PROFILE OF ANTIBODIES AGAINST EPSTEIN-BARR VIRUS IN PATIENTS WITH BLOOD DISORDERS

ANNA ŻUK-WASEK, BOGUMIŁA LITWiŃSKA, AND ZDZISŁAWA TRACZYK

Department of Virology,
National Institute of Public Health-National Institute of Hygiene, 00-791 Warsaw, Poland

Department of Internal Diseases,
Institute of Haematology and Transfusiology, 02-776 Warsaw, Poland

Received for publication July 03, 2008

Abstract

The aim of the study was to investigate the profile of Epstein-Barr virus (EBV) antibodies and to compare this profile with the detectability of EBV DNA and compare this profile in healthy people with the profile in patients with blood disorders. The profile of antibodies was examined in serum from the following groups: 40 patients with blood disorders, but without EBV DNA in their lymphocytes, 44 patients with blood disorders and EBV DNA in lymphocytes, and 46 healthy people. EBV antibodies were detected by using ELISA commercial kits. The obtained results were analysed by Fisher’s exact test. No significant differences in antibody profiles were found between EBV DNA-positive and EBV DNA-negative sera. Between patients and the control group, considerable differences were observed in reactivation and in past infection, and a significant difference was observed in chronic infection. These analyses suggest that EBV reactivation or chronic infection can be related with blood disorders and have an influence on the course of blood disorders.

Key words: humans, Epstein-Barr virus, antibodies, blood disorders.

Epstein-Barr virus (EBV) is widespread among various populations. The infection is generally established in childhood and ranges from asymptomatic course to mononucleosis. EBV might be the causative agent of some cancer diseases, like Burkitt’s lymphoma, Hodgkin’s and non-Hodgkin’s lymphomas, nasopharyngeal carcinoma, post-transplant lymphoproliferative disease, X-linked lymphoproliferative disease, and others (9, 12).

EBV antibody pattern may be used in the diagnostics of EBV infection due to the complex relationship between host reaction and EBV. A profile of antibodies to the different EBV antigens demonstrates a characteristic pattern for primary or persistent latent EBV infection, as well as for each of the EBV-associated diseases. Crucial for the diagnosis of EBV infection is the identification of antibodies to specific EBV protein antigens, which include viral capsid antigen (VCA) and early antigen (EA), or Epstein-Barr nuclear antigen (EBNA). To estimate the antibodies, ELISA is mainly used in laboratory diagnosis, as it is quick and easy to perform (3, 14).

In the course of the disease, the immunological response is different at different phases. It is therefore possible to assess whether the state of infection is current, recent, past, or reactivated (4, 7).

The typical antibody pattern of primary EBV infection is characterised by the presence of both IgM and IgG antibodies to VCA and EA, and by the absence of IgG antibodies to EBNA. VCA IgM antibodies that disappear during convalescence and thus their presence is symptomatic for acute EBV infection, whereas VCA IgG antibodies are maintained for life after recovery. The IgG response to EBNA (mainly EBNA-1) is not usually detectable until convalescence and then persists throughout life. EA IgG antibodies (most frequently EA-D) are detected by IF assay in about 70% of patients with acute infectious mononucleosis (IM) and disappear after recovery. During EBV reactivation, EA IgG antibodies may reappear, frequently with a rise in VCA IgG antibodies and rarely in the presence of VCA IgM antibodies (2, 6, 12).

It is of crucial importance to establish the virological state of patients with growth diseases of the haemogenic system. This reflects especially in the application of chemotherapy, in the preparation for the procedure of haematopoietic cell transplantation, and is of the essence in the development of the post-transplant lymphoproliferative disease.

In the course of our earlier research, we have gathered 113 specimens from patients with various blood disorders (leukaemias, lymphomas and myelomas) and 96 specimens from healthy people.
(blood donors and Institute’s employees). The presence of five herpesviruses (CMV, EBV, HHV6, HHV7, and HHV8) in leukocytes isolated from whole blood was checked. It was demonstrated that the frequency of discovery for the EBV DNA between the group of patients with different blood disorders and healthy people was statistically significant (P<0.05). In light of this research, infection with the EBV virus may turn out to be of more immediate importance in the aetiology of cancer diseases that it is presently considered.

The aim of the present study was to investigate the profile of EBV antibodies and to compare this profile with the detectability of EBV DNA as well as to compare this profile in healthy people with the profile in patients with blood disorders.

**Material and Methods**

**Serum samples.** Serum samples were collected from the following groups:
- 40 patients with blood disorders (lymphoma, myeloma, and leukaemia) but without EBV DNA in their lymphocytes;
- 44 patients with blood disorders and with EBV DNA in their lymphocytes;
- 46 healthy people (control group)

In the group of 84 patients with blood disorders (with or without EBV DNA), 58 people were treated in hospital by immuno- or chemotherapy. Specimens from patients were received from the Warsaw Institute of Haematology.

**ELISA.** EBV antibodies were detected by ELISA using commercial kits. The following antibodies were detected: IgG EA (an early antigen), VCA IgM (a viral capsid antigen), VCA IgG (a viral capsid antigen), and EBNA IgG (a nuclear antigen). ELISA was carried out according to the producer’s instructions.

**Statistical methods:** The obtained results were analysed by the Fisher exact test.

**Results**

The results of serological examinations are summarised in Table 1.

No antibodies were found in the VCA IgM class for the EBV in either of groups of patients with blood disorders. This result excludes the primary infections. In the control group, VCA IgM was found in three sera, in the presence of VCA IgG and EA IgG. The profile of these antibodies testifies to primary infection in convalescent stage.

The presence of VCA IgG, EBNA IgG, and EA IgG indicates the reactivation of infection and this profile was found in 27 (61.4%) samples from EBV DNA-positive patients, in 22 (55.0%) samples from EBV DNA-negative patients, and in three (6.5%) patients from the control group.

In 11 (25.0%) sera from EBV DNA-positive patients, 13 (32.5%) from EBV DNA-negative patients, and 34 (73.9%) from the control group, the VCA IgG and EBNA IgG antibodies were found. This result attests to a past infection. In one (2.3%) serum sample from a patient from the EBV-DNA-positive group and in five (10.9%) sera from the control group, only the VCA IgG antibodies were found.

**Table 1**

Results of serological examinations

<table>
<thead>
<tr>
<th>Determined profile of patient with DNA EBV (n=40)</th>
<th>Profile of antibodies</th>
<th>Number of patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation</td>
<td>EA IgG</td>
<td>VCA IgM</td>
</tr>
<tr>
<td>reactivation</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>past</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>chronic</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>past w. EBNA</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determined profile of patient with DNA EBV (n=44)</th>
<th>Profile of antibodies</th>
<th>Number of patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation</td>
<td>EA IgG</td>
<td>VCA IgM</td>
</tr>
<tr>
<td>reactivation</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>past</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>chronic</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Determined profile of the control group (n=46)</th>
<th>Profile of antibodies</th>
<th>Number of patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpretation</td>
<td>EA IgG</td>
<td>VCA IgM</td>
</tr>
<tr>
<td>reactivation</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>past</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>past w. EBNA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>primary</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>undefined</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

n – number of patients in the group.
This indicates to a past infection without production of the EBNA IgG antibodies, or to a transitional phase in a primary infection. In five (11.4%) samples from EBV-positive patients and in five (12.5%) samples from EBV-negative patients, EA IgG and VCA IgG were found and this profile is interpreted as a chronic infection (5, 8). In one (2.2%) sample from a person from the control group, only EBNA IgG was found and there is no clear interpretation of this result.

In the group of 58 patients with blood disorders, who were treated (by chemo- or immunotherapy) 38 (65.5%) with EA IgG, VCA IgG, and EBNA IgG antibodies were found, and this means a reactivation of infection. In 15 (25.9%) patients, VCA IgG and EBNA IgG antibodies were found and this result attests to a past infection. In three sera, VCA IgG and EA IgG were present, and this result is interpreted as a chronic infection. In two samples, only VCA IgG was found and this could be a past infection without EBNA IgG or a primary infection.

The obtained results of serological tests demonstrated that as much as 67.5% of patients without DNA of the EBV were diagnosed with the current or chronic infection with the virus. These results might testify to a recent past infection, when DNA of the virus was not detected but the antibodies were present in blood of the patients.

Chronic infection was diagnosed only in 10 (11.9%) out of all patients with blood disorders. Reactivation was confirmed in 49 (58.3%) patients and in three (6.5%) healthy people. Past infection was diagnosed in 24 (28.6%) patients and in 34 (73.9%) healthy people. Reactivation was diagnosed almost nine times more often in patients group than in the control group. Past infection was diagnosed two and half times more often in the control group than in the patients group.

Between the EBV DNA-positive and EBV DNA-negative groups, no significant difference in the profiles of all antibodies was found. There was no difference between the positives and negatives groups in VCA IgG level and the difference was not significant at the VCA IgM and EBNA IgG level. The difference between these groups was significant (P=0.038) in EA IgG level.

In the control group, the differences in profiles and in levels of separate antibodies were not significant.

Between patients with blood disorders and the control group extremely significant difference in profile of antibodies was observed (P<0.00001, $\chi^2 = 4.2058$). Between patients and the control group, considerable differences were observed also in reactivation and in past infection, and a significant difference was observed in chronic infection as well.

### Discussion

Serological tests are a common practice in diagnostics of EBV infection (8). For example, Grazi (5) collected sera from 340 patients with EBV-associated clinical symptoms and determined the presence of EBNA IgG, VCA IgG, and VCA IgM, and stage of the infection. Past infection was observed in 59.4% of cases, acute or early primary infection in 5.3%, late primary infection in 2.6%, chronic infection in 2.6%, reactivated infection in 4.2%, and 16.4% of the patients were not infected.

![Fig.1](image_url). Stages of infection in group of patients with blood disorders and healthy people group

?? – undefined.
There are a few articles available about EBV antibodies in lymphoma patients or immunocompromised hosts. Matter et al. (10) have checked the antibody patterns of immunocompromised patients. They found abnormal EBV serological patterns among HIV infected and renal transplant patients. EBNA-1, but not EA-IgG antibody titres were significantly lower among patients with advanced HIV infection than in those with less pronounced immunodeficiency. The IgA EBV antibodies were investigated in 35 people with NPC and 44 people from the control group by Hinderer et al. (6). Thirty (86%) NPC patients were positive versus one (2%) control person. Akiba et al. (1) discussed the role of EBV in gastric carcinogenesis. They noted that antibodies against EBV-related antigens, including EBV capsid antigen (VCA) were elevated in prediagnostic sera, and EBV reactivation was suspected to precede EBV-GC development. EBV infection of gastric cells by lymphocytes with reactivated EBV was suspected to be the first step of EBV-GC development. In other study, Schetter et al. (13) examined the levels of EBNA and VCA in high-risk cohort of gastric carcinoma. They found that EBV reactivation could play possible role at an early phase of gastric carcinogenesis.

To confirm or deny the participation of the virus in the aetiopathogenesis of the disease, the molecular methods are used. The detection of DNA of a suspected virus in investigated materials by PCR is a common practice. In our earlier study (15), the presence of herpesviruses DNA was checked in the lymphocytes isolated from patients with blood disorders. EBV DNA was detected in 39.8% (45 out of 113) patients and only in 9.3% (9 out of 96) people from the control group. Statistical analysis suggested that herpesvirus co-infection can be related to blood disorders. The interesting problem was the stage of the infection. In the case of patients with blood disorders, it is the reactivation of EBV infection or chronic infection.

In our studies, EBV DNA was detected in leukocytes of 10 patients (23.3%) but the profile of antibodies indicated a past infection. Possibly, it was an early stage of reactivation, when EBV was present in the blood, but there was not antibody response yet. Statistical analysis showed that there was no significant difference between the EBV DNA-positive and EBV DNA-negative group. However, serological tests demonstrated that as many as 67.5% of patients, in whom the DNA of EBV was not found, were diagnosed with the current or chronic infection with the virus. These results might testify to a recent past infection, when the DNA of the virus was not detected but the antibodies were present in blood of the patients. All these issues prove that in order to obtain reliable diagnostic results, both methods: serological and molecular should be used.

The Fisher’s exact test showed that an extremely significant difference in the profile of antibodies was observed between patients with blood disorder and the control group. Significant differences between both groups were also observed in the reactivation, past infection, and chronic infection. These analyses suggest that EBV reactivation or chronic infection can be related to blood disorders. The process of infection can influence the course of blood disorders.

References


