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Research Paper

The Relationship Between Environmental Factors and the Profile of Epstein-Barr Virus Antibodies in the Lytic and Latent Infection Periods in Healthy Populations from Endemic and Non-Endemic Nasopharyngeal Carcinoma Areas in China

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ABSTRACT

Our previous study found that smoking was associated with an elevated level of the antibody against VCA in the Epstein-Barr virus (EBV) lytic phase, which was an important predictive marker of the risk of nasopharyngeal carcinoma (NPC). It remained unknown whether environmental factors were associated with the levels of other EBV antibodies, such as Zta-IgA, EA-IgA, EBNA1-IgA, and LMP1-IgA, in the lytic and latent infection periods. We aimed to investigate the possible environmental inducers that could affect EBV antibody levels in two independent healthy male populations from endemic NPC areas in South China ($N = 1498$) and non-endemic NPC areas in North China ($N = 1961$). We performed ELISA and immunoenzymatic assays to test the levels of antibodies specific to the EBV antigens. The seropositive rates of antibodies against the antigens expressed in both the EBV latent and lytic infection periods, namely, LMP1-IgA, EBNA1-IgA, and Zta-IgA, in endemic areas (28.65%, 5.43% and 14.49%, respectively) were significantly higher than those in non-endemic areas (14.43%, 1.07% and 6.32%, respectively). Smoking was associated with higher seropositivity for EBNA1-IgA (OR = 1.47, 95% CI = 1.12–1.93) and Zta-IgA (OR = 1.28, 95% CI = 0.99–1.66), with dose-response effects, while not associated with the levels of LMP1-IgA. In conclusion, smoking was an important environmental factor, which associated with increased levels of EBNA1-IgA, and Zta-IgA.

1. Introduction

EBV is a widespread γ-herpesvirus that causes a life-long, asymptomatic infection in >95% of adults worldwide, remaining in resting memory B cells with a low copy number of episomal virus in the latent phase in healthy individuals (Babcock et al., 1998). EBV can be reactivated periodically and can switch from the latent to the lytic phase with elevated viral particle assembly and release, which could trigger an elevated immune response with higher antibody levels against specific antigens, such as BZLF1 transcription activator protein (Zta), early antigen (EA), and viral capsid antigen (VCA) (Fig. 1).

EBV, identified as a class I oncovirus by the WHO, is associated with the occurrence and development of nasopharyngeal carcinoma (NPC). NPC, which exhibits distinctive geographic and ethnic distributions globally, is rare in most areas of the world, while it is common in South China, where the risk can be 50-fold higher than that in non-endemic areas (Jia et al., 2006; Tang et al., 2016). Numerous studies have shown that NPC patients and individuals at high-risk for developing NPC exhibit distinctive anti-EBV antibody profiles, with elevated IgA antibodies against specific EBV antigens, and the most widely used serological biomarkers have been VCA-IgA, EA-IgA, Zta-IgA and EBNA1-IgA. Previous cohort studies in endemic areas of NPC in South China (Cao et al., 2011; Zeng et al., 1985; Ji et al., 2007; Liu et al., 2012) and Taiwan (Chien et al., 2001) have identified several serological biomarkers for screening and early-diagnosis in high-risk individuals, and those with higher antibody levels may have up to a 20-fold increased NPC risk compared with seronegative individuals. These cohort studies

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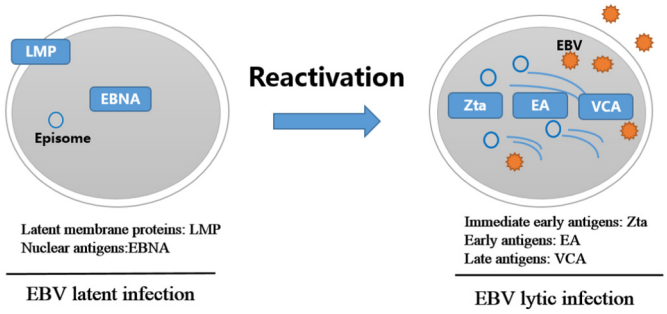

Fig. 1. EBV antigens being expressed during life cycles in latent and lytic infection. During EBV latent infection, a limited number of antigens were expressed, mainly was latent membrane proteins and nuclear antigens. The blue circle represents the episome of EBV. Once EBV was reactivated under certain conditions, large numbers of antigens were expressed, including the immediate early antigens of Zta, early antigens of EA and late antigens of VCA. The orange particles represent the replicated and released EBV particles.

have reported that elevated antibody levels precede the occurrence of NPC and that the serological windows can be detected several years prior to disease onset, which implies that EBV seroreactivity may have a causal role in the occurrence of NPC (Fang et al., 2009).

Although the cause of this phenomenon is not fully understood, identifying environmental inducing factors that trigger elevated levels of IgA antibodies to EBV specific antigens in vivo may be crucial for NPC primary prevention. In our previous molecular epidemiological study (Xu et al., 2012), we observed that among the putative NPC risk factors, smoking is an inducing factor. Smoking was associated with elevated levels of the IgA antibody against VCA, which is expressed in the late stage of lytic infection. However, it is unclear whether the potential environmental factors are associated with other EBV IgA antibodies, such as the antibody against EBNA1, which is expressed in both the latent and lytic stages of infection, or the antibodies against Zta and EA, which are expressed in the early stage of lytic infection. Additionally, few large-scale studies have focused on describing the distribution of the IgA antibody against latent membrane protein 1 (LMP1), which is specifically expressed in latent infections and is essential for EBV-mediated growth transformation.

We conducted this comprehensive study in 3459 males from areas with different NPC risk levels to describe the epidemiology of the profiles of antibodies against EBV antigens that are expressed at different stages of EBV infection, namely, EBNA1-IgA, Zta-IgA, EA-IgA and LMP1-IgA.

2. Materials and Methods

2.1. 21-RCCP Population in South China and Yangquan Population in North China

Two populations, one from South China, where NPC is endemic, and one from North China, where NPC is not endemic, were included in our study. These populations have been previously described in detail (Xu et al., 2012). Briefly, one population was from the areas of Guangdong Province where NPC is endemic. In total, 1498 males from 21 municipalities of Guangdong Province who attended a physical examination center between October 1, 2005 and October 1, 2007 were included in this study (this population is abbreviated as the 21-RCCP population). All participants were without any history of cancer or immunological disease. The other population came from areas of Shanxi Province where NPC is not endemic (this population is abbreviated as the North population). For the North population, 1961 healthy males were enrolled who visited the First General Hospital of Yangquan City between March 1 and August 1, 2010 for a health check-up. An informed consent was signed by every subject before the interview, and the human ethics committee of the Sun Yat-Sen University Cancer Center approved our study.

2.2. Data Collection

In this study, the comprehensive face-to-face interviews were conducted by well-trained interviewers (Xu et al., 2012; He et al., 2015; Jia et al., 2010). The structured questionnaire was mainly based on the questionnaires issued by the University of Arizona Cancer Center (http://uacc.arizona.edu/research/shared-resources/bmisr/questionnaires). The information included demographic data (age, education level), lifestyle behaviors (cigarette smoking, alcohol consumption and consumption of preserved vegetables), and family history (family history of tumors and NPC). For the 21-RCCP population, we also investigated the prevalence of the Cantonese diet of salted fish, slow-cooked soup, tea, and herbal tea. For cigarette smoking, those who had smoked at least 100 cigarettes during their lifetime were considered smokers. Ex-smokers were those who had quit smoking at least 1 year before the interview. Detailed smoking data such as the age at which they started smoking, the number of years they smoked, the average number of cigarettes smoked per day and the type of smoking were also included for further analysis. Pack-years were calculated by multiplying the number of packs smoked per day by the years the person had smoked. Heavy smokers were those with no fewer than 20 pack-years, which was the median value of the smoking pack-years for the total population.

2.3. EBV Antibody Tests

A 5–10 ml blood sample was collected from each subject in the two populations (21-RCCP and North populations). The LMP1-IgA, EBNA1-IgA and Zta-IgA antibody levels were measured with commercial ELISA kits by the same technicians in the same laboratory of the Sun-Yat Sen University Cancer Center (SYSUCC) (Xu et al., 2012; Liu et al., 2012; Cao et al., 2011). LMP1 peptide was derived from Yeast-expressed EBV strain (GD1) 185-366aa; EBNA1 and Zta were produced with purified recombinant peptides specified by EBV BKRF1 (72 kD), BZLF1 (36 + 38-kDa fine doublet) respectively. The serostatuses of LMP1-IgA, EBNA1-IgA and Zta-IgA were defined as seronegative or seropositive according to the ELISA OD values following the manufacturers' instructions (Shanghai Jining Shiye Co., Ltd., Shanghai City, China; Zhongshan Bio-technology Co., Ltd., Zhongshan city, China). For EBNA1-IgA, in addition to the seronegative and seropositive statuses, a weak seropositive status was also defined by the manufacturer's instructions. The EA-IgA antibody was detected by an immunoenzymatic assay performed according to the protocol of the SYSUCC clinical laboratory, which used the Raji cell line and determined the presence of the EA-IgA antibody by titration, with the cut-off value set at 1:10 (Chen et al., 2014). To evaluate the reproducibility for each assay, we randomly selected 90 samples to conduct a test-retest assay for each sero-marker.

2.4. Statistical Analysis

Multivariable unconditional logistic regressions were used to assess the associations between EBV serostatus and environmental factors by adjusting for age (years, continuous variable) and education levels (primary school or less, high school, and university or more). Ordered logistic regressions were used to assess the EBV seropositivity risk among different populations by dividing them into four levels according to the quartile of the corresponding OD (optical density) value of the total population for LMP1-IgA, EBNA1-IgA and Zta-IgA. Linear trend analyses for associations between exposures and EBV antibodies were conducted by treating the categorical variables as continuous variables, such as smoking and consumption of alcohol, tea, herbal tea, salted fish and preserved vegetables. A detailed subgroup analysis was performed