An Overview of Regulatory T cells and their Interpretation in Cancer Liver Patients

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Abstract

Background: Hepatocellular carcinoma (HCC) is the most common primary malignancy of the liver, representing the second leading cause of cancer related death worldwide. Approximately 70%–90% of patients with HCC have an established background of chronic liver disease and cirrhosis, with major risk factors for developing cirrhosis including chronic infection with hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic liver disease, and nonalcoholic steatohepatitis (NASH). In addition to the mechanisms of clonal deletion and energy that physically eliminate or functionally inactivate potentially hazardous self-reactive lymphocytes, there is accumulating evidence that regulatory T cells (Tregs) actively suppress the activation and expansion of self-reactive T cells, thereby preventing autoimmune disease. In cancer, regulatory T cells (Treg) appear to play an important, although somewhat controversial, role. In many human cancers and in most mouse models of tumor growth, the frequency of Treg and their suppressor functions are increased as compared to those reported for healthy subjects.

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Hepatocellular carcinoma

More than 80% of liver cancer patients have hepatocellular carcinoma (HCC), the disease's primary cancerous cell type, which arises from the liver's hepatocytes (1).

HCC is more common between the ages of 55 and 59 in China, and 63-65 in North America and Europe when first diagnosed. Asia, Eastern and Western Africa, and the Middle East all have higher rates of HCC. According to GLOBOCAN's most recent estimates, there were 782,000 new cases and 745,000 deaths due to liver cancer in 2012. As a result, the WHO ranks hepatocellular carcinoma as the second highest cause of cancer-related death (1).

Environmental and genetic factors interact to cause HCC development. Infection with hepatitis B and C viruses as well as liver cirrhosis, excessive alcohol intake, aflatoxin B1 ingestion, and nonalcoholic steatohepatitis are all significant risk factors for the development of hepatocellular carcinoma (2).

Many different etiologic variables can contribute to HCC. These many etiologic factors influence patient features and the course of their disease, making HCC a difficult condition with a dismal prognosis across the globe (3).
Regulatory T cells

Definition and characterization:

In immunology and medicine, one of the most important questions is how immune unresponsiveness to one's own constituents is developed and maintained, as well as how autoimmune disease develops when self-tolerance is lost. Regulatory T cells (Tregs) actively suppress the activation and proliferation of self-reactive T cells, preventing autoimmune illness, in addition to the mechanisms of clonal deletion and energy that physically destroy or functionally inactivate potentially hazardous self-reactive lymphocytes (4).

Types of Tregs include CD4+ CD25+ FoxP3+ Tregs, interleukin-10 (IL10) secreting type1 regulatory T cells (TR1), transforming growth factorβ (TGFβ)-secreting T helper 3 (TH3) cells, CD8+ Tregs, CD28 Tregs, CD3+ Tregs, CD4+ Vα14+ NK Tregs, and γδT- cells (5).

CD4+ T cells are now commonly divided into two distinct lineages: Treg cells and conventional Th cells. Treg cells are defined as T cells in charge of suppressing potentially deleterious activities of Th cells (6).

Immune self-tolerance and homeostasis are maintained by inhibiting excessive or aberrant immune responses damaging to the host by naturally existing CD25+CD4+ regulatory T (Treg) cells constitutively expressing transcription factor Foxp3 (7).

A functionally mature and antigen-primed T cell subset specialising in immunological suppression produces the bulk of Foxp3+ natural Treg (nTreg) cells. Under some circumstances, some of these T cells also diverge from classic T (Tconv) cells in the periphery. One of Foxp3+ nTreg cells' most important functions is to move and inhibit different effector lymphocyte subsets, particularly helper T (Th) cell subsets such as Th1 and Th2 (8).

A subset of CD4+ CD25+ T cells is commonly referred to as a "regulatory T cell" by several researchers. However, it's not obvious how closely the suppressor activity phenotypes and CD25 expression match, especially in non-specific pathogen-free environments. CD25 is expressed by conventional T cells after stimulation, and it has been shown that in human peripheral blood, only the CD4+ CD25(high) T cells are "suppressors" (9).

Although there is limited evidence to support the theory that defective CD4+ CD25+ T cells contribute to the development of autoimmune disorders in humans, the theory is intriguing. As a result, many of the animal models of autoimmunity employed in the research on CD4+ CD25+ T cells are very artificial for naturally occurring autoimmune illnesses, such as neonatal thymectomy or T cell adoption into immune-deficient animals (10).

Even if their importance in cancer is debatable, regulatory T cells (Treg) appear to play a key function. The prevalence of Treg cells and their tumor-suppressing actions is higher in several human diseases and most tumor-growing mice models than in healthy individuals (11).
Despite the widespread belief that tumor associated Treg accumulations indicate a poor prognosis (12), several studies have found a link between increased Treg activity and a better prognosis. Tumor growth, progression to metastasis, and disease outcome are still contested, although there is substantial experimental and clinical evidence that Treg decrease antitumor immune responses, allowing tumors to evade the immune system and spread to other parts of the body (13).

**Development:**

All T cells are derived from bone marrow progenitor cells and become dedicated to a particular clonal lineage in the thymus before leaving the body. As all T cells begin their life, they are CD4-CD8-TCR-cells at the double-negative stage, where each cell rearranges its T cell receptor genes to form a unique and useful functional molecule, which they then test against cells in the thymic cortex for a minimum level of interactivity with their own major histocompatibility complex (MHC). If they receive these signals, they proliferate and express both CD4 and CD8, becoming double-positive cells (14).

The selection of Tregs occurs on radio-resistant haemopoietically-derived MHC class II expressing cells in the medulla or Hassal's corpuscles in the thymus. At the double positive stage, they are selected by their interaction with the cells within the thymus, begin the transcription of Foxp3, and become Tregs cells, although they may not begin to express Foxp3 until the single-positive stage, at which point they are functional Tregs. Tregs do not have the limited T cell receptor (TCR) expression of natural killer T cells (NKT) or gamma delta T cells (γδ); Tregs have a larger TCR diversity than effector T cells, biased towards self-peptides (15).

The process of Tregs selection is determined by the affinity of interaction with the self-peptide MHC complex. Selection to become a Tregs is a "Goldilocks" process-i.e., not too high, not too low, but just right. A T cell that receives very strong signals will undergo apoptotic death; a cell that receives a weak signal will survive and be selected to become an effector cell. If a T cell receives an intermediate signal, then it will become a regulatory cell. Due to the stochastic nature of the process of T cell activation, all T cell populations with a given TCR will end up with a mixture of T effector and Tregs. The relative proportions determined by the affinities of the T cell for the self-peptide-MHC. Even in mouse models with TCR-transgenic cells selected on specific-antigen-secreting stroma, deletion or conversion is not complete (16).

Foxp3+ Tregs generation in the thymus is delayed by several days compared to T effector cells and does not reach adult levels in either the thymus or periphery until around three weeks postpartum. Tregs cells require CD28 co-stimulation and B7.2 expression is largely restricted to the medulla, the development of which seems to parallel the development of Foxp3+ cells. It has been suggested that the two are linked, but no definitive link between the processes has yet been shown. Transforming growth factor 3 (TGF3) is not required for Tregs functionality, in the thymus, as thymic Tregs are insensitive for TGF3 (17).

**Function of regulatory T cells:**

The primary function of Treg cells was originally defined as prevention of autoimmune diseases by maintaining self-tolerance (18).

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Over the years, several additional functions have been suggested and it will be important to clarify what Treg cells actually do in the immune system. Presently, at least 10 nonexclusive functions have been proposed for Treg cells (19).

**Figure (1): Proposed functions of regulatory T cells (19).**

**Control of Treg cell function and generation:**

Foxp3+ Treg cells are generated in the thymus but also in the periphery from naive T cells. In the thymus, Foxp3+ cells are detected with increasing frequency from the late CD4+CD8+ double-positive to CD4+CD8– single-positive stages (20).
Morikawa and Sakaguchi, (21) have shown that the function of Foxp3 is not to determine cell fate of developing thymocytes to the Treg cell lineage but, more likely, to stabilize Treg cell function once the Treg cell fate is determined. However, these cells do not suppress lymphocyte proliferation in vitro. This indicated that the interaction of developing thymocytes with thymic stromal cells activates a transcriptional program in parallel with or upstream of Foxp3 and that, once the Foxp3 gene is switched on, Foxp3 may stabilize and sustain the Treg cell phenotype and confer suppressive activity. It remains to be determined what signal triggers Foxp3 expression.

It has been suggested that Foxp3+ Treg cells are selected on high-affinity self-reactive TCRs, and that high TCR affinity for self-ligand instructs Treg cell fate. Alternatively, the propensity of Treg cells to bear high affinity self-reactive TCRs may be due to greater survival and resistance to negative selection of Treg cells compared with conventional T cells. Another suggestion is that Treg cell development begins at the CD4–CD8– double negative stage, before agonist-mediated selection (20).

Pacholczyk et al. (22) have shown that Treg cell and non–Treg cell TCR repertoires overlap, although the reported extent of the overlap varies. A common finding in current TCR transgenic models is that Foxp3+ Treg cells only develop if thymocytes are allowed to express endogenous chains; therefore, recombination activation gene (RAG)–TCR transgenic mice are devoid of Foxp3+ Treg cells.

This indicates that endogenous TCR chains and the transgenic TCR chain form TCRs sufficiently self-reactive to facilitate Treg cell development. Supporting the notion of Treg cell self-reactivity, in some TCR transgenic mice expressing cognate agonist antigen in the thymus, the opposite occurs in that effector T cells use endogenous TCR chains whereas Treg cells carry the transgenic TCR (23).

However, in RAG-TCR transgenic mice created from TCRs isolated from Foxp3+ Treg cells, most transgenic T cells are deleted in the thymus and very few of them express Foxp3. Interestingly, the frequency of Foxp3+ TCR transgenic cells in the thymus increased in situations in which the TCR transgenic thymocytes are allowed to develop as a fraction of polyclonal thymocytes. These findings suggest the existence of a “Treg niche” that is saturable only at low clonal frequencies of Treg precursors. The TCR specificity contributes to thymic Treg cell generation in an instructive manner but not as the sole determinant (24).

The existence of an overlap in TCR repertoires between Foxp3+Treg cells and conventional Foxp3– T cells makes it difficult to explain thymic Treg cell development and Foxp3 induction solely on the basis of TCR specificity. Besides the TCR, other molecules play critical roles in thymic development of Foxp3+ Treg cells. CD28, CD40L and lymphocyte function associated antigen 1 (LFA1) (CD11a-CD18) on T cells, as well as their respective interaction partners CD8 and CD86, CD40 and intercellular adhesion molecule 1 (ICAM1) also known as cluster of differentiation 54 (CD54) on thymic stromal cells, influence Treg cell development. Indeed, the deficiency of CD28, CD40, CD11a and CD18, or CD80 and CD86 substantially reduces the number of Treg cells in the thymus. Interleukin 2 (IL2) and other cytokines such as IL7 and IL15, which share the common chain receptor, are required for peripheral maintenance of Treg cells and likely for the survival of mature Treg cells in the thymic medulla (25).
Several types of thymic APCs have been implicated in Treg cell development. For example, the absence of mature medullary thymic epithelial cells (MTECs) due to deficiency of necrosis factors (NF) B kinase or tumor necrosis factor receptor associated factor 6 (TRAF6) which transduce CD40 signals, hampers Treg cell development (26).

Yet, in one study, exclusive expression of MHC II on cortical epithelium was enough for Treg cell development. Furthermore, in humans, MTEC derived Hassal's corpuscles produce thymic stromal lymphopoietin, which stimulate thymic dendritic cells (DCs) to promote Treg cell differentiation (27).

A subset of MTECs expresses the Aire protein, which is required for ectopic expression of a set of tissue specific antigens (TSAs) such as insulin. Notably, Treg cell deficiency and Aire deficiency produce a similar spectrum of autoimmune diseases, at least in mice (for example, autoimmune gastritis on the BALB/c background) (27).

In addition to thymic production of natural Treg cells, naive T cells in the periphery can upregulate Foxp3 expression and consequently acquire Treg cell functions and phenotypes in several experimental settings in mice (for example, in vitro antigenic stimulation in the presence of transforming growth factor (TGF), in vivo chronic suboptimal antigenic stimulation and in vivo targeting of antigen to immature dendritic cells (DCs) (28).

With a recent more reliable detection and discrimination of Treg in tissues and the peripheral circulation of patients with cancer (29), it has become apparent that Treg accumulating in at the tumor site are phenotypically and functionally altered relative to circulating Treg (30).

Detection of Tregs:

The best method which is now available to identify Treg cells is flow cytometry. The capture and analysis of these cells requires multiple markers. A common approach to the identification and isolation of Tregs is exploiting CD4+ and CD25+ expression. It is a difficult task to identify Treg cells by flow cytometry, as the most specific marker FoxP3 is localized intracellularly (4). That is, why FoxP3 detection is only possible after cell permeabilization. Permeabilization step is not very advantageous for routine laboratory testing. However, it has been recently recognized that FoxP3+ cells are expressing a subunit of IL-7R, CD127, in a significantly lower density. It was proved experimentally that CD4+/CD25high/CD127low cells are Fox P3+. It is now generally accepted that this phenotype can serve as a surrogate marker for Treg cells (31). For routine testing, CD25high/CD127low/- phenotype is enough specific and sensitive to identify Treg.

Role of regulatory T cells in HCC:

The liver is considered as immune organ and immune escape is one of the mechanisms of hepatocarcinogenesis. The immunological microenvironment is very important for progression of HCC and regulatory T cells (Tregs) are involved in the immunological microenvironment (32). Tregs are a subgroup of CD4 +T cells characterized by expression of CD25, and fork head or winged helix family of transcription factor P3 (FoxP3) is critical for the development and function of Tregs (33).
Tregs are important in maintaining self-tolerance and regulating immune responses in both physiologic and disease states. However, recent studies Pedroza-Gonzalez et al. (34) have revealed that Tregs might play a role in tumor progression. Increased numbers of Tregs have been reported in peripheral blood and tumor tissues of patients with HCC and Tregs can impair CD8+ T-cell function in HCC, which is critical for immune evasion in liver cancer (35).

CD4+ cells that constitutively express CD25, the interleukin-2 receptor α-chain, are generally considered to be natural Treg cells, and constitute 5–10% of peripheral CD4+ T cells in healthy animals and humans (36). These cells are partially anergic, but require activation via their T-cell receptors to become suppressive and to inhibit the proliferation of other T cells.

It has been shown that human CD4+ cells activated with allogeneic stimulator cells in the presence of TGF-B become potent CD25+ suppressor cells with a phenotype and functional properties similar, if not identical, with natural CD4+CD25+ Treg cells (37).

In patients with hepatocellular carcinoma (HCC), one group has quantified CD4+CD25+ Treg cells in the PB of these patients, but information on the numbers of these cells in the liver is not available. The adult human liver contains large numbers of lymphocytes with a unique phenotypic distribution compared with the PB and other organs and has distinctive features in the immune system (38).

Studies have suggested that Tregs have a positive effect on tumor progression through suppression of effective anti-tumor immunity, and removal of CD4+CD25+ Tregs restores the immune response to tumors in vivo (39). Tregs were increased in peripheral blood (PB) and/or tumor in situ in HCC patients and that increased Tregs suppressed CD4 helper T-cell responses and appeared to promote HCC progression (40).

In humans, hepatitis B and C viruses can both cause long-term viral illness. Cirrhosis and hepatocellular cancer are both linked to chronic infection. Chronic infection has been shown in numerous studies to be caused by an ineffective adaptive immune response. CD4+CD25+ Tregs have been implicated in the reduction of virus-specific immunity, according to a slew of recent studies. CD4+CD25+ Treg frequency and functional characteristics in chronic HBV and HCV infections, in particular, may contribute to the formation of chronic virus and influence the course of the disease by reducing antiviral immunity (41).

Hepatocellular carcinoma is a major clinical concern in people with chronic viral hepatitis (CVH), especially because treatment options for HCC are limited. HCC is more likely to occur in men with cirrhosis than in women. It is not known whether the accumulation of intrahepatic T regulatory cells increases the risk of malignancy by inhibiting antitumor responses, but it is clear that T regulatory cells are infiltrated into HCC in patients with chronic viral hepatitis, which increases the risk of HCC in these patients (40).

Several studies as Zhou et al. (42) found that administering an anti-CD25 antibody significantly increased the body's ability to fight tumors. A high Tregs level has been linked to poor outcomes in several studies, according to the data discussed above, making them an intriguing prognostic factor in patients with HCC. (43).
References


