Review Article

Chronic microbial infections: Manipulation of host immunity as interventional approach

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List of Abbreviations

ALT, alanine aminotransferase: anti-HBs, antibody to HBsAg: APC, Antigen-presenting cell: CHB, Chronic hepatitis B: cccDNA, covalently closed circular DNA: CTL, Cytotoxic T lymphocytes: DC, Dendritic cells: HBV, Hepatitis B virus: HBsAg, hepatitis B surface antigen: HCV, Hepatitis C virus: HBV-TM, HBV transgenic mouse, HIV, Human immune deficiency virus: HSV, Herpes simplex virus: HPV, Human papillomavirus: HBV-TM, HBV transgenic mouse: IL, Interleukin: IFN, Interferon Key words: Chronic viral infections, Immune responses, Antiviral agents, Dendritic cells, Immune therapy

Abstract

Chronic viral infections represent major challenges in contemporary medicine, virology and pharmacology. The virus-bearing hosts are commonly found in every parts of the world and it is extremely difficult to manage these patients. In addition, considerable numbers of these patients develop progressive diseases and severe complications. Finally, most of these patients act as permanent reservoirs of virus. Understandings of viral life cycle during the last decade of 20th century and the first decade of 21st century have allowed development of hundreds of antiviral agents for different diseases. But, the clinical efficacy of these drugs is not yet satisfactory. In addition, virologists have provided conclusive evidences suggesting that eradication of most chronic virus from infected hosts may an unachievable goal. In this context, it is essential to develop alternative, novel, and evidence-based therapeutic maneuver for these patients. Manipulation of host immune system may be one of these approaches. We would discuss about scopes, limitations, and strategies for manipulation for controlling of chronic viral infections.

The primary function of the host's immune system is to mount responses that protect the individual from various microbial infections including viruses. Host's immune responses also control the spread and virulence of the viruses [1]. This is applicable to viruses that cause acute infection. After entering the hosts, these viruses are localized in host's tissues, proliferate and induce antiviral immunity. These cellular events may cause damage and destruction of tissues and the host exhibit features of acute inflammatory diseases. However, the viruses are either almost completely eliminated from the hosts or adequately controlled in situ by host's immune systems. However, chronic infection is established by many viruses because the hosts induce improper and uncoordinated immune responses against these viruses. Most viruses cause persistent infection by evading the host immune surveillance mechanism. Both virus-related factors and host-dependent factors are primarily responsible for viral persistency in subjects with chronic viral infections.

Virus-related factors and persistent viral infection:

Different viruses adopt different mechanisms to establish persistent infections. For example, some viruses may hide in some tissues other than main organ of localization and replication. Thus, the virus can avoid surveillance mechanisms of host immune system [2]. Some viruses can cause latent infections in many tissues and remain inactive for long period of time with minimum levels of replication [3]. In addition, many viruses undergo mutations and avoid immune surveillance of the hosts. The viruses can also circulate as a population of closely-related, yet heterogeneous sequences: the quasispecies in many chronic viral carriers [4]. Some viruses develop different type of life cycle so that it can not be eradicated from the hosts [5].

Latent viral infection and viral persistence:

Evolutionary, microbial agents including viruses have evolved with host immune systems and accordingly are

well adapted to their hosts. Tropism to some specific tissues is part of natural life cycles of many viruses. But, most of the viruses also enter, replicate and integrate in tissues other than their main target organs. Viruses in these tissues can avoid immunological surveillance of the hosts. Latent viral infection may result in acute phase of the disease or more slowly progressive diseases or different types of diseases not resembling to original diseases [6]. Latent viruses can become productive under certain circumstances or in specific cell types. Epstein-Barr virus is latent in B cells and can be productive if released from pharyngeal epithelial cells [7]. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two important human viruses that cause chronic liver diseases such as chronic hepatitis and serious complications like liver cirrhosis and hepatocellular carcinoma. Although these two viruses are primarily hepatotrophic, they have also been detected from many tissues and cells including cells of the immune system such as peripheral blood mononuclear cells, T cells, B cells, macrophages, and dendritic cells [8]. Herpes simplex virus (HSV) usually infects the mucocutaneous surfaces and replicate inside the cells and cause cell death. Concurrently, virus is transported to neuronal cells nuclei in the ganglia innervating the mucocutameous zone of infection. The virus may replicate in this site and establish a clinically silent latent infection [9]. If the host becomes immunosuppressed, the virus may enter into a productive phase and may cause pathological lesions. Recent studies indicate that latent viral infection may be an a common features of most viruses.

Escape from immune surveillance by viral mutations

In order to escape from the host immune surveillance, several viruses undergo mutations so that they are not recognized by the immune systems. Different viruses adapt different mechanisms to achieve this. HCV replicate at an enormous rate of 1012 virions/day by the action of its RNA-dependent RNA polymerase that lacks a proof reading function. This favors formation of variant viruses by cellular and humoral immune responses. Analogous to other RNA virus, HCV circulates in an infected individual as a population of closely related, yet heterogeneous sequences, the quasispecies [10, 11]. The progressive evolution of viral diversity in the hypervariable region of E2 of the HCV has been associated with chronically-evolving hepatitis, whereas, stasis is associated with a self-limited course. However, it is possible that viral escape may be the result rather than cause of viral persistence. Human immune deficiency virus (HIV)-1 also adopts genetic variations to escape attack by host's immune system. The nucleoside misincorporation rate of HIV-1 is of order of 10-4 per site per replication cycle [12]. This virus can undergo frequent recombination between two single stranded positive sense RNA genomes that are present in each virus particle. In addition, internal deletions occur frequently during retrovirus replication. All these allow the mutant strains to escape recognition by the cytotoxic T cell (CTL) responses of the host. In case of human papillomavirus (HPV), infections with multiple HPV types may be responsible for HPV persistence. Infection of individuals with HPV 16 and another type of HPV result in longer duration of detectable HPV 16 than did infection with HPV 16 alone [13]. In comparison to retroviruses, the rate of mutations is comparatively low in DNA viruses such as the HBV. However, the HBV undergoes mutations at different regions of the HBV. It has been estimated that 1010 incorrect nucleotides are incorporated into viral particles every day in a HBV-infected individual. This provides a potential reservoir of genomic variants [14].

Altered life cycle of the HBV and viral persistence

Once a person is infected with the HBV, that individual harbors the virus for the rest of his or her life. The HBV adopts a special type of replication cycle. After interaction of the HBV with cellular receptor on the hepatocyte's membrane, the HBV particle fuses with the membrane, and releases the nucleocapsid into the cytoplasm. The viral envelope proteins are shed during this process and the nucleocapsid migrate to the host nucleus where it is transformed into a supercoiled covalently closed circular DNA molecule (cccDNA). The initial formation of cccDNA from incoming virions cannot be prevented using potent nucleoside analogs inhibiting the viral polymerase and plus strand DNA synthesis [15]. The half life of HBV DNA in the hepatocytes is long compared to that in the blood. Reactivation of viral replication from cccDNA supply new HBV virions that infect fresh hepatocytes. Although, altered life cycle of HBV has been cited here, this may be detected in other viruses as well.

Host-related factors and chronic viral infections

The description provided above indicate that chronic viral infection is established in a host due to several virus-related factors such as presence of latent virus, mutation at the immunogenic epitopes of the viruses, presence of viral quasispecies and exceptional nature of replication of the viruses. In addition, viruses may directly cause functional impairment of host's immune system. Induction of an effective immune response after viral infection is dependent on several factors. Different types of cells of the immune system must act in a coordinated manner for the induction of virus-spe-

cific immune response [16-18]. After the entry to the hosts, the viruses or their antigens must be recognized by the host's antigen-presenting cells (APCs). Different types of immunocytes and parenchymal cells possess APC-like functions. Among these, antigen-presenting dendritic cells (DC) are most potent APC and able to induce both innate and adaptive immune responses. DCs are also endowed with immune regulatory capacities. DC first recognizes the virus at the tissue of infiltration or localization of the viruses. After recognition, the virus is internalized in the DCs by various receptors. The virus is cleaved at the endosomal compartments of DCs into immunogenic epitopes, which then migrate to MHC compartments of DCs and finally to the surface of the DCs. DCs undergo maturation due to internalization and processing of viruses. Maturating DCs then migrate to lymphoid tissues to present antigen to clonally-selected lymphocytes. This lead to the production of virus-specific CD4+ T cells, CD8+ T cells including CTL and antibody-forming B cells including plasma cells. Virus-specific T cells migrate to the tissue of localization of the viruses and destroy the virus-infected cells by cytopathic mechanisms or block the replication of virus by noncytopathic manners. Antibody produced by virus-specific plasma cells neutralizes the free viruses. If the magnitude of virus-specific immune responses is strong and coordinated, it will ultimately result in clearance or down regulation of viral replication. An efficient antiviral immune responses can be induced if all the cellular events related to virus/host interactions such as (1) viral recognition (2) viral internalization (3) intracellular migration of viral antigens (4) maturation of DCs after processing of viral antigens (5) migration of DCs expressing antigenic peptides of the virus (6) presentation and formation of virus-specific T and B cells (7) migration of antiviral T cells to virus-infected tissues and (8) production of neutralizing antibodies proceed in a systemic and coordinated manner. However, several studies have shown that the virus down regulate the functions of DCs by various techniques. Viruses such as HIV-1, HCV and HBV infect the DCs and down regulate their capacities to induce virus-specific immunity by destroying DCs, reducing their T cell stimulatory capacities and interfering with their ability to produce various cytokines. Moreover, virus-specific CTL, and CD4+ T cells also show impaired migration to tissues of localization of the viruses. Finally, some viruses down regulate the functions of effecter cells of immune systems. High viral load inhibit or down regulate the functional capacities of CTLs. In addition to DCs, many viruses have been detected in T cells and B cells and the functions of these cells are also compromised by these viruses [18].

Disease patterns during chronic viral infections

Patients chronically infected with different viruses may remain asymptomatic or may develop features of different diseases. The nature of the pathological processes also shows diversities. Again, chronic viral carriers may remain asymptomatic initially for many years and then develop features of different diseases. Some times, the diseases are progressive in nature and serious complications of those diseases are developed. This is especially manifested in cases of chronic infections by non cytopathic viruses like hepatitis viruses. The causes underlying these are not well understood. However, several investigations have pointed that inadequate and uncoordinated immune responses in chronic viral carriers play roles not only for viral persistence but also for diseases progression.

Therapy of chronic viral infections

Therapy against chronic viral infections is a major challenge for all disciplines of medicines and sciences. Several questions regarding purpose of therapy and nature of therapy have remained unanswered. The final goal of treatment of chronic viral carriers is to eradicate the viruses and down regulate the disease processes. The eradication of the viruses from chronic viral carriers should be achieved before irreversible damages of the hosts occur. Accordingly, several antiviral agents have been developed to destroy the viruses from chronic viral carriers. An ideal antiviral agent should have the following criteria: (1) capable of destroying all types of viruses including latent viruses from all tissues, (3) able to destroy the mutant viruses and viral quasispecies, (3) should be effective against intracellular viruses including modified forms of viruses such as the cccDNA forms of the viruses. Unfortunately, antiviral drugs with these properties are not available at present. However, newer antiviral drugs are coming to the markets. Many of these drugs have been developed to tackle some particular viruses after developing insights about the life cycle of those viruses. Several antiviral agents are used in combination in some patients with chronic viral infections. However, adequate therapeutic effects are not detected in most cases. It is elusive whether it is possible to develop ideal antiviral drugs for chronic viral carriers? In this perspective, we will discuss about some agents that are not endowed with direct antiviral properties, but possess antiviral capacities. Already some of these agents are used in clinics and there are ample opportunities to develop newer and more potent agents in future. Indeed, many of these agents may also be used with antiviral drugs to have a synergist effect against chronic viral infections.

Non antiviral agents with antiviral properties

Viruses that establish chronic infection in immune competent hosts affect either the afferent limb or the efferent limb of the host's immune systems. In some cases, these viruses cause impairment of both wings of host's immune system. Chronic viral infection is established and maintained due to virus-related factors and/or host's immune-dependent factors. It appears that factors like viral mutations, viral quasispecies or latent viral infections are virus-related factors causing persistent infections. However, this is also a failure of host's immune system to handle mutant viruses and latent viruses. It is important to assess the target of therapy for patients with chronic viral infections. Circumstantial evidences suggest that complete eradication of viruses from chronic viral carriers may not be an achievable goal for most of the viruses. Accordingly, the aim of the therapy should be to induce reduction of replication of viruses to a degree so that progressive diseases can be minimized or blocked. The approach will vary according to the nature of the viruses. It has been proposed that induction of virus-specific immune responses in chronic viral carriers have antiviral potentiality. Most chronic viral carriers harbor abundant amounts of viruses. However, they fail to induce proper anti viral immune responses due to alteration of their own structures or changes in their life cycle or due to establishment of latent infection. In addition, some viruses directly down regulate the functions of immunocytes including those of DCs.

Rationale for designing virus-specific immune therapy against chronic viral infection:

Impaired immune responses of the host are detected in patients with chronic viral infection. There are two ways to induce immune responses in these hosts: (1) antigen non-specific immune responses by administration of cytokines and immune modulators and (2) antigen-specific immune responses by administration of virus-specific antigens. It is important to clarify why there is need to induce antigen-specific, but not, antigen non-specific immune responses in chronic viral carriers. Comparative data are not available regarding the role of different antigen non-specific and antigen-specific immune responses in chronic viral infections. But recent studies have shown that antigen non-specific immune responses may have destructive, not protective, role in some chronic viral infections such as in patients with chronic HBV and HCV infections [19]. Many mononuclear cells including lymphocytes are accumulated at the liver of these patients. Although these features indicate that there are ongoing immune responses in these subjects, the viruses are not eliminated. On the other hand, the hepatocytes are destroyed in these patients with exacerbation of necroinflammatory liver diseases. Only recently, it has been reported that most of these mononuclear cells at the liver of these patients are antigen non-specific. Moreover, it has been shown that antigen non-specific lymphocytes cause damage of the hepatocytes in these patients [20]. This may be applied for other chronic viral infections, but more information about the immunopathogenesis of those diseases is required. In one hand, complete eradication of the viruses from chronic viral infected persons is unrealistic and unachievable. Induction of antigen non-specific immune response may cause tissue damage and progressive diseases. In this perspective, we opted to induce virus-specific immune responses in chronic viral carriers. The goal of this therapy is to induce a condition like recovered acute infection in which the residual viruses are controlled by host's immune responses. Moreover, there are evidences that antigen-specific immune responses reduce tissue damage and eliminate the viruses or reduce their replication.

Therapeutic vaccination for induction of antigen-specific immune responses in chronic viral carriers

The concept of therapeutic vaccination pre-dates the availability of antibiotics when they were unsuccessfully used to treat invasive bacteria. In 1920s therapeutic immunization against HPV was done with wart extracts. Only recently, new insights have been developed regarding therapeutic vaccine in HPV [21]. In chronic HSV infections, a wide range of therapeutic vaccines have been tried with no apparent success [22]. In HIV infection, various HIV-related antigens have been used as therapeutic vaccines. Various phase I/II trials have been conducted from 1985 [23]. In order to induce antigen-specific immune responses, investigators have administered virus-specfiic antigens. The antigens have been used in different types of adjuvant for different durations. Vandepapeliere et al. have reviewed about therapeutic vaccinations in different types of chronic viral diseases [24]. Several studies have been conducted in chronic HBV carriers to induce antigen-specific immune responses by vaccines. At present it is possible to use hepatitis B surface antigen (HBsAg) as a specific antigen to induce antigen-specific immune responses. Vaccine containing HBsAg has many advantages. It is commercially available in human consumable forms as prophylactic vaccine. However, the scientific rationale was lacking to use this in patients with chronic hepatitis B (CHB). All patients with CHB harbor abundant amounts of HBsAg and it is presumed that HBsAg is a tolerogenic antigen for CHB patients. A series of animal studies paved the way for usage of HBsAg-based vaccine as therapeutic vaccine in CHB pa-

tients [25, 26]. Studies in HBV transgenic mice (HBV-TM) reveled that although HBV-TM expressed HBV DNA and HBsAg in the liver and the sera, administration of HBsAg in complete Freund's adjuvant caused HBsAg-specific immunity and production of HBsAg-specific T cells and anti-HBs in HBV-TM [27, 28]. Vaccine therapy in HBV-TM also caused reduced signals of HBV DNA in the liver. It was hard to explain why HBV DNA was reduced in HBV-TM due to vaccine therapy because there was no evidence of damages of HBV containing hepatocytes due to this therapy. At that time, Chisari and his groups have reported that it is possible to destroy or induce reduced replication of HBV and HBV DNA by a non cytopathic manner by cytokines and immune modulators in another line of HBV-TM [29, 30]. Pilot study about vaccine therapy has been conducted in patients with CHB [31]. The outcome of these studies are variable, but most of the studies have shown that vaccine therapy caused reduced replication of HBV DNA, normalization of liver enzymes and seroconversion to antibody to HBe in some, but not all patients, with CHB [32-34]. However, extensive controlled trials are required regarding the dose of vaccine, the nature of vaccine, and duration of immunization to get antiviral responses from these non antiviral agents in patients with CHB. Recently, we have reported that a combination of antiviral therapy and vaccine therapy have potent antiviral potential inpatients with CHB. A group of CHB patients were given lamivudine, an antiviral agent, daily, for 3 months. Then, these patients received 12 injections with vaccine containing HBsAg. This combination therapy showed potent antiviral capacity and all patients became negative for HBV DNA during the first year of therapy [35]. This therapeutic approach is scientific because high viral load is a major obstacle for induction of natural or therapy-mediated immune responses in chronic viral infections. One of the important causes underlying the impaired immune responses of chronic viral carriers is the inability of APCs to recognize and process viral antigens properly. They are also unable to present antigenic peptide to clonally selected lymphocytes. It is apparently hard to induce antigen-specific immune responses in these subjects by administration of vaccines or antigens. This issue can be addressed by loading antigen on DCs in vitro. This caused processing of antigens by DCs in vitro and there was no need to recognize and process antigens by DCs in situ. Using HBsAg-pulsed DCs, we were able to induce very potent HBsAg-specific immune responses in HBV-TM [36]. Presentation of antigen by antigen-pulsed DCs has already been used by several investigators to induce antigen-specific immune responses in patients with cancers. But, this has not been used to induce antigen-specific immune responses in chronic viral infections. Recently, we have shown that it is possible to prepare HBsAg-pulsed human blood DCs. A study has been reported by us regarding the safety and efficacy of this approach in normal volunteers [37].

Concluding remarks

Chronic viral infection is a major public health problem around the world. There are several hundred million chronic viral carriers in the globe and many of them will develop progressive diseases including serious complications like cancers. On the other hand, all chronic viral carriers are permanent and living sources of viral infections. It is these persons who transmit the viruses to healthy individuals and maintain the transmission cycle of the virus. Extensive studies indicate that chronic viral carriers establish chronic infection by evading the host's immune surveillance. In most of the cases, it is not possible to eradicate the viruses from chronic viral carriers completely by antiviral drugs. But, administration of antiviral drugs cause improvement of clinical conditions and arrest the progression of diseases processes in some cases. Immunotherapy and especially active, specific immunotherapy or therapeutic vaccination is in theory a very promising approach for the treatment of chronic viral infections. Deficient immunological mechanisms are involved in the establishment of chronic infection, in its persistence, and/or in the pathogenesis of clinical disease. In some cases, successful spontaneous control of the infection is associated with profound modifications in the systemic or local immune responses with intense activation of specific immune mechanisms. Usually, those immune responses are T-cell mediated. Both T-helper 1 and CTLs responses have been detected on these conditions. Also, numerous studies in various animal models have delivered positive results with candidate therapeutic vaccines, mainly in HBV, HPV and HSV infections. The concept of therapeutic vaccination is almost one century old. Several clinical trials have been conducted during from the last decade of 1990s. More works are needed to reproduce the findings in laboratory benches to patient's bedside. Therapeutic vaccines to HBV may be the most feasible approaches to control of chronic infections in view of the immune-correlated control of infection observed naturally. All attempts at vaccination in CHB have not shown desired effect, but have shown some therapeutic efficacy. There are many causes underlying this. Practically, the immune approach will have to be adapted to the virological, serological, and clinical status of the infection. The selection of the right antigens will also be critical in the development of therapeutic vaccines. Although several candidate vaccines are promising, the pathogenesis of the infection and disease is so complex that clinical demonstration of efficacy may be very long and difficult. HIV chronic infection will prob-

ably be one of the most challenging targets for the development of therapeutic vaccination. The outcome of HIV infection is precisely a progressive destruction of the immune cells that could have a key role in viral control. Most of the current strategies for an HIV vaccine concentrate on the induction of a CD8+ T-cell response. However, it has been shown recently that a single point mutation can allow a viral escape and development of disease. Therefore, a broader immune response, including a CD4+ T cell and a strong antibody response is likely to be needed, both for prophylactic and therapeutic vaccination.The world is encountering different emerging viruses with potential devastating properties. Bird flu viruses are becoming a major public health challenges. In addition to viruses, many bacterial infections may shatter the ongoing public heath delivery system. Malaria and tuberculosis are two such bacterial infections. Due to emergence of mutant types of strains of these bacteria, it has become difficult to treat these patients by antibiotics. Although, manipulation of host immunity has not been started by immune modulators during bacterial infections, a new strategy of anti-bacterial therapy can be developed by on the basis of this concept. Modulation of host immunity is a traditional approach of management of infectious diseases. The endeavor started with eating nutritious food, taking adequate rest, using of traditional biological response modifier. Now, modulation of host immunity during chronic microbial infections is a reality. Development of proper strategies remains to be addressed.

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Conflict of interest

There is no conflict of interest about this article.

References

1. Zinkernagel RM. Immunology taught by viruses. Science; 1996; 271: 173-178.
2. Borrow P. Mechanisms of viral clearance and persistence. J. Viral. Hepat 1997; 4 (suupl): 16-24..
3. Jarvis, MA, Nelson JA. Mechanisms of human cytomegalovirus persistence and latency. Front. Biosci 2002; 7: 1575-1582
4. Gould, K.G.; Bangham, CR. Virus variation, escape from cytotoxic T lymphocytes, and human retroviral persistence. Semin Cell Dev Biol 1998; 9: 321-328
5. Locarnini S. Molecular virology of hepatitis B virus. Semin Liver. Dis; 2004 (suupl): 24, 3-10
6. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisziewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF, Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. Nature Med 1999; 5: 512-517.
7. Amon, W, Farrell PJ. Reactivation of Epstein-Barr virus from latency. Rev Med Virol 2005; 15:149-156.
8. Hilleman MR. Overview of the pathogenesis, prophylaxis and ther apeusis of viral hepatitis B, with focus on reduction to practical applications. Vaccine 2001; 19: 1837-1848.
9. Schmid SD, Malwe AC. T cell responses to herpes simplex viruses in humans. Rev Infect Dis 1991; 13; S946-S949
10. Forns X, Purcell RH, Bukh J. Quasispecies in viral persistence and pathogenesis of hepatitis C virus. Trends. Microbiol 1999; 7: 402-410
11. Layden TJ, Lam NP, Wiley TE. Hepatitis C viral dynamics. Clin. Liver. Dis 1999; 3: 792-810.
12. Chao L. Fitness of RNA virus decreased by Muller's ratchet. Nature 348; 454-455.
13. Ho GY. Bierman R, Beardsley L, Chang CL, Burk RD. Natural history of cervicovaginal papillomavirus infection in young women. N Eng J Med 1998; 338: 423-428,
14. Gunther S, Li BC, Miska S, Kruger D, Meisel H, Will, H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients J Virol 1995; 69: 5437-5444.
15. Zoulim F. Mechanism of viral persistence and resistance to nucleoside and nucleotide analogs in chronic hepatitis B virus infection. Antiviral Res 2004; 64: 1-15.
16. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. Cell 2001; 106: 255-258.
17. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature 1998; 392: 245-252..
18. Onji M, Akbar SM. Dendritic Cells in clinics. Springer; Tokyo, 2008.
19. Tuttleman JS, Pourcel C, Summers J. Formation of the pool of covalently closed circular viral DNA in hepadnavirus-infected cells. Cell., 1986; 47: 451-460.
20. Maini MK, Boni C, Ogg GS, King AS, Reignat S, Lee CK, Larrubia JR, Webster GJ, McMichael AJ, Ferrari C, Williams R, Vergani D, Bertoletti A. Direct ex vivo analysis of hepatitis B virus-specific CD8(+) T cells associated with the control of infection. Gastroenterology 1999; 117: 1386-1396.
21. Vandepapelière P. In Sexually transmitted diseases: vaccines, prevention and control; LR Stanberry and DI Bernstein, Ed: Academic Press, London, 2000, pp. 273–293.
22. Stanberry LR, Harrison CJ, Bernstein DI, Burke RL, Shukla R, Oti G, Myers MG. Herpes simplex virus glycoprotein immunotherapy of recurrent genital herpes: factors influencing efficacy. Antiviral Res 1989; 11: 203-214.
23. Peters BS. The basis for HIV immunotherapeutic vaccines, Vaccine., 2001; 688-705.
24. Vandepapeliere P. Therapeutic vaccination against chronic viral infections. Lancet Inf Dis 2002; 2: 353-267.

25. Chisari F, Ferrari C. Hepatitis B virus immunopathogenesis.Ann. Rev. Immunol 1995; 13: 29-60.

26. Akbar SM, Onji M. Hepatitis B virus (HBV)-transgenic mice as an investigative tool to study immunopathology during HBV infection. Int J Exp Pathol 1998; 79: 279-291.

27. Akbar SM, Kajino K, Tanimoto K, Michitaka K, Horiike N, Onji M. Placebo-controlled trial of vaccination with hepatitis B virus surface antigen in hepatitis B virus transgenic mice. J Hepatol 1997; 26: 131-137.

28. Akbar SM, Furukawa S, Horiike N, Onji M. Vaccine therapy for hepatitis B virus carrier. Curr Drug Targets Infect Disord 2004; 4: 93-101.

29. Chisari FV, Ferrari C. Ann Rev Immunol 1995; 13: 29.

30. Chisari FV. Cytotoxic T cells and viral hepatitis. J Clin Invest 1997; 99: 1472-1477.

31. Pol S, Nalpas B, Driss F, Michel M, Tiollais P, Denis J, Brechot C and a multicenter study group. Efficacy and limitations of a specific immune therapy in chronic hepatitis B. J. Hepatol., 2001, 34, 917-921.

32. Pol S, Driss F, Michel ML, Nalpas B, Berthelot P, Brechot C. Specific vaccine therapy in chronic hepatitis B infection. Lancet 1994; 344: 342-343.

33. Wen YM, Wu XH, Hu DC, Zhang QP, Guo SQH. Hepatitis B vaccine and anti-HBs complex as approach for vaccine therapy. Lancet 1995; 345: 1575-1576.

34. Horiike N, Akbar SM, Ninomiya T, Abe M, Michitaka K, Onji M. Activation and maturation of antigen-presenting dendritic cells during vaccine therapy in patients with chronic hepatitis due to hepatitis B virus. Hepatol Res 2002; 23: 38-47.

35. Horiike N, Akbar SM, Michitaka K, Joukou K, Yamamoto K, Kojima, N, Hiasa Y, Abe, M, Onji M. In vivo immunization by vaccine therapy following virus suppression by lamivudine: a novel approach for treating patients with chronic hepatitis B. J Clin Virol 2005; 32: 156-161.

36. Akbar SM, Furukawa S, Hasebe A, Horiike N, Michitaka K, Onji M. Production and efficacy of a dendritic cell-based therapeutic vaccine for murine chronic hepatitis B virus carrier. Int J Mol Med 2004; 14: 295-299.

37. Akbar SM, Furukawa S, Onji M, Murata Y, Niiya T, Kanno S, Murakami H, Horiike N. Safety and efficacy of hepatitis B surface antigen-pulsed dendritic cells in human volunteers. Hepatol Res 2004; 29: 136-141.