SUMMARY

EPSTEIN-BARR VIRUS SPECIFIC IgG AND IgA ANTIBODIES DIVERSITY OF NASOPHARYNGEAL CARCINOMA PATIENTS FROM THE NETHERLANDS AND INDONESIA

The Epstein-Barr virus (EBV) is one of the most common viruses in humans. Infection Epstein-Barr virus continues a lifetime after primary contact and the virus is spread throughout the world in about 90% of all humans. In Indonesia, NPC is the most common tumor in the head and neck, constituting 23.8% of all head and neck cancer cases (Adham et al., 2012; Chen et al., 2002). In Europe the incidence of NPC is increasing due to migration of people from endemic countries (Arnold et al., 2013). EBV has a formal taxonomic name as human herpes virus 4 (HHV4) and is part of the gamma herpesvirus subfamily. It is one of the most common viruses and humans are the only natural host for EBV where the virus is transmitted via saliva (Chien et al., 2001). Most people get infected with EBV at some point in their lives and as a carrier of the virus. EBV is able to establish a long term latent infection in memory B-lymphocytes. Upon differentiation to plasma cells latent EBV is triggered into lytic replication, producing new virions. Some lytic infections can be found in the epithelial and myeloid cells (Hadinoto et al., 2009; Thorley-Lawson, 2015).

EBV is extremely well adapted to the human host, because it is able to tightly regulate expression of viral genes to prevent undesired replication, thereby escaping immune surveillance and maintaining viral persistence. EBV exhibits a dual tropism; the virus is able to infect both B-cells and epithelial cells (Figure 2.8). The epithelial compartment is believed to play a major role in lytic replication of EBV and has been postulated to be crucial in the shedding of infectious EBV particles into saliva for viral transmission (Hadinoto et al., 2009). However, the virus preferentially infects B-cells and has its reservoir in memory B-cells (Thorley-Lawson, 2015).

When triggered to become plasma cells, EBV+ B cells express the full coding content of the viral genome to allow virus replication, which is accompanied by virus-driven anti-apoptotic signaling and immune evasion strategies ensuring cell survival for the production of viral progeny (Laichalk and Thorley-Dawson, 2005). During uncomplicated virus persistence, the in-situ detection of EBV latent genes and even more so of viral lytic gene expression has proven extremely difficult (Frangou et al., 2005; Hudnall et al., 2005). However, under various pathological conditions, EBV gene expression can be detected in defined tissues as outlined above. In subclinical situations, such as stress and inflammation, EBV reactivation occurs, which may temporarily lead to increased EBV production, as reflected by elevated EBV DNA levels in blood and saliva as well as elevated antibody responses (Chen and Hudnall, 2006; Glaser et al., 2005).

Infectious mononucleosis (IM) or mono (also glandular fever or kissing disease), is usually caused by primary EBV infection after virus spread in saliva. EBV is orally transmitted and infectious virus can be observed in oropharyngeal secretions (saliva) from IM patients, from immunosuppressed patients and at lower levels from healthy EBV seropositive individuals (Middeldorp at al., 2003). Once infection occurs it is generally lifelong with viral episomes persisting in circulating memory B cells (Wilson et al., 1996). Epithelial coinfection is common leading to persistent virus secretion in saliva.

EBV is causally associated with nasopharyngeal carcinoma (NPC) and aberrant IgG and IgA antibody responses can be detected at early stages of disease development and have diagnostic importance (Fachiroh et al., 2004). NPC is a curable disease if treated early. The problem in South East Asian countries including Indonesia is that patients mostly came to the hospital at a late stage of NPC. This is due to the lack of awareness of the disease's symptoms by both patients and health experts (R.Fles et al., 2010, 2016). Early stage diagnosis is often difficult because the symptoms are not distinctive and the nasopharynx is a difficult area to examine. NPC initiates at hidden areas that are presenting with limited, non-specific signs and symptoms at early age. These conditions have caused improper treatment for NPC patients (Hutajulu et al., 2014). These

antibodies recognize a range of viral antigens, exceeding the "normal" range of reactivity (diversity). Whereas IgG responses are considered to reflect systemic immune control, IgA antibodies reflect mucosal immune responses at body surfaces produced by specialized (Nasal/Gut/Bronchus) mucosa-associated lymphoid tissue (MALT or NALT/GALT/BALT).

In routine diagnostic settings no clear functional aspect is considered for IgG and IgA responses, but it is clear that multiple EBV antigens (proteins and epitopes) are involved in such responses, being either the VCA, EAd or EBNA complex. Previous research has found that Zebra and viral membrane antigens can also be used as marker to detect EBV (Middeldorp, 2015). Serology is the major method to determine status of EBV infection; by measuring antibody(ies) reactivity towards EBV antigen(s), distinct antibody patterns has been determined for individuals with primary infection, latent infected carriers and individuals at disease (Middeldorp, 2015).

The research in this study is performed to analyze the EBV specific antibody diversity between NPC patients of the Netherlands and Indonesian background and has two specific aims, which are: 1) to define correlation between IgG or IgA against VCA P18 and EBNA1 (whether they are related in strength of the response) and IgG vs IgA for these markers (whether they are clonally related in systemic immunity vs mucosal B-cell triggers), and 2) subsequently to compare IgG and IgA Zebra epitope reactivities (peptides vs recombinant) (i.e. what is the dominant peptide for measuring anti-Zebra reactivity).

By using Immunoblot and ELISA techniques this research found that immunoblot techniques shows IgG and IgA antibodies in sera from different individuals recognize different EBV protein antigens including different epitopes, spread out over the entire blot-strip. Especially, from side-by-side comparison it is clear that IgG (G) and IgA (A) antibodies in the same NPC patient frequently have a different antigen recognition pattern (diversity), suggesting these IgG and IgA responses are triggered by different antigen encounters in the same individual.

Elisa techniques confirms that IgA responses to EBV VCA P18 and EBNA1 are independent serological markers for NPC and each having diagnostic value in both Indonesian and Netherlands NPC patient populations. IgG and IgA responses to these proteins do not correlated, indicating independent antigen triggering of systemic (IgG) and mucosal (IgA) B-cells by EBV antigen encounters. This study did not confirm the NPC-diagnostic value of the Zebra protein or its epitopes, as was suggested by others previously. Our epitope analysis indicated that IgG and IgA responses to (sub)epitopes of the Zebra protein are not related suggesting independent clonal triggering. Due to the low immunogenicity of these proteins, antibody responses to Zebra N, Zebra P125, Zebra P130, and Recombinant Zebra are not very effective markers.

IgG and IgA antibody responses to EBV antigens in NPC patients are each triggered by different antigen-epitope recognition events and are not clonally related and the immunodominant epitopes (peptides) are best for use in diagnostic testing of Nasopharyngeal Carcinoma.

Serological EBV-IgA markers are suitable and affordable for NPC risk screening programs. Only prevention and early-stage detection of NPC will be the options in the near future, in a country that cannot afford to build multiple specialized chemo-irradiation centers for its population. The search for (therapeutic) vaccination, and preferably oral (virus)-specific NPC treatment at an early stage is needed to reduce the burden of NPC in endemic regions of the world, including Indonesia.

ABSTRACT

Nasopharyngeal carcinoma (NPC) is the most prevalent head and neck malignancy in Indonesia (Adham et al., 2012). A report based on a five-year analysis indicated that patients usually arrive at the hospital to examine their condition at a late stage of NPC due to the unspecific symptoms in the early stage (Fachiroh et al., 2004). The treatment of NPC is stage dependent and early detection is imperative in order to achieving higher treatment success (Fachiroh et al., 2006). Knowing the markers that can be used to detect NPC at an early stage is essential at this time. NPC corresponds to the highest tumor burden that is associated with the Epstein-Barr virus (EBV) infection (Hutajulu et al., 2014). EBV-based serologies using IgA-based ELISA and IgG immunoblotting as well as EBV DNA viral quantification, which allowed for the development of improved NPC diagnostic tools (Fachiroh et al., 2006). IgG and IgA antibody responses to EBV antigens in NPC patients are each triggered by different antigen-epitope recognition events and are not clonally related and the immunodominant epitopes (peptides) are best for use in diagnostic testing of Nasopharyngeal Carcinoma. These new EBV related diagnostic tools have excellent diagnostic capabilities in screening NPC patients from the population.

Keywords: Epstein-Barr Virus, Nasopharyngeal Carcinoma, Immunoglobulin A, Immunoglobulin G, EBNA 1, VCA P 18, Zebra's Protein