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ANTIBODIES TO EPSTEIN-BARR VIRUS IN NASOPHARYNGEAL CARCINOMA, TONSILLAR CARCINOMA AND CONTROL GROUPS*

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Summary. — In the sera of 17 patients with nasopharyngeal carcinoma (NPC) and of 19 patients with tonsillar carcinoma (TC) the titres of IgA, IgG and IgM antibodies to EBV VCA (viral capsid antigen) and of IgG antibodies to EBV EA (early antigen) were determined by the indirect immunofluorescence (IF) method. Significant difference was observed in the frequency of IgA antibodies to EBV VCA and IgG antibodies to EBV EA between NPC patients and controls. There was also a significant difference between the frequency of IgM antibody to EBV VCA and EBV EA antibody titres in TC patients and controls. The geometric mean titre (GMT) of IgG antibodies to EBV VCA was significantly higher in the NPC and TC patients as compared to controls.

Key words: *Epstein-Barr virus*; *antibodies*; *nasopharyngeal carcinoma*; *tonsillar carcinoma*

Introduction

Epstein-Barr virus (EBV) first detected in cultured lymphoblasts from Burkitt's lymphoma (BL) (Epstein *et al.*, 1964), is considered oncogenic not only in association with BL (Henle *et al.*, 1969; Klein, 1977) but also with NPC and TC. The BL, NPC, and TC biopsies were found positive for EBV DNA (zur Hausen and Schult-Holthausen, 1970; Bornkamm *et al.*, 1976; Andersson-Anvret *et al.*, 1978; Bricháček *et al.*, 1981).

High antibody titres were reported in patients with NPC and in those with TC as compared with controls (De Schryver *et al.*, 1969; Kottarides *et al.*, 1977; Vonka *et al.*, 1977; Pearson *et al.*, 1978; Bricháček *et al.*, 1981). Different antibodies against EBV have been demonstrated by IF test.

Recently, Henle and Henle (1976) reported the frequent presence of IgA class antibodies to EBV antigens in the sera of NPC patients. We determined the serological response to different EBV antigens, cytomegalovirus (CMV), herpes simplex virus type 1 (HSV-1) and adenovirus type 2 in patients with NPC and TC and in control individuals.

*Dedicated to Prof. Joachim Schmidt on his 60th birthday

Table 1. Geometric mean titre of IgG antibodies to EBV VCA and EBNA antibodies in NPC and TC patients as compared to controls

Group	Number of patients	Antibody GMT to EBV VCA (IgG)	Antibody GMT to EBNA
NPC patients	17	182.5	36.8
controls	17	35.1 ($p < 0.01$)	19.2*
TC patients	19	96.5	70.6
controls	19	34.1 ($p < 0.01$)	32.9*

* No significant difference in t-test.

Materials and Methods

Sera were obtained from 17 NPC patients and from 19 TC patients 1—10 years after operation and/or X-irradiation. Their age ranged from 21 to 70 years in the NPC group, and from 22 to 80 in the TC group (the average of 51.9 years in the first group and of 57.1 in the second group). All patients were treated at the clinic of Otorhinolaryngology of the Wilhelm-Pieck-University in Rostock, G.D.R. Control sera were obtained from healthy subjects of the same age and sex. All sera were stored at −20 °C until use.

Cell lines. The B-95 and P3 HR1 and the Raji cell line were maintained in Eagle's MEM medium supplemented with 5% bovine serum.

Serological tests. IgG and IgA antibodies to the viral capsid antigen (VCA) were previously described (Henle and Henle, 1966: Pearson *et al.*, 1971). EBV class IgM antibodies were tested as described by Oberender *et al.* (1979). Sera were examined for the presence of antibodies to EBV-associated nuclear antigen (EBNA) by the anticomplement immunofluorescence (ACIF) test of Reedman and Klein (1973) as modified by Henle *et al.* (1974). Antibodies to the early antigen (EA) of EBV were tested in P3 HR1 infected Raji cells according to Pearson *et al.* (1978). Antibodies to the late antigen (LA) of HSV-1 (Mc Intyre) and to the LA of CMV (Ad 169) and to adenovirus type 2 were tested by IF using coverslip cultures infected with these viruses. The different dilutions of each serum specimen were checked in duplicates.

Results

All NPC and TC patients were positive for IgG antibodies EBV VCA. Table 1 shows GMT of the sera from NPC as well as TC patients compared to controls. The GMT of IgG antibodies to VCA and EBNA were in the group of NPC patients 182.5 and 36.8, and in the group of TC patients 96.5 and 70.6, respectively. A statistically significant difference was observed in GMT for VCA between either patient group and controls.

The percentage of sera showing antibodies to EA as well as IgM and IgA class antibodies to EBV VCA was also higher in NPC and TC patients than in controls (Table 2). A statistically significant difference ($p < 0.05$) was observed in the frequency of EBV-specific IgA antibody titres and anti-EA titres in the NPC patients and of EBV IgM antibody titres and anti-EA titres in the TC patients, respectively, as compared to controls. Table 3 indicates GMT of IgG class antibodies to CMV LA, HSV-1LA and to adenovirus type 2 antigens. No significant differences could be observed between the NPC or TC patients and control group.

Table 2. IgM, IgA class antibodies to EBV VCA and antibodies to EBV EA in NPC and TC patients as compared to controls

Group	Number of patients	IgM to EBV VCA[1])	IgA to EBV VCA[2])	IgG to EA[3])
NPC patients	17	7	8	10
controls	17	2*	1	3
χ^2-test			($p < 0.05$)	($p < 0.05$)
TC patients	19	9	7	13
controls	19	2	3*	5
χ^2-test		($p < 0.05$)		($p < 0.05$)

[1]) No of positive cases with titre of $\geqq 20$; [2]) No of positive cases with titre of $\geqq 10$; [3]) No of positive cases with titre of $\geqq 5$.
* No statistically significant differences.

Discussion

The aim of our study was to obtain serological data on NPC and TC patients in our district. The EBV VCA antibody titres in sera from NPC and TC patients were significantly higher than those in sera from the normal controls. Other reports indicate also higher GMT of anti-VCA in NPC patients than in controls (Kottaridis *et al.*, 1977). The anti-VCA titres of sera from TC patients were lower than those of sera from NPC patients, but higher than those of sera from controls. Our serological findings agree with earlier results obtained by Břicháček *et al.*, (1981). The anti-EBNA titres of sera from NPC and TC patients were higher than those of sera from controls, but the differences were not statistically significant.

The most specific immunological parameter appeared to be the presence of EBV VCA antibodies of the immunoglobulin A class as reported by Henle and Henle (1976). In our studies 8 out of 17 NPC patients and 7 out of 19 TC patients had IgA serum antibodies to EBV VCA. Other authors observed a higher percentage of EBV IgA-positive sera from NPC patients (Ho *et al.*, 1978*a*, Ho *et al.*, 1978*b*). This discrepancy is perhaps due to the fact that our groups included mainly treated patients. These results confirm findings published by Henle and Henle, 1976, which showed that antibody titres to

Table 3. Geometric mean titres of IgG antibodies to CMV LA, HSV LA and to adenovirus type 2 in NPC and TC patients as compared to controls

Group	Number of patients	GMT to		
		CMV	HSV-1	adenovirus type 2
NPC patients	17	8.3	320.0	5.7
controls	17	11.3*	265.4*	6.1*
TC-patients	19	12.6	372.7	5.0
controls	19	17.8*	244.2*	6.6*

* No significant differences in t-test

EBV-associated antigens were generally lower in patients without a clinically active disease than in untreated individuals, suggesting that this parameter might be of prognostic value. In our study, the percentage of anti-EA positive sera was higher in NPC patients and in TC patients than in controls. Our findings confirm those of other studies (Anderson-Anvret *et al.*, 1978). IgM antibodies to EBV VCA could be found more fequently in NPC and TC patients. The difference was statistically significant between the TC and the control group.

The antibody titres to CMV, HSV-1 and to adenovirus showed no differences between the tumour groups and controls. However, the results obtained also show that some patients without any cancer have IgA antibodies to EBV VCA and antibodies to EBV-EA. EBV infections and reactivations seem to be more frequent in patients who spent a longer period in hospital than in those staying at home (Sumaya, 1977; Gallo *et al.*, 1982). These serological findings should be carefully considered; nevertheless, they may help in further studies to determine the potential value of EBV serology in diagnosis and prognosis of NPC and TC.

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