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Analysis and Significance of Anti-Latent Membrane Protein-1 Antibodies in the Sera of Patients with EBV-Associated Diseases

Jingwu Xu,* Ali Ahmad,* Mario D’Addario,* Laurent Knafo,* James F. Jones, † U. Prasad, ‡ R. Dolcetti,§ E. Vaccher,§ and José Menezes*

Anti-latent membrane protein-1 (LMP-1) is an EBV-encoded type III integral membrane protein with oncogenic potential that is expressed most consistently in various EBV-associated malignancies. Unlike many other EBV proteins, LMP-1 Abs have rarely been demonstrated in EBV-associated disease conditions. We established a high level LMP-1-expressing cell clone and used it for the detection, quantitation, and characterization of these Abs in various human sera in immunoblots and ELISA. Our results demonstrate that, in contrast to the commonly held notion, LMP-1 induces significant humoral immune responses in EBV-associated malignant conditions especially in nasopharyngeal carcinoma (NPC) patients in whom >70% sera are positive for these Abs, and their titers correlate with the clinical condition of the tumors. Interestingly, anti-LMP-1 Abs of IgA isotype were found only in NPC patients. These Abs were absent from the sera of infectious mononucleosis and chronic EBV infection patients, whereas a small fraction (~5%) of the healthy, EBV-seropositive individuals were positive for them; however, their OD values were much lower than those of NPC patients. These studies demonstrate, for the first time, the potential significance of LMP-1-specific Abs for the diagnosis and prognosis of EBV-associated malignancies, especially of NPC. The Journal of Immunology, 2000, 164: 2815–2822.

The EBV is a highly prevalent herpesvirus in humans, in whom it may induce several lymphoproliferative diseases (1–3). Primary infections of EBV usually occur in early childhood, and these infections are often self-limiting and present with mild symptoms. The infected hosts, however, never completely eliminate the virus, which persists in them mainly in a latent form for their entire lives. In industrialized western countries, where primary infections are delayed until adolescence, EBV is the major cause of infectious mononucleosis (IM), a mild lymphoproliferative disorder accompanied by clinical symptoms of fever, malaise, pharyngitis, rashes, etc. (2, 3). A proportion of the infected persons develops chronic EBV infections; they present with a variety of disease symptoms and have unusually high titers of Abs to the lytic and latent cycle viral Ags. The virus replication in these individuals remains high. In the individuals with X-linked lymphoproliferative syndrome, EBV infections are usually fatal (2).

EBV has been causally associated with many human tumors, e.g.: nonkeratinizing nasopharyngeal carcinoma (NPC), which is prevalent in Southeast Asia, North Africa and in certain indigenous populations of North America; endemic Burkitt’s lymphoma in Africa and with the classic Hodgkin’s disease (HD) (2, 3). Recently, EBV has been found associated with T cell lymphomas, NK cell granulomas of the nasal septum, gastric tumors, and B cell lymphomas of AIDS patients (1–3). In the latter patients, EBV also causes oral hairy leukoplakia characterized by the localized lesions in the oral cavity, especially on tongue, in which intensive viral replication is taking place (2). In vitro, EBV infects and readily immortalizes human B cells into continuously growing lymphoblastoid cell lines (2).

Latent membrane protein-1 (LMP-1) is one of the limited number of EBV Ags that are expressed in the latent EBV infection. This infection is exemplified by EBV-immortalized B cells (1). In the latently infected cells, EBV exists as an episome that replicates once with each cell division and expresses only a few genes, i.e., LMP-1, -2A, -2B, six EBV nuclear Ags, and two small polyadenylated EBV RNAs (EBERs) (1, 3). In addition to its role in latent infection (see below), LMP-1 (hereafter referred to as LMP) also seems to play a role in lytic EBV infection by acting as an anti-apoptotic protein and thereby delaying the cell death until all the viral structural and nonstructural proteins are expressed, and virions bud off the infected cells (1, 4). Because LMP has been detected in virions, it may also play a role in the viral infection process (5).

LMP is a 63-kDa type III protein which carries six hydrophobic transmembrane regions and occurs as aggregated patches spanning the surface of EBV-infected and/or-immortalized cells. It resembles TNFR family of proteins (6). Its intracellular carboxy-terminal cytosolic region interacts with signaling molecules that are involved

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in the pathways activated by the TNFR family members which include CD40 (3, 7–11). By this virtue, LMP acts as a constitutively activated CD40 receptor in B cells. LMP transforms rodent fibroblasts and is essential for the EBV-induced immortalization of B cells (1, 3, 12). It induces activation of NF-kB, c-JNK, and AP-1, and the expression of antipapoptotic genes Bcl2, Mcl-1, and A20 in different cell types (4, 13, 14). In LMP-transfected cells, it induces several phenotypic changes characteristic of the EBV-infected cells, e.g., CD23, epidermal growth factor receptor, certain adhesion molecules, etc. (3, 13, 14). Recently, LMP was shown to induce the expression of cyclin D2 and hyperphosphorylation of pRb, rendering cells unresponsive to the growth-inhibitory effects of TGF-B1 (15). These studies strongly suggest a role of LMP-1 in the EBV-associated lymphoproliferative diseases and malignancies. This is further supported by the consistent expression of this protein in many EBV-associated tumors.

The EBV-infected subjects mount a vigorous antiviral cellular and humoral immune responses (reviewed in Refs. 16 and 17). These responses, particularly cellular ones, are thought to keep the viral replication under control in healthy subjects. The detection of humoral responses (i.e., Abs) against various viral Ags has been very useful for the diagnosis and prognosis of EBV-associated disease conditions. LMP-specific cellular immune responses have been well documented (16, 17). However, information on anti-LMP Abs is scanty despite the fact that this protein is expressed on the surface of EBV-infected/immortalized cells and may be targeted by such Abs. The detection of these Abs may be helpful not only in the diagnosis and prognosis of EBV-associated diseases but may also provide insights about their pathogenetic processes. We report here our studies on the detection, analysis, and significance of LMP-specific Abs in different EBV-associated disease conditions. LMP-specific cellular immune responses have been well documented (16, 17). However, information on anti-LMP Abs is scanty despite the fact that this protein is expressed on the surface of EBV-infected/immortalized cells and may be targeted by such Abs. The detection of these Abs may be helpful not only in the diagnosis and prognosis of EBV-associated diseases but may also provide insights about their pathogenetic processes. We report here our studies on the detection, analysis, and significance of LMP-specific Abs in different EBV-associated diseases and show for the first time that these Abs can readily be demonstrated in the sera of patients with EBV-associated malignancies and that anti-LMP IgA may be of prognostic value in patients with advanced stages of NPC.

Materials and Methods

Expression of LMP-1 in BJA-B

LMP-1 was expressed in an EBV-genome negative human B cell line BJA-B derived from Burkitt’s lymphoma (18). For this purpose, a pGEM2 (Promega, Madison, WI)-based expression plasmid plgLMP-1 (provided by Dr. Nancy Raab-Traub, University of North Carolina, Chapel Hill, NC) was transfected into these cells by electroporation as described earlier (19). This plasmid has an Ig promoter/enhancer 5’ to two tandemly arranged copies of the 3.0-kb LMP-1 genomic sequences. The transfected cells were cloned by limiting dilution in the selection medium, i.e., RPMI 1640 containing 10% FBS and 1 mg per ml of geneticin (Life Technologies, Burlington, ON, Canada). The transfected cell clones were screened for LMP-1 expression by immunofluorescence using S12 (provided by Dr. Elliott Kieff, Harvard Medical School, Boston, MA) and CS1-4 (Dako, Glostrup, Denmark) Abs. The selected clones were maintained in the selection medium, and their lysates were used to detect anti-LMP-1 Abs in the sera (see below).

Serum samples

 Serum samples for the detection of anti-LMP Abs were obtained after informed consent from the following groups of individuals: category 1, healthy EBV seronegatives (HN), 3; category 2, healthy EBV seropositives (HI), 20; category 3, IM, 15; category 4, chronic EBV infection (CEI), 25; category 5, HD, 15; category 6, nonkeratinizing NPC, 86. These EBV-associated disease conditions have been defined in our recent publications (20, 21). We further divided the NPC patients into different stages based on tumor size (T), lymph node involvement (N), the presence of hematogenous metastases (M), patient death or tumor remission, and AJC stages as described (22).

Radioimmunoprecipitation (RIPA) and Western blots

RIPA was conducted as described by us earlier (19). Briefly, 5 × 10⁶ cells were labeled with 250 μCi ¹³¹I-methionine (NEN/DuPont), lysed in RIPA buffer in the presence of protease inhibitors, and immunoprecipitated using 2 μl of the undiluted ascites fluid of S12 Ab or 40 μl of the serum sample. The immunoprecipitates were resolved on 10% SDS-PAGE and detected by autoradiography. For Western blots, 5 × 10⁶ cells were lysed in 50 mM Tris-HCl (pH 6.8), 2% SDS and sonicated for 15 s. The lysates were clarified by centrifugation (14,000 × g for 30 min at 4°C), and their protein concentrations were determined with a commercial kit (Bio-Rad Laboratories, Hercules, CA). Forty micrograms of the proteins were resolved on 10% SDS-PAGE, electroblotted onto nylon membranes (Immobilon, Millipore, Bedford, MA). The membranes were blocked in 5% skim milk powder in PBS and then incubated with appropriately diluted S12, CS1-4, or serum samples. After washings, blots were developed using alkaline phosphatase (AP)-conjugated secondary Abs (anti-mouse or anti-human IgG; both from Promega) and chromogenic substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Promega). For detection of LMP-1-specific IgA Abs, AP-conjugated rabbit anti-human IgA (α-chain specific; Dako; at 1:1000 dilution) was used in Western blots.

Indirect immunofluorescence

For this purpose, cells were fixed and premeabilized using the Cytofix/Cytoperm Kit (PharMingen, San Diego, CA). They were incubated with CS1-4 or S12 for 45 min at 4°C, washed, and stained with FITC-conjugated goat anti-mouse IgG (Becton Dickinson, Mountain View, CA) for a further 45 min. After three washes, the cells were examined by flow cytometry using FACSscan. A compares the fluorescence profile of LMP-1-expressing cells with control vector-transfected cells when S12 mAb was used, whereas B depicts the same using CS1-4 mAb. In both panels, the y-axis denotes number of cells, and the x-axis depicts fluorescence intensity in arbitrary log units.

ELISA protocol

For the quantitation of anti-LMP-1 Abs, a LMP-1-specific ELISA was developed using a protocol described by us earlier (23) after some modifications. Briefly, plastic ELISA plates (Corning, Corning, NY; catalog number 28805) were coated (at 4°C overnight) with 50 μl of the cell lysates (protein content, 100 μg/ml) from LMP-1-expressing or control vector-transfected BJA-B cells. These lysates were obtained as above for Western blots. The wells were washed three times with wash buffer containing 0.1% gelatin (Bio-Rad) and 0.05% Tween 20 in PBS and blocked with 0.2% gelatin in PBS (200 μl/well) at 4°C overnight. After washing, the wells were incubated with 50 μl of the serum dilution and
incubated at room temperature for 2 h. After three subsequent washings with the wash buffer, 50 μl of 1:7500 diluted AP-conjugated anti-human IgG (heavy and light chain, Promega) was added. After incubation at room temperature for 2 h with the secondary Ab, the plates were washed extensively with the wash buffer, and colors were developed by using 50 μl p-nitrophenyl phosphate (Sigma, St. Louis, MO; catalog number N-9389, 1 mg/ml) dissolved in 0.1 M glycine buffer (pH 10.4) containing 1 mM MgCl₂ and 1 mM ZnCl₂. The reactions were stopped 30 min later with the addition of 50 μl of 3 N NaOH, and OD was measured at 405 nm in an automatic ELISA reader (Easy Reader EAR 400AT, STL Labinstruments, Salzburg, Austria).

**Results**

**Detection of anti-LMP Abs by Western blots**

The cell clones that we used as a source of LMP-1 in these studies could be easily stained and detected by flow cytometry using anti-LMP Abs (CS1–4 or S12) and FITC-conjugated secondary Abs after permeabilization (Fig. 1). These Abs, however, were unable to stain these cells by indirect membrane immunofluorescence without permeabilization (data not shown). With these Abs, LMP-1 could also be demonstrated in these cells by RIPA and Western blots (Fig. 2 and data not shown). Following this, we used sera from EBV-seronegative and healthy EBV-seropositive individuals and from NPC patients in the RIPA and Western blots to see whether they contained anti-LMP Abs. As shown in Figs. 3 and 4, several NPC sera showed positivity for these Abs. However, only a single sample of 20 EBV-seropositive healthy sera showed positivity for anti-LMP Abs. Sera from EBV-seronegative individuals were always negative both in RIPA and Western blots (data not shown). These data strongly suggested the presence of anti-LMP humoral responses in EBV-infected individuals. Interestingly, sera from CEI and IM patients were negative for these Abs. Table I depicts the percent positivity of sera for anti-LMP-1 Abs for various EBV-associated disease conditions. Within NPC patients, the percentage of anti-LMP Ab-positive sera showed a clear increasing trend in higher stages of the tumor (Table II). An overwhelmingly high percentage of the dead NPC patients was positive for anti-LMP Abs (Table II). In the case of HD, all EBER and/or LMP-positive tumors were positive for these Abs (Table III). One
(of six) EBER-negative HD patient also contained these Abs. Taken together, these results illustrate the presence of anti-LMP humoral responses, especially in patients with EBV-associated NPC and HD.

Detection of LMP-specific Abs by ELISA

Although anti-LMP Abs were detected by RIPA and/or Western blots, in many cases the revealed bands were faint, showed background darkness, and were not amenable to quantitative analyses. Therefore, to quantitate these Abs in the sera, we developed ELISA using lysates from LMP-expressing and control BJA-B cells. First, we determined the suitability of these lysates for ELISA (data not shown). We then used pooled sera from EBV-control-transfected cells were used, suggesting specificity of the Abs.

To determine whether these anti-LMP-1 Abs differed in various stages of this tumor, all sera from each category were tested individually in ELISA and average OD values for each category of serum were calculated. As shown in Fig. 6, A–D, in each category, a trend toward higher average OD with increase in tumor size (T0→T4), increased nodal involvement (N0→N3) and with metastasis is evident. Higher average ODs were also seen in sera from dead patients as compared with the patients with remissions. Furthermore, these OD values also increased in advanced stages of the tumor. These results strongly suggest a prognostic value of these Abs.

Detection of LMP-1-specific IgA in NPC sera

Because earlier studies from this and other laboratories have shown that Abs of IgA isotype against several EBV Ags are of diagnostic and prognostic value, especially for NPC (16, 20, 24), we did Western blots using AP-conjugated human α-chain-specific secondary Abs to detect these Abs in these sera. Fig. 7 depicts a typical Western blot, and the results from several blots are shown in Table IV. Only NPC sera (50%) were found to be positive for anti-LMP-1 IgA Abs. The percent positivity for this Ab in NPC sera also tended to increase in advanced stages of the tumor (Table II).


data table image

Table I. Positivity of sera of different groups of subjects for anti-LMP-1 Abs by Western blots

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Sera Tested</th>
<th>Negative</th>
<th>Positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>HI</td>
<td>20</td>
<td>19</td>
<td>1</td>
<td>5.00</td>
</tr>
<tr>
<td>IM</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>CEI</td>
<td>25</td>
<td>25</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>HD</td>
<td>15</td>
<td>6</td>
<td>9</td>
<td>66.67</td>
</tr>
<tr>
<td>NPC</td>
<td>86</td>
<td>25</td>
<td>61</td>
<td>70.93</td>
</tr>
</tbody>
</table>

* Individual sera in each category were tested for anti-LMP-1 Abs on Western blot strips prepared from the lysates of LMP-expressing BJA-B cells. The serum sample was considered positive if it gave a clear band at the location corresponding to the molecular mass of LMP-1 (∼63 kDa). HN, healthy EBV seronegatives.

Table II. Detection of anti-LMP-1 Abs in different NPC stages by Western blots

<table>
<thead>
<tr>
<th>Tumor stages</th>
<th>No. of Patients Tested</th>
<th>Anti-LMP-1 Ab</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0–T1</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>T2–T3</td>
<td>56</td>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>T4</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Lymph node involvements</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>15</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>N1</td>
<td>30</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>N2</td>
<td>26</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>N3</td>
<td>15</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>74</td>
<td>12</td>
<td>52</td>
</tr>
<tr>
<td>M1</td>
<td>12</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>AJC stages</td>
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</tr>
<tr>
<td>0–II</td>
<td>9</td>
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<td>5</td>
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<td>III</td>
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<td>10</td>
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<td>IV</td>
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<td>Clinical stages</td>
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<tr>
<td>Remission</td>
<td>73</td>
<td>16</td>
<td>49</td>
</tr>
<tr>
<td>Dead</td>
<td>13</td>
<td>0</td>
<td>12</td>
</tr>
</tbody>
</table>

* The NPC tumors were classified depending on size (T0→T4), nodal involvement (N0→N3), the presence or absence of hematogenous metastases (M0 or M1), AJC stages (0–IV), or whether the tumor was in remission or the patient died. Each serum was tested for the presence of anti-LMP-1 Abs by Western blots prepared from the lysates of LMP-expressing BJA-B cells. Note the corresponding increase in the percentage of anti-LMP-1 Ab-positive sera in advanced tumor stages.
These data collectively show that anti-LMP IgA Abs are pathognomonic for NPC and have prognostic value.

Discussion

LMP is an EBV-encoded oncoprotein which is expressed very frequently in several EBV-associated malignancies. In these malig-
nancies, the human host mounts strong anti-EBV cellular and humoral immune responses. These humoral (Ab) responses against several EBV Ags, e.g., early Ags, viral capsid Ags (VCA), have been used as important tools in the diagnosis and prognosis of these EBV-associated diseases. Although LMP is thought to play an important role in EBV-associated malignancies and LMP-specific CTL responses have been well documented in EBV-infected individuals, quantitative studies of anti-LMP Ab responses and their significance in EBV-associated diseases have not yet been reported. To our knowledge, this is the first report to quantify these Abs by ELISA using lysates from LMP-1-expressing BJA-B cells. This confirms an earlier report (31) that, unlike Burkitt’s lymphoma, the presence or absence of anti-EBV Abs is not predictive of the EBV status of the tumor cells in HD. Interestingly, sera from IM and CEI patients were negative for these Abs. More importantly, two-thirds or more sera from patients with EBV-associated malignancies (NPC and HD) were positive for these IgA Abs despite the absence of EBV genome in its tumor (Reed-Sternberg) cells. This confirms an earlier report (31) that, unlike Burkitt’s lymphoma, the presence or absence of anti-EBV Abs is not predictive of the EBV status of the tumor cells in HD. Interestingly, sera from IM and CEI patients were negative for these Abs. More importantly, two-thirds or more sera from patients with EBV-associated malignancies (NPC and HD) were positive for these Abs. Furthermore, the OD values of these Abs in ELISA also correlated positively with advanced stages of the tumor in NPC patients. IgA Ab titers for several EBV Ags, e.g., early Ag, viral capsid Ag, gp350/220, have been documented to have both diagnostic and prognostic significance in NPC (2, 24). We also found that anti-LMP Abs of IgA isotype were detectable only in the sera of healthy EBV-seropositive individuals, and lanes 6–10 with NPC sera.

Table IV. Percent positivity of sera for anti-LMP-1-specific IgA in different stages of NPC by Western blots

<table>
<thead>
<tr>
<th>Category</th>
<th>NPC Stage</th>
<th>No. of Individuals Tested</th>
<th>No. of Positive</th>
<th>% Positive</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>T&lt;sub&gt;i&lt;/sub&gt;–T&lt;sub&gt;j&lt;/sub&gt;</td>
<td>10</td>
<td>3</td>
<td>33.00</td>
<td>A vs B 0.1442</td>
</tr>
<tr>
<td>B</td>
<td>T&lt;sub&gt;j&lt;/sub&gt;–T&lt;sub&gt;k&lt;/sub&gt;</td>
<td>15</td>
<td>6</td>
<td>40.00</td>
<td>B vs C 0.0245</td>
</tr>
<tr>
<td>C</td>
<td>T&lt;sub&gt;k&lt;/sub&gt;</td>
<td>9</td>
<td>8</td>
<td>88.89</td>
<td>A vs C 0.0149</td>
</tr>
</tbody>
</table>

*The indicated number of sera was selected at random from patients with different stages of NPC and tested individually for the presence of LMP-1-specific IgA Abs. The percentages between different categories were tested for significance using Fisher’s exact test. In each higher stage of the tumor, a higher percentage of sera became positive for these Abs.*
The LMP-specific mAbs CS1-4 and S12 are known to recognize intracellular parts of LMP (32, 33), and LMP Ab-positive sera stained our LMP-expressing cells in indirect immunofluorescence assays only when these cells were first permeabilized. These Abs and sera, however, were unable to stain these cells without prior permeabilization, suggesting that these Abs recognized only intracellular parts of the protein. Similar observations were made earlier by Rowe et al. (27). These data suggest that extracellular parts of LMP (the three 8- to 12-amino acid-long turns) are not immunogenic and are not targeted by human anti-LMP Abs. This is further supported by our observations that LMP-expressing cells are not killed by NK cells in Ab-dependent cellular cytotoxicity (ADCC) assays as described by us for anti-gp350/220 Abs (Ref. 20 and data not shown). Thus, it appears that anti-LMP humoral response does not play a role in the control of EBV-infected/immortalized cells. Therefore, LMP-expressing malignant cells are not under pressure to accumulate mutations to evade this response. This also reinforces the commonly held notion that anti-EBV cellular immune responses are more important to control EBV infections (16, 17).

LMP is a transforming protein that mimics (but is not identical with) a constitutively activated CD40 receptor (1, 3, 7, 9, 10, 14). It activates several cellular genes, acts as a classical oncogene and is the frequently expressed EBV protein in several EBV-associated malignancies. The expression of LMP-1 in 100% cases of rarely induced tumorigenicity. However, there is no clear in vivo evidence for such a role for this protein. On the contrary, LMP per se is cytostatic or even cytotoxic for human cells (37, 38), and LMP-positive tumors have better prognosis than LMP-negative tumors (39, 40). LMP is targeted by EBV-specific CTL and its expression in Burkitt’s lymphoma makes them susceptible to lysis by these CTL (39). It also enhances expression of MHC class I Ags and costimulatory molecules, e.g., CD80, CD86, and consequently depends on the cell type (3, 35). Antisense oligos to the first five codons of LMP induce apoptosis in EBV-immortalized B cells (36). Taken together, these observations strongly suggest a role for LMP in EBV-induced tumorigenesis. However, there is no clear in vivo evidence for such a role for this protein. On the contrary, LMP per se is cytostatic or even cytotoxic for human cells (37, 38), and LMP-positive tumors have better prognosis than LMP-negative tumors (39, 40). LMP is targeted by EBV-specific CTL and its expression in Burkitt’s lymphoma makes them susceptible to lysis by these CTL (39). It also enhances expression of MHC class I Ags and costimulatory molecules, e.g., CD80, CD86, and consequently increases the presentation of endogenous and exogenous Ags to the immune system (41–43). LMP-1-expressing Burkitt’s lymphomas are less tumorigenic than their LMP-negative counterparts in nude and SCID mice (39). Recently, Cherney et al. (44) demonstrated that expression of LMP augments nonspecific antitumor immunity by inducing the secretion of cytokines and chemokines. The EBV-induced tumors, however, may have evolved strategies to overcome these responses by accumulating mutations in LMP. An LMP gene cloned from an NPC patient was reported to be more tumorigenic and less immunogenic in mice than its B95-8 counterpart (45). Mutations in the promoter region and in the C-terminal cytoplasmic region of LMP have been documented by several workers (46–49). A characteristic 30-bp mutation in the C-terminal part has attracted much attention (49–51). However, there is not yet convincing in vivo evidence of the role of this or any other LMP mutation in increased tumorigenicity. Our results suggest that anti-LMP Abs recognize hidden intracellular parts of LMP and do not mediate ADCC and therefore may not be driving the accumulation of these mutations in EBV-associated tumors. Instead, these mutations may enable tumors to escape LMP-specific CTL, render this protein unable to stimulate nonspecific antitumor immunity, or reduce its cytotoxic/cytostatic effects. Because human anti-LMP-1 Abs recognized only intracellularly located parts of the protein, the immune system may be exposed to LMP-1 on lysis of LMP-expressing EBV-infected cells and/or by virolysis of released virions. The titers of anti LMP Abs, therefore, may reflect the magnitude of this expression. Our data also suggest that anti-LMP Abs increase in advanced stages of NPC. The increase in anti-LMP Abs in advanced NPC stages may simply reflect an enhanced exposure to LMP in these patients. Alternatively, these patients may be mounting disproportionally higher humoral responses as compared with the CTL responses. Because we have not determined LMP-specific cellular immune responses in these patients, further studies will be needed to address these issues.

This quantitation and analysis of anti-LMP Abs using ELISA underscores the potential usefulness of these Abs in the diagnosis and prognosis of EBV-associated malignancies, particularly NPC.

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References


