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Epstein-Barr Virus and Nasopharyngeal Carcinoma

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Abstract The Epstein-Barr virus (EBV) has been studied for over 25 years as a probable cause of certain human cancers, including nasopharyngeal carcinoma (NPC). This is a low-incidence head and neck cancer in Western countries (including the USA), but is the third-leading cancer in males in Southeast Asia. Evidence supporting an etiologic relation between this virus and NPC includes the fact that there is a 100% infection rate in patients with this cancer and that EBV DNA and antigens have been demonstrated in all biopsies examined to date. The determination that EBV is at least a major co-factor in the etiology of NPC has led to the development of new diagnostic and prognostic tests for this disease using anti-viral markers. Of particular importance to the diagnosis of NPC were the findings, initially reported by the Henles [Int J Cancer 17:1–7, 1976], that the serum of patients with NPC contain IgA antibodies to EBV at a high frequency. In general, 80–90% of patients with this disease contain serum IgA antibodies to EBV as opposed to 10–30% of the normal population. This finding has resulted in the development and successful employment of tests measuring this antibody as adjuncts to pathology in the diagnosis of NPC including the occult form. In addition, this finding has resulted in the development of tests for the early detection of this disease. The IgA test for antibodies to EBV is currently employed in large screening programs in Southeast Asia designed to identify those individuals at risk for the development of NPC. Results from these screening programs that have now been ongoing for over 5 years indicate that the incidence of NPC is 100–1000-fold higher in IgA antibody-positive individuals as opposed to IgA anti-EBV antibody-negative individuals, demonstrating the value of this IgA test for identifying individuals at high risk for NPC. The fact that this antibody test can be used reliably for the detection of early NPC should lead to earlier and more successful treatment of this disease.

Key words: Diagnosis, IgA antibodies, Epstein-Barr virus, nasopharygral cancer, early detection

The Epstein-Barr virus (EBV) has been studied as a candidate human cancer virus since its discovery in 1964 [1]. Existing data indicate that this *herpes* virus, which is the etiologic agent of heterophil-positive infectious mononucleosis (IM), is also an important cofactor in the etiology of some human cancers, namely African Burkitt's Lymphoma (ABL), nasopharyngeal carcinoma (NPC) and B-cell lymphomas in immunodeficient populations including individuals with AIDS (Table I). The data supporting these associations have been reviewed by a number of investigators [2–7]. Thus, EBV appears to be a *bonafide* human cancer virus, raising the realistic possibility that it might be possible to prevent EBV-associated diseases, including these cancers, through the employment of a vaccine. In addition, these studies on the etiological relationship of EBV with cancer have resulted in the identification of certain immune response patterns to EBV-associated antigens that are of value to the clinician for the diagnosis and clinical management of patients with EBV-asso-

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TABLE I. Candidate Diseases Associated With Infection by EBV

Infectious mononucleosis[1]
African Burkitt's lymphoma
Nasopharyngeal carcinoma
B-cell lymphomas in immunodeficient populations
Hodgkin's Disease
Sjogren's syndrome
Rheumatoid arthritis
Chronic fatigue syndrome

[1]Etiology has been established

ciated diseases. Some of these anti-viral markers have been used for the early detection of these cancers, as well as metastatic lesions. This paper will review some of the relevant findings with NPC in the context of the major topic of this scientific meeting.

EBV AND NPC

The original data implicating EBV as a factor in the etiology of NPC came from serological studies on African patients [4]. In the initial studies, it was observed that all African patients with NPC were infected with EBV and had antibody titers to a variety of EBV antigens which were substantially higher than control populations. These patterns were similar to those described for patients with ABL, raising the possibility that EBV was also an etiologic agent in this disease. These findings were confirmed by similar studies on Chinese patients with NPC [8–10]. Since these initial observations, it has been established that all patients studied to date with NPC have been infected with EBV regardless of the patient's geographic origin. This observation differs from that on patients with Burkett's lymphoma (BL) since it was found that only in African cases were the majority (97%) of individuals infected with EBV [11]. The incidence of EBV in individuals with BL in low-incidence areas such as the U.S.A. was significantly lower in comparison with Africa. Thus, with the exception of heterophil-positive IM, NPC is the only EBV-associated disease with a 100% infection rate. This consistent association provides strong support for the hypothesis that this virus is a major factor in the etiology of this malignant disease.

The association of EBV with NPC was strengthened by other studies which demonstrated a relationship between anti-EBV antibody titers and disease course. For example, it has been reported by different investigators that antibody titers to different EBV antigens increase progressively with stage of disease, indicating that the virus antigens reside in the growing tumor [3,8,9]. In addition, it was demonstrated that antibodies to different EBV antigens were prognostic in patients following treatment, thereby providing evidence for the presence of the virus in the tumor [3,8,9]. In general, antibody titers to different antigens remained stable or increased in those patients unsuccessfully treated. In contrast, antibodies tended to decrease to low or undetectable levels in successfully treated patients and increased with recurrent disease often before metastatic cancer was clinically evident. These antibody patterns again indirectly placed the virus genetic material in the tumor cells themselves. In fact, the expression of a variety of EBV antigens

TABLE II. Frequency of IgA-Positive Sera in Different Disease Categories

Disease	No. Sera Positive/ No. Tested	Positive (%)
NPC		
WHO1	5/51	10
WHO2 and 3	206/249	83
Other head and neck cancers	51/324	15
Benign head and neck diseases	57/428	13
Other malignancies including lymphomas	7/73	10
Normal	18/405	5

has now been demonstrated directly in NPC biopsies [12].

In an effort to directly demonstrate the presence of EBV DNA in the carcinoma cells, hybridization studies using viral DNA as the probe were successfully performed by a number of laboratories. These studies demonstrated that the viral DNA was indeed present in the tumor cells of all NPC biopsies regardless of the WHO histopathological classification [13]. In addition, EBV DNA has now also been identified in cell lines successfully established from NPC biopsies. These findings, along with the results of the serological investigations, strongly support the hypothesis that EBV is, at minimum, an important cofactor in the etiology of NPC.

How does the virus get into the epithelial cells? Until very recently, the answer to this question was unknown. One possibility was that fusion occurred between epithelial cells in the nasopharynx and EBV genome-positive B lymphocytes. Since the WHO2 and WHO3 histopathological types of NPC are heavily infiltrated with lymphocytes *in vivo*, fusion was clearly a possible explanation for the mechanism of infection and transformation of epithelial cells. In fact, hybrid cells expressing EBV-associated antigens could be formed *in vitro* by cell fusion [14]. Although this mechanism might indeed function *in vivo*, it has now been established that a small percentage of epithelial cells in the nasopharynx do indeed have EBV receptors and are therefore directly infectable with EBV [15, 16]. Therefore, it is likely that direct infection of epithelial cells is the major mechanism of entry.

EARLY DETECTION OF NPC

As already noted, serological studies on patients with NPC identified anti-viral markers that varied with disease course and therefore were of clinical significance. In addition, certain antibodies to EBV antigens appeared to be useful adjuncts to pathology in the diagnosis of NPC (including the occult form) and for screening of populations at high risk for the development of this disease, such as those in Southeast Asia. The most disease-specific of these markers was the presence of IgA antibodies to certain EBV antigens. This outstanding feature of NPC was originally reported by the Henles [17] who noted that greater than 90% of the patients with NPC from high-incidence areas were positive for IgA antibodies to EBV as compared to a much lower frequency of positivity (10%) in control populations. These controls included individuals with EBV-associated lymphomas. The original findings of the Henles have now been reproduced in a number of different laboratories working with patients from both high- and low-incidence areas for this cancer [18,19]. These include North American patients with NPC [19]. In general, over 75% of the North American patients who have been studied to date have been positive for this antibody at diagnosis in comparison with 10–15% of various

control groups, including patients with other head and neck cancers. Interestingly, when the diagnostic serum antibody profile was related to histopathology according to the WHO classification, there was a striking difference between the well-differentiated WHO1 tumors compared with the less-differentiated WHO2 and WHO3 tumor types (Table II). The frequency of IgA antibodies to EBV was approximately 10–15% in the sera of patients with WHO1 tumors and various control groups. In contrast, over 80% of the sera from patients with WHO2 and WHO3 tumors were positive for this antibody. The reason for this difference is still unknown; it has been established that all three histopathological types of NPC contain the EBV genome [13]. It is possible that the degree of lymphoid infiltration in these different histopathological types of NPC is responsible for this result, but this has not yet been determined. Nevertheless, these findings established that the measurement of IgA antibody to EBV was a useful marker to diagnose WHO2 and WHO3 histopathological types of NPC, which comprise 75% of all cases, and might also be of value in identifying individuals at risk for the development of this disease. Studies have demonstrated that this test could also be used successfully to diagnose occult NPC and to detect metastatic disease before the appearance of clinical signs [20–22].

Because of the specificity and sensitivity of the IgA marker for detecting minimal tumor load, these assays have now been employed in screening tests in Southeast Asia in an effort to identify individuals at risk [23]. Results from a 10-year prospective study in Wuzhow City in China, involving approximately 21,000 individuals over 40 years of age, are promising in this regard. At a recent international meeting, Zeng Yi [24] reported that the incidence of NPC in the IgA antibody-positive population over this 10-year period was approximately 200-fold higher than in the IgA antibody-negative population. The NPC detection rate in these two populations was 4666 per 10^5 in the IgA antibody-positive group versus 20 per 10^5 in the IgA antibody-negative population. Similar results were noted in a prospective study in Zangwa county. These findings demonstrate quite convincingly that the IgA antibody to EBV is a reliable marker for the detection of early NPC and therefore could be employed for identifying candidates for early intervention studies against this disease.

CONCLUSIONS

The studies on the relationship of EBV to NPC over these past 25 years have established that this virus is at least an essential cofactor in the etiology of this disease. The serological studies have also identified antibody response patterns to EBV antigens that have clinical significance. Of particular importance to early intervention studies are the observations that IgA antibodies to EBV herald the presence of early disease. Using this marker in screening programs in high risk areas should therefore increase the probability of identifying individuals with minimal disease, thereby offering the physician the opportunity to initiate early treatment for successful eradication.

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