Combating Hepatitis B and C through immunological approach

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Abstract. Infections with hepatitis B and C viruses are the main factors contributing to the development of chronic liver disease and have been known as the major global health problems. This paper examines evidence that demonstrates the involvement of host immune responses in hepatitis B and C, particularly in the protection against immune-mediated liver injury. The proposed mechanisms of protection range from T cell responses that facilitate spontaneous resolution during acute infection and prevent persistent infection to immunoregulatory cytokines that inhibit destructive immune responses. Regulatory T cells (Tregs), TGF-β1, IL-4, and IL-10 are the main components of the immune system that play an important role in the protection mechanisms against the detrimental effects of hepatitis B and C viruses in liver tissues. Thus, factors contributing to increased Tregs activity and immunoregulatory cytokines should be elaborated. Recent studies reported factors that facilitate the development of Tregs during hepatitis C viral infection include HCV epitope, expression of miR 146a in monocytes and the Tim-3/Gal-9 pathway. On the other hand, the generation of Tregs is inhibited by IL-6 produced during inflammation. These findings suggest that immunomodulation strategy should be further developed and applied in the management of hepatitis B and C.

1. Introduction
Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the blood-borne viruses with the potential to cause chronic infection and severe liver damage, including cirrhosis and cancer, which lead to liver failure. Currently, there are 257 million and 71 million of people living with chronic liver disease...
caused by HBV and HCV, respectively, leading to more than a million of fatality per year due to liver failure [1, 2]. Until now, hepatitis B and C cannot be cured as the antiviral therapy can only slow down the disease progression and the vaccine is available only for hepatitis B. At the end stage of liver failure, transplantation is the only treatment for life-saving and this is practically difficult even though in developed countries. The nature of persistent infection, a few available treatment, and poor treatment responses complicate the management of hepatitis B and C. Hence, a new strategy for combating hepatitis B and C is urgently needed.

The host defense responses to HBV and HCV viruses mainly involve subpopulation of white blood cells named T lymphocytes (T cells). T cells responses are critical for spontaneous resolution of HBV and HCV acute infection as well as preventing persistent infection [3]. Unfortunately, T cells also contribute to the immune-mediated liver injury through unknown mechanisms [4]. It is likely that liver damage in patients with chronic hepatitis is caused by pro-inflammatory cytokines produced by non-specific T cells during the viral clearance in liver tissue [5] so that regulatory/inhibitory cytokines are needed to prevent liver destruction. Therefore, from an immunological perspective, restoring the effective functions of T cells and controlling cytokines levels may be the key to preventing liver damage caused by HBV and HCV.

Regulatory T cells (Tregs) are a group of T cells that suppress the activity, proliferation, differentiation, and effector function of immune cells, including T and B-lymphocytes, natural killer (NK) cells and dendritic cells (DCs) [6, 7, 8, 9]. In patients with HBV and HCV infections, Tregs protect liver tissues from the damage by suppressing the immune responses against viral replication [10]. Although there is an increased number of studies focusing on Tregs amongst hepatitis B and C patients within the last decade, the exact mechanisms of protection and the best strategy to modify Tregs’ roles in the course of hepatitis B and C infections remain unclear. This review aims to deepen our understanding on the roles of Tregs and various cytokines involved in HBV and HCV infections so that the best strategy for modulating immune system for the management of hepatitis B and C can be developed.

2. Regulatory T Cells and Immunity

Immune system develops mechanisms for protecting the host from pathogen invasion. Unfortunately, such mechanisms may cause excessive immune responses or target self-antigens and cause tissue damage and death. Tregs have an important role in regulating immune responses to specific self and non-self antigens, specifically for maintaining tolerance. This role is conducted by limiting the undesired immune responses that could be harmful to the host; for instance: severe inflammation, autoimmunity, and allergy [11]. Thus, the presence of Tregs is important to provide appropriate immune responses with sufficient reactivity against pathogen and assure self-tolerance.

2.1. Development of Tregs

Regulatory T cells are produced by the normal immune system and specialized for immune suppression. These cells are being part of CD4+ T cells population and express high levels of IL-2 receptor α chain (CD25), cytotoxic T-lymphocyte antigen-4 (CTLA-4), and the transcription factor Foxp3 [12]. Foxp3 is the main regulator of Treg development and function. In human, Foxp3 is highly expressed in Tregs but up-regulated transiently and in lower quantities in activated conventional T cells. Foxp3 deficiency or mutation can cause autoimmune disease in various tissues and the manifestations include massive lymphoproliferation, diabetes, exfoliative dermatitis, thyroiditis and enteropathy [13].

Activated nonregulatory T cells produce IL-2 that is critical for maintaining immune homeostasis through negative feedback mechanism of immune responses. This substance is important for the development of natural Tregs and maintaining their survival and function whilst limit the development of nonregulatory T cells. This negative feedback control is essential for Tregs suppressive activity as Tregs do not produce IL-2 themselves and rely on the use of IL-2 secreted by nonregulatory T cells.
Reduced IL-2 production interferes Tregs’ development and function of which consequently may precipitate autoimmunity and promote the development of inflammatory diseases [14, 15, 16].

Tregs are divided into two major subgroups: naturally occurring Treg (nTreg) cells and peripherally induced Tregs.

2.1.1. Naturally Occurring Treg Cells or Thymus-Derived Tregs
This type of Tregs is developed during maturation process of T cells in thymus. Central T cell tolerance in thymus involves the process of “negative selection” during which self-reactive T cells are destroyed (Figure 1). However, some developing CD4+ T cells that recognize self-peptides survive and these are naturally occurring regulatory T cells (nTregs). These cells account for 5–10% of peripheral T cells and are present in healthy individuals from birth [11].

Figure 1. The Development of Regulatory T Cells in Thymus
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2.1.2. Peripherally Induced Treg Cells
These cells are generated from naïve T cells under the stimulation of appropriate antigens and cytokines either naturally (e.g., during inflammatory processes) or experimentally generated. These cells are essential in peripheral self-tolerance when there is a lack of self antigen-specific nTreg. A major substance that can induce the transformation of CD4+CD25+ T cells into Tregs is transforming growth factor-β (TGF-β) either alone or in conjunction with retinoic acid, or 2,3,7,8-tetrachlorodibenzo-p-dioxin, a ligand for the aryl hydrocarbon [11, 14]. Retinoic acid plays an important role in TGF-β–dependent immune responses by inhibiting IL-6. Interleukin-6 poses detrimental effects to the host as it can induce the differentiation of proinflammatory Th17 cells and inhibit the differentiation of anti-inflammatory Tregs during chronic inflammation [17, 18, 19].

2.2. Mechanisms of Tregs-Mediated Suppression
Regulatory T cells migrate to and become activated in inflamed tissues, infectious sites, tumours, and in regional lymph nodes where the specific antigens are presented. Following their activation and proliferation, antigen-activated Tregs contact DCs then modulate DC function to prevent the activation of other T cells (Figure 2). After this, Tregs may then secrete granzyme/perforin or immunosuppressive cytokines [15]. As the results, Tregs maintain peripheral tolerance by suppressing the expansion and function of conventional T cells. Tregs can also suppress antigen presenting cells (APCs), macrophages, DCs, NK, and B cells [14]. From a functional perspective, it has been suggested that Tregs’ suppression activities use four mechanisms: (a) Suppression by inhibitory cytokines (i.e., IL-10, IL-35 and TGF-β); (b) Suppression by cytology or killing mechanisms that depend on granzyme A, granzyme B and perforin; (c) Suppression by metabolic disruption mediated by CD25, cyclic AMP (cAMP), CD39 and/or CD73 and adenosine receptor 2A; and (d) Suppression
by modulation of DC maturation or function which involves lymphocyte-activation gene 3 (LAG3 or CD223), major histocompatibility complex (MHC) class I, CTLA4, CD80/CD86 and indoleamine 2,3-dioxygenase (IDO) [20].

Figure 2. Tregs-Mediated Suppression
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3. The Roles of Tregs in Responses to HBV and HCV Infections
When the microbes entering host, cells of immune system are recruited to the site of infection and is often resulting various degree of inflammation. Regulation of immune response is required to contain or eliminate the pathogen as well as develop tolerance to non-pathogenic agents. A dysregulation of Tregs-mediated suppression mechanisms is harmful because it can lead to excessive inflammation causing severe tissue damage.

It has been reported that during HCV infection, several factors contribute to the development of Tregs, including HCV epitope [21], expression of miR-146a in monocytes [22], the Tim-3/Gal-9 pathway [23, 24] and decline of miR-124 in myeloid cells [25]. Tregs can suppress hepatitis antiviral responses [26] so it can be hypothesized that the decrease of Tregs numbers may increase host’s capacity in viral clearance, which is important during the acute stage of hepatitis. In contrast, increasing the numbers of Tregs in chronic hepatitis may be advantageous to hamper immune-mediated liver damage [27].

4. The Roles of Cytokines in Hepatitis B and C
Cytokines are important mediators of immune responses. They are secreted by immune cells and give feedback effects to immune cells. For example, T cells switch to Tregs in peripheral tissues as a result of exposure to TGF-β, and then the resulting Tregs produce TGF-β and Foxp3. There are immunoregulatory/inhibitory cytokines that regulate/inhibit immune responses (e.g., TGF-β1, IL-4 and IL-10) and inflammatory or effector cell-associated cytokines (e.g., IFN-γ, IL-17 and TNF-α) [28]. In the course of illness, however, the functions of cytokines are often ambiguous and need to be further investigated.

Hepatitis B and C viruses are both non-cytopathic, meaning that the viruses themselves cannot cause liver injury. Instead, the liver injury during infections is the result of specific immune responses
controlled by cytokines. A previous study reported that there was a strong correlation between viral load and IL-4 or IL-6 in hepatitis B and IL-6 or IL-10 in hepatitis C, indicating the association of these cytokines with persistent infection [29].

Some cytokines are useful for combating HBV and HCV infections. Interleukin-4 has been shown to have direct antiviral effect on HBV [30] while IFN-α, IL-1β and IL-6 play an important role in inhibiting HBV entry [31, 32, 33]. Other studies demonstrated that IL-6, IL-1β and TGF-β repress expression of essential HBV transcription factors [34, 35, 36, 37]. Interferon-α disrupts transcription process through epigenetic control of HBV DNA minichromosomes [38, 39, 40] thus inhibit HBV replication as well as inhibit HCV protein translation [41]. Furthermore, IFN-γ, TNF-α and TGF-β could induce HBV DNA degradation [42, 43]. At post-transcriptional level, HBV replication could be inhibited by IFN-γ, IFN-β and TNF-α [44, 45, 46]. Finally, IFN-α has been shown to block virion secretion from an inducible HBV replicating cell line [47].

Infection with HCV activates both innate and adaptive immune responses [48]. Innate immune response is initiated by the synthesis of type I interferon (IFN-α/β) which induces NK and NK T cells (NKT) to release perforin, granzyme β, IFN-γ and TNF-α. This is followed by the expression of Fas ligand (FasL or CD95L) and causes the death of infected hepatocytes. The adaptive immune response is mediated by interactions between DC and CD4⁺ T cells, resulting in the release of various cytokines (i.e., IFN-γ, TGF-β, TNF-α, IL-2, IL-4, IL-10 and IL-13). Currently, IFN-α is the only cytokine used for the treatment of hepatitis C.

5. Conclusions
Management of hepatitis B and C is challenging since currently available treatments can only control HBV and HCV replication but cannot eliminate the infection, leading to chronic inflammation and liver failure. Tregs’ are essential to maintaining peripheral tolerance and regulating effector cells in the immune system so that immune-mediated liver injury can be prevented. Various cytokines have also been shown to control viral replication and contribute to the cure of hepatitis B and C. Therefore, in addition to modulating Tregs, the roles of cytokines associated with Tregs should be considered in order to provide adequate treatment to the patients. In the future, modulation of Tregs and cytokines should be further developed for management of hepatitis B and C in conjunction with antiviral therapy and vaccination.

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