HLA antigen expression in enteropathy associated T cell lymphoma

M Ashton-Key, N Singh, L X Pan, M E F Smith

Abstract
Aims—To investigate the occurrence of abnormal patterns of HLA-ABC and HLA-DR expression in enteropathy associated T cell lymphoma and to relate such abnormalities to the Epstein Barr virus (EBV) status of the tumours.

Methods—Eleven enteropathy associated T cell lymphomas were immunostained with HC10 (HLA-ABC heavy chain) and TAL 1B5 (HLA-DR alpha chain) monoclonal antibodies and polyclonal anti-β2 microglobulin (β2m, the HLA-ABC light chain) antibodies. In situ hybridisation for EBV using EBER probes was performed on all cases.

Results—Tumour cells of two of 11 patients were EBER positive. One of these showed partial, and the other, complete loss of β2m. HLA-DR expression was undetectable in both patients. Of the remaining nine EBER negative tumours, two were HLA-ABC heavy chain negative or showed only occasional positive cells and five of nine showed partial or complete loss of the HLA-ABC light chain, β2m. Seven of the nine cases were either negative for HLA-DR or showed weak expression in a proportion of tumour cells.

Conclusions—These data show that low or absent HLA-ABC and HLA-DR antigen expression occurs commonly in enteropathy associated T cell lymphoma. These abnormal patterns of HLA expression may be associated with escape from immune attack which, in a minority of patients, could be directed against EBV antigens.

(Keywords: enteropathy associated T cell lymphoma, HLA-ABC, HLA-DR, EBV)

Loss of HLA-ABC molecules is a common finding in many types of tumour. Such loss may confer a selective growth advantage on the tumour cells by preventing recognition of foreign antigens by cytotoxic T cells. In some tumours the foreign antigen may be of viral origin rather than a tumour specific antigen. As examples, the Epstein Barr virus (EBV) infected Burkitt lymphoma cell line, Daudi, does not express HLA-ABC molecules at its cell surface and the Reed–Sternberg cells of Hodgkin's disease frequently harbour EBV but fail to express HLA-ABC molecules.

The tumour cells of enteropathy associated T cell lymphomas have been shown to be infected by EBV in up to 36% of patients. We have investigated the expression of HLA-ABC and HLA-DR antigen expression in a series of enteropathy associated T cell lymphomas and have correlated their expression with tumour EBV infection.

Methods

MATERIAL
Formalin fixed, paraffin wax embedded material from 11 enteropathy associated T cell lymphomas located in the small intestine was retrieved from the files of the Histopathology Department of UCL Medical School, London, and their histology reviewed. CD3 and CD30 immunostaining had been performed previously and was available for review in all cases. Four of these cases had been included in a previous study.

IMMUNOHISTOCHEMISTRY
Paraffin wax sections were heated in sodium citrate buffer (pH 6.0, 0.01 M) in a pressure cooker at 130°C for two minutes prior to immunostaining with HC10 (anti-HLA-ABC heavy chains, preferentially recognising HLA-B chains) and TAL 1B5 (anti-HLA-DR alpha chain; Dako, High Wycombe, UK) monoclonal antibodies and polyclonal anti-β2 microglobulin (β2m) (Dako) antibodies, using a Streptavidin-biotin complex method. Positive (tonsil) and negative (placenta) tissue controls were included for each antibody, as were negative controls omitting the primary antibody.

The intensity of immunostaining on tumour cells was assessed semiquantitatively as follows: absent (−), weak (+), intermediate (++), and strong (+++), where strong staining was equivalent to that of normal small mantle zone lymphocytes.
Table 1  HLA antigen and EBER1 expression in enteropathy associated T cell lymphoma

<table>
<thead>
<tr>
<th>Case number</th>
<th>EBER status</th>
<th>HLA-ABC heavy chain (HC10)</th>
<th>β2m</th>
<th>HLA-DR (TAL 1B5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Negative*</td>
<td>++</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>++</td>
<td>–</td>
<td>++</td>
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<tr>
<td>3</td>
<td>Negative</td>
<td>++</td>
<td>+/-</td>
<td>+/–</td>
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<tr>
<td>4</td>
<td>Negative</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>+++</td>
<td>+++</td>
<td>–/+</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>++</td>
<td>–/+</td>
<td>+/+</td>
</tr>
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<td>7</td>
<td>Negative</td>
<td>+</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Negative</td>
<td>+</td>
<td>+/–</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>Negative</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>Positive†</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>Positive</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Tumour cells EBER negative; † tumour cells EBER positive; cytoplasmic rather than membrane staining. Positive staining scored from + (weak staining) to +++ (strong staining).
+/- = Mostly positive with focal negative staining.
-/+ = Mostly negative with focal positive staining.

Results

Table 1 summarises the results.

All cases showed the typical morphology of enteropathy associated T cell lymphoma (fig 1). The tumour cells of all cases were CD3 positive and those of seven of the 11 cases were CD30 positive (table 1, cases 2, 3, 5–7, 9, and 11).

Tumour cells of two cases were EBER positive. One of these tumours showed complete and the other partial loss of β2m and in neither case was HLA-DR expression detected (fig 2).

Of the nine EBER negative cases, five showed complete or partial loss of β2m, and two were negative or showed only occasional positive cells for the HLA-ABC heavy chain. Abnormal

IN SITU HYBRIDISATION

Recombinant plasmid containing the EBV EBER1 cDNA fragment was kindly provided by R Ambinder.15 RNA probes to EBV EBER1 were generated using a digoxigenin-11-dUTP transcription kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer’s instructions. In situ hybridisation for EBER 1 RNA on paraffin wax sections, washing, and immunodetection were performed as described previously.13 Sections were counterstained with haematoxylin.
patterns of cytoplasmic immunostaining without associated membrane staining were seen for $\beta_2m$ in two cases. Seven of the nine EBER negative cases showed complete or focal loss of HLA-DR expression.

In summary, all 11 cases showed either complete loss or only very low levels of expression of $\beta_2m$ or HLA-DR, or both.

**Discussion**

The majority of enteropathy associated T cell lymphomas showed low expression of either the light or the heavy chain of the HLA-ABC molecule. Thus, in seven of 11 tumours we either failed to detect the HLA-ABC light chain, $\beta_2m$, and/or the HLA-ABC heavy chain, or detected only weak expression of one of them in a minority of tumour cells. Other studies have reported the HLA-ABC antigen status of non-enteropathy associated T cell neoplasms. Thus, Medeiros et al. did not report the absence of HLA-ABC antigens in any of the nine large cell T cell lymphomas studied. Jones et al. detected levels of HLA-ABC antigen expression equivalent to those of normal T lymphocytes in 19 samples of non-enteropathy associated T cell lymphoma post-thymic T cell neoplasms, including samples of T cell lymphoma and leukaemia. Although direct comparisons are difficult because of differences in methodology and assessment, it would seem that absence or low expression of HLA-ABC molecules occurs far more commonly in enteropathy associated T cell lymphoma than in many other groups of non-enteropathy associated T cell neoplasms. As normal T cells strongly express HLA-ABC antigens the low expression of these molecules in enteropathy associated T cell lymphoma probably represents a loss of the molecule during neoplastic development or progression. In two of our cases there was evidence of EBV infection in the tumour cells as shown by EBER1 in situ hybridisation. Both of these EBV positive cases showed loss of $\beta_2m$ making the HLA-ABC heavy chain non-functional. It
is possible that EBV infected tumour cells may acquire a selective growth advantage through their loss of HLA-ABC antigens, a change which would enable them to avoid HLA-ABC directed cytotoxic T cell attack against EBV determinants. Abnormalities of HLA-ABC antigen expression have been reported in other EBV infected tumour cells, such as the Burkitt lymphoma cell line Daudi, which also lacks β₂m. However, EBV infection cannot explain the altered HLA-ABC expression in the majority of our cases. Six of nine EBV negative cases showed loss of HLA-ABC expression. In these cases, however, it is still possible that the altered HLA expression conferred protection from immune attack, directed either against another type of virus or against tumour specific antigens. Tumour specific antigens have been identified in several types of tumour, including melanomas and carcinomas of the breast, pancreas and lung.\(^\text{15,\text{16}}\)

HLA-DR was detected in the tumour cells of only four of the 11 enteropathy associated T cell lymphomas studied and in two of these expression was weak and focal. These low levels of expression contrast with much higher levels reported in some non-enteropathy associated T cell lymphomas. Thus, Jones et al.\(^\text{17}\) detected HLA-DR expression in all 16 T cell lymphomas studied, though in two cases less than 20% of tumour cells were positive. Medeiros et al.\(^\text{18}\) detected HLA-DR in 20 of 24 large cell T lymphomas. HLA-DR antigens were detected in all 10 T cell lymphomas studied by Smith et al.,\(^\text{19}\) although in two cases less than 5% of tumour cells were positive. These data and that of the present study suggest, therefore, that lack of HLA-DR antigens occurs much more frequently in enteropathy associated T cell lymphoma than in many other types of T cell neoplasms.

HLA-DR antigens are not expressed by resting T cells, but are present on activated T cells.\(^\text{1}\) The high grade cytology of enteropathy associated T cell lymphoma cells and their frequent expression of the activation marker CD30 both suggest that they should have an activated phenotype. The likelihood is, therefore, that the lack of HLA-DR expression seen in tumour cells in the vast majority of enteropathy associated T cell lymphoma represents a loss of HLA-DR rather than the presence of a non-activated phenotype. Lack of HLA-DR could confer a growth advantage on enteropathy associated T cell lymphoma cells by several mechanisms. HLA-DR is necessary for the recognition of foreign antigen by CD4 helper T cells, which in turn have a role in the activation of CD8 cytotoxic T cells. If viral or tumour specific antigens are expressed by the malignant T cells of enteropathy associated T cell lymphoma, an absence of HLA-DR could prevent recognition of these antigens by helper T cells and may be associated with escape from immune attack. Alternatively, a more direct mechanism would be evading attack from the subgroup of cytotoxic T lymphocytes that are HLA-DR restricted.\(^\text{19}\)

In conclusion, our data indicate that low or absent HLA-ABC and HLA-DR expression occur commonly in enteropathy associated T cell lymphoma, and probably more commonly than in the majority of non-enteropathy associated subtypes of T cell lymphoma. These abnormalities in HLA expression may permit escape from immune attack which, in a minority of cases, may be directed against EBV antigens.

6 Nilsson K, Ehrn P-E, Welsh KI. Production of β₂m, microglobulin by normal and malignant human cell lines and peripheral lymphocytes. Transplant Proc 1974;6:2153–84.