Expression of B7 (CD80) and CD40 antigens and the CD40 ligand in Hodgkin’s disease is independent of latent Epstein–Barr virus infection

P G Murray, J Oates, G M Reynolds, J Crocker, L S Young

Abstract

Aim—to examine the expression of CD40 and B7 (CD80) antigens and the CD40 ligand in Hodgkin’s disease.

Methods—Antigen and ligand expression was studied in 17 cases of Hodgkin’s disease using immunohistochemistry. The study included 11 cases of Hodgkin’s disease, in which latent Epstein-Barr virus (EBV) infection could be demonstrated within tumour cells by in situ hybridisation for the EBV encoded early RNAs (EBERs). Results—In all cases, irrespective of EBV status, Reed-Sternberg cells and their variants (HRS cells) showed strong expression of both B7 and CD40 antigens. CD40 ligand expression was not shown in HRS cells but was confined to a subset of small lymphocytes some of which were seen to be in intimate contact with HRS cells.

Conclusions—As CD40 and B7 expression are features of professional antigen presenting cells, these results provide further evidence that HRS cells may have antigen presenting properties and that this may contribute to the characteristic recruitment and activation of non-malignant lymphocytes which is a feature of Hodgkin’s disease. The ability of HRS cells to activate Th cells may in turn contribute to their own survival through the induction of the gp39/CD40 pathway.

Keywords: Hodgkin’s disease, B7 (CD80) antigen, CD40 antigen, CD40 ligand.

Methods

SPECIMENS

Lymph node biopsies from a total of 17 cases of Hodgkin’s disease were available as frozen and paraffin embedded tissue samples. A further 60 cases were available only as paraffin embedded specimens. All specimens were obtained from either Birmingham Heartlands Hospital or the Queen Elizabeth Hospital, Birmingham. Haematoxylin and eosin stained sections from each block were reviewed by a single observer and subtyped according to the Rye classification system (table 1).

SECTION PREPARATION

Frozen sections were cut on a cryostat at 5 μm, allowed to air dry for 5 minutes, and then fixed for 20 minutes in acetone at 4°C. Paraffin wax sections were cut at 4 μm onto adhesive coated
slides (Frotissuer, Binding Site Ltd, Birmingham) and were left at 60°C overnight before immunostaining. Sections for Epstein-Barr virus encoded early RNA (EBER) in situ hybridisation were prepared under RNAse-free conditions.

Table 1 Expression of Epstein-Barr virus encoded early RNAs (EBERs) and B7 and CD40 antigens within HRS cells of the initial 17 cases of Hodgkin’s disease examined

<table>
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<th>Case number</th>
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<th>EBER</th>
<th>B7 antigen</th>
<th>CD40 antigen</th>
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<td>+</td>
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<td>Totals</td>
<td></td>
<td>11/17</td>
<td>17/17</td>
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</table>

EBER in situ hybridisation

EBER in situ hybridisation was employed on both paraffin and frozen sections to identify those cases in which Epstein-Barr virus (EBV) was present within tumour cells. Digoxigenin labelled sense and antisense probes to EBER1 and EBER2 sequences were generated from the plasmids BSJ[1]1 and BSJ[2]2 and were used as a cocktail in a rapid in situ hybridisation assay as previously described. Positive controls consisted of a known EBV positive Hodgkin’s disease specimen and a lymphoblastoid cell line grown as a tumour in a SCID mouse.

Immunostaining

Paraffin wax sections were deparaffinised and transferred to Tris buffered saline (TBS), pH 7-6. Frozen sections were transferred directly to TBS.

Two mouse monoclonal antibodies were employed separately on each specimen to identify expression of the CD40 antigen. G28-S was used at a dilution of 1/100 and S2C-6 at a dilution of 1/50. Both were equally effective in demonstrating CD40 expression in both paraffin wax and frozen sections. Normal tonsil provided a suitable positive control for CD40 expression.

Similarly the mouse monoclonal antibodies dol-1 and BB1 were employed to detect the B7 antigen at dilutions of 1/50 and 1/100 respectively. Both antibodies were effective on frozen sections of control lymphoblastoid SCID tumours and acetone fixed cytospin preparations of the Hodgkin’s cell line KMH2. However, paraffin sections yielded only weak signals and B7 expression could therefore not be reliably assessed in paraffin wax tissue sections.

The monoclonal antibodies M90 and 5c8 (Immunex) (Dr S Lederman, Columbia University, New York, USA) were employed to detect the CD40 ligand. Both antibodies were used at a dilution of 1/50 and were effective on frozen sections of tonsil specimens. They were not found to be suitable for use on paraffin wax sections.

Selected cases were also analysed for the expression of the EBV encoded latent membrane protein-1 (LMP-1) using the CS1-4 monoclonal antibody preparation at a dilution of 1/200. Microwave pretreatment for 20 minutes in citrate buffer, pH 6-0, was employed before immunostaining for LMP-1. All primary antibodies were diluted in TBS containing 10% normal sheep serum.

Bound primary antibodies were detected either by the standard APAAP technique in the case of frozen sections or by a peroxidase based avidin-biotin method for paraffin sections. Negative controls consisted of substitution of the primary antibodies with non-immune serum of the same immunoglobulin subclass.

Results

EBV was detected by EBER in situ hybridisation in a total of 21 of 77 cases of Hodgkin’s disease examined. In all cases the hybridisation signal was confined to the nuclei of HRS cells and in some cases showed the characteristic nucleolar sparing. Expression of LMP-1 in the cytoplasm of HRS cells in EBER...
positive specimens was confirmed in all cases. CD40 was shown equally well in both frozen and paraffin tissue and with both monoclonal antibodies. In normal tonsil, germinal centre B cells and follicular dendritic cells showed both membrane and cytoplasmic staining with the CD40 antibodies (fig 1). Occasional interdigitating reticulum cells and immunoblastic cells in interfollicular regions were also stained. In Hodgkin’s disease, CD40 was strongly expressed on the membrane and within the cytoplasm of HRS cells in all cases examined irrespective of the presence of latent EBV infection (fig 2). CD40 was also strongly expressed on follicular dendritic cells within germinal centres in a few cases of Hodgkin’s disease where residual normal lymph node tissue remained.

B7 antigen could only be reliably demonstrated on frozen material. Therefore, only the 17 cases in which frozen biopsy material was available could be assessed for B7 expression. B7 antigen was demonstrated both within the cytoplasm and on the cell membrane of HRS cells in all 17 cases irrespective of the presence of latent EBV infection (fig 3). The EBV negative Hodgkin’s cell line KMH2 also strongly expressed B7. B7 expression could also be demonstrated within infiltrating macrophages found in high numbers in some of the cases of Hodgkin’s disease examined. In some Hodgkin’s disease specimens some residual normal lymphoid tissue remained. In these cases B7 expression could be shown in B cells and macrophages within normal germinal centres. These results are summarised in tables 1 and 2.

CD40 ligand expression was performed only on those cases for which frozen sections were available. In all cases CD40 ligand expression was confined to a subset of small lymphocytes some of which were occasionally seen to be intimately associated with HRS cells (fig 4). However, only a small number of the reactive lymphocytes immediately surrounding HRS cells were CD40 ligand positive in any given case.

Discussion
In this study we have shown the expression of the B7 antigen (now designated CD80) within HRS cells in all cases of Hodgkin’s disease examined, irrespective of subtype or the presence of latent EBV infection within tumour cells. The B7 antigen is expressed on professional antigen presenting cells and is the natural ligand for CD28 on T cells. Binding of B7 to CD28 provides an important costimulatory signal for T cell activation after engagement of the T cell receptor, leading to T cell proliferation and the secretion of cytokines including interleukin-2 (IL-2), tumour necrosis factor α (TNFα), and γ interferon (IFNγ). HRS cells express the IL-2 receptor1 and their ability to induce IL-2 expression on surrounding lymphocytes might provide a means by which HRS stimulate their own proliferation. HRS cells have also previously been shown to express MHC class II molecules and the adhesion molecules ICAM1 and LFA3, and cells from the Hodgkin’s disease cell line LA28 are able to present soluble antigen to T cells in an MHC restricted fashion.11 Taken together, these results suggest that HRS cells express a number of the molecules necessary to engage and activate CD4+ cells. An antigen presenting role for the HRS cell might explain the presence of large numbers of non-malignant cells, including T cells, which are characteristically found in close association with HRS cells in many forms of Hodgkin’s disease. Some of these T lymphocytes have also been shown to express CD28.12

Previous studies have reported the expression of B7 antigen by HRS cells but not in relation to the presence of latent EBV infection of tumour cells. There is evidence that EBV can induce B7 expression in B lymphocytes and B7 is strongly expressed by lymphoblastoid cell
presenting cells, been shown that EBV in protein-I from or intimately regulated by inducer of important expression.2

lymphokines.2 growth the difficulty

defects either implies the normal cytotoxic cell. Several the normal relevance of the HRS cell and its cognition,3 of the B7/B7-1 antigen expressed by Reed-Sternberg cells of Hodgkin's disease and contributes to the stimulatory capacity of Hodgkin's disease-driven cell lines. Blood 1993;82:2845–52.


Ranheim EA, Kaaks TJ, Activated T cells induce expression of B7/B7-1 on normal or leukemic B-cells through a CD40 dependent signal. Exp Med 1997;177:925–35.


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