CHAPTER 1

Introduction

1.1 Statement and significance of problem

Human immunodeficiency virus (HIV) infection causes serious problem worldwide, including Thailand. World Health Organization (WHO) reported that 36.9 million [34.3 - 41.4 million] people were living with HIV as at the end of 2014 globally [1]. HIV-infected (HIV+) individuals, who persistently expose to viral antigens, manifest several impairments of the immune system compared to healthy individuals [2]. The loss of number and/or function of CD4+ helper T cells, which are necessary to activate both B cells and cytotoxic CD8+ T cells, are crucial for these defective immune responses [3-5]. As other immunocompromised populations, HIV+ individuals are more susceptible to infections and are at increased risk to severe disease than normal populations. These include influenza and hepatitis B viruses (HBV) infections. Vaccination is a cost effective approach for long-term prevention against the diseases caused by these viruses.

The antibody responses to 2009 H1N1 influenza A and hepatitis B vaccination in HIV+ populations have been widely studied. Lower antibody responses to H1N1 influenza A and hepatitis B vaccinations in people living with HIV compared with healthy people have been reported [6-10]. Poor antibody responses are associated with high HIV viral load and low CD4+ T cell counts. On the contrary, the mechanism of cell-mediated immune responses (CMI) to H1N1 influenza A and hepatitis B vaccines, both in healthy and HIV+ populations, is less well understood. T cell responses to the viruses, particularly to cross-reactive epitopes of the viruses, may have the capacity to attenuate the course of diseases.

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This study aimed to investigate CMI responses to the 2009 H1N1 influenza A and hepatitis B after vaccination in HIV-infected individuals. Whether modification of hepatitis B vaccination schedule and/or doses in HIV+ individuals would improve

immune responses to the vaccines was also investigated. The knowledge from this study may provide fundamental information addressing important issues for vaccine development.

1.2 Objectives of study

1.2.1. To characterize the phenotypes and functions of antigen-specific CD4+ and CD8+ T cells after 2009 H1N1 influenza A vaccination.

1.2.2. To characterize the cellular immune response after HBV vaccination.

1.2.3. To compare cellular immune responses between HIV-infected and healthy vaccinees.

1.3 Education/application advantages

1.3.1. The cellular immune responses after 2009 H1N1 influenza A and hepatitis B vaccination in HIV-infected and healthy individuals are identified.

1.3.2. The understanding of cellular immune response to vaccination in HIV-infected and healthy vaccinees are established.

1.3.3. This study may provide fundamental information addressing important issues for vaccine development.

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1.4 Literature reviews

1.4.1. Biology of viruses

1) Human immunodeficiency virus (HIV)

HIV is classified as Lentivirus which belongs to the family of Retroviridae and the subfamily of Lentivirinae. Mature HIV virions have spherical morphology of 100-120 nm in diameter. An HIV particle consists of an envelope surrounds core-shape nucleoprotein (core) which contains genomic ribonucleic acid (RNA) molecules, viral protease (PR), reverse transcriptase (RT), integrase (IN), and regulatory proteins [11, 12] (Figure 1.1).

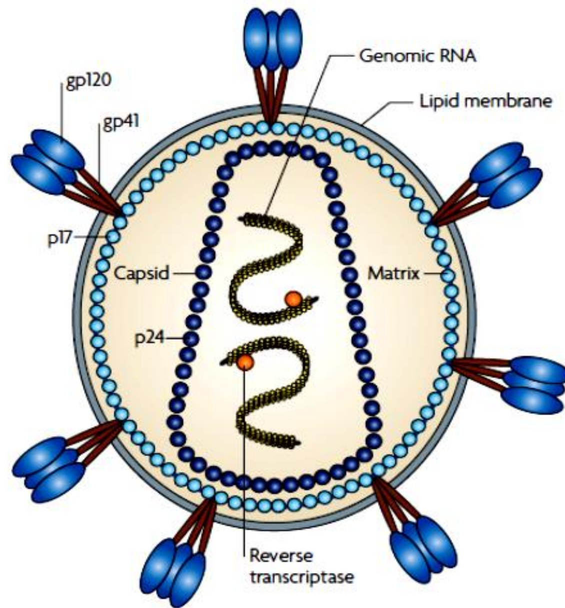

Figure 1.1. Schematic diagram of HIV-1 virus
(source: modified from Karlsson Hedestam GB. et al, Nat Rev Microbiol, 2008 [13])

The viral envelope is an outer bi-layered membrane consisting of two major viral glycoproteins which derived from the host cell, these are gp41 and gp120. These viral glycoproteins are generated by enzymatic cleavage of the viral precursor protein gp160 [14]. gp120 and gp41 play an important role in viral binding and fusion to the target cell membrane, respectively. The HIV structural proteins forming the core consist of matrix protein (p17), capsid protein (p24) and nucleocapsid protein (p7 and 9). The p17 proteins form a shell anchored to the inner side of the envelope. The assembly of p24 proteins constitutes the capsid structure. The p7 nucleocapsid protein is associated with the formation and stabilization of the genomic RNA dimers and with the nucleocapsid assembly, whereas p9 nucleocapsid proteins do not covalently attached to the viral RNA [11, 12].

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The genomic RNA in HIV particle is comprised of two copies of positive-sense, single-stranded RNA (ssRNA). Its genome is approximately 10 kb in length and encodes 16 distinct proteins. These proteins are encoded by the gag (group-specific antigen) pol (polymerase) and env (envelope) genes. HIV also encodes two

regulatory proteins, the transcriptional transactivator (Tat) and the regulator of virion gene expression (Rev). Additional four accessory proteins encoded by the virus are negative effector (Nef), viral infectivity factor (Vif), the viral proteins r (Vpr) and u (Vpu). These proteins are essential for HIV infection, replication and production [11].

2) Influenza virus

Influenza viruses are negative-sense ssRNA viruses of the family Orthomyxoviridae that causes regular seasonal epidemics in humans, other mammalian species and birds. Influenza viruses are spherical or filamentous in shape, with the spherical form of 100 nm in diameter and the filamentous form often in excess of 300 nm in length [15]. Three phylogenetically and antigenically distinct viral types, designated as influenza A, B and C, circulate globally in human populations. However, type A viruses exhibit the greatest genetic diversity, the widest range infection of host species and the vast majority cause of severe disease in humans as well as the great pandemics infection [16]. Influenza A viruses have eight gene segments that encode 11 viral proteins: hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), polymerase acidic protein (PA), polymerase basic protein 1-F2 (PB1-F2), two matrix proteins (M1 and M2), two non-structural protein (NSP1 and NSP2 or nuclear export protein (NEP)) and two polymerase basic protein (PB1 and PB2) [17]. Base on the antigenicity of HA and NA, influenza A viruses are divided into 16 HA (H1–H16) and 9 NA (N1–N9) subtypes (or serotypes) [18, 19].

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The influenza A viral particle comprises a lipid bilayer envelope, which is derived from the host cell membrane during the viral budding process, containing three viral proteins, HA, NA, and M2 (Figure 1.2).

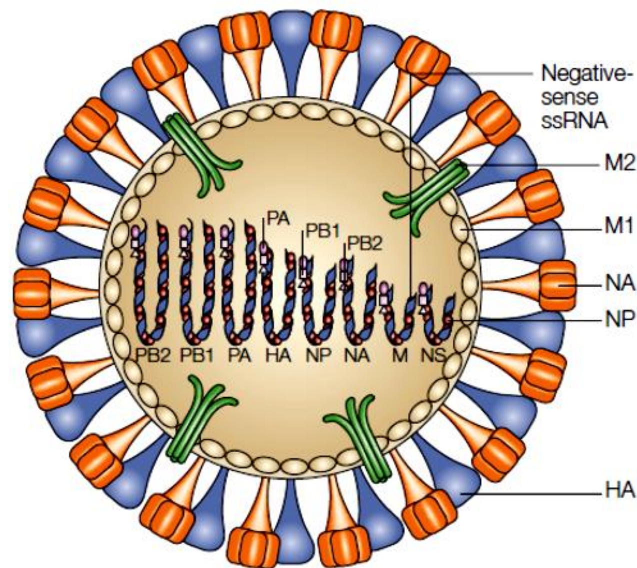

Figure 1.2. Schematic diagram of an influenza A virion
(source: modified from Horimoto T, Kawaoka Y., Nat Rev Microbiol, 2005 [20])

The HA forms a homotrimer, which is responsible for the receptor binding and membrane fusion. The function of NA homotetramer is to destroy receptors by hydrolysing sialic acid groups from glycoproteins and to release the viral progeny. M2 protein generates the homotetramer, which functions as an ion channel for the acidification of the interior of the viral particle during viral infection. M1 protein, which is associated with both ribonucleoprotein and envelope, forms a layer under the viral envelope, defined as capsid [11, 12]. All eight influenza viral RNA segments are bound to the NP and three viral polymerases (PA, PB1 and PB2) to form ribonucleoprotein (RNP) complexes.

3) Hepatitis B virus (HBV)

HBV, a partially double stranded DNA (dsDNA) virus, is a prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting the liver cells, but small amounts can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extrahepatic disease. HBV virions are 40 to 42 nm in diameter of double-shelled

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particles. The outer shell is lipoprotein envelope that contains three related envelope glycoproteins (or surface antigens). Within the envelope is the viral nucleocapsid or core that contains the viral genome and polymerase, which is responsible for the synthesis of viral DNA in infected cells. In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter. These subviral particles contain only envelope glycoproteins and host-derived lipids and typically outnumber virions by 1000:1 to 10,000:1. [21].

HBV envelope consists of lipid bilayer of host cell membrane and three different membrane-spanning viral surface proteins, termed large (L), middle (M), and small (S) proteins (Figure 1.3).

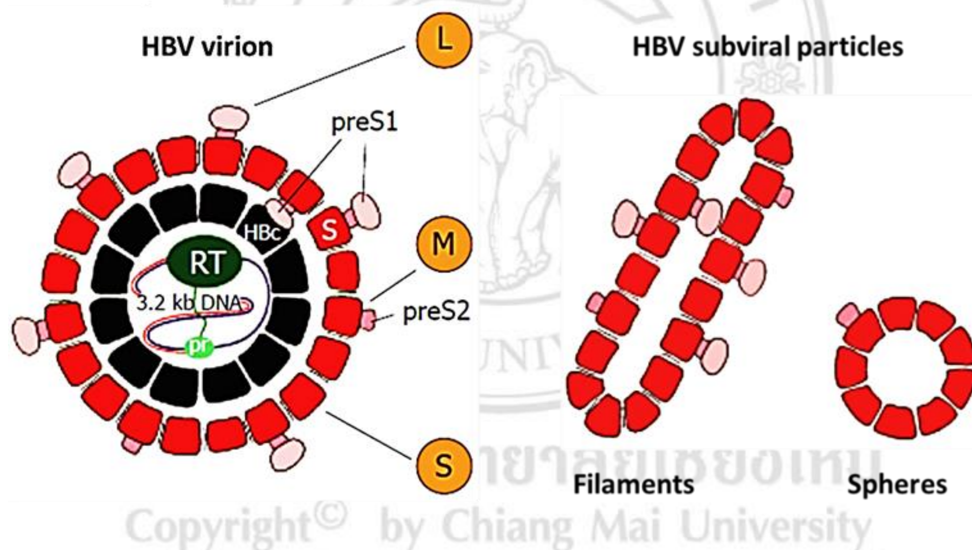

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Figure 1.3. Schematic diagram of HBV virion and subviral particles
(source: modified from Glebe D, Urban S., World J Gastroenterol., 2007 [22])

L-protein consists of an S domain, preS1, and preS2 domains. The preS1 and preS2 domains of L-protein are localized either at the viral surface or inside the virion. M-protein contains only the S and preS2 domains and S-protein, which is known as HBsAg, consists of the S domain alone. The assembly of viral core proteins (HBcAg) forms an icosahedral structure capsid, which encapsidates the viral genome.

The core probably interacts with the internally localized pre-S domain of HBsAg. The HBV genome is a relaxed circular DNA of ~3,200 nucleotides and consists of a full-length of negative strand and a shorter positive strand.

1.4.2. Immunity against viral infections in healthy population

1) Immunity against influenza virus infection

Influenza virus infection is the most common causes of acute respiratory disease with mild morbidity with fever to mortality with ultimately respiratory failure [23]. Both innate and adaptive immunity play a crucial contribution to the clearance of virus infected cells and the resolution of pulmonary inflammation and injury.

1.1) Innate immunity against influenza virus infection

The innate immune system is the first line of defence against influenza virus infection that endeavours to control viral replication, prevent infection of respiratory epithelial cells and initiate the adaptive immune response. Influenza virus infection is firstly detected within the infected respiratory epithelial cell through the recognition of pathogen-associated marker pattern (PAMP) of virus by germline-encoded receptors of the innate immune system known as pattern recognition receptors (PRRs) [24]. The three main classes of PRRs involved in influenza virus recognition include the Toll-like receptors (TLRs), the retinoic acid–inducible gene (RIG)–like receptors (RLRs), and the nucleotide-binding oligomerization domain (NOD)–like receptors [25].

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The TLRs are the first groups of PRRs to recognize influenza viral infection in the respiratory epithelial cell [26]. After infection, influenza virus replicates within the epithelial cell, then double-stranded RNA (dsRNA) is generated [27]. Viral dsRNA in the endosome is recognized by TLR3 [28] (Figure 1.4 A). During the spreading of influenza virus from epithelial cells to alveolar macrophages and dendritic cells (DCs), TLR7, an intracellular receptor inside acidified endosomes recognizes ssRNA from the degraded ribonucleoprotein complex without the requirement of viral replication [29, 30] (Figure 1.4 B). Signalling through TLR3and TLR7 induces the

production of pro-inflammatory cytokines and type I interferons (IFNs) via the activation of nuclear factor-κB (NF-κB) or IFN-regulatory factor (IRF), respectively [28, 30-32]. The recognition of TLR3 induces an antiviral state via the recruitment of damage-inducing inflammatory cells, and the induction of IFN response via the recognition of TLR7 leads to blocking of viral replication and promoting antibody responses [25].

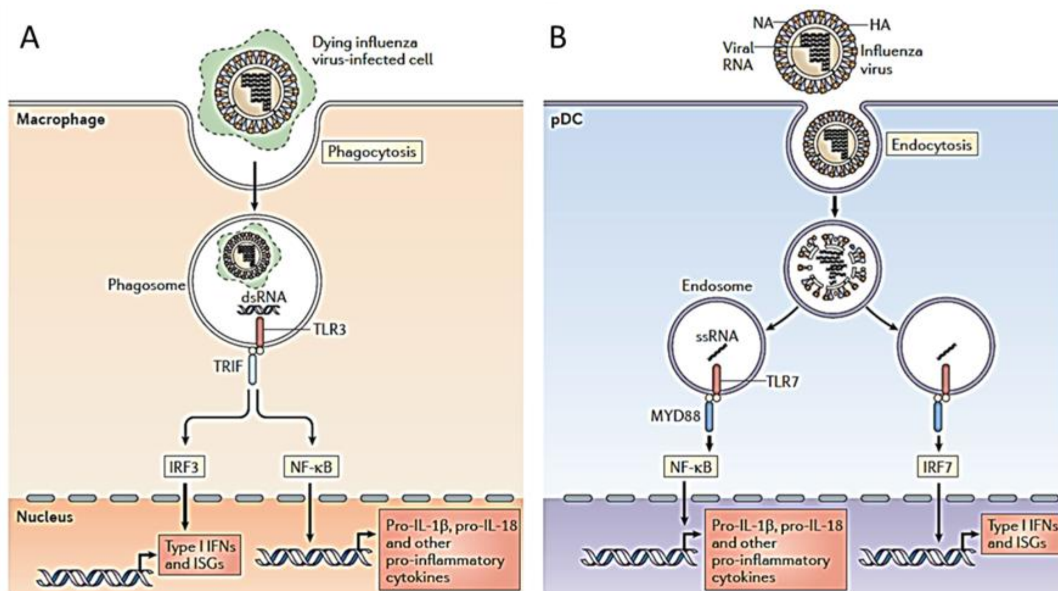

Figure 1.4. Innate sensing of influenza virus infection via TLR3 (A) and TLR7 (B). (source: modified from Iwasaki A. and Pillai P. S., Nature Reviews, 2014 [25])

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RIG-I, a member of the RIG-like helicase receptor family, is also essential for viral detection in infected epithelial cells (Figure 1.5), conventional DCs and alveolar macrophages [33]. Inside the cytosol of influenza virus infected cells, RIG-I recognizes the intact genomic and shorter genomic segment ssRNA bearing the 5′-triphosphate that is generated during viral replication [34-37]. Upon recognition of 5′-triphosphate viral ssRNA, the ATP-binding RIG-I forms complex with caspase recruitment domains to bind to its adaptor mitochondrial antiviral signalling protein (MAVS) [38-40]. MAVS signalling initiates NF-κB and IRF3 signalling to result in

pro-inflammatory cytokine production and type I IFN production, respectively. RIG-I activation is essential to efficiently produce IFN and control influenza virus replication [36]. Moreover, activation of RIG-I can strongly increase germinal centre (GC) reactions and T follicular helper cell (Tfh) responses, resulting in the induction of long-lasting antibodies, enhancement of antibody affinity and augmentation of antibody-secreting cells (ASCs) [41].

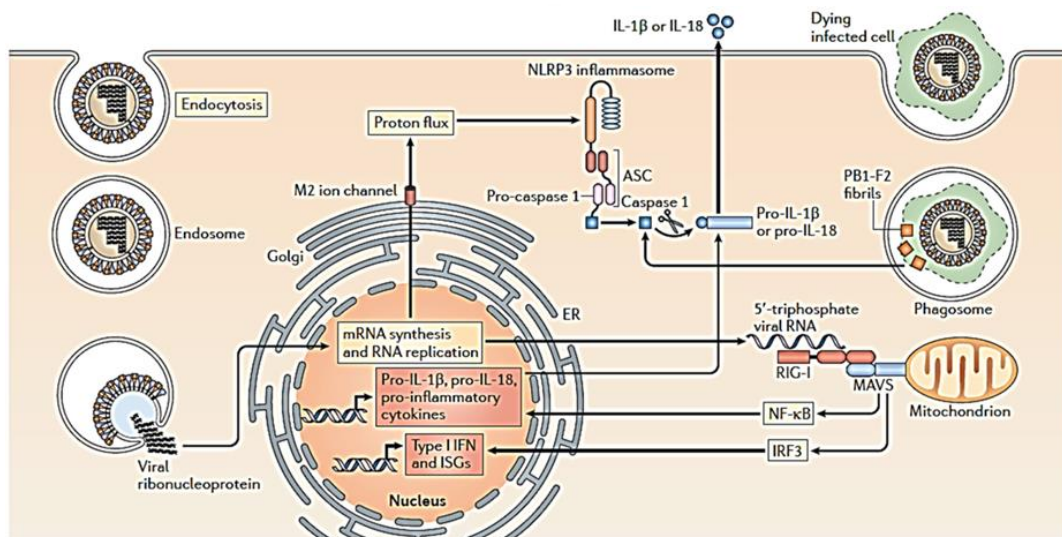

Figure 1.5. Innate sensing of influenza virus infection via RIG-I and NLRs-pyrin domain-containing 3.

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NOD-like receptors (NLRs) is also crucial for detection of microbial invasion and cellular injury. Activation of NLRs induces the assembly of multimeric protein to generate inflammasome complex (Figure 1.5). The NLRs family, NLRs-pyrin domain-containing 3 (NLRP3) is expressed by myeloid cell types including monocytes, DCs, neutrophils, macrophages [42] and human bronchial epithelial cells [43]. NLRP3 inflammasome complex consists NLRP3which is a PRR, the adaptor protein apoptosis-associated speck-like protein containing a caspase recruitment domain and activated caspase-1. Activated inflammasome complex is required for the proteolytic maturation

of the pro-inflammatory cytokines, pro-interleukin (IL)-1β and pro-IL-18 into IL-1β and IL-18, respectively [25, 44]. Furthermore, inflammasome activation can elicit pyroptosis (an inflammatory form of programmed cell death) of infected cells [45]. Host protection by activated NLRP3 involves in the increasing of disease tolerance but not antiviral resistance [46, 47] via the recruitment of leukocytes into the lungs and tissue-repaired induction [48].

The recognition of PAMP by PRRs in epithelial cells, alveolar macrophages and DCs induce the production of type I IFN, including IFN-α, IFN-β, IFN-κ, IFN-ε, and limitin, and type III IFNs, including IFN-λ1, IFN-λ2, and IFN-λ3 [49, 50]. Type I IFN stimulates the expression of IFN-stimulated genes (ISGs) in neighbouring cells, which induce antiviral functions. Mx protein is the first ISGs identified capable of inhibiting influenza virus infection [51]. MxA protein locating in the cytosol binds viral nucleocapsid and prevents nuclear import of the virus [52-54]. Mx1protein is present in the nucleus to prevent viral transcription [51, 55, 56]. The 2′–5′ oligoadenylate synthetases (OAS) family is expressed in the cytosol to produce 2′–5′ oligoadenylate from ATP, which activates RNase L to cleave viral RNA [57]. Serine/threonine kinase is also expressed in the cytosol to inhibit cellular translation machinery [58].

In addition to IFNs that induce antiviral effector molecule, various cytokines are also comprised in influenza virus infection responses. These include tumour necrosis factor -α (TNF-α), IL-1α, and IL-1β, IL-6, IL-18 and a variety of chemotactic cytokines such as IL-8 (a neutrophil attractant), monocyte chemoattractant proteins (MCPs), and macrophage inflammatory proteins (MIPs) [59]. TNF-α has a direct inhibitory effect on the replication of influenza viruses and activates neutrophil and macrophage functions [60]. IL-1 induces the migration of DCs from lung to mediastinal lymph nodes [61]. The pleiotropic cytokine IL-6 promotes protective adaptive immune responses and prevents fatal immunopathology [62], and IL-18 induces CD8+ T cells to produce IFN-γ [63]. IL-8 and MCP-1 function as chemoattractant to recruit neutrophil and monocytes, respectively [64]. The function of MlP-1 and MIP-2 are recruiting of monocytes, lymphocytes and neutrophils. MIPs also

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functions in haematopoiesis regulation and stimulation of other inflammatory mediators related to the production IL-1and TNF-α [65].

DCs, the professional antigen-presenting cells, can detect and opsonize the neutralized-virions and apoptotic bodies from infected cells and at the same time can be infected themselves. After recognizing virions and infected cells, DCs migrate to the draining lymph node via the afferent lymphatic system then present the influenza virus-derived antigen to T cells for activation [65].

Natural killer (NK) cells are also one of important effector cells in the innate immune response. NK cell-mediating cytolysis consists of 3 mechanisms. These include (a) exocytosis of cytoplasmic granules containing perforin and granzyme, (b) Fas ligand (FASL)–mediated induction of apoptosis, and (c) antibody-dependent cellular cytotoxicity (ADCC) [66]. NK cells express three major families of molecules, transmembrane natural cytotoxicity receptors (NCRs), KIR receptors (humans), and the Natural killer cell group (NKG)-2 receptors that involved in regulation of NK cells functions. The engagement of NKp44 to HA of influenza virus [67] and NKp46 to HA of influenza virus-infected cells [68] trigger the function of NK cell to lyse the infected cells.

1.2) Adaptive immunity against influenza virus infection

The adaptive immune system establishes the second line of defence against influenza virus infection. This system comprises humoral immunity mediated by specific antibodies from differentiated B cells, defined as plasma cells and cellular immunity mediated by both antigen-specific CD4+ and CD8+ T lymphocytes.

1.2.1) Humoral Immune response

B cells are important for humoral immunity, being the precursors to antibody-producing plasma cells. The virus-specific B cell response is generated generally in lymphoid tissues, either in regional draining lymph nodes or in mucosa-associated lymphoid tissue [69]. When encountered the infectious virus, mature DCs take up the virus and migrate to the secondary and tertiary lymphoid tissues and present specific viral antigen to B cells. Antigen recognition, B cells migrate to the border of the

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lymphoid follicle where proliferation occurs. Contact between CD4+ helper T (Th) cells and B cells via the recognition of T cell receptor (TCR) on Th cells and antigen presented by the major histocompatibility complex (MHC) class II on B cells together with the interaction between CD40 and CD40L and cytokine signalling via IL-4, IFN-γ and transforming growth factor (TGF)-β, induces immunoglobulin class switching from IgM to IgG or IgA, and proliferation of B cells [70, 71]. Non-GC short-lived plasmablasts differentiate at this stage, while some B cells progress to form the secondary follicle with CD4+ Tfh cells and follicular DCs that initiate somatic hypermutation and affinity maturation in the germinal centre (GC) reaction [72, 73]. Following multiple rounds of selection in the GC reaction, non–antibody-secreting memory B cells and high-affinity antibody-secreting cells are generated. Memory B cells are composed in order to generate a greater and a more rapid secondary response upon re-infection with the virus. On the other hand, high-affinity antibody-secreting cells are responsible for the sudden increase in antibody titres during infection, however this population compresses after antigen clearance, leaving a small number of long-lived plasma cells [74]. The leftover long-lived plasma cells migrate to survival niches that include the bone marrow and respiratory mucosa, and they are responsible for the maintenance of long-term antibody titres for many years [75, 76]

HA is the viral receptor that binds to the host's epithelial cell receptor, allowing the virus to enter the host cell. The binding of specific neutralizing antibodies to HA on viral surface obstructs the viral attachment and its entry into the target cells [77]. In addition to neutralizing viruses, the ligation of Fc receptors by neutralizing antibodies can also trigger the complement cascade and ADCC mechanism [78]. The enzymatic activity of NA to cleave the sialic acid residues on the cell surface results in facilitating the release and spread of newly formed virus particles [79]. The NA-specific antibodies do not block infection, but they inhibit enzymatic activity of the virus resulting in the reduction of pulmonary virus titres and limiting virus spread [80]. Furthermore, both HA or NA-specific antibodies also facilitate ADCC mechanism that contributes to clearance of virus-infected cells [80]. Matrix protein 2 (M2) is a third viral membrane protein with highly conserved among influenza viruses of different subtypes. M2-specific antibodies facilitate the ADCC mechanism [80, 81] and are likely to afford heterosubtypic immunity [82-84]. NP, a highly conserved antigen between

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influenza A virus subtypes, is an important antigen for protective T cells activation. NP-specific antibodies are also induced after infection [85]. NP-specific antibodies are non-neutralizing but they may contribute to protection against influenza virus infection [86, 87]. Although the exact mechanism of protection remains to be elucidated, NP-specific antibodies can accelerate ADCC mechanism of infected cells, improve T cell responses and induce complement mediated cell lysis of infected cells [87-89].

Most antibody-mediated protection against influenza has been determined in serum by which Immunoglobulin (Ig) G is the main class. However, peripheral blood is probably not the site of antibody response to prevent the respiratory virus infection. Serum IgGs specific to influenza are predominantly found in the respiratory tract and afford long-lived protection [90]. In fact, the elimination of respiratory virus by immune system occurs mainly at the respiratory mucosa. In this mucus membrane, virus-specific antibodies operate in concert with the physical barrier and antiviral substances secreted by respiratory epithelium [91]. Mucosal antibodies specific to influenza, particularly in the upper respiratory tract, are mostly in the form of locally produced dimeric secretory IgA [92], and lack of IgA are extremely susceptible to influenza virus infection [93].

1.2.2) Cellular immune response

The initiation of adaptive immunity depends on DCs, with the ability to activate naïve CD4+ and CD8+ T cells [94]. DCs are a key element in viral infection by their ability to respond rapidly and secrete high levels of anti-viral type I interferons [95]. DCs exist in the epithelia of the upper respiratory tract which is the entry site of influenza A virus, and are rapidly mobilized to this area in response to infection [96, 97]. There is low evidence of influenza A virus replication in lymphoid tissue, therefore the main source of virus antigen is thought to be DCs that exit the respiratory tract and travel to lymphoid tissue where adaptive immune responses are initiated [98, 99].

(1) Role of CD4+ T cells

Mature naïve T cells migrate and entry to secondary lymphoid tissue by expression of lymphoid homing receptors (CD62L also known as L-selectin) and

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chemokine receptor (CCR7). In lymphoid tissues, influenza-specific naïve CD4+ T cells interact with mature DCs bearing viral antigens via TCR on CD4+ T cells and antigen-specific MHC class II on mature DCs, resulting in activation of CD4+ T cells. These activated CD4+T cells undergo several rounds of proliferation and polarize into distinct effector CD4+ T cell subsets that differentially orchestrate protective immune response. The differentiation of polarized effector CD4+ T cells is organized by the cytokine environment and unique sets of transcription factors that make specific cytokine-releasing CD4+ T cells during their activation.

There are various effector CD4+ T cells subsets in response to viral infection [100]. Th1 cells, mainly generated in response to viral infection produce large amounts of IFN-γ and TNF-α, and express T-bet transcription factor. This phenotype classically depends on the exposure of T cells to high levels of IL-12, type I IFNs and IFN-γ in the priming milieu [101]. Th2 cells express GATA3 transcription factor and produce predominantly IL-4 to drive optimal humoral immune response [102]. Tfh cells accomplish the generation and maintenance of high affinity B cells and antibody responses and produce IL-21 at the most frequency [103]. Regulatory T (Treg) cells, involving in the suppression of the immune responses, express Foxp3 transcription factor and produce IL-10 as a key suppressive function [104, 105].

(2) Role of CD8+ T cells

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In general, CD8+ T cells play a key role against viral infections. Following influenza infection, DCs bearing influenza antigens from respiratory tract migrate to draining lymph nodes to encounter naïve CD8+ T cells [117]. The activation of CD8+ T cells initiates by recognition of specific antigen on TCR by antigen-presenting MHC class I on DCs or APCs, serving as a primary signal. The interactions of co-stimulatory molecules and its ligands, which are CD28, CD27 and CD40 on T cells to B7 (CD80/86), CD70 and CD40L on APCs, respectively act as a second signal to induce their clonal expansion [118, 119]. The third signal is delivered by cytokines such as IL-12, type I interferons and IL-15 that manipulate the magnitude of the primary response (effector cells) and the formation of memory cells [120].

Effector CD8+ T cells eliminate viral infected cells through direct cytotoxicity, cytokine production or recruitment of other effector cells to the infection area via chemokine-dependent manner. Cytotoxicity is mediated via two major pathways, granule-dependent and ligand-ligand induced cell death mechanisms [106]. The competency of memory CD8+ T cell responses is dependent on several signals during the priming phase, including availability of CD4+ T cell help [107]. Lytic granules of CD8+ T cells consist of perforin and granzymes [106]. Granzyme B plays an important role in inducing apoptosis, and perforin facilitates the entry of granzymes into the target cells. These two molecules are contained in membrane-bound lysosomes coated with lysosomal-associated membrane proteins (LAMPs or CD107). LAMPS are not usually present on the surface of T cells but are exposed only during degranulation. Different populations of effector CD8+ T cells in response to infection can be identified by detection of LAMPs [108]. Ligands interaction induced cell death is also involved in the induction of apoptosis. FAS-FASL and TNF-related apoptosis-inducing ligand (TRAIL)-TRAIL-Receptor (death receptor, DR) are the two interaction molecules that important for cytotoxic activity. Effector CD8+ T cells express FasL and TRAIL whereas target cells express their ligand, Fas and DR, respectively. These cells are susceptible to apoptosis mediated by caspases cascade machinery [109].

Pre-existing virus-specific CD8+T cells correlates with protection against disease severity caused by infection with 2009 H1N1 influenza A viruses in humans [110]. Human cytotoxic CD8+T cells (CTL) induced by influenza virus infection are mainly directed against highly conserved epitopes between different subtypes of influenza A virus such as NP, M1 and PA proteins [111-114]. After activated by APCs in lymph nodes, protective influenza-specific CD8+ T cells require secondary MHC-restricted antigen-dependent interaction with pulmonary DCs in lung [115]. Normally, the destructions of target cells by CD8+ T cells utilize the cytolytic contact-dependent via perforin and Fas pathway as a primary mechanism [116]. After influenza virus infection, virus-specific effector and memory CD8+T cells are also found in the lung apart from secondary lymphoid organs for several months [117-119]. This lung resident memory CD8+ T cells are recruited from circulating memory T cells to the lung through CCR5 [120], CXC chemokine receptor (CXCR) 3 [121] and the

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retention of lung resident memory T cells by the collagen-binding integrin, namely very late antigen (VLA)-1 [122].

2) Immunity against HBV infection

2.1) Innate immunity against HBV infection

Liver, the target organ of HBV infection, contains parenchymal hepatocytes and non-parenchymal cells (NPCs) which involved in local innate immune responses to HBV infection. NPCs are composed of liver sinusoidal endothelial cells (LSEC), liver resident macrophages or Kupffer cells (KCs), hepatic stellate cells (HSCs) and intrahepatic immune cells including DCs, NK cells, NKT cells, lymphocytes and circulating monocytes and neutrophils [123]. The enrichment of innate immune cells support the notion that the liver is an immunologic organ fully competent for immune sensing [123, 124].

Several PRRs, including TLRs, RIG-I and melanoma differentiation associate gene (MDA) 5 are expressed by liver-resident LSECs, KCs, HSCs and immune cells [125, 126], as well as the hepatocytes [127, 128]. The mRNA of NLRs family has also been detected in liver cells, though pattern of expression of NLRs in hepatocytes remains unclear [129]. The activation of PRRs by viral components, such as viral nucleic acids, oligomers of envelope proteins and nucleocapsids induces innate immune responses via signalling pathway. The activation of signalling pathways via IRF3 or IRF7 and NF- κB results in type I IFN and pro-inflammatory cytokines production. These cytokines and chemokines have an effect on the viral replication and spreading before the more specific and powerful adaptive immune responses commence [130].

2.2) Adaptive immunity against HBV infection

The outcome of HBV infection is determined by the host immune response against the virus. Major HBV-specific adaptive immune cells including B cells, CD4+ T cells and CD8+ T cells are the key elements influencing the outcome of HBV infection. B cells are responsible for the production of neutralizing antibody. CD4+ T cells function as a helper to facilitate CD8+ T cells and B cells responses via a

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robust cytokines production. Activated CD8+ T cells operate cytotoxic function to eliminate the HBV-infected cells.

2.2.1) Humoral Immune response

The information from routine monitoring of serological patterns of HBV infected patients arise the current knowledge of the humoral immune response. The patterns of the humoral response against the structural HBcAg and HBsAg proteins of the virus provide the distribution into distinct clinical profiles. HBcAg-specific IgM antibodies are the marker of early infection, whereas antibodies against HBsAg and HBeAg appear much later and indicate a favourable outcome of the infection [131, 132]. Both HBcAg-specific and HBsAg-specific IgG antibodies remain for life after clinical recovery. Anti-HBcAg antibodies do not exhibit protective activity but represent a reliable marker of all patients who have ever been exposed to HBV. In contrast to anti-HBcAg antibodies, antibodies specific to HBsAg are virus neutralizing antibodies and mediate protective immunity by both neutralizing free viral particles and preventing the uptake of uninfected hepatocytes [133]. Anti-HBsAg antibodies also can be detected in HBV-vaccinated individuals. Anti-HBeAg antibodies appear late during infection and indicate a favourable outcome of infection. The loss of serum HBeAg and seroconversion to anti-HBeAg are associated in clinical recovery in acute hepatitis patients. In chronically evolving hepatitis, clearance of serum HBeAg and development of anti-HBeAg antibodies indicate the transition from high replicative to low replicative inactive hepatitis B [134].

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2.2.2) Cellular immune response

(1) Role of CD4+ T cells

The facilitation of HBV-specific CD4+ T cells are required to support efficient cytotoxic CD8+ T cell responses and T cell-dependent B cell responses against HBV infection. Patients in acute phase who subsequently develop chronic infection can not generate the CD4-mediated protective immune response [135]. CD4+ T cell response in the acute phase of self-limiting infection patients is significantly more frequent, strong and multi-specific than that observed in the chronic patients [136-138].

These CD4+ T cells have a preferential Th-1 profile, specific to multiple epitopes targeting HBcAg (which mostly shared epitopes with HBeAg) rather than HBsAg [131, 138-140] and able to persist for decades after the resolution of infection serving as long-term immunological memory response [141, 142]. The response of CD4+ T cell specific to HBcAg are found within weeks during the incubation phase of acute hepatitis B [143]. At the time of maximal liver damage, those CD4+ T cells are still present but much lower frequency than the incubation phase [143]. Then, the decreases in the frequency of these specific CD4+ T cells are observed after clinical resolution of infection [143, 144]. Therefore, CD4+ T cells contributions to the immune response are probably essential for the effective control of viremia and seem to be associated with HBV clearance.

(2) Role of CD8+ T cells

It is widely believed that CD8+ T cells are the main cellular subset responsible for viral clearance. The development of a vigorous polyclonal CD8+ T cell response during acute infection is associated with the efficient resolution of HBV infection [134]. These polyclonal cytotoxic CD8+ T cells in peripheral blood recognize several epitopes within HBcAg, HBsAg, HBV polymerase and HBx proteins and persist for decades after clinical recovery from acute infection and remain present after spontaneous resolution of chronic infection [142, 145, 146]. Patients with chronic infection and progressive liver injury tend to have weak and narrowly focused CD8+ T cell responses [135-137, 142]. CD8+ T cells serve a dual function in HBV control which are the elimination of HBV-infected cells via their cytotoxicity and antiviral by secreting antiviral cytokines such as IFNs and TNF-α without cytolytic activity [147-149]. Similar to CD4+ T cells, HBcAg-specific CD8+ T cells appear to represent an effective correlate of protection. Amino acid mutations within HBcAg epitope, leading to a functional loss of the corresponding CD8+ T cell response, have been detected n chronically infected patients, whereas mutations within HBsAg and polymerase epitopes are rare [150-152].

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1.4.3. Immunity against viral infection in HIV+ individuals

Infection with HIV of immune cells such as CD4+ T cells, macrophages and DCs results in impairment of cell functions. This ultimately causes the immune system to lose the capacity to control infections, defined as acquired immunodeficiency syndrome (AIDS), leading to life-threatening illnesses. It is believed that both humoral and cellular arms of the immune system are affected.

Plasmacytoid DCs (pDCs) express high levels of CD4 and CCR5 and CXCR4, which are HIV co-receptors. The interaction between HIV envelope protein and CD4 on pDCs induce endocytosis and the production of IFN-α by DCs via endosomal acidification dependent mechanism [32, 153, 154]. However, HIV-stimulated pDCs express low levels of co-stimulatory CD86 molecule and express Indoleamine 2,3-dioxygenase, which is IFN-inducible enzyme that suppresses adaptive T-cell immunity, suggesting that they do not differentiate into potent APCs [155, 156] and HIV-exposed pDCs also inhibit CD4+ T cell proliferation [157, 158]. Besides, monocytes and macrophages are affected being unable to present antigens, phagocytose, kill intracellular pathogens, migrate in response to stimulus and produce cytokines [159-163]. Impairment in the antigen presentation and stimulation capacity of antigen presenting cells has been reported in HIV-infected patients [159].

The major target cells of HIV infection is activated CD4+ T cells [128] and the progressive depletion of CD4+ T cells is a hallmark of this viral infection. HIV infection induces elimination of CD4+ T cells by direct infection [164], and bystander effects of syncytia formation [165], immune activation, proliferation, and senescence [166]. Additionally, the dysregulation of CD4+ T cell responses is also a crucial event in the failure of immune control of chronic infections [167, 168]. T cells manifest with progressive loss of effector functions, sustained upregulation and co-expression of multiple inhibitory receptors (such as programmed cell death protein 1 (PD1) and Fc-receptor-like-4 (FcRL-4)), altered expression of key transcription factors and a failure to acquire antigen-independent memory T cell homeostatic responsiveness, known as T cells exhaustion [2, 169, 170]. Exhausted CD4+ T cells display reduced production of effector cytokines, such as TNF-α and IFN-γ and express high levels of PD1 [171, 172].

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As CD4+ T cells play an important role in the establishment of CD8+ T cell function, CD8+ T cell dysfunction would be more pronounced in HIV infected patients [159]. Lack of CD4+ T cell recovery in long-term treated HIV infection results in skewed, senescent T cell maturation profile, insufficient T-helper function and poor HIV-specific CD8+ T cell response [173]. The viral antigen persistence is associated with exhaustion of CD8+ T cells, resulting in impairment of cytokine production, proliferation and effector functions [174]. HIV-specific CD8+ T cells are arrested at a late differentiated memory phenotype which express low levels of perforin and are unable to increase a cytolytic response [175].

Humoral immunity against various viral antigens such as measles, mumps, rubella, varicella zoster virus and hepatitis B in patients with HIV infection is impaired [176, 177]. As B cells activation needs help from helper CD4+ T cells, HIV-induced CD4+ T cell lymphopenia drives the expansion of immature transitional B cell populations [178]. Moreover, the expression of inhibitory receptor, FcRL-4 is enriched on peripheral blood-derived mature B cells of HIV-viraemic individuals [179]. In addition to the expression of inhibitory receptors, exhausted memory B cells up-regulate their expression of CXCR3 and down-regulate chemokine receptors CCR7, CD62L and CXCR4, which are important for B cells developing into long-lived plasma cells [179, 180]. Down-regulation of these molecules may lead to recruitment of B cells to inflamed tissues and their differentiation into short-lived memory B cells.

1) Immunity against influenza virus infection in HIV+ individuals

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HIV+ individuals are well recognized as being at greater risk of influenza virus infection [181, 182]. Studies in a high HIV-prevalence setting have shown that HIV infection is a major risk factor for influenza hospitalization and severe disease [183-185]. This risk of influenza infection is decreased following the widespread introduction of highly active antiretroviral therapy (HAART) [185, 186]. However, HIV+ persons who receiving antiretroviral therapy and being not severely immunosuppressed, appear to be hospitalized, and having symptoms, severity, and clinical outcomes comparable to HIV-uninfected persons [187, 188]. High levels of CXCL10, which is associated with a poor control of viral infection by the immune

system [189] have been reported in HIV+ individuals, suggesting that that influenza virus clearance may be affected and may lead to a lower antibody response [190].

2) Immunity against HBV infection in HIV+ individuals

HIV shares the routes of transmission with HBV, leading to high incidence of acute and chronic HBV infection in HIV-infected individuals. Co-infection with HBV of HIV-infected persons induces extensive damage to the liver leading to significant liver disease and mortality. The persistence of immune activation in patients with chronic HBV infection cans upregulate HIV replication [191]. HBx protein plays an essential role to induce ongoing replication and long-term repeated transcription of HIV by synergizing with kappa B-like enhancer and T-cell activation [192, 193]. HBV coinfection is an independent predictor of immunologic progression, defined as the occurrence of CD4 cell count < 350 cells/μL at 3 months or more after diagnosis of primary HIV infection in adults patients [194]. However, the interactions of HBV among HIV-infected individual who had a seroconversion window of ≤ 3 years increase the hazards ratio for an AIDS or death event [195]. In HIV cohort study, patients who tested positive for HBsAg have significantly impaired CD4+ T cells recovery during the first 3 years of combination anti-retroviral therapy (cART) compared to patients without HBV infection [196]. Individuals with HIV infection have a higher risk of chronicity after acute HBV infection compared to HIV-uninfected subjects [197]. A higher proportion of chronic persistence of HBsAg in blood has been observed in HIV-infected patients, possibly due to the destruction of CD4+ T cells by HIV which subsequently diminishes host immunity against HBV [198].

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1.4.4. Immune response against vaccination in healthy individuals

1) Immunity against influenza virus vaccination

The use of influenza vaccine may result in reduced overall burden and reduced disease severity. Resistance to infection with seasonal influenza virus strains correlates directly with both serum haemagglutination inhibition (HAI) and neutralizing antibody levels [199]. Measurements of serum HAI and neutralizing antibodies are used to assess the immunogenicity of both seasonal and pandemic influenza vaccines [200].

Serum HAI titres of 1:40 or greater are thought to be associated with the degree of protection, defined as seroprotection. Seroconversion are defined as pre-vaccination HAI titre < 1:10 and post-vaccination HAI titre ≥ 1:40 or pre-vaccination HAI titre ≥ 1:10 and a minimum of 4-fold rise in post-vaccination HAI titre. The degree of protection after vaccination is dependent on the antigenic match between the vaccine strains and those circulating in the community, the age of the vaccine recipient and previous history of influenza [201]. The vaccine can only protect 59% of adults aged 18–65 years from laboratory confirmed influenza despite the presence of pre-existing immunity [202]. However, vaccine effectiveness against influenza-like illness is reduced to 39%, and 49% against laboratory-confirmed influenza in among over 65 year old population [203]. The combination of adjuvant can improve vaccine efficacy in case of both induction of seroprotection and duration of protective antibody [204].

The generation of virus-specific antibodies represents the primary correlate of immunity, whereas cell-mediated immunity can contribute to reduce the clinical symptoms [205]. The influenza-specific IL-21+ ICOS1+ CD4+ T cells, specialized in helping B lymphocytes, can be measured in human blood and increase in frequency after vaccination correlating to the increase of HAI titre [206]. Non-adjuvanted inactivated whole virus vaccine also induced a strong and robust type 1 CD4+ T cell response, which was maintained over a 6-month periods [207]. Adjuvanted formulation of influenza vaccine enhances antibody persistence and induces stronger T- and B-cell responses. Cross-reactive and polyfunctional virus-specific CD4+ T cells are also amplified after vaccination [208]. Anti-influenza virus immunity of CD4+ T cells that simultaneously secrete IFN-γ, IL-2, or TNF-α (multifunctional T cells) are functionally superior than single cytokine producers [209]. Both trivalent live-attenuated or inactivated influenza vaccines (LAIV or TIV, respectively) are well tolerated and induced similar HAI responses, whereas, only LAIV has an influence on induction of CD4+ and CD8+ T cells specific to highly conserved influenza peptides [210].

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2) Immunity against HBV vaccination

Success of the induction of immune responses at the initial vaccination is a key factor for the persistence of antibodies against hepatitis B surface antigens (anti-HBs Abs) [177, 211, 212]. The protection of HBV infection correlates with anti-HBs Ab titre of 10 IU per litre (IU/L) or higher [213, 214]. Vaccinees who have anti-HBs Ab titre ≥ 10 IU/L after vaccination are defined as responder. In contrast, people who have anti-HBs Ab titre < 10 IU/L after 3 doses vaccination are defined as non-responder. More than 90 % of normal adults achieve protective HBsAb titre after completion of the standard 3 doses vaccination [10]. The protective response is approximately 30-55% of adult age < 40 year after the first dose, 75% after the second dose and > 90% after the third dose. However, the protective titres in people age older than 40 years decline below 90%, and 75% by age 60 years. In addition to age, other host factors, such as smoking, obesity, genetic factors and immunosuppression are involved to the decrease of vaccine response [215]. The durability of an effective anti-HBs antibody titre strongly relate to higher antibody titre after the primary vaccination [216]. Besides the key role of neutralizing antibodies for protection against HBV, T cell–mediated immunity is considered essential for control and clearance of HBV infection [134]. Vaccinations also induce both virus-specific cytokine-producing CD4+ and CD8+ T cells [217, 218].

1.4.5. Immune response against vaccination in HIV+ individuals

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1) Immunity against influenza virus vaccination

Lower seroprotection and seroconversion following influenza virus vaccination in HIV-infected individuals compared to uninfected persons has been observed [8, 219]. Most HIV-infected individuals fail to generate durable seroprotective antibody response to 2009 H1N1 influenza A virus [6]. However, the data on antibody response to influenza A vaccination in HIV+ individuals is still controversial. Comparable effective humoral immune responses against influenza vaccination between HIV+ and uninfected population have been reported, although HIV-infected patients showed a profoundly impairment of cellular response [220, 221]. The antibody response to influenza antigens in HIV+ individuals can be recovered after treated with HAART,

causing functional improvement of antigen specific CD4+ T helper cell responses [222]. However, other study reported that antibody response in HIV+ individuals is lower, whereas cellular immune response was similar between healthy and HIV+ individuals [223]. Immunological responses in HIV+ population need further investigation in order to improve vaccination strategies.

2) Immunity against HBV vaccination

Vaccination against HBV is recommended for HIV+ population who have no serologic evidence of HBV exposure [224]. However, substantial proportions of HIV-infected population do not respond to HBV vaccine, and antibody titres against HBV often wane relatively rapidly in individuals who respond to the vaccine [177, 225]. The rate of response to hepatitis B recombinant vaccine with standard regimen (3 doses of 20 μg of HBsAg) in patients infected by HIV is significantly lower than in the healthy control group [226]. The response rate to hepatitis B vaccine of HIV-infected individuals is only 18 - 71% after completion of 3 doses primary vaccination [10]. Several studies have reported the improvement of responses to HBV vaccine by using higher doses [227, 228], increasing frequencies [211, 228], and increasing doses and frequencies [177, 228, 229]. HIV+ individuals on HAART treatment have improved responsiveness to HBV vaccine compared to untreated patients [230]. The combination of CpG to HBV vaccination is capable of inducing cellular immune responses to hepatitis B vaccine antigen in effectively treated HIV+ adults [231]. Investigation on induction of effective cellular immune responses is warranted which may provide a key to developing a vaccine that generate better T cell responses capable of attenuating and clearing HBV infection.

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This study purposed to investigate CMI responses to 2009 H1N1 influenza A and HBV vaccination in HIV-infected adults, and to 2009 H1N1 influenza A in HIV-infected children. Specific aims and strategies are described in the following chapters. We hope to be able to understand the mechanisms which contribute to the development of CMI to HBV and influenza A vaccination in HIV-infected population. The fundamental information from this study may address important issues for vaccine development and may generate long-term value not only to the HIV setting, but also to the populations that these vaccines will be used.