In Vivo Expression of the B7 Costimulatory Molecule by Subsets of Antigen-Presenting Cells and the Malignant Cells of Hodgkin’s Disease


The B-lymphocyte/accessory-cell activation antigen B7 (BB1) has been shown in vitro to stimulate T-lymphocyte proliferation and cytokine production via CD28 present on the latter cells. In this study, benign lymphoid tissues, lymphomas, and extralarymphoid inflammatory sites were examined immunohistochemically using anti-B7 and other relevant monoclonal antibodies. B7 was expressed by benign transformed germinal center B cells, as it was by B cells of follicular lymphomas. B7 was also expressed by a subpopulation (a mean of 31% to 85%) of macrophages and dendritic cells in a variety of lymphoid tissues. It was present in abundance on all macrophages constituting sarcoid granulomas in lymph nodes. In extralarymphoid inflammation, 17% to 35% of macrophages expressed B7 only. Cases of Hodgkin’s disease showed expression of B7 by the majority of Reed-Sternberg cells or malignant mononuclear variants, a phenomenon that potentially contributes to the lymphocytic accumulation that is a feature of this condition. CD28+ T cells were seen in all areas where T cells were present. B7+ and CD28+ cells co-localized in, for example, lymphoid follicles, lymph node paracortex, sarcoid granulomas, and Hodgkin’s disease tissue, indicating a potential for cellular interaction via these molecules at these sites.

IMMUNE RESPONSES depend on T-cell activation, rapid proliferative expansion, and lymphokine secretion. For antigen-specific T-cell activation to occur, there is a requirement for signaling mediated by the T-cell receptor/CD3 complex, which recognizes foreign peptide antigen. Also required for T-cell activation is one or more second, ie, costimulatory, signals. One central costimulatory molecule on antigen-presenting cells appears to be the B7 antigen (also known as BB1), which has M, of 44/54 kD and is a member of the Ig gene superfamily. Although B7 was initially described on activated B cells, subsequently it was also noted to be inducible on monocytes by interferon-γ and to be present on peripheral blood dendritic cells, and more recently it has been identified on chronically stimulated T lymphocytes. B7 acts as a ligand for CD28 present on T lymphocytes; on ligation of CD28, in conjunction with engagement of the T-cell receptor complex, there is proliferation of T cells and expression of cytokine genes. T-cell CTLA-4 may also function as a ligand for B7.

In light of the potential clinical relevance of the B7/CD28 costimulatory pathway, we wished to determine whether previous in vitro observations were consistent with in vivo findings in humans. In the present study, we assessed the expression of B7 and CD28 in benign lymphoid tissues as well as in lymphoid neoplasms and inflammatory tissues. In the results reported below, we show that B7 is expressed in vivo by transformed B lymphocytes and other professional antigen-presenting cells. Moreover, B7 is expressed by macrophages in sarcoid granulomas and by Reed-Sternberg and related cells in Hodgkin’s disease. This study of B7 expression in inflammatory and neoplastic states may help to provide understanding of the pathophysiology of these conditions.

MATERIALS AND METHODS

Blocks of human tissues, as listed below, were collected from surgical specimens, or from autopsies performed within 24 hours of death. Systematic study has shown that leukocyte antigens are well preserved during, and indeed beyond, this postmortem interval. Permission for tissue collection was obtained from the Human Subjects Protection Committee of the Brigham and Women's Hospital. Benign lymphoid specimens (number of cases in parentheses) were:

- Nonlymphoid tissue:
  - Sarcoid lymph node: 35%
  - Reactive lymph node: 31%
  - Peyer’s patch: 17%
  - Normal (3) and rheumatoid synovium: 7.3%
  - All skin: 7.3%
  - Dermal: 7.3%
  - Normal: 7.3%
  - Appendix: 7.3%
  - Normal (3) and dermal: 7.3%

- Lymphoid tissue:
  - Normal lymph node (3) in abundance on all macrophages constituting sarcoid granulomas in lymph nodes. In extralarymphoid inflammation, 17% to 35% of macrophages expressed B7 only. Cases of Hodgkin’s disease showed expression of B7 by the majority of Reed-Sternberg cells or malignant mononuclear variants, a phenomenon that potentially contributes to the lymphocytic accumulation that is a feature of this condition. CD28+ T cells were seen in all areas where T cells were present. B7+ and CD28+ cells co-localized in, for example, lymphoid follicles, lymph node paracortex, sarcoid granulomas, and Hodgkin’s disease tissue, indicating a potential for cellular interaction via these molecules at these sites.

© 1994 by The American Society of Hematology.
IN VIVO B7 EXPRESSION

Table 1. Follicular and Paracortical/Interfollicular B7 Expression in Selected Lymphoid Microenvironments

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent B7+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diffuse Follicular (B cells)</td>
<td></td>
</tr>
<tr>
<td>Resting lymph node</td>
<td>66.7 ± 33.3</td>
</tr>
<tr>
<td>Reactive lymph node</td>
<td>66.7 ± 21.1</td>
</tr>
<tr>
<td>Peyer’s patch</td>
<td>100.0 ± 0.0</td>
</tr>
<tr>
<td>Tonsil</td>
<td>79.2 ± 17.0</td>
</tr>
<tr>
<td>B-cell follicular lymphoma</td>
<td>93.1 ± 17.0</td>
</tr>
<tr>
<td>Paracortical/Interfollicular Cells</td>
<td></td>
</tr>
</tbody>
</table>

The first column of results refers to the percentage of follicles showing diffuse B-cell staining. Numbers of B7+ individual interfollicular cells stated are the percentage of the CD68+ cells (see Materials and Methods). The antibody used recognizes both macrophages and interfollicular dendritic cells.18

CA); B4-78 (anti-CD20, provided by Tom Tedder, Division of Tumor Immunology, Dana-Farber Cancer Institute); EBM/11 (anti-CD68, macrophages and dendritic cells, Dako Corp); and 4B10 (anti-CD28, provided by Kanji Sugita and Chikao Morimoto, Division of Tumor Immunology, Dana-Farber Cancer Institute). Also used was a control IgG1 antibody (Coulter Corp, Hialeah, FL), at a three times stronger concentration than 104. This control immunoglobulin did not produce immunostaining of cytospin preparations or tissue sections. Thymuses studied (stated ages, 6 to 20 months postnatal) were additionally assessed using a combination of two monoclonal antibodies (AE1/AE3, both IgG1, Boehringer-Mannheim Corp, Indianapolis, IN) that recognize thymic epithelial cells.17 Germinal centers in sections of tissues containing them were assessed for diffuse (B lymphocyte) B7 expression. The number of EBM/11+ mononuclear cells (at least 30) were counted in random fields of relevant areas and compared with the number of B7+ cells (with non-lymphocytic morphology) in parallel sections; these included dendritic cells identified on the basis of their morphology. The mean proportion of accessory cells expressing B7 was thus obtained, and the standard error of the mean additionally calculated using standard techniques. In cases of Hodgkin’s disease, 25 (unless otherwise indicated) Reed-Sternberg or related malignant mononuclear cells identified in randomly selected fields were assessed for B7 expression. The relative intensity of immunohistochemical staining, when present, of the cells in this condition was tabulated after determination using an arbitrary scale as follows: −, negative; +, weak; ++, moderate; ++++, strong.

RESULTS

Cytospin preparations of B7 cDNA-transfected CHO cells uniformly bound anti-B7 (Fig 1).

B7 expression by benign and malignant follicular B cells. B7 was diffusely expressed by the activated B cells in most germinal centers of reactive lymph nodes, tonsils, and Pey-

er’s patch (Fig 2, Table 1). The staining appeared to be of moderate degree in tonsils and Peyer’s patch, but tended to be strong in peripheral lymph nodes showing reactive changes (Fig 2). CD28+ T cells were present in germinal centers. Expression of B7 by mantle zone B cells of secondary follicles was not identified, although B cells forming primary lymphoid follicles (without germinal centers) in resting lymph nodes did show weak expression in two of three cases. In addition, B cells in the central region of splenic white pulp showed weak expression, and peripheral white pulp B cells (including marginal zone B cells) were negative. B7 was not identified on plasma cells. Six cases of follicular B-cell non-Hodgkin’s lymphoma examined all showed weak staining of most of the malignant follicles.

Macrophage and dendritic cell B7 expression in normal and inflamed lymphoid tissues. A proportion of accessory cells expressed B7 in various lymphoid microenvironments. These included the germinal center, where macrophages often stood out (as scattered large, polygonal, and sometimes more strongly staining cells) against a background of B-cell staining. Expression of B7 by follicular dendritic cells was not identified, although such staining could in theory be obscured by the extensive staining of B cells. B7+ macrophages and cells with dendritic morphology were identified in the paracortical regions of lymph nodes (Fig 2, Table 1). Strong B7 expression was further noted on a proportion of macrophages and dendritic cells at the periphery of splenic white pulp (65% ± 12% compared with the total number of cells in the mantle and marginal zones positive using anti-CD68), although fewer red pulp cells of these types weakly expressed this antigen (32.9% ± 13.4% were positive).

Fig 1. Immunohistochemistry on cytospin preparations of CHO cells transfected with B7 cDNA. (A) Control antibody does not produce staining. (B) Anti-B7 antibody stains cells.

Fig 2. B7 and other relevant antigen expression in lymphoid tissues. (A) Strong staining with anti-B7 of reactive lymph node germinal center B cells, although mantle zone (m) B cells are negative. (B) Paracortex with B7+ cells having the morphology of macrophages and dendritic cells, eg, cells indicated by arrowheads. (C) Thymic medulla stained with antikeratin monoclonal antibodies. These identify a network of epithelial cells and, to the left, part of a Hassel’s corpuscle (h). (D) Parallel section stained with anti-B7. Scattered cells are positive.

Table 2. Expression of B7 in Hodgkin’s Disease by Reed-Sternberg Cells and Mononuclear Malignant Variants

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Subtype</th>
<th>Reed-Sternberg Cells and Mononuclear Malignant Variants Positive</th>
<th>Intensity of Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mixed cellularity</td>
<td>24/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>2</td>
<td>Lymphocyte predominant</td>
<td>22/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>3</td>
<td>Nodular sclerosis</td>
<td>24/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>4</td>
<td>Mixed cellularity</td>
<td>19/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>5</td>
<td>Nodular sclerosis</td>
<td>16/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>6</td>
<td>Nodular sclerosis</td>
<td>21/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>7</td>
<td>Nodular sclerosis</td>
<td>15/22</td>
<td>+ to +++</td>
</tr>
<tr>
<td>8</td>
<td>Nodular sclerosis</td>
<td>15/25</td>
<td>+ to +++</td>
</tr>
<tr>
<td>9</td>
<td>Nodular sclerosis</td>
<td>*</td>
<td>+++</td>
</tr>
</tbody>
</table>

Number of cells positive and range of relative intensity of expression by cells that were positive.

* In case 9, virtually all malignant cells were present in clusters and appeared to show uniformly strong B7 expression.

Antibodies used to study thymuses included anti-B7, anti-CD68, and antikeratin antibodies, the latter recognizing thymic epithelial cells (Fig 2). The antikeratin antibodies bound to all Hassal's corpuscles and, additionally, showed a cortical and medullary network of cells connected by lengthy processes. The anti-CD68 antibody identified scattered cortical and medullary mononuclear cells, including a few within Hassal's corpuscles. Anti-B7 recognized cells with similar morphology to those that bound anti-CD68, although the B7+ cells were present in lesser numbers and were virtually all present within the medulla (Fig 2); as a proportion of cells identified here using anti-CD68, these B7+ cells formed 56.2% ± 8.8%. Occasional B7+ cells here had short dendritic processes. As with anti-CD68, a few cells within Hassal's corpuscles stained with anti-B7, but most here did not.

All granulomas (a total of 119) in three cases of sarcoidosis affecting lymph nodes showed widespread strong expression of B7 (Fig 3). This was attributed to the expression of this antigen by epithelioid macrophages (histiocytes) that comprise these. CD28+ cells were also present within and adjacent to granulomas (Fig 3). In various lymphoid sites and conditions, T cells were present in variable numbers and, in all instances, a subset of them bound anti-CD28.

Assessment of B7 expression in extralymphoid sites. B7 was expressed by only rare cells with the morphology of macrophages in normal appendix wall, skin, and synovium (mean positive in various sites 0% to 5.1%). In extralymphoid inflammation (appendix wall and serosa, skin, synovium), and in contrast to the stronger accessory cell B7 expression in lymphoid tissues, relatively few macrophages or cells with dendritic morphology expressed B7 (17.2 ± 5.8, 35.4 ± 17.1, and 16.5 ± 11.3% of CD68+ population, respectively), generally only weakly (Fig 3). Small aggregates of lymphocytes identified in two of six cases of rheumatoid arthritis that were confirmed as B cells using anti-CD20 on parallel sections, also weakly expressed B7. B7 was not identified on the variable numbers of scattered T lymphocytes that were seen in inflamed tissues, or indeed on cells with lymphocytic morphology in T-cell areas (eg, paracortex) of lymphoid tissues.

B7 expression by the malignant cells of Hodgkin's disease. The majority of malignant Reed-Sternberg or mononuclear variant tumor cells (Hodgkin's cells) in nine of nine cases of Hodgkin's disease expressed B7 (Fig 4, Table 2). This expression was typically weak to moderate in degree, although within individual cases there was variability in the intensity of staining and most cases had at least some malignant cells showing strong expression. In one case (number 9), the malignant cells were present in clusters and, in this instance, all appeared to show uniformly strong staining. CD28 was expressed with generally moderate intensity on T cells present in the regions where tumor cells were identified, contrasting with the generally weak expression seen in other conditions. The CD28+ cell population included cells immediately adjacent to the malignant cells (Fig 4).

DISCUSSION

This study shows the expression of B7 by transformed B cells, macrophages, and dendritic cells in vivo. B7 was seen on transformed germinal center B cells, in keeping with the presence of B7 on transformed B lymphocytes in vitro. On the basis of the presence of CD28+ cells in follicles, the extensive B7 expression by B cells, and the recognized physical interactions between B and T cells here, a potential for these cells to interact via B7 and CD28 here would appear to be very likely. However, it is noted that occasional follicles, for unknown reasons, had no B7+ B cells, and that the follicular lymphoma B cells consistently showed only weak expression of B7.

Expression of B7 by interfollicular dendritic cells indicates that an interaction between accessory cell B7 and T-cell CD28 can occur in the sites where these cells colocalize, and there is evidence that dendritic cells derived from human blood support T-cell activation with such a mechanism. Macrophages constituting sarcoid granulomas expressed abundant B7 and it would be of interest to study different granulomatous conditions to see whether strong B7 expression is a general feature of granulomas. The fact that B7 may participate in stimulating T-lymphocyte proliferation has, along with other knowledge, raised the possibility that it may be involved in positive selection of developing T cells, a phenomenon considered to be potentially mediated by epithelial cells in the thymus. Previously, researchers have concluded that certain thymic nonlymphocytic (therefore, potentially epithelial) cells may express the B7 antigen. In the present study, B7+ cells had the morphology of macrophages or dendritic cells and a pattern of expression comparable to that seen with antiepithelial cell antibodies was not identified. This calls into question the concept that B7 on thymic epithelial cells supports positive selection; however, possibilities for which there is some evidence are that B7 on hematopoietic cells supports positive selection or that molecules similar to B7 on epithelial cells interact with B7 ligands in the thymus.

In Hodgkin's disease, the bulk of the relevant tissue is typ-
ically composed of reactive non-neoplastic cells, including T lymphocytes, which are found close to Reed-Sternberg cells and variants in all forms of Hodgkin’s disease. The tumor cells have a physical interaction with lymphocytes, which show evidence of in situ proliferation. It would seem possible that B7, which is known to costimulate T lymphocyte proliferation (and cytokine elaboration) in vitro, could have a similar role in sites involved by Hodgkin’s disease. It is not clear what role, if any, abundant expression of B7 might have in leading to the defect in T-cell function that characterizes both Hodgkin’s disease and sarcoidosis.

In conclusion, these studies indicate the microenvironments where B7 and CD28 may potentially interact and support T-cell activation and proliferation, for example in germinal centers in the case of B-cell-T-cell interactions, or in lymph node paracortex or sarcoid granulomas in the case of accessory-cell-T-cell interactions. Reed-Sternberg and related cells in Hodgkin’s disease expressed B7, possibly contributing to the accumulation of lymphocytes that occurs in this condition.

ACKNOWLEDGMENT

The authors thank Heather Ladyman and Mary Ritter for helpful comments.

REFERENCES


In vivo expression of the B7 costimulatory molecule by subsets of antigen-presenting cells and the malignant cells of Hodgkin’s disease

JM Munro, AS Freedman, JC Aster, JG Gribben, NC Lee, KK Rhynhart, J Banchereau and LM Nadler