role as well Calnexin, calreticulin, and CypA are also involved.

4: Genome replication: HSP90, some members of HSP40 co-chaperones, TRiC/CCT, FKBP38, SigR1, and some Cyps are involved in viral genome replication. Core and NS3 may play some roles in genome replication as well.

5: Packaging and assembly: HSC70, PDI, and MTTP are the principal chaperones involved in infectious virion assembly, and Cyps also play important roles.

6: Morphogenesis and secretion: MTTP which is involved in the VLDL pathway also plays important roles in viral particle maturation and secretion. Cyps are also involved.

(Abbreviations: Cyp: Cyclophilin; ER: Endoplasmic reticulum; FKBP: FK506-binding protein; HCV: Hepatitis C virus; HSC70: Heat shock cognate protein 70; HSP 40: Heat shock protein 40; MTTP: Microsomal triglyceride transfer protein; NS: Non-structural; PDI: Protein disulfide isomerase; SigR1: Sigma non-opioid intracellular receptor 1; TRiC/CCT: TCP-1 ring complex/chaperonin-containing TCP-1; VLDL: Very low-density lipoprotein.) (Khachatoorian *et al.*, 2016).

Cytokines:

Cytokines are small secreted glycoproteins which is released by cells have a specific effect on the interactions and communications between cells. Cytokines are made by many cell populations, but the predominant producers are helper T cells (Th) and macrophages. Cytokine is a cosmopolitan name other names including site of production such as lymphokine made by lymphocytes, monokine made by monocytes, chemokine with chemotactic activities, and interleukin made by one leukocyte and acting on other leukocytes. Viral contagion is an acute reaction by the infected cell, which allows activation of a preexisting antiviral defense system, allegiance to apoptosis and production of specific cytokines that's why these consequences leads to the reduction of viral replication and to the limitation of viral spread. For a range of viruses it has now been elucidated that the simple interaction of the viral surface proteins with the cellular surface proteins starts a cellular reaction which leads to the first wave of cytokine production after infection. Many viral surface proteins are not present in the infectious particle but produced during the course of the life cycle of virus and are able to affect cellular signaling in a way leading to cytokine production.

Cytokines bind to specific receptors on the membrane of target cells which trigger signal-transduction pathways that finally alter gene expression in the target cells. The susceptibility of the target cell to a particular cytokine is influenced by the presence of

specific membrane receptors. In general, the cytokines and their receptors exhibit very high affinity for each other, with dissociation constants ranging from 10⁻¹⁰ to 10⁻¹² M. Many cytokines are referred as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. Interleukins 1–25 have been identified. In hepatitis C virus (HCV) infection, cytokine levels may influence the outcome of acute HCV infection. Polymorphisms in cytokine genes have been associated with different expression levels in response to HCV infection.

According to Mosmann *et al.*, 1996 two distinct patterns of cytokines production may occur. Type 1 responses are characterized by the production of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF- α), and interferon-gamma (IFN- γ), which are prime, maintain antigen-specific cellular immunity and are important in defense against viruses. Type 2 responses are characterized by IL-4, IL-5, and IL-10, which promote humoral immune responses (Biron *et al.*, 1994; Tough *et al.*, 1996). An imbalance in helper T-cell type 1 (Th1) and type 2 (Th2) cytokines is suggested to play an significant role in the pathogenesis of chronic hepatitis C. The progressive liver injury seen in chronic HCV infection is associated with the upregulation of intrahepatic Th1-like cytokines. Intrahepatic IFN- γ and IL-2 mRNA expression is upregulated in chronic hepatitis C, while the expression of IL-10, a Th2-like cytokine, is downregulated. IFN λ was identified during the recent years and classified into a new group, type III IFN. The IFN λ gene family is composed of three distinct genes: IFN λ 1 (IL29), IFN λ 2 (IL28A), and IFN λ 3 (IL28B) (S. V. Kotenko, *et al.*, 2003; Sheppard *et al.*, 2003).

IL-10 gene:

The human IL-10 gene is located on chromosome 1 and encodes 5 exons (5.1 kb) and The IL-10 promoter is highly polymorphic with two informative microsatellites, IL10.G and IL10.R, located 1.2 and 4 kb upstream of the transcription start site (Eskdale J, *et al.* 1996). Interleukin-10 (IL-10) is an important immunoregulatory cytokine mainly produced by monocytes, macrophages and T cells (Moore *et al.* 2001). Its main biological function seems to be the limitation and termination of inflammatory responses and the regulation of differentiation and proliferation of several immune cells such as T cells, B cells, antigen-presenting cells, mast cells, natural killer cells, and granulocytes. IL-10 is produced by various cell types in

the liver including hepatocytes, sinusoidal endothelial cells, Kupffer cells, hepatic stellate cells, and liver-associated lymphocytes. Infection with the HCV is characterized by a broad spectrum of possible outcomes. Although the mechanisms underlying the production of IL-10 are not clear yet, inherited factors might play an important role.

Genetic polymorphisms affecting IL-10 production might be responsible for the neoplastic derangement of the lymphocytes and the high IL-10 production influences the clinical expression of the HCV infection (Persico *et al.* 2006). Therefore, being a central player in the differential expression of this cytokine, IL-10 polymorphism determination may be crucial for predicting the probability of disease occurrence and disease progression. Most of these polymorphisms are in the cytokine gene regulatory regions and are accordingly involved directly in controlling the transcription rates of these genes.

Studies have shown that cytokine gene polymorphism plays an important role in the natural clearance of hepatitis C virus. Interleukin 10 (IL-10), a Th2 cytokine, is one of the many cytokines that seems to play a vital role in immune response against HCV and it shifts the Th1/Th2 balance by down regulating the Th1 responses and by suppression of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF α) and interferon gamma (IFN γ) secretion (Thio C 2008). A number of studies have examined the IL10 promoter region SNPs and their associations with HCV susceptibility and some of these studies indicate that there is a positive IL10 association with susceptibility to chronic hepatitis C infection and resistance to combined antiviral therapy (Vidigal *et al.*, 2002; Knapp *et al.*, 2003; Yee *et al.*, 2001; Edwards-Smith *et al.*, 1991) while in others the studies the association between IL10 promoter region SNPs and viral clearance was not found (Barrett *et al.*, 2003; Constantini *et al.*, 2002; Abbas *et al.*, 2003), IL-10 gene polymorphisms may lead to an imbalance between the pro-inflammatory and anti-inflammatory cytokine responses which may in turn influence the susceptibility to HCV infection (Muhammad *et al.*, 2011). In those HCV patients, whom interferon treatment fails and patients treated with IL-10 were found to have less hepatic inflammation and fibrosis, therefore, this cytokine might be a useful therapeutic option (Heydtmann *et al.*, 2001). In another study which was done by Elizabeth *et al.*, conclude that

biologically relevant polymorphisms at IL-10 do not contribute to successful clearance of HCV infection. SNPs of the IL-10 gene 819 (rs3021097)TT genotype can be used for predicting response to treatment before patients are prescribed the expensive pegylated interferon- α /ribavirin therapy (Shaker *et al.*, 2013).

IL28B Gene:

Interleukin 28A (IL28A) and interleukin 29 (IL29) are the closely related cytokine genes that form a cytokine gene cluster on a chromosomal region mapped to 19q13. Expression of the cytokines encoded by the three genes can be induced by viral infection. Genome-wide association studies have established that genetic variations in the region near the interleukin-28B (IL28B) gene, which encodes interferon- λ 3 (IFN- λ 3), are associated with chronic HCV treatment response (Ge *et al.*, 2009; Rauch *et al.*, 2010).

The IL28B gene is involved in the immune response to certain viruses, including hepatitis C. There are three IL28B subtypes also called as genotypes: CC, CT, and TT. People with the CC genotype have a stronger immune response to HCV infection than people with the CT or TT genotypes. This immune response makes people who have a CC genotype more likely to clear HCV without treatment, within months of becoming infected (Thomas *et al.*, 2009). The polymorphism of IL28B which was previously associated with HCV treatment response also has a dramatic impact on natural clearance of HCV and may have been under selection in human history. According to Thomas *et al.*, 2009 it is now a priority to determine the mechanisms through which IL28B promotes viral defense and the full range of viruses affected by these mechanisms.

CONCLUSION:

The accurate function of the immune response in patients with HCV infection, in finicking the relationship between the levels of inflammatory/regulatory cytokines and the course of HCV infection, is still unclear. Cytokines can actuate trenchant patterns of protective or immunopathological responses and that they are involved in the establishment of chronic HCV infection, the balance of proinflammatory and regulatory cytokines seems to be important in determining the course of HCV infection. Interleukin 10 may be a major regulator of innate and adaptive immunity in vitro and

in vivo, but its role in human infections is still unclear. Further studies should continue to address the effect of these polymorphisms on IL-10 expression. According to Jiménez-Sousa *et al.*, 2013, IL28B polymorphisms influence both IFN treatment outcomes and the natural clearance of HCV infection, although author cannot provide a biological explanation and his findings indicate the most adequate genetic marker seems to vary depending on ethnicity, HCV genotype, and type of viral infection. IL28B SNP as a pharmacogenetic marker of IFN-based treatments can be considered in treatment decision making.

Search strategy and selection criteria:

We did an extensive Medline and PubMed search of publications. Only English language papers were considered. Search terms included cytokines, IL-10, IL28B and hepatitis C virus. We also searched using the names of eminent scientists in the field and used PubMed.

Conflicts of interest: The authors stated that no conflicts of interest.

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