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Immune Response to the Terminal Repeat Protein of Epstein-Barr Virus in Patients with Undifferentiated Nasopharyngeal Carcinoma

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Screening of 205 patients suffering from various diseases and healthy donors for the presence of serum antibodies to terminal repeat protein (TP) of Epstein-Barr virus was carried out using the indirect immunofluorescence technique. TP antibodies were detected exclusively in patients with undifferentiated nasopharyngeal carcinoma (in 30-40% of patients within this group). Evaluation of TP antibody titers before and after treatment revealed a correlation between the dynamics of these antibodies and the clinical prognosis of patients.

Key Words: *Epstein-Barr virus; undifferentiated nasopharyngeal carcinoma; terminal repeat protein*

Epstein-Barr virus (EBV), a widespread human herpes virus, is the etiological agent of infectious mononucleosis and is also associated with human malignant B-cell lymphomas and undifferentiated nasopharyngeal carcinoma (uNPC) [1].

Differential diagnosis of uNPC is fraught with difficulty due to the complex anatomic-topographic interrelationships in the corresponding region, the patterns of the clinical course and diagnostic criteria making it possible to differentiate between various malignant and benign lesions of this region. The conventional assay for IgG and IgA antibodies to the proteins of EBV lytic infection (viral capsid and early antigen complexes - VCA and EA) is fairly informative as a significant additional criterion in the differential diagnosis of uNPC [4]. However, this assay is of limited prognostic value, since the titers of the above-mentioned antibodies can remain high for a long time and therefore usually fail to reflect the development of the disease.

It is established that EBV persists in transformed cells in latent form, so that only a part of the viral genome is expressed. EBV transcription has been characterized in detail mostly in lymphoid cell lines. At present, nine latent viral proteins have been identified. Six are associated with the cell nucleus (EBNA 1-6), and three are localized in the cell membrane (LMP and two recently described proteins of the terminal repeat, TP1 and TP2) [7,8]. Transcripts of both TPs are

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highly homologous and differ only in one exon, i.e., the N-terminal exon of TP1, which is absent in TP2 [5,6,10].

Recently preliminary reports have appeared indicating TP to be a principal marker of uNPC, since antibodies to this protein are found in the serum of uNPC patients exclusively [2]; moreover, the finding of TP antibodies appears to be related to an unfavorable course of the disease [3].

The goal of the present study was to analyze the immune response to TP in order to evaluate both the diagnostic and prognostic significance of TP antibodies in uNPC. It is worth noting that our collection of sera provided us with a unique opportunity of studying for the first time the anti-TP immune response both in long-term survivors (more than 5 years of remission) and in patients who had died from progression of the disease.

MATERIALS AND METHODS

Sera from 90 patients with head and neck cancer who had been under treatment at the Cancer Research Center during the last 2.5 years were tested. A retrospective analysis of sera from 115 patients with various diseases, including uNPC, who had been examined earlier in our Department, was also carried out.

Screening of sera for antibodies to VCA and EA was performed using the indirect immunofluorescence reaction as described earlier [4]. TP antibodies were determined using the SF158 insect cell line infected with recombinant baculovirus that has inserted practically full-length TP1 gene derived from the M-ABA/CBL1 EBV cDNA library [2]. The recombinant virus was a generous gift of Prof. Mueller-Lantzsch (Germany). SF158 cells infected with wild-type baculovirus served as the control. Cells were grown in TC-100 medium (Gibco BRL) supplemented with 10% fetal calf serum and 80 μg/ml gentamicin. Three days after infection the cells were washed in phosphate-buffered saline, placed on glass slides, and dried. The slides were fixed with acetone precooled to -20°C and stored at -20°C before use. The indirect immunofluorescence reaction on slides was performed by incubating: the slides with tested human serum for 30 min at 37°C and then with FITC-conjugated sheep anti-human immunoglobulin (Sigma) for 40 min.

RESULTS

In order to analyze the distribution and frequency of the humoral immune response to TP, we tested 115 EBV-positive sera from our collection. The

TABLE 1. Individual Serological Characteristics of uNPC Patients Observed in the Cancer Research Center during the Last 2.5 Years

Patient No.	Stage	Time of testing	VCA IgG	EA IgG	VCA IgA	TP IgG	Duration of remission, years
TP antibody–negative patients							
1	IV	Before treatment	1:5120	1:2560	1:640	n.d.	1.5
		After treatment	1:2560	1:80	1:320	n.d.	
2	IV	Before treatment	1:640	1:160	1:20	n.d.	1.5
		After treatment	n.t.	n.t.	n.t.	n.t.	
3	III	Before treatment	1:1280	1:80	1:80	n.d.	2
		After treatment	1:1280	1:80	1:40	n.d.	
4	IV	Before treatment	1:1280	1:160	1:80	n.d.	0.75
		After treatment	1:640	1:160	1:80	n.d.	Local relapse
TP antibody–positive patients							
5	IV	Before treatment	1:1280	1:40	1:10	1:2560	1.2
		After treatment	1:1280	1:10	n.d.	1:640	
6	IV	Before treatment	1:1280	1:320	1:320	1:160	1
		After treatment	n.t.	n.t.	n.t.	n.t.	
7	IV	Before treatment	1:2560	1:640	1:320	1:320	No remission
		After treatment	n.t.	n.t.	n.t.	n.t.	
8	IV	Before treatment	1:640	1:160	1:20	1:20	No remission
		After treatment	1:320	1:160	1:10	1:80	

Note. Here and in Table 2: VCA: viral capsid antigens; EA: early antigens; n.d.: antibodies to TP are not detected in the serum; n.t.: not tested.

TABLE 2. Serological Characteristics of Earlier Observed uNPC Patients

Group of patients	Time of testing	Mean geometric titer of antibodies				
		VCA IgG	EA IgG	VCAIgA	TP IgG	N/M
Stable remission	Before treatment	1:1280	1:160	1:110	1:20	2/7
	After treatment	1:900	1:110	1:110	1:2	2/7
	Remission	1:280	1:45	1:30	n.d.	0/12
Progressive disease	Before treatment	1:1020	1:100	1:115	1:28	2/6
	After treatment	1:2100	1:140	1:115	1:51	6/6

Note. N/M: ratio of the number of TP antibody–positive patients to total number of tested patients.

group included 20 healthy donors, 20 patients with lymphadenopathy of various etiology, 20 with rheumatoid arthritis, 8 with infectious mononucleosis, 7 with Hodgkin's disease, and 40 with uNPC. Antibodies to TP were found exclusively within the uNPC group, in 30% of cases (12/40).

In the course of differential diagnosis 49/90 (54%) patients with head and neck cancer manifested an elevated level of IgG and IgA antibodies to EBV lytic infection proteins. This is a characteristic feature of uNPC. Diagnosis of uNPC was subsequently confirmed in this group of patients by morphological criteria. The other 41 patients were found to suffer from squamous-cell nasopharyngeal carcinoma (26, 28.9%), lymphosarcoma (5, 5.5%), squamous-cell carcinoma of the tonsils, larynx, and oropharynx (7, 7.8%), and metastases to cervical lymph nodes without a detectable primary tumor (3, 3.3%). As in the retrospective analysis, TP antibodies were found exclusively in uNPC patients, specifically in 41% of cases (20/49).

Thus, our studies have shown that TP antibodies can be detected only in uNPC patients, and, moreover, only in 30-40% of these. These data are in agreement with earlier reports [2,3]. This phenomenon is unique, since antibodies to other EBV latent proteins (EBNA, LMP) are found both in patients with various EBV-associated diseases and in healthy donors [9].

In order to assess the prognostic value of TP antibodies, patients were examined in dynamics, i.e., before and after treatment. Table 1 presents individual serological characteristics of certain uNPC patients observed in the Cancer Research Center over 2.5 years. All patients received chemoradiotherapy. In initially antibody-negative patients (Nos. 1-4) complete tumor regression was achieved; moreover, the follow-up of these patients (with the exception of patient № 4) revealed no recurrence during 1-2 years (the period of observation). Only patient № 4 developed a local relapse 9 months after completion of therapy.

Two of 4 initially antibody-positive patients have a poor prognosis due to the development of distant metastases. Two other patients are in remission, without any sign of recurrence or metastasis. However, these patients initially had advanced disease (intracranial invasion in patient № 5 and involvement of distant lymph nodes in patient № 6). Thus, the pretreatment presence of TP antibodies appears to correlate with the severity of the clinical status of the disease.

In further studies we analyzed the material collected in our department over several years. This enabled us for the first time to perform a comparative analysis of the immune response to TP in uNPC patients with long-term remission and in those who had died of the disease progression. Twelve patients with remission lasting more than 5 years were assigned to the first group. The second group consisted of 6 patients who had died of disease progression. Three of these patients had failed to achieve remission due to incomplete success of treatment, the other three had died due to recurrence. In the first group 67% (8/12) of patients were in stage III, and 33% (4/12) in stage IV. In the second group one patient was in stage III, and 5 (83%) in stage IV.

Before treatment, TP antibodies occurred in both groups with similar frequency (Table 2). However, in the first group TP antibody titers dropped after treatment and fell to an undetectable level during remission. In contrast, in the second group the titers of TP antibodies tended to rise after treatment; moreover, in certain patients a seroconversion seemed to take place, i.e., initially antibody-negative patients developed TP antibodies after treatment.

It is worth noting that patients with a progressive course of disease who had not achieved even a short-term remission initially manifested a very low level of humoral immune response to both EBV lytic infection antigens and to TP. This phenomenon may explain the negligible differences in the initial mean geometric titers of TP antibodies between the two mentioned groups. However, in the patients who had at first achieved remission and subsequently died of recurrence, the antibody titers were at a higher level. This is probably an indirect

indication of a profound deterioration of the immune system of patients who had no remission at all.

Thus, our investigations have shown that: a) regarding patients with head and neck cancer, antibodies to TP are determined exclusively in the uNPC group; b) within the uNPC group antibodies are detected in 30-40% only; c) preliminary data point to a relationship between the dynamics of TP antibodies in the course of treatment and the prognosis of the disease. Studies in this field are in progress.

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Detection of STLV-1 Integration in DNA Extracted from Formalin-Fixed and Paraffin-Embedded Specimens of *M. Arctoides* Lymphomas Using the Polymerase Chain Reaction

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The feasibility of using the polymerase chain reaction to analyze of DNA extracted by the conventional method from formalin-fixed and paraffin-embedded specimens of monkey lymphoid tissue was investigated. Using HTLV-1 primers that can amplify DNA fragments of various length (159 to 717 base pairs), we determined the size of formalin-destroyed DNA which appeared to be up to 500 base pairs in length. Analysis of four specimens of *M. arctoides* lymphomas that had been stored since 1969 revealed integrated STLV-1 provirus.

Key Words: *polymerase chain reaction; DNA; formalin fixation; primer; STLV-1*

The polymerase chain reaction (PCR) [11,13] has become one of the main methods of detecting various viral agents [3,4,8,15]. PCR-amplified products are easily visualized and can be used in a broad spectrum of analytical studies [6,9]. The high sensitivity of the PCR method in the detection of specific DNA sequences is attractive regarding retrospective investigations. However, formalin-induced fragmentation of conventionally extracted

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