A review article on IFN-γ and FOXP-3 in Hepatitis C virus infection.

Abstract:

Hepatitis C virus (HCV), which belongs to the *Hepacivirus* genus in the *Flaviviridae* family and contains a single-stranded positive-sense RNA genome, has emerged as a common cause of liver-related disorders worldwide. The genome of HCV is approximately 9,600 bases long and contains a large open reading frame (ORF). This ORF is linked to 5' and 3' untranslated regions (UTRs). The immune system, both the innate and adaptive responses, plays a vital role in HCV infection. These immune responses towards HCV infection include different cytokines and immune cells. During HCV infection, Interferon-gamma (IFN-γ) and Fork-head box protein P3 (FOXP3) genes play a significant role in managing the immune responses. IFN-γ, being a type-II cytokine, promotes the control of viral replication and clearance of infected hepatocytes during HCV infection. Studies have reported that higher expression of IFN-γ is linked with a better prognosis, and there is more likelihood of viral clearance. On the other side, FOXP3 is a transcription factor that is essential for the development and functioning of regulatory T cells (Tregs), which are important for maintaining immune tolerance and preventing autoimmunity. FOXP3+Tregs play a dual role in HCV infection. They downregulate excessive immune responses and prevent liver damage by controlling effector T cell activity. The efficiency of antiviral response is limited by the immunosuppressive function of FOXP3. In conclusion, IFN-γ and FOXP3 have a vital role during HCV infection, focusing on the dynamic and complex nature of the host–virus interaction. The IFN-γ is important for the antiviral immune response in HCV infection. However, the activity of IFN-γ must be controlled to prevent immunopathology. Similarly, FOXP3+Tregs play a protective role in reducing liver inflammation as well as damage to liver cells. FOXP3 can also facilitate the virus to persist in the liver cells, which can lead to a chronic HCV infection.

Keywords:

Hepatitis C, IFN-γ, FOXP3, Immune regulation, T-cell response, Cytokine imbalance.

Introduction:

Globally, Hepatitis C virus (HCV) infection has emerged as a common cause of liver-related diseases [1]. The innate and adaptive immune responses play a complex and challenging role in the immune response against HCV infection. These immune responses towards HCV infection include different cytokines and immune cells. The non-structural protein (NS) of the virus is primarily targeted by these cytokines along with CD4+ and CD8+ T cells [2]. During HCV infection, Interferon-gamma (IFN-γ) and Fork-head box protein P3 (FOXP3) genes play a significant role in managing the immune responses.

IFN-γ is a critical cytokine involved in innate immunity as well as activation of adaptive immune response. This cytokine is largely produced by natural killer (NK) cells and cytotoxic T lymphocytes (CTLs). It plays a significant role in the non-cytolytic clearance of HCV-infected hepatocytes. This clearance is induced by interferon-stimulated genes (ISGs) and by activating antiviral pathways [3]. Additionally, IFN-γ enhances the cytolytic activity of NK cells and CTLs. This mechanism helps in the removal of infected hepatocytes [3, 4]. IFN-γ also plays an important role in controlling HCV replication and promoting viral clearance [4].

FOXP3 is a transcription factor for the development and functioning of regulatory T cells (Tregs). During HCV infection, the Tregs have a very important role in maintaining immune tolerance and preventing excessive immune-mediated liver damage [5, 6]. Tregs, by limiting the activity of effector T cells, help in reducing immunopathology while allowing the development of virus-specific immune responses [5]. The FOXP3 gene regulates the balance

between Tregs and effector T cells. This regulation is crucial for an effective but controlled immune response against HCV infection [7, 8].

This review article aims to provide valuable information regarding the interaction between IFN-γ and FOXP3 in HCV infection. This will include immune regulatory mechanisms and potential therapeutic targets for antiviral immunity while minimising liver damage.

An overview of the Hepatitis C Virus (HCV):

HCV belongs to the *Hepacivirus* genus in the *Flaviviridae* family [9]. It is an enveloped virus with a positive-sense single-stranded RNA [10]. The genome of HCV is approximately 9,600 bases long and contains a large open reading frame (ORF). This ORF is linked to 5' and 3' untranslated regions (UTRs) [11]. The transcription and translation of viral proteins are mediated by the single-stranded RNA genome of the virus. The ORF of the 5' region has four domains, domains I to IV. Domain – I have a short stem-loop structure, and other domains II to IV form the internal ribosomal entry site (IRES), which is a highly structured element to regulates the binding of viral RNA to the host ribosomal subunit. This IRES plays a central role in translating the ORF region [12, 13].

The HCV genome consists of three structural proteins, i.e. Core, E1 and E2 and seven non-structural proteins i.e. P7, NS2, NS3, NS4A, NS4B, NS5A and NS5B (Figure 1) [14].

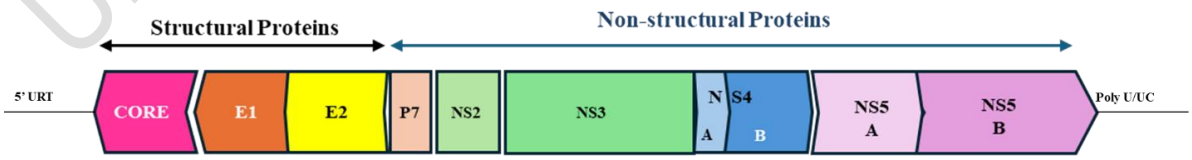

Figure 1: Hepatitis C virus genome proteins (Structural proteins and Non – Structural proteins) [14].

The HCV core is the first protein that translates from the genome and forms the viral capsid [15, 16]. As a structural part of nucleocapsid, the HCV core protein promotes the binding of HCV RNA to the host-presented lipid membrane [15, 17].

The E1 and E2 are envelope glycoproteins. These proteins are highly glycosylated transmembrane proteins. They form a heterodimer that is essential for the HCV replication cycle [18, 19]. The E1 protein primarily attaches the virus to the host cell, aids the fusion of the endosome-lipid membrane, and also assists the E2 protein in maintaining a conformation suitable for receptor binding [20–22]. Studies also suggest that the E1 has a role in binding to host apoproteins, CD36 [23]. The heterodimers of E1–E2 are fixed in the lipid membrane derived from the host and facilitate the formation of the viral envelope [20, 24]. The entry of the virus, as well as fusion with the endosomal membrane, is facilitated by the E2 protein bound to host receptors, which include CD81 and scavenger receptor class B type 1 (SR-B1). This allows the release of HCV RNA into the cytoplasm [18–20].

The p7 protein of the HCV genome is a hydrophobic transmembrane protein. It is also involved in the assembly of the virus and its release. The oligomerized hexamer of the p7 protein has the property of being an ion channel in host cell membranes [25, 26]. Though p7 is a part of non-structural proteins in HCV viral proteins, in hepatocytes, it has a structural role [27]. The p7 protein works along with other proteins of the virus to deliver core proteins used for the assembly of the capsid at the endoplasmic reticulum while structuring the viral genome. The activity of the p7 channel protects the viral glycoprotein inactivation from by the low pH of secretory compartments of cells [26, 28].

The non-structural protein 2 (NS2) works as a dual-functioning protein involved in cysteine protease and acts as a cofactor during assembly of the HCV genome [29]. The protease

domain of the NS2 protein, along with the N-terminal region of NS3, helps to catalyse the cleavage between the NS2 and NS3 proteins. This cleavage plays a vital role during HCV RNA replication. During HCV viral assembly, the NS2 protein with other viral proteins, plays an important role, though it is not associated with HCV RNA replication [30, 31].

The non-structural 3 (NS3) protein acts as a bifunctional enzyme, i.e. helicase activity and serine protease [32]. The Helicase region of the NS3 protein plays an important role in HCV RNA replication by unwinding the viral RNA. The viral polyprotein is cleaved by the NS# region of serine-type protease. NS3 also weakens the innate host immune response by inactivating host cell factors. [33, 34].

The non-structural protein 4a (NS4A) is one of the smallest non-structural proteins of the HCV genome. The outer part of the endoplasmic reticulum and the outer membrane of mitochondria are anchored by a complex of NS3 and NS-4A proteins [35]. The NS4A protein acts as a cofactor for serine protease and catalyses the helicase activity of NS3A. NS4A also regulates NS5A hyperphosphorylation as well as HCV replication of the virus. The NS4A with NS4 B controls replication of the HCV genome, and with NS3, it plays a vital role in assembly of the virus [36, 37]. Non-structural protein 4B (NS4 B) incorporates changes in the cytoplasmic membrane and resolves the interaction between the virus and host [38]. Along with endoplasmic reticulum and other NS proteins, this NS4 B forms the complex of replication. For viral replication, this NS4 B protein is essential to create a microenvironment within the cytoplasm [39, 40].

Non-structural protein 5A (NS5A) is critical in the HCV replication complex. The NS5A protein works together with NS5B and NS4B along with viral RNA and cellular proteins of the host, e.g. kinases, cyclophilin A, helps to regulate replication of the virus and its assembly [41]. This non-structural protein also has a significant role in the endoplasmic

reticulum-derived structure of double-membrane vesicles (DMVs). NS5A protein also promotes the pathogenesis of HCV by modulating virus propagation and cell signalling pathways [42].

Non-structural protein 5B (NS5B) is an RNA-dependent RNA polymerase (RdRp), which has a significant role in replication of the virus by catalysing polymerisation of ribonucleoside triphosphates (rNTPs). NS5B polymerase inhibits nucleotide analogues simulating the natural substrate and influences termination of the chain into a new RNA. The non-nucleotide inhibitors allosterically bind to the sites of the enzyme and weaken its function [43, 44].

Interferon-gamma (IFN-γ):

The structure of IFN-γ is a homodimer, and it is the only class II interferon in the cytokine family. The activity of IFN-γ is influenced by binding with its receptor complex, composed of IFN-γ R1 and R2 genes. These receptor genes are located in human chromosomes at 6q23–q24 and 21q22.11 locations [45]. This class II interferon is a major cytokine, which participates in innate as well as adaptive arm of immune responses. In innate immune response, T-cells, natural killer cells (NK cells) are the main inducers for the production of IFN-γ, whereas in adaptive immune response, CD8+ and CD4+ T-cells are major sources for the production of IFN-γ [46, 47]. The JAK-STAT pathway is the primary pathway for the signalling of IFN-γ. This pathway is very much essential for various growth factors, cytokines, and hormones to regulate their associated genes [48]. The IFN-γ receptors, i.e., IFN-γR1 and IFN-γR2, interact with the Janus tyrosine kinase (JAK) and activate JAK1 and JAK2, subsequently phosphorylase signal transducer and activator of transcription (STAT) 1. The STAT1 self-associates to form a homodimer, then moves towards the cell nucleus, then binds with the IFN-γ-activated site (GAC). The IFN-γ-regulated genes consist

of these GAC elements at the promoter region, activating the classical JAK-STAT signalling pathway and subsequently initiating transcription of various genes [49, 50].

IFN-γ has antiviral activities against HCV through various mechanisms that improve the immune response in the host and inhibit replication of the virus. This mechanism includes modulation of cellular receptors, induction of antiviral proteins, and enhancement of immune cell activity, collectively contributing to controlling HCV infection [51, 52]. It reduces the expression of a key receptor for HCV entry, i.e., claudin-1, thus disrupting the viral entry into host cells. This downregulation results in altered obstructive function in epithelial cells, which makes them less susceptible to HCV infection [53]. IFN-γ stimulates expression of interferon-stimulated genes (ISGs), e.g. PKR, ISG20, and viperin. These ISGs inhibit replication of the HCV genome, non-cytopathically, specifically targeting viral components and restricting the replication process [54]. IFN-γ also helps in the proliferation and activation of HCV-specific T cells, which improves the immune response against the virus. Recent studies also suggested that IFN-γ facilitates the immune cells to migrate into the liver by promoting the expression of various markers [55].

Studies have supported that increased expression of IFN-γ in HCV-infected patients is associated with various clinical findings that indicate the disease severity and treatment outcomes. Higher IFN-γ levels were associated with advanced fibrosis stages (F2–F3, F4) and hepatocellular carcinoma (HCC), with a statistical significance of $P < 0.0001$ in comparison with healthy controls [56]. An increase in the level of IFN–γ is also associated with key markers of liver dysfunction, which include albumin levels, platelet counts, and total bilirubin [57]. Studies also revealed that increased IFN-γ levels in patients with specific genetic backgrounds, e.g., interleukin-28B rs8099917TT carriers, are related to treatment failure while treated with peginterferon/ribavirin [58]. The lower levels of IFN–γ at treatment initiation

are predictive of achieving sustained virological response (SVR), which highlights its efficiency during treatment. The rise in IFN-γ levels is often linked with adverse clinical outcomes, studies also suggest that it may show a protective role in viral clearance, indicating a complex relationship between the levels of cytokines and disease progression in HCV infection [59].

Fork-head box protein 3 (FOXP3):

The FOXP3 gene has 12 exons, which code for 431 amino acids of the FOXP3 protein. This FOSP3 protein contains a C2H2 zinc finger (Cys2-His2), a C-terminal forkhead (FKH) domain and an essential leucine domain [60]. This gene has a significant role in preserving immune tolerance through its function in regulatory T cells (Tregs). It is a crucial transcription factor for the development of Tregs in the thymus, essential for suppressing excessive immune responses and preventing autoimmunity. Studies have shown that mutations in this gene can lead to autoimmune diseases, which highlights its central role in immune regulation [61]. The anti-inflammatory cytokines produced by FOXP3+ Tregs suppress immune responses and inhibit the activation of effector T cells [62]. FOXP3 has a role in Tregs' function as it can form higher-order multimers, allowing it to bind to DNA and regulate gene expression effectively [63]. In clinical implications, it has been reported that lower levels of FOXP3 splice variants are associated with poor outcomes in kidney transplant recipients [64]. The FOXP3+ Tregs' functions are being explored to improve the safety and efficiency of therapies like Adeno-Associated Virus (AAV) gene therapy [65].

The maturation and development of Tregs primarily occur in the thymus (Figure 2). In the thymus, T-cell receptor (TCR), CD4 and CD8 double-positive cells obtained from hematopoietic cells differentiate into CD8 and CD4 single-positive thymocytes. The CD4+ cells differentiate into pTregs when stimulated by antigen stimulation, along with the

expression of transforming growth factor-β (TGF-β) and IL-2. However, in the thymus, CD4+ cells differentiate into tTregs upon upregulation of IL-2Rα/CD25, FOXP3, cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR) [66–68].

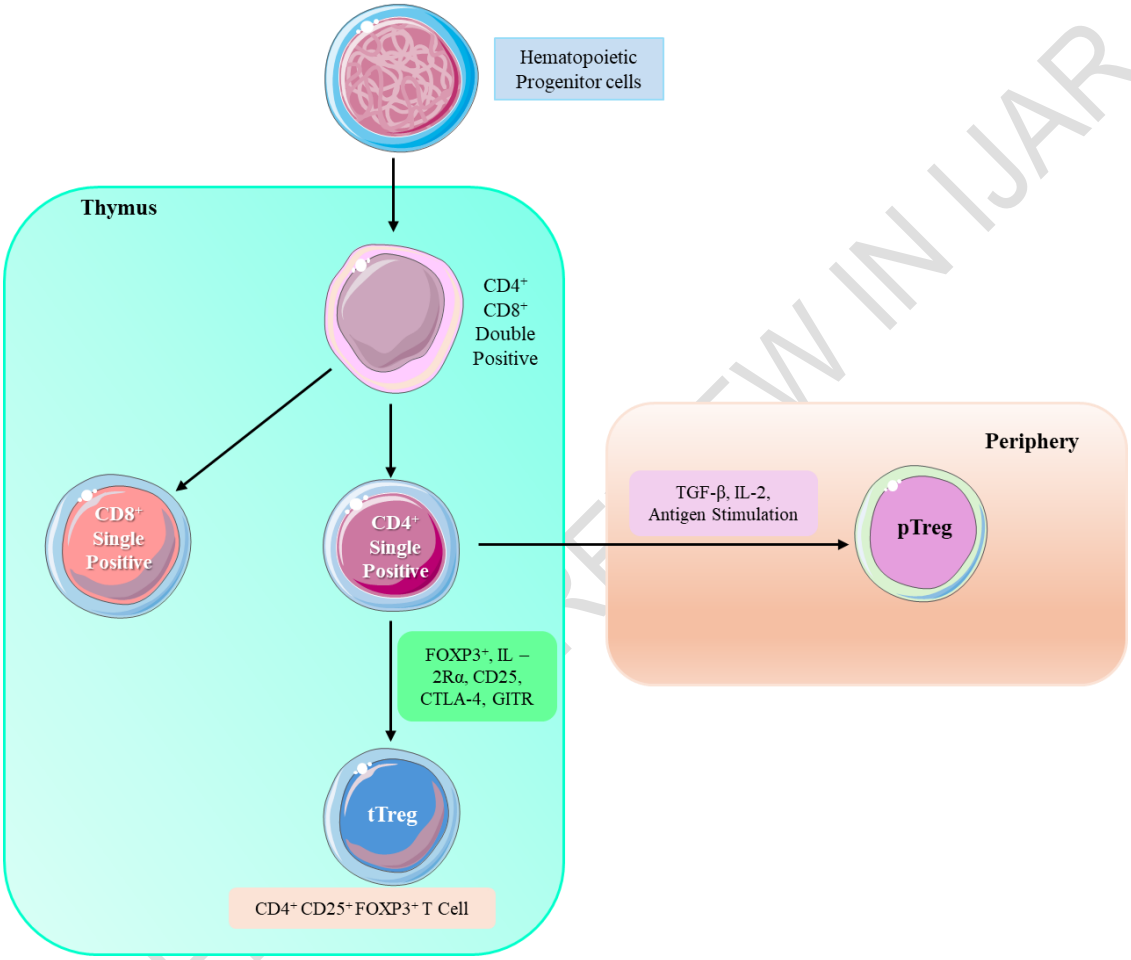

Figure 2: Schematic diagram of Treg development.

The differentiation of FOXP3+ Tregs requires activation of the phosphatidylinositol-3-kinase (PI3K) signalling pathway, mediated by the TCR-CD28 complex [69]. Recent studies also reported that Treg development markers like CTLA-4 and CD25 are influenced by the insulin receptor substrate 1 (IRS1) signalling pathway. The reduced expression of Treg phenotypic markers and FOXP3 can be observed when there is an overexpression of IRS1 [70].

The Tregs play a multifaceted role during HCV infection, primarily involving the regulation of immune tolerance and suppressing excessive immune activation. Tregs are crucial in maintaining a balance between viral replication and immune responses, which can influence disease progression and persistence of the virus. Studies suggest that HCV-infected patients exhibit a higher frequency of CD4+CD25+CD127- cells and low Tregs in comparison with healthy controls (8.2% vs. 5.4%). These elevated levels may correlate with impaired antiviral immunity, suggesting a potential role for Tregs in promoting viral persistence [71]. Tregs express immunosuppressive cytokines such as IL-10, which can inhibit effector T cell responses, which limits the ability to clear the virus. The upregulation of Tim-3 on Tregs during chronic HCV infection increases their immune-suppressive functions, which further limits the virus-specific T-cell response [72]. Genetic polymorphisms in FOXP3 and TGF-β1 have been shown to modulate immune responses in viral hepatitis, which indicates that genetic factors can also influence Treg activity and the overall immune response [73]. The Tregs play a protective role in averting excessive liver inflammation during hepatitis infection. However, the overactivity of Tregs can hamper an effective antiviral response. This may lead to a chronic infection in patients with HCV infection. This dual nature of Tregs highlights the complexity of its functions in HCV infection.

Interaction between IFN-γ and FOXP3 in HCV infection:

The interplay between IFN-γ and FOXP3 expression significantly influences immune responses and regulatory T cell (Treg) dynamics, which impacts the progression of liver diseases caused by HCV. Being a critical factor for transcription, FOXP3 controls the stability and suppressive functions of the Tregs, which are vital for controlling immune response and preventing excessive inflammation during infections like HCV. Studies show

elevated FOXP3 expression is related to chronic HCV infection, where it plays a crucial role in immune tolerance and disease severity [74]. Studies also supported that elevated levels of FOXP3+Tregs have a strong association with HCV viral load in chronic HCV infections with a *p-value* less than 0.001 [75]. The FOXP3+Tregs play a dual role in antiviral immunity. While they are necessary to prevent intense immune responses that can lead to tissue damage, they can also suppress the effector T cells' activity, which is crucial for clearing viral infections, leading to the chronicity of the disease [76, 77]. On the other side, IFN–γ has a crucial role in the activation of the immune response during HCV infection. It also impacts the expression of FOXP3 during HCV infection. Studies showed lower expression of FOXP3 on HCV-specific CD4 T cells, while there are cases where elevated levels of FOXP3 expressions are associated with a loss of HCV – specific T – cell proliferation and reactivation of the virus [78]. IFN-γ also plays a significant role in the introduction of FOXP3 and the alteration of CD4+ T cells to Tregs [79]. The balance between IFN-γ production and FOXP3 expression may regulate the consequence of HCV infection, influencing both inflammation and progression of fibrosis [80].

Conclusion:

The roles of IFN-γ and FOXP3 in HCV infection underscore a complex interaction of both innate and adaptive immune responses. These immune responses significantly influence the progression and outcome of the disease. The IFN-γ and FOXP3 play a vital role to control as well as to clear the HCV infection immunologically. The HCV genome itself has a vital role in these immunological interactions. The genome of the virus encodes numerous proteins. These proteins can interfere with immune responses in the host. The NS proteins of HCV, specifically NS3 / 4A and NS5A, show inhibition of IFN-γ signalling. Whereas the HCV core protein can control Treg function. This property of core protein enhances their

suppressive activity and promotes immune tolerance. These strategies of the HCV virus help to persist in the host cells and contribute to a chronic infection.

IFN-γ is a type II cytokine. This cytokine has a critical role in the immune response against HCV infections. The primary producers of IFN-γ are T cells and NK cells. The antiviral properties of IFN-γ are mediated through activation of macrophages, enhancement of antigen presentation, and promotion of Th1 responses. IFN-γ promotes the control of viral replication and clearance of infected hepatocytes during HCV infection. Studies have reported that higher expression of IFN-γ is linked with a better prognosis, and there is more likelihood of viral clearance. However, prolonged production of IFN-γ can be a vital cause of liver inflammation as well as tissue damage, which can lead to chronic HCV infection.

FOXP3 is being a transcription factor which is very essential for the development and functioning of Tregs. Tregs are important for maintaining immune tolerance and preventing autoimmunity. FOXP3+Tregs play a dual role in HCV infection. On one side, they downregulate excessive immune responses and prevent liver damage by controlling effector T cell activity. The efficiency of antiviral response is limited by the immunosuppressive function of FOXP3. This activity allows HCV to persist and cause a chronic infection. The interaction between IFN-γ and FOXP3 in HCV infection is multifaceted. This interaction presents a delicate balance between immunopathology and viral clearance. HCV has recognized multiple ways to escape these immune responses. These include the modulation of cytokine production and the induction of Treg activity. HCV can also downregulate IFN–γ signalling pathways. The result of this altered signalling can reduce the antiviral efficacy of the immune response towards the virus. In addition, HCV infection can increase the expression of FOXP3+Tregs. This activity can suppress the activation and function of the

HCV-specific T cells. The increased expression of FOXP3+Tregs can lead to a chronic infection.

In conclusion, IFN-γ and FOXP3 have a vital role during HCV infection, focusing on the dynamic and complex nature of the host–virus interaction. The IFN-γ is important for the antiviral immune response in HCV infection. However, the activity of IFN-γ must be controlled to prevent immunopathology. Similarly, FOXP3+Tregs play a protective role in reducing liver inflammation as well as damage to liver cells. However, it can also facilitate the virus to persist in the liver cells. This activity can lead to a chronic HCV infection. The role of FOXP3 in liver-related disorders presents new prospects for better treatments by targeting FOXP3 and Tregs, involving pathways. This approach could improve immune responses against viral infections. The reduction in inflammation, as well as damage to the hepatocytes can improve the management of chronic HCV-related liver disease. Understanding the delicate balance between these immune regulators and their interaction with the HCV genome is very important for the future development of effective therapeutic strategies to achieve viral clearance while minimizing injury towards the hepatocytes. Future research should focus on resolving the detailed mechanisms by which HCV controls these key immune regulators and on identifying potential targets for immunomodulatory therapies.

References:

Imran M, Manzoor S, Ashraf J, Khalid M, Tariq M, Khaliq HM, Azam S (2013) Role of viral and host factors in interferon based therapy of hepatitis C virus infection. Virol J 10:1–12

Shahid I, Jabeen Q (2023) Hepatitis C Virus-Host Interactions and Therapeutics: Current Insights and Future Perspectives. Hepatitis C Virus-Host Interactions and Therapeutics: Current Insights and Future Perspectives. https://doi.org/10.2174/9789815123432123010 1

Chigbu D, Loonawat R, Sehgal M, Patel D, Jain P (2019) Hepatitis C Virus Infection: Host–Virus Interaction and Mechanisms of Viral Persistence. Cells 8:376

Boisvert M, Shoukry NH (2016) Type III interferons in hepatitis C virus infection. Front Immunol 7:240324

Stross L, Günther J, Gasteiger G, et al (2012) Foxp3+ regulatory T cells protect the liver from immune damage and compromise virus control during acute experimental hepatitis B virus infection in mice. Hepatology 56:873–883

De Castro GLC, Bichara CDA, Santiago AM, et al (2020) Polymorphisms in the TGFB1 and FOXP3 genes are associated with the presence of antinuclear antibodies in chronic hepatitis C. Heliyon 6:e04524

Rios DA, Valva P, Casciato PC, et al (2017) Chronic hepatitis C liver microenvironment: role of the Th17/Treg interplay related to fibrogenesis. Sci Rep 7:13283

Casares N, Rudilla F, Arribillaga L, et al (2010) A Peptide Inhibitor of FOXP3 Impairs Regulatory T Cell Activity and Improves Vaccine Efficacy in Mice. The Journal of Immunology 185:5150–5159

Fields BN, Knipe DM (David M, Howley PM (2013) Fields virology, 6th ed. Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia

Toygar Deniz M, Akhan S (2023) Hepatitis C Virus Structure and Diagnostic Methods. Hepatitis C - Recent Advances. https://doi.org/10.5772/intechopen.1000863

314. Adams RL, Pirakitikulr N, Pyle AM (2017) Functional RNA structures throughout the
315 Hepatitis C Virus genome. Curr Opin Virol 24:79

316. Dubuisson J, Cosset FL (2014) Virology and cell biology of the hepatitis C virus life cycle -
317 An update. J Hepatol 61:S3–S13

318. Catanese MT, Uryu K, Kopp M, Edwards TJ, Andrus L, Rice WJ, Silvestry M, Kuhn RJ,
319 Rice CM (2013) Ultrastructural analysis of hepatitis C virus particles. Proc Natl Acad Sci U S
320 A 110:9505–10

321. Seng-Lai Tan (2006) Hepatitis C Viruses: Genomes and Molecular Biology, 1st ed. Horizon
322 Bioscience, Norfolk (UK)

323. Gawlik K, Gallay PA (2014) HCV core protein and virus assembly: what we know without
324 structures. Immunol Res 60:1–10

325. Eng FJ, El-Shamy A, Doyle EH, Klepper A, Muerhoff AS, Branch AD (2017) Newly
326 discovered hepatitis C virus minicores circulate in human blood. Hepatol Commun 2:21

327. Akuta N, Suzuki F, Kawamura Y, et al (2007) Amino acid substitutions in the hepatitis C
328 virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–
329 1364

330. Law JLM, Chen C, Wong J, et al (2013) A Hepatitis C Virus (HCV) Vaccine Comprising
331 Envelope Glycoproteins gpE1/gpE2 Derived from a Single Isolate Elicits Broad Cross-
332 Genotype Neutralizing Antibodies in Humans. PLoS One 8:e59776

333. Freedman H, Logan MR, Law JLM, Houghton M (2016) Structure and Function of the
334 Hepatitis C Virus Envelope Glycoproteins E1 and E2: Antiviral and Vaccine Targets. ACS
335 Infect Dis 2:749–762

336 Vieyres G, Dubuisson J, Pietschmann T (2014) Incorporation of Hepatitis C Virus E1 and E2
337 Glycoproteins: The Keystones on a Peculiar Virion. Viruses 2014, Vol 6, Pages 1149-1187
338 6:1149–1187

339 Tong Y, Lavillette D, Li Q, Zhong J (2018) Role of hepatitis C virus envelope glycoprotein
340 E1 in virus entry and assembly. Front Immunol 9:387256

341 Haddad JG, Rouillé Y, Hanoulle X, Descamps V, Hamze M, Dabboussi F, Baumert TF,
342 Duverlie G, Lavie M, Dubuisson J (2017) Identification of Novel Functions for Hepatitis C
343 Virus Envelope Glycoprotein E1 in Virus Entry and Assembly. J Virol.
344 https://doi.org/10.1128/JVI.00048-17/ASSET/C219441D-02E9-4F4B-A74A-
345 8082FCA2D51F/ASSETS/GRAPHIC/ZJV9991825120011.JPEG

346 Moustafa RI, Haddad JG, Linna L, et al (2018) Functional Study of the C-Terminal Part of
347 the Hepatitis C Virus E1 Ectodomain. J Virol. https://doi.org/10.1128/JVI.00939-
348 18/ASSET/FAB44D8C-710D-4212-AA29-
349 F1F9BC1BEAD2/ASSETS/GRAPHIC/ZJV0201839340010.JPEG

350 El Omari K, Iourin O, Kadlec J, Sutton G, Harlos K, Grimes JM, Stuart DI (2014)
351 Unexpected structure for the N-terminal domain of hepatitis C virus envelope glycoprotein
352 E1. Nature Communications 2014 5:1 5:1–5

353 Madan V, Bartenschlager R (2015) Structural and Functional Properties of the Hepatitis C
354 Virus p7 Viroporin. Viruses 2015, Vol 7, Pages 4461-4481 7:4461–4481

355 Ouyang B, Xie S, Berardi MJ, Zhao X, Dev J, Yu W, Sun B, Chou JJ (2013) Unusual
356 architecture of the p7 channel from hepatitis C virus. Nature 2013 498:7455 498:521–525

357 Atoom AM, Taylor NGA, Russell RS (2014) The elusive function of the hepatitis C virus p7
358 protein. Virology 462–463:377–387

358 359. Gentzsch J, Brohm C, Steinmann E, Friesland M, Menzel N, Vieyres G, Perin PM, Frentzen
360 A, Kaderali L, Pietschmann T (2013) Hepatitis C Virus p7 is Critical for Capsid Assembly
361 and Envelopment. PLoS Pathog 9:e1003355

362 363. Jones CT, Murray CL, Eastman DK, Tassello J, Rice CM (2007) Hepatitis C Virus p7 and
363 NS2 Proteins Are Essential for Production of Infectious Virus. J Virol 81:8374–8383

364 365. Lorenz IC (2010) The Hepatitis C Virus Nonstructural Protein 2 (NS2): An Up-and-Coming
365 Antiviral Drug Target. Viruses 2010, Vol 2, Pages 1635-1646 2:1635–1646

366 367. Lorenz IC, Marcotrigiano J, Dentzer TG, Rice CM (2006) Structure of the catalytic domain
367 of the hepatitis C virus NS2-3 protease. Nature 2006 442:7104 442:831–835

368 369. Brass V, Berke JM, Montserret R, Blum HE, Penin F, Moradpour D (2008) Structural
369 determinants for membrane association and dynamic organization of the hepatitis C virus
370 NS3-4A complex. Proc Natl Acad Sci U S A 105:14545

371 372. Raney KD, Sharma SD, Moustafa IM, Cameron CE (2010) Hepatitis C virus non-structural
372 protein 3 (HCV NS3): A multifunctional antiviral target. Journal of Biological Chemistry
373 285:22725–22731

374 375. Morikawa K, Lange CM, Gouttenoire J, Meylan E, Brass V, Penin F, Moradpour D (2011)
375 Nonstructural protein 3-4A: the Swiss army knife of hepatitis C virus. J Viral Hepat 18:305–
376 315

377 378. Brass V, Berke JM, Montserret R, Blum HE, Penin F, Moradpour D (2008) Structural
378 determinants for membrane association and dynamic organization of the hepatitis C virus
379 NS3-4A complex. Proc Natl Acad Sci U S A 105:14545–14550

380. Lindenbach BD, Prágai BM, Montserret R, Beran RKF, Pyle AM, Penin F, Rice CM (2007) The C Terminus of Hepatitis C Virus NS4A Encodes an Electrostatic Switch That Regulates NS5A Hyperphosphorylation and Viral Replication. J Virol 81:8905–8918

383. Phan T, Kohlway A, Dimberu P, Pyle AM, Lindenbach BD (2011) The Acidic Domain of Hepatitis C Virus NS4A Contributes to RNA Replication and Virus Particle Assembly. J Virol 85:1193–1204

386. Esser-Nobis K, Romero-Brey I, Ganten TM, et al (2013) Analysis of hepatitis C virus resistance to Silibinin in vitro and in vivo points to a novel mechanism involving nonstructural protein 4B. Hepatology 57:953

389. Gouttenoire J, Penin F, Moradpour D (2010) Hepatitis C virus nonstructural protein 4B: a journey into unexplored territory. Rev Med Virol 20:117–129

391. Paul D, Hoppe S, Saher G, Krijnse-Locker J, Bartenschlager R (2013) Morphological and Biochemical Characterization of the Membranous Hepatitis C Virus Replication Compartment. J Virol 87:10612–10627

394. Bukh J (2016) The history of hepatitis C virus (HCV): Basic research reveals unique features in phylogeny, evolution and the viral life cycle with new perspectives for epidemic control. J Hepatol 65:S2–S21

397. Dustin LB, Rice CM (2007) Flying under the radar: The immunobiology of hepatitis C. Annu Rev Immunol 25:71–99

399. Love RA, Parge HE, Yu X, et al (2003) Crystallographic Identification of a Noncompetitive Inhibitor Binding Site on the Hepatitis C Virus NS5B RNA Polymerase Enzyme. J Virol 77:7575–7581

402. Boyce SE, Tirunagari N, Niedziela-Majka A, et al (2014) Structural and Regulatory Elements
403 of HCV NS5B Polymerase – β-Loop and C-Terminal Tail – Are Required for Activity of
404 Allosteric Thumb Site II Inhibitors. PLoS One 9:e84808

405. Savan R, Ravichandran S, Collins JR, Sakai M, Young HA (2009) Structural conservation of
406 interferon gamma among vertebrates. Cytokine Growth Factor Rev 20:115–124

407. Burke JD, Young HA (2019) IFN-γ: A cytokine at the right time, is in the right place. Semin
408 Immunol. https://doi.org/10.1016/J.SMIM.2019.05.002

409. Lieberman LA, Hunter CA (2002) Regulatory pathways involved in the infection-induced
410 production of IFN-γ by NK cells. Microbes Infect 4:1531–1538

411. Schroder K, Hertzog PJ, Ravasi T, Hume DA (2004) Interferon-γ: an overview of signals,
412 mechanisms and functions. J Leukoc Biol 75:163–189

413. Platanias LC (2005) Mechanisms of type-I- and type-II-interferon-mediated signalling.
414 Nature Reviews Immunology 2005 5:5 5:375–386

415. Huang M, Jiang J-D, Peng Z (2014) Recent advances in the anti-HCV mechanisms of
416 interferon. Acta Pharm Sin B 4:241–247

417. Kang S, Brown HM, Hwang S (2018) Direct Antiviral Mechanisms of Interferon-Gamma.
418 Immune Netw. https://doi.org/10.4110/IN.2018.18.E33

419. Wei X, Jia ZS, Lian JQ, et al (2009) Inhibition of Hepatitis C Virus Infection by Interferon-γ
420 Through Downregulating Claudin-1. https://home.liebertpub.com/jir 29:171–178

421. Krieger SE, Zeisel MB, Davis C, et al (2010) Inhibition of hepatitis C virus infection by
422 anti-claudin-1 antibodies is mediated by neutralization of E2–CD81–Claudin-1 associations.
423 Hepatology 51:1144–1157

424. Jiang D, Guo H, Xu C, Chang J, Gu B, Wang L, Block TM, Guo J-T (2008) Identification of
425 Three Interferon-Inducible Cellular Enzymes That Inhibit the Replication of Hepatitis C
426 Virus. J Virol 82:1665–1678

427. Shin E-C, Protzer U, Untergasser A, Feinstone SM, Rice CM, Hasselschwert D, Rehermann
428 B (2005) Liver-Directed Gamma Interferon Gene Delivery in Chronic Hepatitis C. J Virol
429 79:13412–13420

430. Attallah AM, El-Far M, Zahran F, et al (2016) Interferon-gamma is associated with hepatic
431 dysfunction in fibrosis, cirrhosis, and hepatocellular carcinoma. J Immunoassay
432 Immunochem 37:597–610

433. Afify M, Hamza AH, Alomari RA (2017) Correlation Between Serum Cytokines, Interferons,
434 and Liver Functions in Hepatitis C Virus Patients. https://home.liebertpub.com/jir 37:32–38

435. Lu MY, Huang CI, Dai CY, et al (2016) Elevated on-treatment levels of serum IFN-gamma is
436 associated with treatment failure of peginterferon plus ribavirin therapy for chronic hepatitis
437 C. Scientific Reports 2016 6:1 6:1–11

438. Wandrer F, Falk CS, John K, Skawran B, Manns MP, Schulze-Osthoff K, Bantel H (2016)
439 Interferon-Mediated Cytokine Induction Determines Sustained Virus Control in Chronic
440 Hepatitis C Virus Infection. J Infect Dis 213:746–754

441. Georgiev P, Charbonnier LM, Chatila TA (2019) Regulatory T Cells: the Many Faces of
442 Foxp3. Journal of Clinical Immunology 2019 39:7 39:623–640

443. Golzari-Sorkheh M, Zúñiga-Pflücker JC (2023) Development and function of FOXP3+
444 regulators of immune responses. Clin Exp Immunol 213:13–22

Tong X, Kim SH, Che L, Park J, Lee J, Kim TG (2024) Foxp3+ Treg control allergic skin inflammation by restricting IFN-γ-driven neutrophilic infiltration and NETosis. J Dermatol Sci 115:2–12

Zhang W, Leng F, Wang X, Ramirez RN, Park J, Benoist C, Hur S (2023) FOXP3 recognizes microsatellites and bridges DNA through multimerization. Nature 2023 624:7991 624:433–441

Muñoz-Melero M, Biswas M (2024) Role of FoxP3+ Regulatory T Cells in Modulating Immune Responses to Adeno-Associated Virus Gene Therapy. Hum Gene Ther 35:439–450

Saleh QW, Mohammadnejad A, Tepel M (2024) FOXP3 full length splice variant is associated with kidney allograft tolerance. Front Immunol 15:1389105

Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, Naji A, Caton AJ (2001) Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nature Immunology 2001 2:4 2:301–306

Sebzda E, Wallace VA, Mayer J, Yeung RSM, Mak TW, Ohashi PS (1994) Positive and negative thymocyte selection induced by different concentrations of a single peptide. Science (1979) 263:1615–1618

Hogquist KA, Tomlinson AJ, Kieper WC, McGargill MA, Hart MC, Naylor S, Jameson SC (1997) Identification of a naturally occurring ligand for thymic positive selection. Immunity 6:389–399

Sauer S, Bruno L, Hertweck A, et al (2008) T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc Natl Acad Sci U S A 105:7797–7802

466 Lee WH, Kim GE, Hong KJ, Kim HS, Lee GR (2023) Insulin Receptor Substrate 1 Signaling
467 Inhibits Foxp3 Expression and Suppressive Functions in Treg Cells through the mTORC1
468 Pathway. Int J Mol Sci 24:2551

469 Asadipour M, Khansalar S, Rezaei Kahmini F, Eshkevar Vakili M, Ataollahi MR, Ali-
470 Hassanzadeh M, Shams K, Faghih Z, Kalantar K (2024) Evaluation of circulating
471 CD4+CD25+CD127−/low regulatory T cells in newly diagnosed hepatitis C-infected
472 patients. Eur J Inflamm.
473 https://doi.org/10.1177/1721727X241242701/ASSET/IMAGES/LARGE/10.1177_1721727X
474 241242701-FIG2.JPEG

475 Nieves-Rosado HM, Banerjee H, Kane LP (2023) Tim-3 expression in Treg controls viral
476 persistence and effector T cell response during chronic LCMV infection. The Journal of
477 Immunology 210:236.03-236.03

478 Kao HH, Yu RL, Chuang WL, Huang JF, Dai CY, Tan CH (2021) Genetic polymorphisms of
479 regulatory T cell-related genes modulate systemic inflammation induced by viral hepatitis.
480 Kaohsiung J Med Sci 37:1000–1009

481 Da Silva Graça Amoras E, Gomes STM, Freitas FB, et al (2016) Intrahepatic mRNA
482 Expression of FAS, FASL, and FOXP3 Genes Is Associated with the Pathophysiology of
483 Chronic HCV Infection. PLoS One 11:e0156604

484 El-Refaei KER, Zaky DSE, Attia FAK, Hendy OM, Kawzae A-AM (2019) Role of FoxP3+T
485 Regulatory Cells in Chronic HCV and Its Relation to Disease Severity. Clinical Medicine and
486 Diagnostics 9:74–78

487. Smyk-Pearson S, Golden-Mason L, Klarquist J, Burton JR, Tester IA, Wang CC, Culbertson
488 N, Vandenbark AA, Rosen HR (2008) Functional suppression by FoxP3+CD4+CD25(high)
489 regulatory T cells during acute hepatitis C virus infection. J Infect Dis 197:46–57

490. Li S, Floess S, Hamann A, et al (2009) Analysis of FOXP3+ Regulatory T Cells That Display
491 Apparent Viral Antigen Specificity during Chronic Hepatitis C Virus Infection. PLoS Pathog
492 5:e1000707

493. Heeg MHJ, Ulsenheimer A, Grüner NH, et al (2009) FOXP3 expression in hepatitis C virus-
494 specific CD4+ T cells during acute hepatitis C. Gastroenterology.
495 https://doi.org/10.1053/J.GASTRO.2009.06.059

496. Wang Z, Hong J, Sun W, Xu G, Li N, Chen X, Liu A, Xu L, Sun B, Zhang JZ (2006) Role of
497 IFN-γ in induction of Foxp3 and conversion of CD4+ CD25– T cells to
498 CD4+ Tregs. J Clin Invest 116:2434–2441

499. Li S, Jones KL, Woollard DJ, Dromey J, Paukovics G, Plebanski M, Gowans EJ (2007)
500 Defining target antigens for CD25+FOXP3+IFN-γ− regulatory T cells in chronic
501 hepatitis C virus infection. Immunol Cell Biol 85:197–204.