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- β_2 microglobulin (β_2 m, 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 β_2 m. 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, β_2 m. 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.

(J Clin Pathol 1996;49:545-548)

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

Major histocompatibility complex antigen expression is essential for the presentation of foreign antigens to the immune system. HLA-ABC (MHC class I) and HLA-DR (MHC class II) antigens are surface membrane glycoproteins with a non-covalently bound heterodimer structure. HLA-ABC antigens are strongly expressed by most cells in the body and CD8 cytotoxic T lymphocytes can only recognise foreign antigen when it is complexed to these molecules. HLA-DR antigens have a more restricted tissue distribution, including activated T cells.¹ The HLA-DR/foreign antigen complex is recognised by CD4 helper T lymphocytes.

Loss of HLA-ABC molecules is a common finding in many types of tumour.²⁻⁵ 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 surface⁶ and the Reed-Sternberg cells of Hodgkin's disease frequently harbour EBV but fail to express HLA-ABC molecules.⁷

The tumour cells of enteropathy associated T cell lymphomas have been shown to be infected by EBV in up to 36% of patients.⁸ 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.⁸

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)⁹ and TAL 1B5 (anti HLA-DR alpha chain; Dako, High Wycombe, UK) monoclonal antibodies and polyclonal anti- β_2 microglobulin (β_2 m) (Dako) antibodies, using a Streptavidin-biotin complex method.^{10 11}

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.

Department of
Histopathology,
University College
London Medical
School,
University Street,
London WC1E 6JJ
M Ashton-Key
N Singh
L X Pan

Department of
Histopathology,
Derriford Hospital,
Derriford Road,
Plymouth PL6 8DH
M E F Smith

Correspondence to:
Dr M E F Smith.

Accepted for publication
21 March 1996

Table 1 HLA antigen and EBER1 expression in enteropathy associated T cell lymphoma

Case number	EBER status	HLA-ABC heavy chain (HC10)	β_2m	HLA-DR (TAL 1B5)
1	Negative*	++	++	-
2	Negative	++	-	+++
3	Negative	++	+/-	+/-
4	Negative	+++	-	-
5	Negative	+++	+++	-/+
6	Negative	++	-/+‡	‡
7	Negative	-	+++‡	-
8	Negative	-/+	+/-	-
9	Negative	+	+++	-
10	Positive†	+++	-/+	-
11	Positive	++	-	-

*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.

IN SITU HYBRIDISATION
Recombinant plasmid containing the EBV EBER1 cDNA fragment was kindly provided by R Ambinder.¹² 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.¹² Sections were counterstained with haematoxylin.

Results

Table 1 summarises the results.

All cases showed the typical morphology of enteropathy associated T cell lymphoma (fig 1).¹³ 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 β_2m and in neither case was HLA-DR expression detected (fig 2). Of the nine EBER negative cases, five showed complete or partial loss of β_2m , and two were negative or showed only occasional positive cells for the HLA-ABC heavy chain. Abnormal

Figure 1 Case 7. (A) Pleomorphic tumour cells typical of enteropathy associated T cell lymphoma. (B) The tumour cells are negative for HLA-ABC heavy chain (HC10, immunoperoxidase). (C) The tumour cells show strong cytoplasmic staining for β_2m (anti- β_2m , immunoperoxidase). (D) There is no expression of HLA-DR by the tumour (TAL 1B5, immunoperoxidase).

Figure 2 Case 10. (A) The tumour cells are positive for EBER1 by in situ hybridisation. (B) The tumour cells are positive for HLA-ABC heavy chain (HC10, immunoperoxidase). (C) Focal β_2m positivity is evident (anti- β_2m , immunoperoxidase). (D) There is no expression of HLA-DR by the tumour cells. Endothelial cells and macrophages are positive (TAL 1B5, immunoperoxidase).

patterns of cytoplasmic immunostaining without associated membrane staining were seen for β_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 β_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, β_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*⁶ did not report the absence of HLA-ABC antigens in any of the nine large cell T cell lymphomas studied.

Jones *et al*¹⁴ 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 β_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 β_2m .⁶ 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.^{15 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*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*⁸ 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*,¹⁸ 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.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.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 abnormal patterns of HLA expression may permit escape from immune attack which, in a minority of cases, may be directed against EBV antigens.

1 Ko H-S, Fu SM, Winchester RJ, Yu DT, Kuntel HG. Ia determinants on stimulated human T lymphocytes. Occurrence on mitogen- and antigen-activated T cells. *J Exp Med* 1979;150:246-55.

2 Moller P, Herrmann B, Moldenhauer G, Momburg F. Defective expression of MHC class I antigens is frequent in B-cell lymphomas of high-grade malignancy. *Int J Cancer* 1987;40:32-9.

3 Smith MEF, Marsh SGE, Bodmer JG, Gelsthorpe K, Bodmer WF. Loss of HLA-A, B, C allele products and lymphocyte function-associated antigen 3 in colorectal neoplasia. *Proc Natl Acad Sci USA* 1989;86:5557-61.

4 Medeiros LJ, Gelb AB, Wolfson K, Doggett R, McGregor B, Cox RS, *et al*. Major histocompatibility complex class I and class II antigen expression in diffuse large cell and large cell immunoblastic lymphomas. Absence of a correlation between antigen expression and clinical outcome. *Am J Pathol* 1993;143:1086-97.

5 Cordon-Cardo C, Fuks Z, Drobnjak M, Moreno C, Eisenbach L, Feldman M. Expression of HLA-A, B, C antigens on primary and metastatic tumour cell populations of human carcinomas. *Cancer Res* 1991;51:6372-80.

6 Nilsson K, Evrin P-E, Welsh KI. Production of β_2 microglobulin by normal and malignant human cell lines and peripheral lymphocytes. *Transplant Rev* 1974;21:53-84.

7 Poppema S, Visser L. Absence of HLA class I expression by Reed-Sternberg cells. *Am J Pathol* 1994;145:37-41.

8 Pan L, Diss TC, Peng H Lu Q, Wotherspoon AC, Alerio Thomas J, Isaacson PG. Epstein-Barr virus (EBV) in enteropathy-associated T-cell lymphoma. *J Pathol* 1993; 170:137-43.

9 Stam NJ, Spits H, Ploegh HL. Monoclonal antibodies raised against denatured HLA-B locus heavy chains permit biochemical characterization of certain HLA-C locus products. *J Immunol* 1986;137:2299-306.

10 Guesdon JL, Ternynck T, Avrameas S. The use of avidin-biotin interaction in immunoenzymatic techniques. *J Histochem Cytochem* 1979;27:1131-9.

11 Norton AJ, Jordan S, Yeomans P. Brief high temperature heat denaturing (pressure cooking): A simple and effective method of antigen retrieval for routinely processed tissues. *J Pathol* 1994;173:371-9.

12 MacMahan EME, Glass JD, Hayward SD, Mann RB, Becker PS, Charache P, *et al*. Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet* 1991;338:969-73.

13 Isaacson P, Wright DH. Malignant histiocytosis of the intestine. Its relationship to malabsorption and ulcerative jejunitis. *Hum Pathol* 1978;9:661-77.

14 Jones RA, Scott CS, Katz FE, Child JA. MHC class I and class I-like gene product expression by malignant T cells: relationships between CD1a, HLA-ABC and β_2 -microglobulin. *Clin Exp Immunol* 1988;74:454-8.

15 Boon T, Cerottini JC, Van den Eynde B, van der Bruggen P, Van Pel A. Tumour antigens recognised by T lymphocytes. *Annu Rev Immunol* 1994;12:337-65.

16 Weynants P, Lethe B, Brasseur F, Marchand M, Boon T. Expression of MAGE genes by non-small-cell lung carcinomas. *Int J Cancer* 1994;56:826-9.

17 Jones DB, Wright DH, Paul F, Smith JL. Phenotypic heterogeneity displayed by T-non Hodgkin's lymphoma (T-NHL) cells dispersed from diagnostic lymph node biopsies. *Hematol Oncol* 1986;4:219-26.

18 Smith MEF, Holgate CS, Williamson JMS, Grigor I, Quirke P, Bird CC. Major histocompatibility complex class II antigen expression in B and T cell non-Hodgkin's lymphomas. *J Clin Pathol* 1987;40:34-41.

19 Krensky AM, Reiss CS, Mier JW, Strominger JL, Burakoff SJ. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4+. *Proc Natl Acad Sci USA* 1982;79:2365-9.