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Dendritic cells, regulatory T cells and the pathogenesis of chronic hepatitis C

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Abbreviations: CCL, chemokine ligand; CCR, chemokine receptor; FoxP3, transcription factor forkhead box P3; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; iT_{reg} cell, inducible regulatory T cell; mDCs, myeloid dendritic cells; nT_{reg} cell, natural regulatory T cell; NS, non-structural; pDCs, plasmacytoid dendritic cells; T_{eff} cells, effector T cells; TLR, toll-like receptor; TGF-β, transforming growth factor-beta

Hepatitis C virus (HCV) is a small, enveloped RNA virus and a major cause of chronic liver disease. Resolution of primary HCV infections depends upon the vigorous responses of CD4$^+$ and CD8$^+$ T cells to multiple viral epitopes. Although such broad-based responses are readily detected early during the course of infection regardless of clinical outcome, they are not maintained in individuals who develop chronic disease. Ostensibly, a variety of factors contribute to the diminished T cell responses observed in chronic, HCV-infected patients including impaired dendritic cell function and the induction of CD4$^+$FoxP3$^+$ regulatory T cells. Overwhelming evidence suggests that the complex interaction of dendritic cells and regulatory T cells plays a critical role in the pathogenesis of chronic hepatitis C.

Introduction

Hepatitis C is an emerging infectious disease caused by hepatitis C virus (HCV). HCV is a small enveloped virus whose RNA genome consists of a large open reading frame that encodes an ~3,000 amino acid poly-protein precursor, which is cleaved by cellular and viral proteases to yield the core, envelope (E1 and E2) and nonstructural (NS) proteins (NS1–NS5) (**Fig. 1**).[1] HCV is a major cause of chronic liver disease and a principal reason for liver transplant; approximately 170 million people worldwide are chronically infected.[2,3] HCV is primarily contracted through the transmission of infected blood or body fluids. The risk of transmission via blood and blood products in the US is negligible due to the advent of reliable and mandatory blood screening. Isolated (nosocomial/iatrogenic) outbreaks of hepatitis C continue to occur in US medical facilities, however, emphasizing the vigilance required to enforce universal precautions and proper sterilization of medical devices.[4] The infrastructure and technology required to ensure a safe blood supply are elusive for most developing countries where prevalence of HCV infection can be as high as 20% of the population.[5] Unfortunately, while only 20% of patients resolve infection spontaneously, the vast majority develops chronic disease.[3] Standard hepatitis C treatment consists of the combined administration of PEGylated interferon (IFN) and ribavirin. The response to conventional therapy varies widely, however, and is often accompanied by significant side effects. Only an estimated 45–50% of all patients infected with HCV genotype 1 experience a sustained virologic response upon treatment withdrawal.[6] Treatments that include new protease inhibitors, e.g., telaprevir and boceprevir, in conjunction with PEGylated IFN and ribavirin increase the sustained virologic response up to 80% in patients infected with HCV genotype 1.[7]

HCV-related premature death and morbidity in US patients under 65 y of age are expected to increase 2- to 3-fold between the years 2010 and 2019 at a societal cost exceeding $55 billion.[8] Spontaneous clearance is affected by a number of factors that include: age, ethnicity, sex, hepatitis B virus or human immunodeficiency virus co-infection and host genetics (e.g., HLA haplotype and *IL28B* gene variants).[9-13] The majority of new cases in the US occur among young, intravenous drug users.

Resolution of primary HCV infections is associated with the vigorous responses of HLA class I-restricted (CD8$^+$) and class II-restricted (CD4$^+$) T cells to multiple epitopes derived from both structural and non-structural proteins.[14] Although such broad-based responses are readily detected early during the course of infection regardless of clinical outcome, they are not maintained in patients who develop chronic disease.[15] Thus, while individuals who spontaneously clear infection continue to exhibit a proliferative response to a wide range of class I- and class II-restricted epitopes, chronically infected patients respond to a limited number only.[16] A variety of factors purportedly contribute to the diminished T cell responses observed in chronically infected patients including: viral mutation and escape linked to both CD4 and CD8 T cell failure, CD4 T cell anergy, CD8 T cell exhaustion, induction of FoxP3$^+$ regulatory $T_{(reg)}$ cells and/or impaired dendritic cell function.[17-26]

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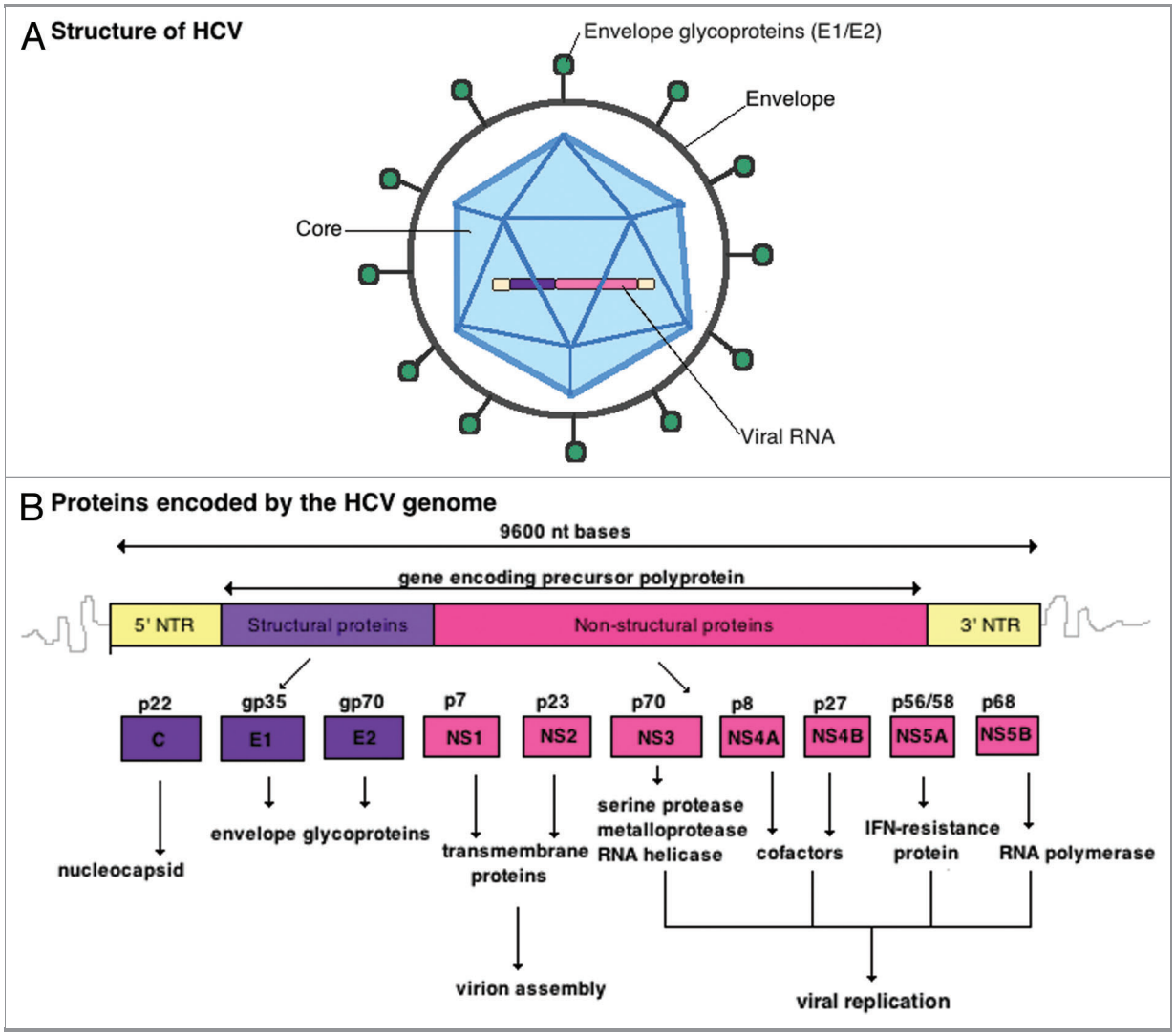

Figure 1. Hepatitis C virus: structure and genomic organization. The viral genome is enclosed within the core protein and surrounded by an envelope composed of lipid and glycoproteins (**A**). HCV is a small, enveloped virus with a single-stranded, positive-sense RNA genome that is ~9,600 nucleotide bases long and consists of a single open reading frame. The 5′, ~340 nucleotide long, non-translated region (NTR) functions as an internal ribosome entry site that binds ribosomes in close proximity to the translation start codon. The HCV genome encodes a ~3,000 amino acid poly-protein precursor that is cleaved co- and post- translationally by cellular and viral proteases into ten protein products: C, core; E1 and E2, envelope; NS, nonstructural proteins (NS1–NS5). The function of each cleavage product is shown (**B**).

Dendritic Cells (DCs)

DCs are professional antigen presenting cells characterized by their potent capacity to elicit primary T cell responses.[27] Two major subsets of DCs are readily purified from human peripheral blood: plasmacytoid (p)DCs and conventional or myeloid (m) DCs.[28-30] Each subset represents 0.3–0.5% of the normal human peripheral blood mononuclear cell (PBMC) population.[28,31] mDCs and pDCs originate from myeloid and lymphoid precursors, respectively, residing in the bone marrow.[32] pDCs have a round morphology similar to secretory lymphocytes and closely resemble plasma cells.[33-35] mDCs, on the other hand, exhibit the typical dendritic cell morphology with prominent cytoplasmic protrusions and veils. Human pDCs and mDCs are further distinguished by the cell surface expression of CD45R/B220^{+}CD123^{bright}CD303^{+} and CD11c^{+}CD1a^{+}CD1c^{+}, respectively.[28-30] mDCs are short lived relative to pDCs, which have a slow turnover rate and a relatively long half-life.[35]

pDCs and mDCs differ markedly in their ability to capture, process and present antigens, express co-stimulatory molecules and produce cytokines.[34] Freshly isolated pDCs express only moderate, heterogeneous levels of HLA-DR, ingest antigens poorly and exhibit diminished allogeneic T cell stimulatory activity in mixed lymphocyte reactions.[28] By comparison, mDCs are 10–50 times more efficient in their ability to capture, process and present HLA class I- and class II-restricted antigenic determinants (epitopes) to CD8^{+} and CD4^{+} T cells.[36]

pDCs and mDCs also differ substantially in terms of Toll-like receptor (TLR) expression.[33,34,37,38] pDCs strongly express TLR-7 and TLR-9 in the endosomal compartment and respond to single stranded RNA and unmethylated CpG-containing DNA ligands, respectively. Consequently, pDCs are potent mediators of antiviral immunity and unique in their capacity to secrete large quantities of type 1 IFN following virus infection.[33-35,39-42] Upon TLR ligation, pDCs upregulate HLA-DR and the cell surface expression of co-stimulatory molecules (e.g., CD80 and CD86), secrete massive amounts of IFN-α/β, acquire T cell stimulatory activity, and induce T_{h1} cell polarization and the production of IFN-γ.[35,39-41] mDCs, on the other hand, recognize viral ligands (e.g., HCV core and NS3) via TLR-2, exhibit elevated HLA-DR levels, specialize in IL-12 production, polarize $CD4^+$ T cells toward T_{h1} and demonstrate potent allogeneic T cell reactivity.[28,37,43-45] In addition, recognition of double-stranded RNA viruses via TLR-3 stimulates the release of large quantities of IL-1, IL-6 and IL-12, and small amounts of type 1 IFN.[46]

Contribution of DCs to the Pathogenesis of Hepatitis C

Despite extensive investigation, there is no general consensus regarding the effects of HCV on DC function.[47] The failure of most patients to manifest clinical symptoms during the acute phase of infection suggests, however, that DC functions are normal at the onset. Moreover, it is generally agreed that the numbers comprising both the pDC and mDC subsets circulating in the peripheral blood are reduced substantially in chronic HCV-infected patients.[39-41,47-50] Indeed, the numbers of circulating DCs correlate inversely with serum alanine aminotransferase levels and the severity of liver disease in chronically infected patients.[51] It is interesting to note, however, that while the numbers of mDCs and pDCs in the peripheral blood decrease, there is a significant increases in both populations found in the livers of chronic, HCV infected patients.[50,52,53] Furthermore, the ratio of mDCs/pDCs is higher in the liver than the peripheral blood of these chronically infected patients suggesting preferential migration of mDCs to areas of liver inflammation.[51,52] This has led investigators to speculate that active trafficking and intrahepatic compartmentalization of DCs constitutes the principal mechanism responsible for decreased circulating DCs in chronically infected patients.[42,50-52] While the mechanism(s) remains to be clarified, immature DCs in the bloodstream express inflammatory chemokine receptors, e.g., CCR5, and migrate to the liver in response to HCV infection and the elevated production of chemokines, e.g., RANTES, MIP-1α and MIP-1β.[53,54] Normally, upon maturation in healthy tissues, DCs downregulate CCR5 and upregulate CCR7 expression enabling the cells to respond to the cognate CCR7 chemokine (CCL21) and traffic to the T cells areas of lymphoid tissues.[54,55] In sharp contrast to healthy individuals, however, recent studies demonstrated the expression of CCR5, but not CCR7, by pDCs and mDCs circulating in the blood of HCV-infected patients.[42,52,55] Moreover, HCV E2 protein rendered CCR7-expressing DCs unresponsive to CCL21.[52] Thus, the attenuated anti-viral response characteristic of chronic, HCV-infected patients appears due in part to the inability of DCs trapped in the liver to home the lymph nodes.[50,52]

Regardless of their distribution (i.e., liver vs. peripheral blood), contradictory data indicate that functions of pDCs and mDCs are either intact[56-63] or impaired[36,39,42,44,48,64-74] in patients with chronic hepatitis C. Functional impairments include: decreased IFN-α and IL-12 secretion, increased IL-10 production, lowered expression of HLA-DR and costimulatory molecules such as CD86, decreased allostimulatory activity and increased ability to prime T_{reg} cells.[36,39,42,44,48-50,64,65,67-69,75-79] Additional studies reported that DCs obtained from chronically-infected patients were phenotypically immature and failed to upregulate maturation (costimulatory) markers in response to stimuli such as TNF-α.[66,73] Circulating pDCs in chronic HCV-infected patients exhibit: diminished HLA-DR expression, a markedly reduced capacity to secrete IFN-α and, consequently, decreased anti-viral potency.[36,39,44,67,68,80] mDCs in chronic HCV infections secrete significantly lower levels of IL-12 and increased concentrations of IL-10, which tends to skew the immune response toward tolerance and a reduced ability to induce T-cell proliferation and T_{h1} polarization.[36,47,49,67,69,72,75-77,81] The stimulatory potential of mDCs and the alloreactive response of $CD4^+$ T cells are also diminished in chronic HCV infections.[44] Although the literature addressing mDCs function during acute HCV infection is limited, patients who exhibit decreased mDC allostimulatory activity seem to exhibit an increased chance of developing viral persistence.[71] In sharp contrast to chronically infected patients in whom DCs are impaired, DCs derived from the peripheral blood of healthy individuals and patients who spontaneously cleared HCV infection were phenotypically comparable and functionally normal.[42,66]

The mechanisms underlying DC dysfunction during HCV infection are not fully understood. The core, NS3, NS4 and NS5 proteins impair DC function by diminishing the HLA and co-stimulatory molecule expression, reducing cytokine production, inhibiting TLR signaling, and decreasing allostimulatory activity.[82] The HCV core protein induces IL-10/TNF-α secretion by monocytes, which triggers pDC apoptosis and, indirectly, reduces IFN-α secretion.[49,83] Similarly, IL-10 produced by monocytes in response to core, NS3 and/or NS4 proteins impairs DC maturation, reduces allostimulatory activity, suppresses IL-12 production and, consequently, interferes with T_{h1} polarization.[72,77,84-86]

Conflicting evidence, however, shows that DCs in chronic HCV infected patients are neither functionally nor phenotypically impaired and exhibit normal activity.[56,57,87] DCs in chronically infected patients express typical maturation markers and CD83, CD86, CD54 and HLA-DR levels similar to those expressed by healthy patients.[56,88] Additionally, DCs derived from healthy subjects and patients with chronic HCV infections demonstrate comparable abilities to prime allogeneic T cells.[56,60,88] Healthy and infected patients secrete similar quantities of IL-12 and IFN-α.[57-60] DCs derived from chronically-infected patients exhibited normal antigen uptake, peptide-MHC complex formation, and antigen trafficking suggesting that the capacity to present antigen is preserved during HCV infection.[63]

A variety of factors contribute to these discrepant results including: (1) the limited number of patients enrolled in studies; (2) patient related factors such as age, gender, co-infection and alcohol use; (3) duration of infection, liver inflammation and disease progression; (4) tissue source of DCs analyzed (i.e., peripheral blood vs. liver) and (5) whether the data originate from freshly isolated DCs or monocyte-derived DCs obtained from chronic HCV-infected patients.[47] Indeed, given the paucity of DCs in the bloodstream and the challenge of tissue acquisition, most human studies are conducted with DCs generated in vitro from $CD14^+$ monocyte precursors present in the peripheral blood. The differentiation of monocyte-derived DCs in vitro resembles the development of mDCs in vivo; notably, pDCs are not readily expand or derive in vitro.[71,89]

Regulatory T Cells

$CD4^+$ regulatory $T_{(reg)}$ cells, characterized by the transcription factor forkhead box P3 (FoxP3) and the constitutive, cell-surface expression of the interleukin (IL)-2 receptor α chain (CD25), represent one of the major mechanisms underlying immunological self-tolerance and homeostasis.[90] Mutations in FoxP3 and T_{reg} cell dysregulation cause severe autoimmune disease and immunopathology leading to death in both mice and humans.[91,92] T_{reg} cells also constitute an important factor in moderating effector immune responses to pathogens that can potentially cause serious organ and tissue damage to the host if left unabated.[93] Though critical for maintaining immune homeostasis, T_{reg} cells can also suppress effective immune responses to autologous tumors, e.g., metastatic melanoma and hematological malignancies, and contribute to the persistence of infections by a wide variety of human pathogens.[93-95]

Two types of T_{reg} cells are commonly described in the literature and classically distinguished by site of origin: natural (n)T_{reg} cells generated by high-avidity selection in the thymus; and adaptive or inducible (i)T_{reg} cells derived from antigen-stimulated conventional ($CD4^+CD25^-FoxP3^-$) T cells in the periphery (**Fig. 2**).[96,97] nT_{reg} cells can induce "infectious tolerance" by converting conventional T cells into iT_{reg} cells directly by cytokine (IL-10, TGF-β or IL-35)-dependent mechanisms or -independent mechanisms mediated by contact with DCs.[98,99] It has been suggested that nT_{reg} and iT_{reg} cells express complementary, immune functions, that nT_{reg} cells and iT_{reg} cells are dedicated to preventing autoimmunity and to maintaining a non-inflammatory environment, respectively.[96] Notably, there are no definitive markers that identify T_{reg} cells, or distinguish nT_{reg} and iT_{reg} subsets. While FoxP3 is a transcription factor common to both subsets, it is also expressed transiently by activated, conventional human T cells that do not exhibit immunosuppressive activity.[97] Moreover, while $CD25^+$ T cells isolated from naïve mice are almost exclusively nT_{reg} cells; CD25 expression in humans is much more heterogeneous.[97]

Once activated, T_{reg} cells are able to suppress the activity of a wide range of immune cell types including both $CD4^+$ and $CD8^+$ T cell subsets, B cells, NK cells, NKT cells, macrophages and DCs (a phenomenon called bystander suppression).[100-105] Suppression can be mediated by multiple mechanisms although it is generally agreed that the effects of nT_{reg} cells are primarily mediated by direct, cell-to-cell contact.[105]

Inhibitory cytokines constitute a principal contact-independent mechanism by which T_{reg} cells suppress T_{eff} cell activity (**Fig. 3**). Soluble and membrane-bound transforming grow factor (TGF)-β, for example, play a critical role both in inducing and maintaining nT_{reg} and iT_{reg} cells, and in blocking the activation of conventional T_{eff} cell.[103,106-108] Similarly, T_{reg} cell-dependent production of interleukin 10 (IL-10), plays an important role in suppressing the responses of $CD4^+$ T_{eff} cells to a variety of pathogens both used in animal models and implicated in human disease.[93]

Contact-independent mechanisms of T_{reg} cell-mediated suppression also involve the constitutive, high-level expression of the IL-2 receptor (CD25). T_{reg} cells are capable of relatively little IL-2 production and, consequently, require IL-2 derived from an exogenous source.[109] As such, the rapid consumption of IL-2 by T_{reg} cells deprives T_{eff} cell populations of IL-2 necessary for activation and proliferation.[109] Cell surface nucleotidase activity (i.e., CD39 and CD73) constitutes an additional mechanism by which T_{reg} cells disrupt the metabolic activity of T_{eff} cells.[110] These ectonucleotidases rapidly degrade extracellular ATP released by activated or damage cells and abrogate the proinflammatory response that would otherwise occur.[110] In addition, adenosine generated as a product of ATP degradation binds A2A receptors expressed by T cells and prevents effector cell activation.[110-112]

T_{reg} cells also exhibit the ability to suppress T_{eff} cell function by a number of contact-dependent mechanisms. Both mouse and human T_{reg} cells, for example, exhibit cytotoxic activity and the ability to induce apoptosis by T_{eff} cells dependent upon granzyme A or B and perforin.[108,113] Galectin-1, a member of a highly conserved family of β-galactosidase-binding proteins, has also been implicated in the immunosuppressive activity exhibited by T_{reg} cells.[114] Galectin-1, expressed on the cell surface, inhibits cell proliferation and promotes apoptosis by activated T_{eff} cells

In addition to regulating T_{eff} cell functions directly, T_{reg} cells can suppress conventional T cell activity indirectly by inhibiting DC maturation and immunostimulatory activity.[108] In a process called trans-endocytosis, for example, cytotoxic T lymphocyte-4 (CTLA-4) molecules on the surface of T_{reg} cells capture, internalize and degrade CD80 and CD86 expressed by DCs rendering the latter tolerogenic and limited in their capacity to sensitize naïve T_{eff} cells.[115] Similarly, lymphocyte activation gene-3 (LAG-3 of CD223), a CD4 homolog that binds MHC class II molecules, suppresses DC maturation and co-stimulatory molecule expression.[116,117] Neuropilin (Nrp-1), a molecule also expressed by T_{reg} cells, promotes long-term T_{reg} cell interaction with immature DCs and, thus, inhibits immune recognition by naïve $CD4^+$ helper T cells.[118] Notably, the suppressor functions of T_{reg} cells undoubtedly rely upon a combination of all the factors described. Moreover, it is likely that one or more of these factors affect both T_{eff} cells and DCs, as well as other immune cell types.

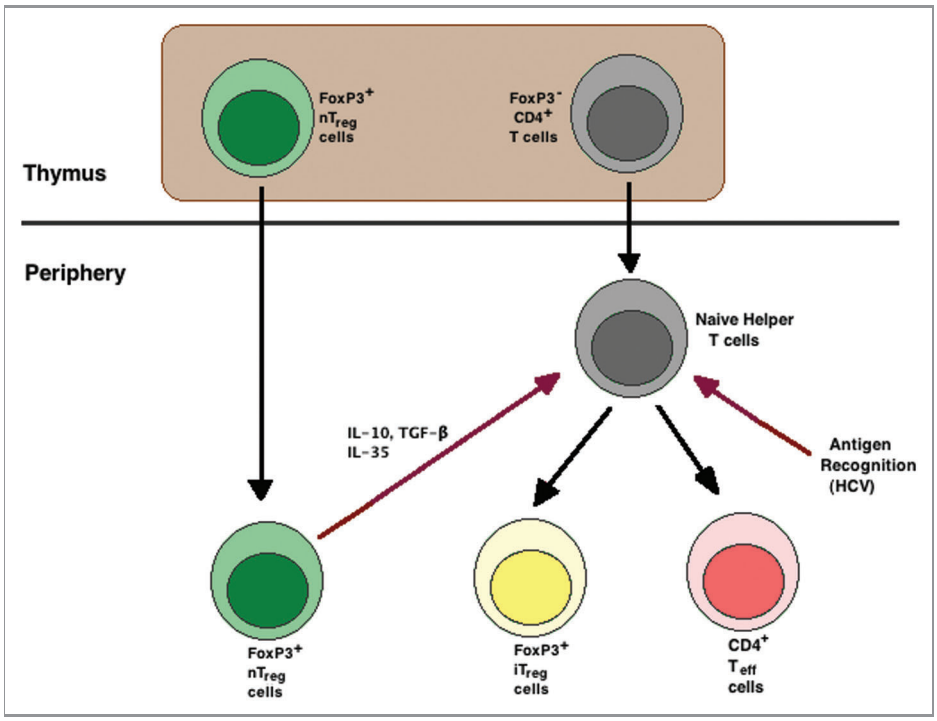

Figure 2. FoxP3$^+$ T_{reg} cell production in the thymus and periphery. Natural (n)T_{reg} cells are generated by high-avidity selection in the thymus. Inducible (i)T_{reg} cells derive from antigen-stimulated naïve T cells in the periphery. nT_{reg} cells can promote iT_{reg} cell development by cytokine-dependent mechanisms (infectious tolerance).

Contribution of T_{reg} Cells to the Pathogenesis of Hepatitis C

T_{reg} cells play a prominent role in the pathogenesis of chronic HCV infection. This role was first suggested based upon the increased frequency of T_{reg} cells found in the liver and circulating in the peripheral blood of chronically-infected patients compared with uninfected individuals or patients who spontaneously cleared the virus.[23,24,93,119-123] Moreover, CD4$^+$CD25$^+$ T cells isolated from the blood of HCV-infected patients suppressed virus-specific CD8 T cell responses, while depletion of the CD25$^+$ T cell population enhanced the proliferation of cells that remained.[23,123] Until recently, however, it remained unclear whether this increase in T_{reg} cells represented an HCV antigen-specific response or occurred as a nonspecific consequence of chronic inflammation and liver disease. Furthermore, descriptions in literature of the phenotype and function of the T_{reg} cell populations are not consistent.

HCV-encoded T_{reg} cell epitopes have now been identified in both structural (i.e., core) and non-structural (NS3, NS4 and NS5b) proteins.[119,120,124-127] Utilizing dye-conjugated, HCV peptide-loaded MHC class II tetramers to stain the cell, Ebinuma and coworkers were the first to report HCV antigen-specific recognition by FoxP3$^+$ T_{reg} cells contained among the PBMCs derived from chronically infected patients.[124] A number of studies have demonstrated an increase in the HCV-antigen specific FoxP3$^+$/CD25^{high} cell population (phenotypically similar to nT_{reg} cells expressing an anergic cytokine profile) in response to stimulation with HCV T regulatory epitopes.[119,121,124] In one study, global gene expression analysis comparing FACS-sorted CD4$^+$/CD25^{high} T cells (nT_{reg} phenotype) from HCV-infected and uninfected individuals found only minimal differences between the two populations.[124] In contrast, other investigators observed an increase in HCV antigen-specific T_{reg} cells phenotypically consistent with iT_{reg} cells, which induced suppression via induction of IL-10 or TGF-β.[120,126] Regardless, the consensus seems to be that the expanded T_{reg} cell population in chronically infected HCV patients is heterogeneous, composed of both nT_{reg} and iT_{reg} cell subsets. Conceivably, the initial HCV epitope-specific response of nT_{reg} cells to infection induces the expansion of, and subsequent suppression by, iT_{reg} cells (i.e., infectious tolerance).[98]

The response of nT_{reg} cells, which normally function to suppress autoimmunity, suggests that HCV encodes peptide (epitope) sequences homologous to self-antigen.[119,128] Indeed, a BLAST search of the viral genome identified a number of T_{reg} cell-epitopes published in the literature that exhibited extensive human homology (Ardito and Losikoff, unpublished observation). While these epitopes elicited a response by T_{reg} cells circulating in the bloodstream of chronic, HCV-infected patients, they failed to elicit a similar response by cells obtained from healthy controls.[119] This seems to imply that these HCV-specific nT_{reg} cell populations expand in vivo during chronic disease.[122,129] It is relevant to note, therefore, that many published epitopes recognized by HCV-specific T_{reg} cells are located adjacent to or overlapping with T_{eff} cell epitopes associated with viral clearance.[119,124,130]

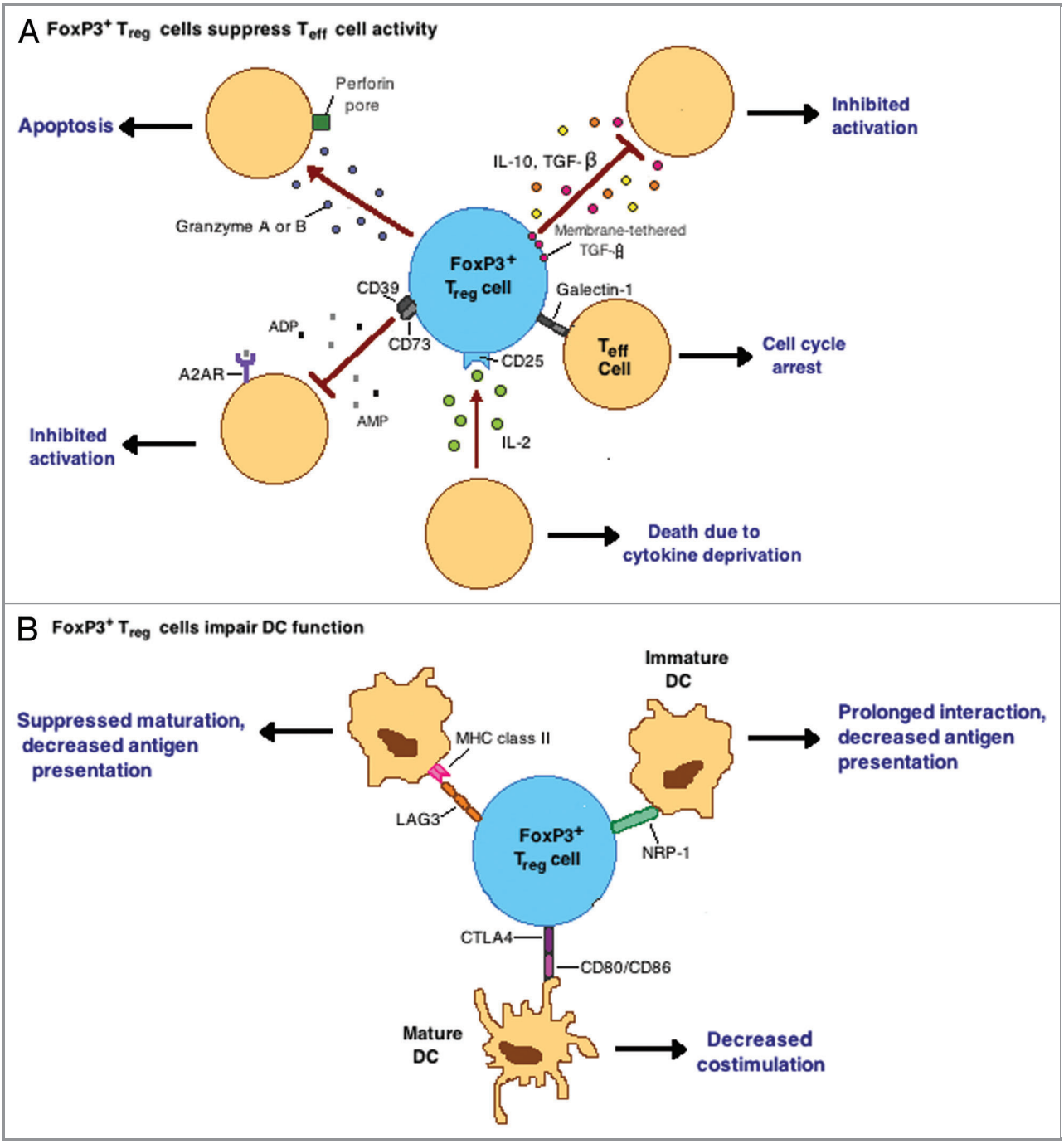

Figure 3. Mechanisms mediating T_{reg} cell suppression. FoxP3$^+$ T_{reg} cells suppress T_{eff} cell activity (**A**). T_{reg} cells inhibit T_{eff} cell activity by multiple contact-dependent and -independent mechanisms described in the text. FoxP3$^+$ T_{reg} cells impair DC function (**B**). Similarly, T_{reg} cells impair DC maturation and function by mechanisms described in the text.

Several cross-sectional studies demonstrated a strong correlation between increased T_{reg} cell numbers and chronic HCV infection.[23,123,124] The specific role of T_{reg} cells in viral persistence could not be determined, however, since the patients were previously infected at some undetermined date. In this regard, two longitudinal studies involving patients acutely infected with HCV reported no difference in the frequency or function of circulating T_{reg} cells irrespective of whether the patient ultimately cleared virus or developed chronic disease.[121,130] In one study, T_{reg} cells derived from patients in whom viremia persisted exhibited significantly more suppressive activity in vitro than did cells obtained from patients who spontaneously cleared the virus.[121] The authors concluded that maintaining an elevated T_{reg} cell population regulated (suppressed) the T_{eff} cell response and promoted the development of chronicity.

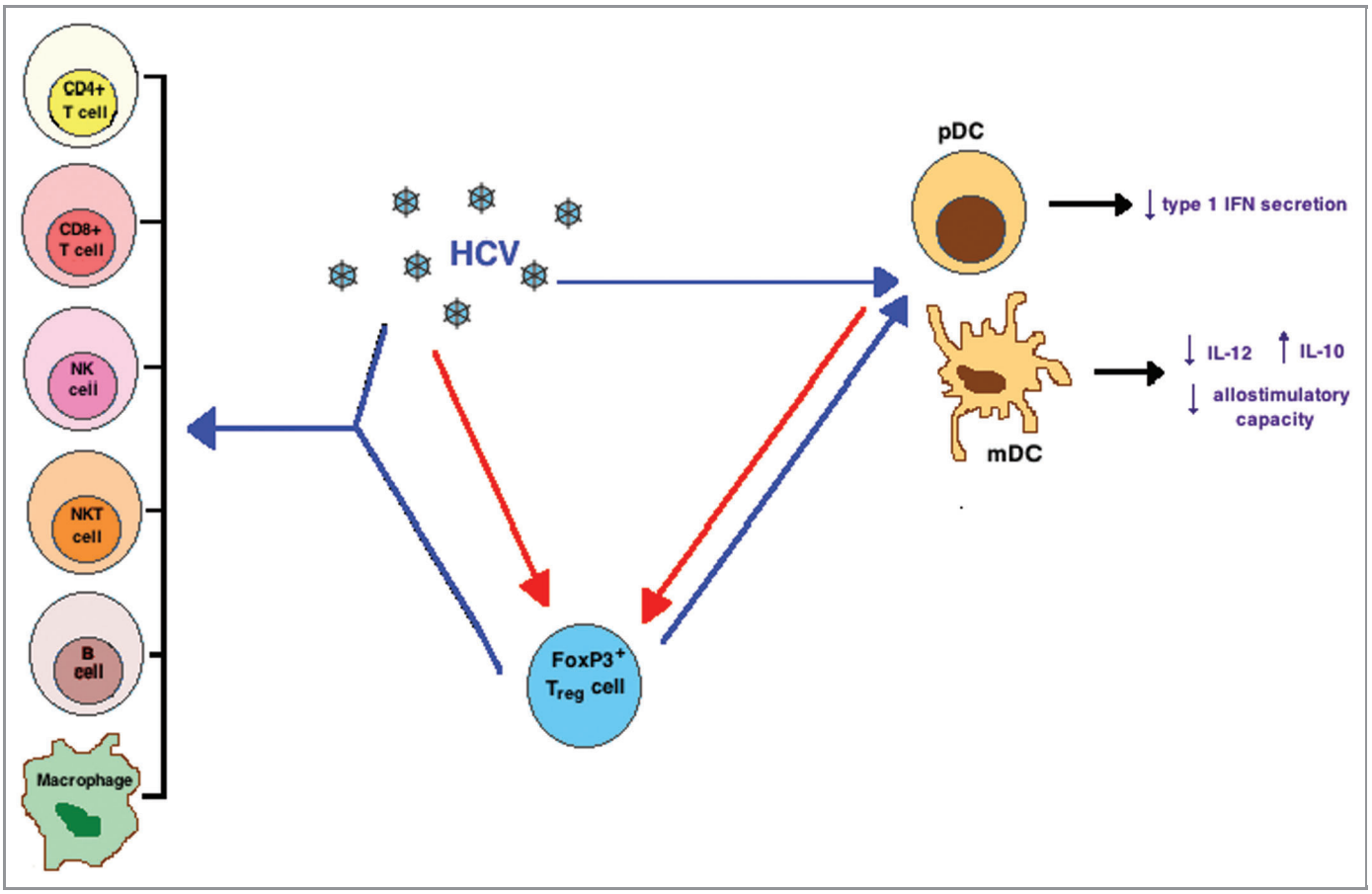

Figure 4. Immune suppression mechanisms in HCV infection. Both the number and functions of circulating mDCs and pDCs are diminished in cases of chronic hepatitis C. Immature DCs promote the expansion and function of FoxP3$^+$ T_{reg} cells. T_{reg} cells, in turn, suppress CD4$^+$ and CD8$^+$ T cell, NK cell, NKT cell, B cell, macrophage and DC activities. Red and blue arrows indicate activation and suppression, respectively.

Recently, Cusick et al. reported that a variant of an immunodominant HCV epitope emerged during the course of persistent HCV infection.[127] This epitope variant induced a population of Foxp3$^+$CD4$^+$ T_{reg} cells that was able to suppress the response of T_{eff} cells to the cognate, but not an unrelated, peptide. Thus, variants of MHC class II-restricted epitopes arise as a consequence of immune pressure to evade host defenses, and these variants induce epitope-specific T_{reg} cells that attenuate conventional CD4$^+$ T cell help required to clear virus infection. In cases of chronic disease, T_{reg} cells suppress virus-specific CD4 T cell activity, impairing maintenance of T_{eff} cell function and promoting viral persistence.

Impaired DCs, T_{reg} Cells and the Pathogenesis of Chronic HCV

It is not entirely clear whether HCV affects the response of T_{reg} cells to infection directly or indirectly, dependent upon the intermediary role of some other cell type. There is accumulating evidence to suggest, however, that DCs play a key role in the expansion of T_{reg} cells during chronic HCV infection. Indeed, HCV proteins (core, NS3, NS4 and NS5) exert a marked, inhibitory effect on the capacity of DCs derived from healthy individuals to express HLA and cell surface co-stimulatory molecules, synthesize proinflammatory cytokines such as IL-12 and induce allogeneic T cell proliferation.[82] These same viral proteins failed to affect T cell proliferation directly, supporting the contention that the effect of HCV on T_{eff} cell function depends upon the intermediary role of DCs.[82] Recent studies suggest, however, that both DCs and conventional T_{eff} cells are susceptible to HCV infection.[131-133] In the former case, the binding and subsequent internalization of viral particles are mediated by the interaction of envelope glycoproteins E1 and E2 with dendritic cell-specific C-type lectin (DC-SIGN), a cell surface receptor expressed by DCs.[133] Consequently, the authors speculated that even in the absence of a productive infection, HCV E1 and E2 bound to DC-SIGN might transmit a signal that promoted tolerance and T_{reg} cell formation. In the case of CD4$^+$ T cells, Dominguez-Villar and coworkers reported that the Jurkat T cell line transfected with an HCV core-expressing vector upregulated FoxP3 and CTLA-4, and acquired the ability to suppress the non-specific proliferative response of both CD4$^+$ and CD8$^+$ T cells.[134] This latter finding implies the potential capacity of HCV infection alone to induce T_{reg} cells formation.

While the virus may infect T cells and DCs directly, impaired DC function appears to play a key role in the induction and maintenance of HCV-specific T_{reg} cell responses. In this regard, overwhelming evidence supports the role of DCs in establishing and maintaining immunological tolerance to foreign, as well as self, antigens.[135,136] In particular, the liver provides a prototypical tolerogenic environment in which interactions that include DCs and T_{reg} cells foster development of chronic infectious diseases, e.g., viral hepatitis.[137] While the underlying mechanisms remain to be delineated fully, it is generally agreed that immature DCs promote T_{reg} cell function.[135-139] It is pertinent to note, therefore, that DCs purified from human liver tissue primarily express an immature, myeloid phenotype characterized by the low-level or negligible expression of cell-surface co-stimulatory molecules: CD40, CD80, CD83 and CD86.[137] Moreover, compared with DCs purified from peripheral blood, liver DCs produce significantly higher levels of IL-10 and lower proinflammatory cytokine (i.e., IL-1β, IL-6 and TNF-α) levels following TLR4 ligation. Finally, compared with DCs in the blood, liver DCs stimulated the IL-10-dependent production of more $CD4^+CD25^+FoxP3^+$ T_{reg} cells in co-cultures that contained naïve, allogeneic $CD4^+$ T cells.[138] Similar results were found when allogeneic T cells were co-cultured with mDCs obtained from healthy control and chronic, HCV-infected patients.[79] Relative to the controls, mDCs derived from infected patients stimulated a marked expansion of T_{reg} cells.

Summary

DCs circulating in the peripheral blood of chronic, HCV-infected patients are functionally impaired. Both pDCs and mDCs are reduced in number and exhibit decreases in: cytokine production, cell-surface co-stimulatory molecule expression and/or allostimulatory activity, resulting in an inability to prime naïve T_{eff} cells and, consequently, a reduction in anti-viral activity (**Fig. 4**). On the other hand, the number and function of T_{reg} cells in the blood of these same patients are increased correlating with the development of persistent viral infection. T_{reg} cells can suppress the activity of other immune cell types including T_{eff} cells directly by contact-dependent and -independent mechanisms, or indirectly by inhibiting DC maturation and immunostimulatory capacity. As such, recent studies indicate that immature DCs are a critical factor in the pathogenesis of chronic hepatitis C by failing to induce adequately naïve T_{eff} cell function and, conversely, promoting the development and activity of T_{reg} cells.

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