Brief report

Galectin-1 mediated suppression of Epstein-Barr virus–specific T-cell immunity in classic Hodgkin lymphoma

Maher K. Gandhi,1,3 Guido Moll,1 Corey Smith,1 Ujjwal Dua,1 Eleanore Lambley,1 Olivier Ramuz,2 Devinder Gill,3 Paula Martin,9 John F. Seymour,4 and Rajiv Khanna1

1Australian Centre for Vaccine Development, Tumor Immunology Laboratory, Division of Infectious Diseases and Immunology, Queensland Institute of Medical Research, Brisbane; 2Molecular and Cellular Pathology, School of Medicine, University of Queensland, Brisbane; 3Department of Haematology, Princess Alexandra Hospital, Brisbane; and 4Haematology Service, Peter MacCallum Cancer Centre, and University of Melbourne, Melbourne, Australia

In Hodgkin lymphoma (HL), the malignant Hodgkin Reed-Sternberg cells interact with the host microenvironment to create an immunosuppressive network that protects the lymphoma from immune attack. These mechanisms are not fully understood. We examined the role of the immunomodulatory protein galectin-1 (Gal-1) on Epstein-Barr virus-specific CD8+ T cell responses in HL. Initial studies indicated Gal-1 expression in all in vitro established Hodgkin Reed-Sternberg cell lines. In situ analysis revealed Gal-1 expression in 26 of 42 classic HL, whereas Gal-1 was uniformly negative in nodular lymphocyte predominant HL. Gal-1 expression was associated with male gender, older patients, reduced CD8+ T cell infiltration at the tumor site, and most importantly, an impaired latent membrane protein 1 and 2-specific CD8+ T-cell responses. In vitro exposure to recombinant Gal-1 inhibited proliferation and interferon-γ expression by Epstein-Barr virus-specific T cells. These observations provide an important link between the Gal-1-mediated immunomodulatory networks and loss of antigen-specific T-cell function in classic HL. (Blood. 2007;110:1326-1329)

Introduction

Hodgkin Reed-Sternberg (HRS) cells constitute a minority of the diseased lymph node. Evidence suggests that HRS cells influence the surrounding cellular infiltrate to resist antigen-specific immunity. Latent Epstein-Barr virus (EBV) proteins, found within HRS cells in 40% of classic Hodgkin lymphoma (cHL),1,4 represent a highly characterized model by which to study T-cell immunity.3 Although antigen processing and presentation remains intact in EBV-positive Hodgkin lymphoma (HL),6 an impaired interferon-gamma (IFN-γ) response to the virus-encoded CD8+ T-cell epitopes is observed in cases of cHL.7 Normal immunologic function in these T cells is restored after remission induction. The mechanisms by which HRS cells evade latent membrane protein (LMP)-specific T-cell immunity are not understood.

Galectin-1 (Gal-1) is a soluble beta-galactoside binding lectin that is expressed at low levels in normal lymphoid tissue.8 It is implicated in the modulation of the T-cell immune response by a variety of mechanisms, including inhibition of antigen-specific proliferation,9 induction of caspase-independent apoptosis in activated T cells10,11 and lymphoid tissue,12 and as a mediator of suppression by regulatory T cells.12,13 Targeted inhibition of Gal-1 expression in tumor cells has been shown to potentiate antitumor effector T cells.14 We examined the potential link between the Gal-1 protein and the loss of EBV-specific T-cell function in cHL.

Patients, materials, and methods

Forty-seven patients with histologically confirmed HL were included in this study (Table 1), which was approved by the human ethics committee of Queensland Institute of Medical Research Peter MacCallum Cancer Centre and Princess Alexandra Hospital. Informed consent was obtained in accordance with the Declaration of Helsinki. Biopsy samples from these patients were used for various histologic studies. All tissue staining was carried out on sections of routinely fixed, paraffin-embedded material. EBV-encoded small RNA (EBER) in situ hybridization and LMP1 and Gal-1 immunohistochemistry (Dako, Carpinteria, CA, and Peprotech, Rocky Hill, NJ, respectively) were performed as per published guidelines.3,14 Biopsies underwent central review by a lymphoma pathologist.

Peripheral blood mononuclear cells (PBMC) from 21 of 47 patients were available (before initiation of therapy) and were assessed for EBV-specific T-cell immunity using IFN-γ enzyme-linked immunosorbent assay (ELISPOT) assay.15 In these assays, 21 different EBV epitopes derived from latent (LMP1/L2, EBV-associated nuclear antigen[EBNA]3A/6) or lytic (BamH fragment Z leftward frame 1 [BZLF1] and BamHI-M leftward reading frame 1 [BMLF1]) proteins and presented by a wide range of HLA class I alleles were used as previously outlined.7

For Western blotting, cells from human HL cell lines (L540, L1236, HDLM2, L428(16)) were washed and lysed by standard procedures and proteins resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Separated proteins were blotted onto a nitrocellulose membrane and probed with rabbit antigalectin-1 antibody (Peprotech) at 1:8000. Blots were incubated with a peroxidase-labeled antirabbit IgG (Dako) and developed with chemiluminescence detection. Recombinant Gal-1 (rGal-1) (R&D Systems, Minneapolis, MN) and a melanoma cell line were used as positive controls. Equal loading was checked with anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Sigma, St. Louis, MO). In addition, enzyme-linked immunosorbent assays were performed to detect circulating Gal-1 on plasma samples from newly diagnosed patients with HL using standard methods.

For in vitro proliferation assays, carboxy fluorescein diacetate succinimidyl ester–labeled PBMCs (Molecular Probes, Eugene, OR) from EBV-seropositive subjects were preincubated with rGal-1 at 1 μg/mL or
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Galectin\textsuperscript{a}</th>
<th>Galectin\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (sex)</td>
<td>32 (18 female:14 male)</td>
</tr>
<tr>
<td>Mean age at diagnosis</td>
<td>29 years (SE 1.9)</td>
</tr>
<tr>
<td>Advanced clinical stage*</td>
<td>11/30 (37%)</td>
</tr>
</tbody>
</table>

Histology

- Nodular sclerosis: 22/32 (69%) vs 11/15 (73%)
- Mixed cellularity: 4/32 (13%) vs 2/15 (13%)
- Lymphocyte-rich: 1/32 (3%) vs 1/15 (7%)
- Hodgkin's unclassified: 5 (16%) vs 0 (0%)
- Positive EBV serology\textsuperscript{†}: 31/31 (100%) vs 15/15 (100%)
- Positive EBV tissue status: 12/32 (38%) vs 7/15 (47%)

No significant differences observed between galectin-1\textsuperscript{a} and galectin-1\textsuperscript{b} cases except for sex (P = .016) and age (P = .019).

*Advanced clinical stage defined as Ann Arbor (with Cotswold modification) IIB, III, or IV. Clinical stage was not available in 2 galectin-1\textsuperscript{a} cases.

\textsuperscript{†}SeroLOGY undetermined in one galectin-1\textsuperscript{a} case.

Results and discussion

Initially, we examined the expression of Gal-1 in a panel of in vitro established HRS cell lines. Data presented in Figure 1A show that a high level of Gal-1 protein was detected in all 4 HL cell lines tested (lanes 1-4). The level of expression was comparable with the melanoma cell line, SKMel28\textsuperscript{13} (Figure 1A, lane 6). Three of the 4 HRS cell lines also showed the expression of a dimeric form of Gal-1. In situ analysis of Gal-1 by immunohistochemistry revealed that nodular lymphocyte-predominant HL cases were uniformly negative (0 of 5), whereas 26 of 42 (62%) CHL cases expressed Gal-1 (Figure 1B). All the tissue sections were independently scored by visually estimating the proportion of HRS cells staining with Gal-1 as 0% (16 of 42), 1% to 19% (6 of 42), 20% to 79% (5 of 42), and 80% to 100% (15 of 42). Gal-1 overexpression was defined as Gal-1\textsuperscript{hi} (80% or more) or Gal-1\textsuperscript{lo} (below 80%). Interestingly, we also detected low levels (mean 1.1 ng/mL) of Gal-1 in fresh plasma samples from newly diagnosed patients with HL (data not shown).

The regulation of Gal-1 within HRS cells remains unclear. Recent work has identified a putative Gal-1 enhancer element that is regulated by activator protein 1.\textsuperscript{18} The activator protein 1 components cJUN and JUN-B are known to be overexpressed in HL. Interestingly, transforming growth factor beta regulates Gal-1 expression in a dose-dependent manner within breast cancer cells.\textsuperscript{19} Transforming growth factor beta is present in two thirds of primary CHL tumors\textsuperscript{20} and within HRS cell lines.\textsuperscript{21}

Gal-1 was also observed (at low frequency) in the extracellular matrix, small lymphocytes, occasional blastoid lymphocytes, follicular dendritic cells, macrophages, and endothelium. Gal-1\textsuperscript{hi} expression in HRS cells was associated with male sex (P = .016) and older patients (P = .019); both are poor prognostic features in the Hasenclever/Diehl index.\textsuperscript{22} Whether Gal-1 expression has independent prognostic relevance is unclear. Although more Gal-1\textsuperscript{hi} patients presented with advanced (clinical stages IIB/III/IV) HL, this did not approach statistical significance (P = .111). There were no associations between Gal-1 and histologic subtype or EBV tumor status. Given that there was multilocal accrual, treatment was not uniform and no evidence linking the expression of Gal-1 to clinical outcome can be provided. Well-designed prospective studies are required to determine whether such an association is present. Within HRS-rich areas, Gal-1\textsuperscript{hi} was associated with significant reduction of CD8\textsuperscript{+} infiltrating T cells compared with tissue samples expressing Gal-1\textsuperscript{lo} (mean 54, SE = 31.2 versus mean 153, SE = 27.0; P = .048) (Figure 1C).

Ex vivo EBV-specific HLA class I-restricted CD8\textsuperscript{+} T-cell responses were assessed in 21 newly diagnosed patients with HL before initiation of therapy by IFN-γ ELISPOT assays (Gal-1\textsuperscript{hi}:Gal-1\textsuperscript{lo} = 8:13) (Figure 1D). Patients with Gal-1\textsuperscript{hi} expression in HRS cells showed a selective loss of IFN-γ expression by CD8\textsuperscript{+} T cells specific for the subdominant LMP1 and 2 epitopes (mean spot-forming cells per 10\textsuperscript{6} PBMC = 18.0, SE = 7.0) compared with patients with Gal-1\textsuperscript{lo} expression (mean spot-forming cells per 10\textsuperscript{6} PBMC = 68.0; SE = 22.9, P = .046). Interestingly, CD8\textsuperscript{+} T cell responses directed toward epitopes derived from EBNA3/4/6 and lytic antigens showed were comparable ELISPOT responses in patients with Gal-1\textsuperscript{hi} and Gal-1\textsuperscript{lo} expression (mean spot-forming cells per 10\textsuperscript{6} PBMC = 286.0; SE = 148.1, versus 228.4; SE = 65.8, P = .728, respectively). The number of assays performed per subject reflects the HLA class I type. We minimized this limitation by using 21 different EBV peptides (9 LMP1/2; 9 EBNA3/4/6; 3 lytic) presented by a wide array of major histocompatibility complex class I alleles such that all 21 patients had at least 2 informative alleles (with a median of 6 informative HLA class I-restricted EBV-specific epitopes per patient). An alternate approach would be to use pooled peptides spanning each individual EBV protein, thus enabling us to determine effector T cell function irrespective of HLA restriction. Because we used this methodology to map many of the peptides used in this study, it is unlikely to have yielded additional data.\textsuperscript{23}

To evaluate the contribution of Gal-1 to the proliferative activity of EBV-specific memory CD8\textsuperscript{+} T cells, we incubated carboxy fluorescein diacetate succinimidyl ester-labeled PBMCs from healthy EBV-seropositive subjects with rGal-1 in the presence of autologous EBV-transformed lymphoblastoid cell lines. CD8\textsuperscript{+} T cells from PBMC treated with Gal-1 showed significantly reduced cell proliferation (less than 50%) relative to negative controls (Figure 1E). Furthermore, pretreatment with rGal-1 reduced the IFN-γ expression (60%-80%) by EBV-specific CD8\textsuperscript{+}, CD4\textsuperscript{+}, and LMP2-specific T cells (Figure 1F).

Taken together, these studies suggest that Gal-1\textsuperscript{hi} expression in HRS cells is an important negative regulator of HL tumor-associated EBV antigen-specific CD8\textsuperscript{+} T-cell immunity in HL and thus enables HRS cells to avoid T cell-dependent immune attack.
The Gal-1-mediated immunosuppressive pathway may represent a target to enhance efficacy of immunotherapeutic strategies for relapsed/refractory HL.

Acknowledgments

This work was supported by a Research Fellowship (R.K.) from National Health and Medical Research Council (NHMRC) and a Career Development Award (M.G.) from NHMRC. This study was supported by funding from NHMRC, Queensland Cancer Fund, and NCRI (SR21CA106172-02) and conducted under the auspices of the Australasian Leukemia and Lymphoma Group. Fresh plasma samples were supplied by the Australasian Leukemia and Lymphoma Group Tissue Bank. We thank Kristina Harej and Laurie Kear for their kind help in coordinating the clinical aspects of this study.

Authorship

Contribution: R.K. and M.G. designed this study and wrote the manuscript; M.G., G.M., U.D., E.L., O.R., and C.S. conducted various experimental studies; and D.G., P.M., and J.S. were responsible for the clinical management of patients with Hodgkin lymphoma and contributed toward the writing of this manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Rajiv Khanna, Queensland Institute of Medical Research, Bancroft Centre, 300 Herston Rd., Brisbane, Australia 4029; e-mail: rajiv.khanna@qimr.edu.au.
References


Galectin-1 mediated suppression of Epstein-Barr virus–specific T-cell immunity in classic Hodgkin lymphoma

Maher K. Gandhi, Guido Moll, Corey Smith, Ujjwal Dua, Eleanore Lambley, Olivier Ramuz, Devinder Gill, Paula Marlton, John F. Seymour and Rajiv Khanna

Updated information and services can be found at:
http://www.bloodjournal.org/content/110/4/1326.full.html

Articles on similar topics can be found in the following Blood collections
- Brief Reports (1930 articles)
- Immunobiology (5464 articles)
- Neoplasia (4182 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml