EDITORIAL

APPROPRIATE INDUCTION OF T REGULATORY CELLS AND HBV TOLERANCE: IMPLICATIONS FOR IMMUNOTHERAPY OF AUTOIMMUNE DISEASES

Khalid Eltahir Khalid [1,2], Mohammed El Imam M. Ahmed[3*], Ahmed M Musa[4], Jingwu Zang [2, 5, 6]

1- Department of Biochemistry and Nutrition, Faculty of Medicine, University of Gezira, Wad Medani, Sudan.
2- Joint Immunology Laboratory of Institute of Health Sciences and Shanghai Institute of Immunology, Shanghai JiaoTong University School of Medicine and Shanghai Institutes of Biological Sciences, Chinese Academy of Sciences; China.
3- Urology and Transplantation, Faculty of Medicine, University of Gezira, Wad Medani, Sudan.
4- Department of Clinical Pathology & Immunology, Institute of Endemic Diseases, University of Khartoum, Sudan.
5- Department of Neurology and Immunology, Baylor College of Medicine, Houston, USA.
6- E-Institutes of Shanghai Universities, Shanghai, China.

*Corresponding author:
Mohammed El Imam M Ahmed; MBBS, MD, SIUF.
Faculty of Medicine, Gezira University, Medani, Sudan
Tel:+249912362293 Fax:+249511543415
e-mail: mohammeimam@hotmail.com

Key words: *Hepatitis B, Regulatory T cells, HBV Dane particles, Immune tolerance, autoimmune diseases.*

Abstract

Opposed to autoimmune diseases the immune tolerance to hepatitis B virus (HBV) has been related to inadequate HBV-specific immune response in addition to the high frequency and over expression of CD4+CD25+ T regulatory cells (Treg); however the exact mechanisms behind the induction of Treg cells in HBV infection are yet to be addressed. Generally Treg cells consist of diverse lymphocyte populations that include CD4+ cells, CD8+ cells, and other minor T cell populations. Several different mechanisms have been evolved to inhibit different immune responses, while many studies imply a cytokine-independent suppressive role of Treg cells *in vitro*, however accumulating evidence suggest the *in vivo* immunosuppressive activity of these cells against infection as well as autoimmunity needs cytokines. It is well documented that HBV Dane particles play a vital role in the immunopathogenesis of HBV infection and it has been associated with increased Treg cells which express various memory or activation markers. Based on that, alternative or indirect way of inducing Treg cells which could be a useful tool to ameliorate and/or treat autoimmune diseases is the top research priority. In this review we attempt to emphasize the role of HBV Dane particles (virions) on the induction of Treg cells and immune tolerance in HBV and we

EDITORIAL

also discuss the relevance to search for new immunotherapeutic targets for autoimmune diseases.

Introduction

Hepatitis B virus (HBV) infects more than 350 million people worldwide with considerable morbidity and mortality. Annually, about one million people die due to chronic hepatitis B (CHB), cirrhosis and hepatocellular carcinoma related to HBV infection. [(1)] Understanding of the immunopathogenesis of hepatitis B virus infection is crucial to design immunopreventive and immunotherapeutic modes of control. The currently approved mode of treatment for chronic HB is IFN-α and lamivudine, with each agent having inherent limitations particularly toxicity and the cost. Although, antiviral treatments with neoclos(t)ide analogs is one of the underway therapeutic options for patients with HBV [(2)] The clinical trials on DNA vaccines against HBV infection are still underway and focus mainly on investigating the effects of primary prophylaxis, but few have passed the efficacy phase (*phase III clinical trial*) both in terms of prevention and treatment.[(3,4,5)] Immune responses induced by invading pathogens are antigen-specific in their induction but are nonspecific in their outcomes. Consequently, they are potentially damaging to the host that induces them. In addition, the immune system can respond specifically to self antigens, thereby giving rise to autoimmune diseases. A number of regulatory mechanisms have evolved to prevent such adverse effects. One of these has been shown to rely on a particular subset of $CD4^+$ T cells that appears to have evolved specifically for this protective role. These cells are termed regulatory T cells. The isolation and characterization of T regulatory cells in clinical trials is under focusing, [(6)] and the adoptive transfer of so called natural-like T regulatory cells generated ex vivo by IL-2 and TGF-β as a treatment tool for autoimmune diseases may have sustained, long term beneficial effects, [(7)] excluding the problems associated with *in vitro* culture needed, and the deleterious adverse effects of TGF-β, and IL-10 generated by these cells. CD4+CD25+ regulatory T (Treg) cells are subsets of T cells that maintain the peripheral tolerance against self and foreign antigens. The functional role of Treg cells in tissue damage mediated by self-reactive T cells in human diseases is also under investigation. [(8, 9)] It has been [(10-13)] found that patients with CHB had a significantly higher percentage of T regulatory cells compared with convalescent HB patients and healthy controls. In addition, the frequency of T reg cells were found to be correlated with positive hepatitis B pre-core antigen (HBeAg) and negative hepatitis B pre-core antibody (HBeAb) status as well as HBV viral load [(14)]. These studies indicate an association between T regulatory cells with viral clearance. The establishment of mice models with human viral hepatitis B, and the successful development of vectors expressing HBsAg, HBcAg, and HBxAg, [(14-20)] should facilitate the studies of the molecular mechanism of HBV tolerance associated with high frequency of regulatory T cells. Here we review recent advances of the role of HBV Dane particles (particularly HBsAg and HBeAg) in the induction of Treg cells in HBV, which can be targeted as a useful chemotherapeutic tool against autoimmune diseases.

The Hepatitis B Virus

Classification, structure and replication

Hepatitis B virus is the prototype virus of the hepadnaviridae. The family includes two genera. The genus Orthohepadnavirus contains members that infect mammals. [(21)] Hepatitis B virus can be further classified into eight major genotypes (A to H) based on the nucleotide diversity of > 8%. [(22)] The nucleoprotein of the hepatitis B virus (HBV) exists in two structural forms. The nucleocapsid, designated as the hepatitis core antigen (HBcAg), is an intracellular 21-kDa protein that self-assembles into particles that encapsidate the viral genome and polymerase and is essential for the maturation and the function of the virion. A unique feature of the HBV is the production of a non-particulate second form of the nucleoprotein designated as

EDITORIAL

the pre-core or hepatitis B e antigen (HBeAg). The pre-core and core proteins are translated from 2 distinct RNA species that have different 5 initiation sites. [23] The infectious virion or Dane particle has an outer envelope, which consists of the hepatitis B surface antigen (HBsAg) in a lipid bilayer. This in turn encloses the nucleocapsid core of the virus, within which lies the viral genome. The latter is a relaxed circular, partially double-stranded DNA molecule of 3.2 kb in length, and contains four partially overlapping open reading frames (ORFs). [24] The Pre-S/S ORF encodes the three envelope glycoproteins that are known as the large (L), middle (M) and small (S) HBsAgs. Until now, there is limited data about HBV internalization and transport to the hepatocyte nucleus, however a compelling study has reported that the virion is internalized and uncoated in the cytosol, whence the genome translocates to the nucleus, where it is converted into a double-stranded covalently closed circular DNA (cccDNA) molecule, following completion of the shorter positive (+) strand and repair of the nick in the negative (–) DNA strand. [23-25] In this form, cccDNA serves as the template for all viral mRNAs , and its formation indicates a successful initiation of infection. The life cycle of the virus begins with its attachment to the appropriate hepatocyte receptor, which still remains unknown. [3] It has been suggested that the attachment site of HBV located in the Pre-S1 of the large hepatitis B surface antigen (LHBs) may be involved in attachment to the hepatocyte, and a domain within the medium S protein (MHBs) involved in bringing the virus particle into close contact with the cell membrane, while the short S protein (SHBs) has a role in virus secretion. [25, 26]

Regulatory T cells

Phenotype

In human, approximately 30% of the CD4+ T cell population expresses T regulatory cells. [27] The majority of these cells express CD25 with low-to-intermediate intensity ($CD25^{int}$) and only between 1 and 3% of the CD4+ T cells express CD25 with high intensity ($CD25^{high}$). [27] Thymus-derived CD4+CD25+ Treg cells which have a contact-dependent, cytokine-independent mechanism of action had received a considerable attention. [7, 28, 29] Three other CD4+ Treg subsets are generated in the periphery. Two of them have cytokine-dependent mechanisms of action. One of them, called Tr1 cells, produce predominantly IL-10, [30, 31] whereas the other, called Th3 cells, produce predominantly TGF-β. [32,33] Furthermore; another CD4+CD25+ Treg subset has been described that has a functional profile similar, if not identical, to thymus-derived CD4+CD25+ cells. [33] It is known that CD4+CD25+ Treg cells express various memory or activation markers. The most specific marker of CD25+ Treg is the fork head transcription factor Foxp3 or scurfin, in addition to CD25, OX40, 4-1BB, CD62L, cytotoxic T lymphocyte-associated antigen 4 (CTLA4), and glucocorticoid-induced tumor necrosis factor receptor family-related gene (GITR). Foxp3 is a key factor in murine CD25+ Treg development and function in vivo, conferring suppressive phenotype in naive CD25- cells upon forced expression. [34]

Suppressive function

T regulatory (Treg) cells are currently believed to broadly control T cell reactivity. [8, 28, 29] The mechanisms of immune suppression by CD4+CD25+ T regulatory cells (Treg) have been addressed using many in vitro and in vivo conditions. In vitro studies of sorted $CD25^{int}$ and $CD25^{high}$ cells have shown that it is the $CD25^{high}$ population that functions as suppressor cells. Consequently, $CD25^{int}$ cells are most likely memory cells with CD25 expression resulting from encounter with foreign antigens. Generally Treg cells consist of diverse lymphocyte populations that include CD4+ cells, CD8+ cells, and other minor T cell populations. Several different mechanisms have been evolved to inhibit different immune responses, while many studies

EDITORIAL

imply a cytokine-independent suppressive role of Treg cells *in vitro*, however accumulating evidence suggest the *in vivo* immunosuppressive activity of these cells needs cytokines. (35) The control of autoimmune diseases by Treg cells seems mainly to require the expression of membrane bound TGF-β. (35) It has been found that Treg cells express high levels of cytotoxic T lymphocytes-associated protein-4 (CTLA-4), which may have a role in immune suppression in infectious diseases. (8, 36)

Regulatory T cells and HBV

The generation and differentiation of T cell immunity is subject to control by multiple cellular and molecular events such as the type of the interacting immune cells, co-stimulatory molecules, and cytokines in the milieu. (37) T cell anergy is a tolerance mechanism in which the T cell is functionally inactivated following an initial antigen encounter but remains alive in a hypoactivated state. Two broad categories of T cell anergy have been defined as *clonal anergy*, principally a growth arrest state, and adaptive tolerance, or *in vivo anergy*, which represents a more generalized inhibition of proliferation and effector functions such as cytokine production. In the most successful chronic infections, viral antigens either evade or tolerate the host immune response. The potential role of Treg cells in human liver disease is just beginning to be defined. They may limit liver injury by controlling inflammation or promote transplant tolerance; also they may promote persistent infection by HBV, HCV or control tumor growth. This is supported by the fact that HBV-specific T cell responses are deeply depressed in patients with an established chronic HBV infection, (3,10,38,39) The potential role of CD25+ Treg cells in HBV infection was examined by Stoop and colleagues,(10) who found that the frequency of CD25+CD45RO+CTLA4+ Treg subset among the circulating CD4 T cells was significantly greater in chronically HBV infected than in uninfected subjects. Increased CD25+ Tregs frequency in HBV infected patients was accompanied by increased Foxp3 mRNA expression in peripheral lymphocytes. As for their suppressive function, HBV specific T cell proliferation and IFN-γ secretion in peripheral lymphocytes was enhanced by CD25+ depletion and directly suppressed by CD25+ Treg cells in a dose-dependent manner. These results suggest that $CD25^{+}CD45RO^{+}CTLA4^{+}$ T cells in HBV infected patients represent true immunsuppressive function of CD25+ Treg cells, rather than activated T cells, and it may promote inadequate HBV specific immune response leading to persistent infection. Recently, the proportion of CD4+ CD25+ Treg in CHB patients is increased compared to the healthy blood donor, as well as, the proliferative capacity of CD4 T cells is inhibited by the presence of CD4+ CD25+ Treg. (40) Also CD25+ Tregs was found to suppress the priming and/or expansion of antigen-specific CD8+ T cells during DNA immunization and are involved in the contraction phase of the CD8+ T cell response and may affect the quality of memory T cell pools. (41) In another compelling study by Franzese et al., (11) indicated that the immune modulating effect of CD25+ Tregs was also demonstrated but without a difference in the frequency between HBV infected and uninfected subjects. However, the discrepancy in the frequency CD25+ Treg cells is most likely due to the different criteria used for CD25+ Treg cells detection. CD25+ Treg cells suppressed HBV specific T cells in both HBV infected and convalescent individuals, consistent with a regulatory (if not a pathogenetic) role for CD25+ Treg cells in HBV infection.

It seems clear that the absence of HBV specific T cells could contribute to an inadequate immune response against the virus, leading to chronic infection, (10) moreover, HBV specific T cells may play a role in maintaining tolerance in hepatitis B virus (HBV) transgenic mice (Tg). (42) While cytotoxic T lymphocytes (CTLs) contribute to the control of hepatitis B virus (HBV) infection by inhibiting viral replication and by either killing (41) or non killing infected cells, (43) CD25+ Treg cells may play a role in viral persistence by suppressing HBV specific T cell and CTL responses. This account is supported by the weak or undetectable CTL response to HBV in chronically infected patients. (34-36) Despite all the evidence described above, the mechanism responsible for T cell hypo-responsiveness or tolerance to HBV proteins in patients with chronic HBV infections are still not completely understood. (41) Probably, CD25+Tregs have an immunosuppressive effect on HBV-specific T helper cells, and the insufficient CD4 help and defective CD8 repertoire may play a role at the early stages of infection in influencing HBV persistence. (44)

HBV Dane particles and immune intolerance

Hepatitis B surface antigen (HBsAg)

Hepatitis B surface antigen has a role in virus attachment and secretion into hepatic cells and in the modulation of immune response against HBV infection. Controversial results have been reported regarding the induction of T cells type, and the demonstration of DNA encoding HBsAg in healthy volunteers develops protective antibody titers, and favorably elicited Th1 cells. (45) Other studies in a number of experimental animal models and in HBV-cirrhotic patients proved the induction of Th2 (46, 47) or balanced Th1/Th2 (48) in addition to CD4+ T cell compartment that is associated with a defect in CD8+ T cell effectors *in vivo*. (49) Interestingly, inhibition of CTL response to HBV antigens was conferred by CD4+ T cells when adoptively transferred to C57BL/6 transgenic mice immunized with recombinant vaccinia virus expressing HBsAg. (42) It was reported that HBsAg of T cell origin may have a role in suppressing HBsAb production, which favors persistence of HBV leading to carrier state. (50)

Hepatitis B core antigen (HBcAg)

It has been shown that nuclear HBcAg and cytoplasmic HBeAg are associated with high levels of HBV replication, and that cytoplasmic HBcAg is associated with active liver disease in contrast to cytoplasmic HBeAg. (51,52) *In vitro* culture of rHBcAg with T cells from patients with chronic active hepatitis B is characterized by diminished Th1 and Th2 cytokines secretion, (53) decreased in IL-17 production, (2) induction of IL-18 secretion in normal individuals and HBV patients, (54) on the other hand, injection of HBcAg and HBeAg at low doses into mice primed Th1 immunity and Th2 respectively. (18) It seems that HBcAg has a role in suppressing anti-viral immune responses leading to persistence of infection through over expression of IL-10, this can be explained by that IL-10 produced in response to HBcAg stimulation may prime dendritic cells and monocytes to induce anergic CD4+ and CD8+ T cells in chronic HBV. (55,56) Patients with core promoter mutations have more severe inflammation and fibrosis compared with patients without core promoter mutations, furthermore patients with and without core promoter mutations have comparable serum HBV DNA levels, intrahepatic HBV DNA levels and amount of HBcAg positive hepatocytes, (54-56) plasmids as DNA vaccines have been used by many investigators to investigate the immunogenicity of HBc gene, which elicits high titer of antibodies and specific CTL responses in mice and monkeys. (57-59) Recently, an *in-vitro* study has indicated the Treg of chronic HBV patients had a more potent suppressive effect on response to HBcAg. (41, 60)

Hepatitis B pre-core antigen (HBeAg)

The function of HBV pre-core or HBeAg is largely unknown because it is not required for viral assembly, infection, or replication, however it has a much greater effect on cellular gene expression in comparison with the core protein, and it may have diverse effects on cellular functions and equally different roles in modulating HBV pathogenesis. It has been proposed that HBeAg is significantly efficient than HBcAg in eliciting T cell tolerance, (50) and therefore contributing to HBV persistence. (61) High HBeAg serum levels in human correlate with high rates of virus replication, high levels of viremia, high infectivity, (11) and increased percentage of CD25+Treg cells in chronic HBV patients. (10) This finding supported by other

EDITORIAL

observation that HBeAg can elicit T cell tolerance in murine experimental studies. (62, 63) Because HBeAg is secreted, elicited HBeAg-specific Th2 cells may cross regulate HBcAg-specific Th1 cells, or the secreted HBeAg may preferentially behave as a tolerogen and inactivate HBcAg-specific T cells through deletion or clonal anergy in the periphery, (63,64) in addition to T cell clonal ignorance. (48) Apparently, there was a role of this antigen in the carcinogenesis associated with hepatitis B. (65) It was found that the neonatal infection with HBeAg negative mutant virus results in acute fulminant rather than chronic HBV infection, further more different pre-core mutations have been reported, which correlated with active liver disease and high level viremia, especially in mediterranean region, but generally data is conflicting. (66) These findings propose that the HBeAg can function as tolerogen or at least exerts an anti-inflammatory influence, as immunogen. Probably HBeAg enhances the immune suppressive activity of Treg cells, but the exact mechanism which favors this suggestion is poorly understood and needs to be visited.

Regulatory T cells and human autoimmune diseases

The crucial role of regulatory T cells in self tolerance and autoimmunity has been clearly established in numerous types of regulatory cells, the majority of which are CD4+ T cells. Much focus has been placed on thymically-derived CD4+CD25+ regulatory T cells, given that the depletion of this subset in murine models results in the spontaneous development of autoimmune diseases. These naturally occurring regulatory T cells functionally mature in the thymus, and exert suppression in a contact-dependent manner, thus prevent activation of auto-reactive T cells. T cell mediated suppression of autoimmune disease was first described by Nishizuka and Sakakura 30 years ago. (67) They discovered that thymectomy on day 3 of life (d3Tx) results in organ specific autoimmunity. However, the cells responsible for the inhibition of autoimmune diseases were not discovered until mid 1990s when Sakaguchi and co-workers identified a subpopulation of CD4+ T cells expressing IL-2 receptor subunit (CD25). (35) CD4+CD25+ T cells from patients with multiple sclerosis showed a defective function that includes reduced inhibition to CD4+CD25- proliferation. (34) Despite that few studies looked at the role of regulatory T cells in rheumatoid arthritis (RA); with the exception that the CD4+CD25+ T cell frequency is higher in the synovial fluid than peripheral blood; it is generally accepted that there is no significant difference in the overall frequency of regulatory T cells between RA patients and normal controls. But, the fact that regulatory T cells from RA patients are unable to suppress cytokine production like TNF-α and IL-10 supports the role of competent Treg cells in modifying some autoimmune diseases. (25) Likewise, despite the similarity in frequency of regulatory T cells, the suppressor function of Tregs as demonstrated in proliferation experiments, were significantly reduced in patients with autoimmune polyglandular syndrome (APS). (68) On the other hand, regulatory T cells frequency were found lower in type I diabetes mellitus (IDDM), (69,70) and systemic lupus erythromatosus (SLE), (71) and autoimmune lymphoproliferative syndrome (ALPS). (72)

Concluding remarks

An imbalance in Th1/Th2 or cytokine profile, induction of anergy, depletion of immune effectors, and the activation of CD4+CD25+ regulatory T (Treg) cells, was proposed one of the multiple mechanisms underline viral immunosuppression. Despite the protective effect of T regulatory cells as in HBV it also controls the reactivity of a potentially harmful, self-reactive T cells, help to limit collateral tissue damage caused by a vigorous antiviral immune response, as well as prevents autoimmune diseases as in multiple sclerosis and rheumatoid arthritis. The early studies on potential impact of Treg cells in liver diseases combined with the evolving knowledge about regulatory T cells define a new direction in studying the pathogenetic mechanisms for various liver diseases, including viral hepatitis. Despite the convincing *in vivo* and *in vitro* evidence of the close relation of T lymphocyte activation and the immunopathogenesis of HBV,

EDITORIAL

and the indirect role of the virus, [73,74] the molecular mechanisms of these complex virus-host interactions are yet to be scrutinized, however understanding of HBV Dane particle processes at the molecular and cellular levels in HBV will be the key to unlocking the functional repertoire of these virions. The establishments of HBV models and the availability of the HBV mediated immunization as a new approach in addition to the knowledge gained concerning the pathogenic mechanism by which a potent HBV virions (particularly HBeAg), we hypothesize an alternative or indirect way of inducing Treg cells which could be other useful tool to ameliorate and/or treat auto-immune disease.

References:

1. Lee, W. M. (1997). Hepatitis B virus infection. New England Journal of Medicine; 337, 1733–45.
2. Zhang JY, Song CH, Shi F, Zhang Z, Fu JL, Wang FS. (2010). Decreased ratio of Treg cells to Th17 cells correlates with HBV DNA suppression in chronic hepatitis B patients undergoing entecavir treatment. PLoS One; 5(11):13869-15.
3. Peter Karayiannis (2003). Hepatitis B virus: old, new and future approaches to antiviral treatment. Journal of Antimicrobial Chemotherapy; 51, 761–785.
4. Kriegel MA et al. (2004). Defective suppressor function of human CD4+CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. J Exp Med; 199(9): 1285–91.
5. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK, et al. (2001) Type 1 T regulatory cells. Immunol Rev; 182: 68–79.
6. Hoffmann P, Boeld TJ, Eder R, Albrecht J, Doser K, Piseshka B, Dada A, Niemand C, Assenmacher M, Orso E, Andreesen R, Holler E, Edinger M. (2006). Isolation of CD4+CD25+ regulatory T cells for clinical trials. Biol Blood Marrow Transplant; 12(3):267-74.
7. David A. Horwitz, Song Guo Zheng, J. Dixon Gray, Ju Hua Wang, Kazuo Ohtsuka, Satoshi Yamagiwa. (2004). Regulatory T cells generated ex vivo as an approach for the therapy of autoimmune disease. Seminars in Immunology; 16 135–143
8. Sakaguchi, S., N. Sakaguchi, M. Asano, M. Itoh, and M. Toda. (1995). Immunologic self tolerance maintained by activated T cells expressing IL-2 receptor alpha chains (CD25). Breakdown of a single mechanism of self tolerance causes various autoimmune diseases. J. Immunol; 155:1151–1164.
9. Baecher-Allan C, Hafler DA. (2004). Suppressor T cells in human diseases. J Exp Med; 200(3):273–6.
10. Stoop JN, van der Molen RG, Baan CC, van der Laan LJW, Kuipers EJ, Kusters JG, et al. (2005). Regulatory T cells contribute to the impaired immune response in patients with chronic hepatitis B virus infection. Hepatology; 41:771-778.
11. Franzese O, Kennedy PT, Gehring AJ, Gotto J, Williams R, Maini MK, Bertoletti A. (2005). Modulation of the CD8 T cell response by CD4+CD25+ regulatory T cells in patients with hepatitis B virus infection. J Virol; 79:3322-3328.
12. Cabrera R, Tu Z, Xu Y, Firpi RJ, Rosen HR, Liu C, et al. (2004). An immunomodulatory role for CD4+CD25+ regulatory T lymphocytes in hepatitis C virus infection. Hepatology; 40:1062-1071.
13. Tobias Boettler, Hans Christian Spangenberg, Christoph Neumann-Haefelin, Elisabeth Panther, Simonetta Urbani, Carlo Ferrari, Hubert E. Blum, Fritz von Weizsäcker, and Robert Thimme. (2005). T Cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus specific CD8+ T cells during chronic hepatitis C virus infection. J Virol; 79 (12): 7860–7867.

EDITORIAL

14. Barboza L, Salmen S, Goncalves L, Colmenares M, Peterson D, Montes H, Cartagirone R, Gutiérrez Mdel C, Berrueta L. (2007). Antigen-induced regulatory T cells in HBV chronically infected patients. Virology;368(1):41-9.
15. Gao LF, Sun WS, Ma CH, Liu SX, Wang XY, Zhang LN, Cao YL, Zhu FL, Liu YG (2004) Establishment of mice model with human viral hepatitis B. World J Gastroenterol;10(6):841-846.
16. Bai GQ, Liu Y, Cheng J, Zhang SL, Yue YF, Huang YP, Zhang LY. (2005) Trans-activating effect of complete S protein of hepatitis B virus and cloning of genes trans-activated by complete S protein using suppression subtractive hybridization technique. World J Gastroenterol; 11(25):3893-3898.
17. Musacchio A, Ernesto G. Rodriguez, Antonieta M. Herrera, Diogenes Quintana, and Verena Muzio. (2001) Multivalent DNA-Based immunization against Hepatitis B Virus with plasmids encoding surface and core antigens. Biochemical and Biophysical Research Communications; 282: 442–446.
18. Riedl P, Stober D, Oehninger C, Melber K, Reimann J, and Schirmbeck R. (2002). Priming Th1 immunity to viral core particles is facilitated by trace amounts of RNA bound to its Arginine-Rich domain. The Journal of Immunology; 168: 4951–4959.
19. Bulut Y, Bulut H, Doymaz MZ. (2003.) Immunization of mice with plasmid DNA encoding Hepatitis B Virus core antigen (HBcAg). Turk J Med Sci; 33 61-63.
20. Zhang SJ, Chen HY, Chen ZX, Wang XZ. (2005). Possible mechanism for hepatitis B virus X gene to induce apoptosis of hepatocytes. World J Gastroenterol; 11(28):4351-4356.
21. Schäfer, S., Tolle, T., Lottmann, S. & Gerlich, W. H. (1998). Animal models and experimental systems in hepatitis B virus research. In Hepatitis B Virus: Molecular Mechanisms in Disease and Novel Strategies for Therapy (Koshy, R. C. & Caselmann, W. H., Eds), pp. 51–74.
22. Stuyver L, De Gendt S, Van Geyt C, et al. (2000). A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol; 81:67–74
23. Ganem, D. and R. J. Schneider. (2001). Hepadnaviridae and their replication p. 2923–2970. In D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, B. Roizman, M. A. Martin, and S. E. Straus (ed.), Fields virology, 4th ed. Lippincott Raven Publishers, Philadelphia, Pa.
24. Seeger, C. & Mason, W. S. (2000). Hepatitis B virus biology. Imperial College Press, London, UK. Microbiology and Molecular Biology Reviews, 64, 51–68.
25. Ehrenstein MR, et al. (2004) Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti TNF alpha therapy. J Exp Med; 200(3):277–85.
26. Xuanyong Lu, Timothy Block. (2004). Study of the early steps of the Hepatitis B Virus life cycle. Int. J. Med. Sci. 1(1): 21-33.
27. Wing K, Suri-Payery E & Rudin A. (2005). CD4+CD25+ Regulatory T Cells from Mouse to Man. Scandanavian Journal of Immunology; 62; 1–15.
28. McHugh RS, Shevach EM. (2002). The role of suppressor T cells in regulation of immune responses. J Allergy Clin Immunol; 110:693–702.
29. Shevach EM. (2002). CD4+CD25+ suppressor T cells: more questions than answers. Nat Rev Immunol; 2:389–400.
30. Groux H, O'Garra A, Bigle M, Rouleau M, Antonenko S, de Vries JE, et al (1997). A CD4+ T cell subset inhibits antigen specific T cell responses and prevents colitis. Nature; 389:737–42.
31. Levings MK, Roncarolo MG. (2000) T regulatory cells: a novel subset of CD4 T cells with immunoregulatory properties. J Allergy Clin Immunol; 106:S109-12.
32. Weiner HL. (2001). Induction and mechanism of action of transforming growth factor-beta-

EDITORIAL

secreting Th3 regulatory cells. Immunol Rev; 182:207–14.
33. Yamagiwa S, Gray JD, Hashimoto S, Horwitz DA. (2001). A role for TGF-beta in the generation and expansion of CD4+CD25+ regulatory T cells from human peripheral blood. J Immunol; 166:7282–9.
34. Hori S, Nomura T, Sakaguchi S. (2003) Control of regulatory T cell development by the transcription factor Foxp3. Science; 299:1057-1061.
35. Maloy KJ, Powrie F. (2001) Regulatory T cells in the control of immune pathology. Nat Immunol; 2:816-822.
36. Belkaid Y, Piccirillo CA, Mendez S, Shevach EM, and Sacks DL. (2002). CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature; 420:502-507.
37. Suvas S, Kumaraguru U, Pack CD, Lee S, and Rouse BT. (2003). CD4+CD25+ T Cells Regulate Virus-specific Primary and Memory CD8+ T Cell Responses. J. Exp. Med. 6, 889–901.
38. Bertoletti A, Ferrari C. (2003) Kinetics of the immune response during HBV and HCV infection. Hepatology; 38:4-13.
39. Rehermann B, Chang KM, McHutchinson J, Kokka R, Houghton M, Rice CM, et al. (1996). Differential cytotoxic T lymphocyte responsiveness to the hepatitis B and C viruses in chronically infected patients. J Virol; 70: 7092-7102.
40. Zhang HH, Guo F, Fei R, Ma H, Cong X, Wei L, Chen HS. (2008). Inhibition of CD4+ CD25+ regulatory T cells in chronic hepatitis B patients. Zhonghua Yi Xue Za Zhi;88(8):511-5.
41. Yoshihiro Furuichi, Hirotake Tokuyama, Satoshi Ueha, Makoto Kurachi,Fuminori Moriyasu, Kazuhiro Kakimi (2005). Depletion of CD25+CD4+T cells (Tregs) enhances the HBV-specific CD8+ T cell response primed by DNA immunization immunization. World J Gastroenterol; 11(24): 3772-3777.
42. Suijin R and Kilhyoun Kim. (2003). overcoming tolerance in Hepatitis B Virus transgenic mice; a possible involvement of regulatory T cells. Microbiol. Immunol; 47 (6): 453-460.
43. Guidotti LG, Ando K, Hobbs MV, Ishikawa T, Runkel L, Schreiber RD, Chisari FV. (1994). Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc Natl Acad Sci U S A. 91(9):3764-8.
44. Urbani S, Boni C, Amadei B, Fisicaro P, Cerioni S, Valli MA, Missale G, and Ferrari C. (2005). Acute Phase HBV-Specific T cell Responses Associated with HBV Persistence after HBV/HCV Co-infection. Hepatology; 41:826-831.
45. Roy MJ, Wu MS, Barr LJ, Fuller JT, Tussey LG, Speller S, Culp J, Burkholder JK, Swain WF, Dixon RM, Widera G, Vessey R, King A, Ogg G, Gallimore A, Haynes JR, Heydenburg FD. (2000). Induction of antigen specific CD8+ T cells, T helper cells and protective levels of antibody in humans by particle mediated administration of a hepatitis B virus DNA vaccine. Vaccine; 19: 764-778.
46. Li WY, Jiang YF, Jin QL, Zhang H, Feng XW, Niu JQ. (2010). Immunologic characterization of posthepatitis cirrhosis caused by HBV and HCV infection. J Biomed Biotechnol; 2010:138237-14.
47. Haynes JR, Fuller DH, McCabe DE, Swain WF, Widera G. (1996) Induction and characterization of humoral and cellular responses elicited via gene gun mediated nucleic acid immunization. Adv Drug Deliv Rev; 21:3–18.
48. McCluskie MJ, Brazolot Millan CL, Gramzinski RA, et al. (1999). Route and method of delivery of DNA vaccine influence immune responses in mice and non human primates. Mol Med; 5:287–

EDITORIAL

300.
49. Chen M, Sällberg M, Hughes J, Jones J, Guidotti LG, Chisari FV, Billaud JN, and Milich DR. (2005).Immune Tolerance Split between Hepatitis B Virus Precore and Core Proteins. J Virol. 79(5): 3016–3027.
50. Nagaraju N, Naik SR, and naik S. (1997). Functional implications in hepatitis B surface antigen (HBsAg) in the T cells of Chronic HBV carrier. Journal of Viral Hepatitis; 4: 221-230.
51. Chu CM, Yeh CT, Chien RN, Sheen IS, Liaw YF. (1997). The degrees of hepatocyte nuclear but not cytoplasmic expression of hepatitis B core antigen reflect the level of viral replication in chronic hepatitis B virus infection. J Clin Microbiol; 35: 102–5.
52. Naoumov NV, Portmann BC, Tedder RS, et al. (1990) Detection of hepatitis B virus antigens in liver tissue. A relation to viral replication and histology in chronic hepatitis B infection. Gastroenterology; 99: 1248–53.
53. Szkaradkiewicz A, Jopek A, Wysocki J, Grzymislawski M, Małecka I, Wo´zniak A, (2003). HBcAg specific cytokine production by CD4 T lymphocytes of children with acute and chronic hepatitis B. Virus Research. 97; 127–133.
54. Manigold T, Bocker U, Chen J, Gundt J, Traber P, Singer MV, and Rossol S. (2003). Hepatitis B core antigen is a potent inductor of interleukin-18 in peripheral blood mononuclear cells of healthy controls and patients with hepatitis B infection. Journal of Medical Virology; 71:31–40
55. N Hyodo, M Tajimi, T Ugajin, I Nakamura, M Imawari (2003). Frequencies of interferon-gamma and interleukin-10 secreting cells in peripheral blood mononuclear cells and liver infiltrating lymphocytes in chronic hepatitis B virus infection. Hepatology Research; 27: 109-116.
56. Hyodo N, Nakamura I & Imawari M. (2004). Hepatitis B core antigen stimulates interleukin-10 secretion by both T cells and monocytes from peripheral blood of patients with chronic hepatitis B virus infection. Clin Exp Immunol; 135:462–466
57. Huang ZH, Lu S, Liu N. (1999). Humoral and cellular immunogenecity of genetic vaccine on core gene of hepatitis B virus in mice. Zhonghua Yixue Zazhi; 79: 456-459
58. Lee YS, Yoon SJ, Kwon TK, Kim YH, Woo JH, Suh MH, Suh SI, Baek WK, Kim HJ, Ahn SY, Choe BK, Park JW. Immune response induced by immunization with Hepatitis B virus core DNA isolated from chronic active hepatitis patients. (2001) Immunol Lett; 78: 13-20.
59. Xing YP, Huang ZH, Wang SX, Cai J, Li J, Chou TH, Lu S. (2005). Novel DNA vaccine based on hepatitis B virus core gene induces specific immune responses in Balb/c mice. W J Gastroenterol. 11(29): 4583-4586.
60. Locarninia S, Shaw T, Dean J, Colledge D, Thompson A, Li K , Lemon SM, Lau GGK, Beard MR. (2005). Cellular response to conditional expression of the hepatitis B virus precore and core proteins in cultured hepatoma (Huh-7) cells. Journal of Clinical Virology; 32 113–121
61. Milich DR, Jones JE, Hughes JL, Price J, Raney AK, McLachlan A. (1990). Is a Function of the secreted hepatitis B e antigen to induce immunologic tolerance in utero? Proc Natl Acad Sci USA; 87:6599–603.
62. Chen MT, Billaud JN, Sallberg M, Guidotti LG, Chisari FV, Jones J, et al. (2004).A function of the hepatitis B virus precore protein is to regulate the immune response to the core antigen. Proc Natl Acad Sci USA; 101: 14913-14918.
63. Milich DR, Chen MK, Hughes JL, Jones JE. (1998). The secreted hepatitis B precore antigen can modulate the immune response to the nucleocapsid: a mechanism for persistence. J Immunol;

EDITORIAL

160:2013-2021.

64. Milich, D. R. (1997). Influence of T-helper cell subsets and cross regulation in hepatitis B virus infection. J. Viral. Hep; 4:48–59.
65. Terazawa S, Kojima M, Yamanaka T, Yotsumoto S, Okamoto H, Tsuda F, Miyakawa Y, and Mayumi M. (1991). Hepatitis B virus mutants with precore region defects in two babies with fulminant hepatitis and their mothers positive for antibody to hepatitis B e antigen. Pediatr. Res; 29:5–9.
66. Milich D and Liang TJ. (2003) Exploring the Biological Basis of Hepatitis B e Antigen in Hepatitis B Virus Infection. Hepatology; 38:1075-1086.
67. Nishizuka Y, Sakakura T. (1969).Thymus and reproduction: sex-linked dysgenesia of the gonad after neonatal thymectomy in mice. Science; 166:753–5.
68. Kriegel MA et al. (2004) Defective suppressor function of human CD4+CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. J Exp Med; 199(9): 1285–91.
69. Roncarolo MG, Bacchetta R, Bordignon C, Narula S, Levings MK, et al. (2001) Type 1 T regulatory cells. Immunol Rev; 182: 68–79.
70. Kukreja A, Cost G, Marker J, Zhang C, Sun Z, Lin-Su K, et al. (2002). Multiple immunoregulatory defects in type-1 diabetes. J Clin Invest; 109(1):131–40.
71. Crispin JC, Martinez A, Alcocer-Varela J. (2003). Quantification of regulatory T cells in patients with systemic lupus erythematosus. J Autoimmun; 21(3):273–6.
72. Bleesing JJ, Brown MR, Straus SE, Dale JK, Siegel RM, Johnson M, et al. (2001). Immunophenotypic profiles in families with autoimmune lymphoproliferative syndrome. Blood; 98(8):2466–73.
73. Guidotti LG, Matzke B, Schaller H, Chisari FV. (1995). High level hepatitis B virus replication in transgenic mice. J Virol; 69: 6158-6169
74. Kao JH, Chen PJ, Lai MY, Chen DS. (2003). Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology; 124: 327-3