DETECTION OF IgG AND IgA ANTIBODIES TO EPSTEIN-BARR VIRUS MEMBRANE ANTIGEN IN SERA FROM PATIENTS WITH NASOPHARYNGEAL CARCINOMA AND FROM NORMAL INDIVIDUALS

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IgG and IgA antibodies to Epstein-Barr virus (EBV) membrane antigen (MA) were detected in sera from 96 NPC patients and normal individuals by the indirect immunofluorescence test. For MA/IgG antibody, 100% of NPC patients were positive with a GMT of 1:439.7 and 97.9% of normal individuals were positive with a GMT of 1:94.7. In contrast, for MA/IgA antibody, 58.3% of NPC patients were positive with a GMT of 1:7.3 and none of the normal individuals were positive. There was no difference in the detection of antibodies to EBV MA when other P3HR-I or B95-8 cell lines, differing in their major membrane antigen, were used.

Klein et al. (1966) first demonstrated EBV MA in cells from Burkitt lymphoma by the indirect immunofluorescence test, and then proved that the MA was specific to EBV by a direct blocking test (Klein et al., 1969). Other studies have shown that EBV MA exists on both the EBV envelope and the membrane of cells which carry EBV genomes and produce intact EBV particles (Sugawara and Osato, 1970; Silvestre et al., 1971). These data indicate that the antibody titer to MA correlates well with that of neutralizing antibody to EBV (Pearson et al., 1970). There are no published reports concerning IgA antibody to EBV MA. A hypothesis links blocking of ADCC with the appearance of IgA antibodies mainly directed to VCA or EA (Mathew et al., 1981). However, no data have been presented on the reactivity of IgG and particularly IgA antibodies in sera from NPC patients and normal individuals to the membrane antigen by indirect immunofluorescence tests.

MATERIAL AND METHODS

Sera

Sera were obtained from 48 NPC patients and 48 normal individuals, and stored at -20°C.

Indirect immunofluorescence test

The target cells used for detection of MA/IgG and MA/IgA antibodies were P3HR-1 or B95-8 cells. They were cultured in RPMI 1640 medium with 20% newborn calf serum.

P3HR-1 or B95-8 cells were activated for 48 hr by 4mM n-butyrate and 500 ng/ml of croton oil. The activated cells were washed 3 times with Hanks’ solution and adjusted to 1 x 10⁶ cells/ml. Then 1 x 10⁵ cells in 100 μl were added to each well of 96-well U-shaped hemagglutination plates. The sera were diluted from 1:10 to 1:640 in 2-fold dilution and then placed in a humidified chamber at 37°C for 45 min. After 3 washes with Hanks’ solution, cell smears were prepared on slides, air-dried and fixed with cold acetone.

FITC-conjugated sheep antibodies diluted 1:10 and directed to human IgG or IgA were added and the slides were kept at 37°C for 30 min. The smears were again washed 3 times with 0.01M PBS, pH 7.6. After counter-staining with 0.006% Evans’ blue for 10 min, they were examined under an Olympus fluorescence microscope. Cell membranes stained with a specific green color were considered to be positive. The number of cells positive for MA was measured with the test described above, a mixture of several sera being used as first antibody.

Immunoenzymatic test

The test was performed as described by Zeng et al. (1979).

RESULTS

Comparison of the EBV-MA-positivity in P3HR-1 and B95-8 cell lines

The positivity of the EBV MA in untreated P3HR-1 and B95-8 cells was 9.1% and 11.2% respectively. The numbers increased to 62.1% and 63.4% respectively after activation with croton oil and n-butyrate for 48 hr. There was no further increase in MA positivity after activation for 72 hr and more fragmented cells were found (Fig. 1).

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TABLE I - COMPARISON OF POSITIVITY RATE OF IgG AND IgA ANTIBODIES TO VCA AND MA FROM NPC PATIENTS AND NORMAL INDIVIDUALS

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>MA/IgG 1</th>
<th>MA/IgA 1</th>
<th>VCA/IgA 2</th>
<th>EA/IgA 2</th>
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</thead>
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<tr>
<td>NPC patients</td>
<td>48</td>
<td>48</td>
<td>100</td>
<td>28</td>
<td>58.3</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48</td>
<td>100</td>
</tr>
<tr>
<td>Normal individuals</td>
<td>48</td>
<td>47</td>
<td>97.9</td>
<td>0</td>
<td>0</td>
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<tr>
<td></td>
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</table>

1MA/IgG and MA/IgA detected by immunofluorescence test.-2VCA/IgA and EA/IgA detected by immunoenzymatic test.

Comparison of the prevalence rate of EBV MA/IgG, MA/IgA, VCA/IgA and EA/IgA antibodies in sera from NPC patients and normal individuals

Sera from 48 NPC patients and from 48 normal individuals were tested for EBV MA/IgG and MA/IgA antibodies by the immunofluorescence test, and for VCA/IgA and EA/IgA antibodies by the immunoenzymatic test. The positivity of the above 4 antibodies was 100%, 58.3%, 100% and 64.6% respectively, in NPC patients, and 97.9%, 0%, 0% and 0% respectively, in normal individuals (Table I).

Comparison of the distribution of EBV MA/IgG and MA/IgA antibody titers in NPC patients and normal individuals

As shown in Figure 2, the range of the MA/IgG antibody titers for NPC patients was from 1:40 to 1:1,280 with a GMT of 1:439.7, and the range for normal individuals was from 1:10 to 1:640 with a GMT of 1:94.7. The range of MA/IgA antibody titers for NPC patients was from 1:10 to 1:160 with a GMT of 1:7.3; only 52% of the patients had antibody titers higher than 1:20, but no such antibodies could be found in normal individuals. In most NPC cases the VCA/IgA antibody titer was higher than that of MA/IgA antibodies (Fig. 3); this difference was less significant in a comparison with EA/IgA antibodies (Fig. 4).

FIGURE 2 - Comparison of MA/IgG and MA/IgA antibodies from NPC patients and normal individuals.

FIGURE 3 - Relationship between MA/IgA and VCA/IgA antibodies in sera from NPC patients.

FIGURE 4 - Relationship between MA/IgA and EA/IgA antibodies in sera from NPC patients.

DISCUSSION

It has been shown that the detection of IgA antibody to VCA and EA of EBV is of value for the diagnosis of NPC (Henle and Henle, 1976; Zeng et al., 1979a, b; 1980; 1983b). The positivity of MA/IgG antibody was
very high both in NPC patients and in normal individuals. Although the GMT of MA/IgG antibody is much higher in NPC patients than in normal individuals, this test is of no value for the diagnosis of individual cases. Of NPC patients, 58.3% had MA/IgA antibody, while all normal individuals lacked IgA antibodies to this antigen. The situation is similar to that seen with EA/IgA antibodies in NPC patients and in normal individuals (Zeng et al., 1983b), hence detection of MA/IgA antibody can be used as a marker for the diagnosis of NPC.

The positivity of MA in P3HR-1 and B95-8 cells was similar, although the major membrane glycoprotein differs in both cell lines (Edson and Thorley-Lawson, 1983) suggesting that both lines can be used as targets for the detection of EBV MA/IgA antibodies. For a higher expression of MA, cells could be activated with croton oil and n-butyrate for 48 hr before use.

Detection of EBV MA/IgA antibody is more specific, but not as sensitive for the diagnosis of NPC as detection of VCA/IgA antibodies. However, a more sensitive technique for the detection of EBV MA/IgA antibodies should considerably reduce the false negatives and give an even better diagnostic value (Jilg and Wolf, 1985).

The predictive value of MA/IgA antibodies for the prognosis of patients is under investigation. An economical production of MA, using genetic engineering technology, should prove helpful for the development of simpler tests which would allow screening of large quantities of serum.

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REFERENCES


