Mechanisms of Action of Intravesical Bacille Calmette-Guérin: Local Immune Mechanisms

Stephen Prescott, Andrew M. Jackson, Simon J. Hawkyard, Anton B. Alexandroff, and Keith James

Department of Surgery, University of Edinburgh, Edinburgh, Scotland

The local immune response to mycobacteria is complex, but mycobacterial antigen presentation by phagocytes to T helper cells is the pivotal interaction. Bacille Calmette-Guérin (BCG) vaccination is associated with the development of antituberculosis immunity but not necessarily with antitumor immunity. Animal studies have shown that an intact host immune system is required for the antitumor activity of BCG. Immunosuppressed and, particularly, T cell–depleted individuals fail to respond to BCG immunotherapy. Clinical and laboratory evidence suggest that the antitumor activity is concentrated at the site of BCG administration, which reinforces the view that local immune mechanisms are responsible for this phenomenon.

Studies of the immunological mechanism of BCG therapy show that an intact immune system, particularly the cellular system, is required for antitumor activity. The administration of T cells to athymic animals has been shown to restore their ability to respond to BCG therapy [1]. Clinical and laboratory evidence show that BCG interaction with the immune system produces systemic immunity to BCG. The antitumor activity of BCG appears to be a local phenomenon confined to the site of administration [2, 3].

The local immune response to mycobacteria is complex, but mycobacterial antigen presentation by phagocytes to T helper cells is the pivotal interaction. The presentation of antigen to T cells requires a series of cell surface interactions, such as processed antigen and T cell receptor, class II major histocompatibility complex (MHC) antigen and CD4, lymphocyte function antigen 1 (LFA-1) and intercellular adhesion molecule 1 (ICAM-1), and CD28 and CD80. During and after intravesical BCG therapy, the bladder cancer cell takes on some of the features of a BCG-infected phagocyte. This may be an important factor for tumor cell killing.

Immunohistochemical assessment of serial cold cup bladder biopsies from patients with carcinoma in situ who are undergoing intravesical BCG therapy shows that there is, in addition to the obvious polymorphonuclear cell presence, a significant increase in the density of the mononuclear cell infiltrate in the lamina propria for 3–6 months after therapy has been completed [4, 5]. This is dominated by T cells, particularly of the CD4 phenotype, and macrophages. The inflammatory response is associated with change in the phenotype of the bladder tumor cells (table 1). Before BCG therapy, bladder tumor cells express the class I MHC antigen (HLA-ABC), but the class II MHC molecule (HLA-DR) is expressed weakly or not at all. ICAM-1 and ICAM-2 are not expressed. After BCG therapy, the bladder cancer cells express HLA-ABC, HLA-DR, and ICAM-1 [4–6].

This change in phenotype has been shown to have a significant impact on the function of tumor cells. IFN-γ produced by the T cell response to BCG seems to be a key cytokine for producing the tumor cell phenotypic changes seen in vivo. IFN-γ released in the local immune response to BCG can render bladder tumor cells capable of acting as both lymphokine-activated killer (LAK) cell sensitive targets and antigen presenting cells for BCG.

The proximity of the bladder wall to the urine has allowed for an indirect assessment of immune activity in this area by measurement of cytokine levels in the urine. Several groups of investigators, including researchers at the University of Edinburgh, have examined BCG-induced cytokines both qualitatively and quantitatively [7–10]. They have observed the following: (1) significant increases to biological important levels of the proinflammatory cytokines IL-1, IL-2, IL-6, IL-8, and IL-12, IFN-γ, and TNF-α; (2) no detectable levels of the Th2 cytokine IL-4, whose role is to terminate the cellular immune response; and (3) the secretion of a soluble form of ICAM-1 after all instillations in which cytokines have been detected (the significant secretion has occurred during the first 12 h after BCG instillation). This in vivo information about the local immune response to intravesical BCG has been applied to human bladder cancer cell lines to provide an in vitro model of BCG treatment of bladder cancer (table 2). The panel of cell lines were RT4 and UM-UC-3 (low grade); RT112 and 5637 (intermediate grade); and MGH-UI, EJ18, J82 and SD (high grade). The surface antigen expression of the cell lines differed from fresh tumor cells because ICAMs were spontaneously expressed, with ICAM-2 alone on both low grade tumor cell lines and ICAM-1 alone on all the remaining cell lines [11].
Researchers were able to induce or enhance the expression of both HLA-DR and ICAM-1 but not ICAM-2 on the cell lines by adding IFN-γ alone or, to a lesser extent, TNF-α or IL-1α to the culture medium in a dose dependent fashion [12, 13]. These effects were also seen if urine from BCG-treated patients were added to the cell culture medium and could be largely abolished by the preincubation of the urine with anti-IFN-γ antibody [14].

The functional importance of the tumor cell phenotypic changes observed in vivo is illustrated in 2 studies[15, 16]. These studies show that LAK cells are important mediators of the antitumor response that kills tumor cells in an MHC-unrestricted fashion and are probably effector cells in the BCG-induced antitumor response. LAK cells generated by stimulation of peripheral blood lymphocytes with IL-2 and TNF-α in vitro show significant activity against bladder tumor cell targets [15]. Binding to the target is a requirement, which is achieved by interaction between the LFA-1 molecule on the LAK cell and ICAMs on the tumor cells.

These researchers have found conjugates between T cells that express LFA-1 and transitional cell carcinoma (TCC) cells that express ICAM-1 in the urine of BCG treatment patients. These findings support the clinical importance that these phenotype changes. In the TCC cell line model, researchers also have found that the degree of spontaneous LAK killing of a tumor cell line directly correlates with the level of tumor cell surface expression of ICAM-1 [17]. Moreover, in a given cell line, there is a direct relationship between the level of upregulation of expression of ICAM-1 by IFN-γ stimulation and the level of LAK killing [17]. This enhanced killing can be partially abrogated by the use of anti-ICAM-1 antibodies and, more completely, by the use of anti-LFA-1 antibodies [17]. In a murine model of bladder cancer developed by Lattime et al. [16], the MB49 cell line was shown to be capable of presenting BCG antigen to BCG-specific CD4 T cells only when it had been induced to express HLA-DR by culture with IFN-γ. The researchers did not look for ICAM expression. These studies show that the IFN-γ released in the local immune response to BCG can render bladder tumor cells capable of acting as both LAK cell sensitive targets and antigen presenting cells for BCG.

The cytostatic properties of bladder tumor cells may be affected by other properties of cytokines present in the post-BCG inflammatory response. Both IFN-γ and TNF-α suppress tumor cell growth in vitro in a dose-dependent manner, particularly with the low and intermediate grade cell lines, but IL-1α has no effect. At higher doses, IFN-γ is actually cytotoxic to RT4 and RT112 [18, 19].

There is a tendency to assume that the bladder tumor cells are passive bystanders in the immune response to BCG, but there is mounting evidence that these cells are very active in several ways. First, it has been shown [20] that BCG organisms in vitro and in vivo bind to and, in some cases, are phagocytosed by bladder cancer cells. This is an energy-dependent process that can be inhibited by cell membrane poisons. There is also the suggestion that tumor cells are capable of degrading the BCG organisms and behave as if they were professional phagocytes, such as macrophages. Lattime et al. [16] also show that tumor cells are capable of antigen presentation, another function normally ascribed to macrophages, and are also secretors of cytokines. Unpublished results from work at the University of Edinburgh show that the majority of TCC cell lines secrete IL-1α and/or IL-6, IL-8, and IL-10 under normal culture conditions (table 3). These researchers believe that IL-1α behaves as an autocrine factor responsible for constitutive ICAM-1 expression [21].

These functions strongly suggest that tumor cells are very active in the immune responses occurring in the bladder wall after BCG therapy. The possibility therefore exists that the BCG–tumor cell interaction is as important as the BCG-immunocompetent cell interaction.

The question that must be asked, then, is what happens if the tumor cell lines are incubated with BCG organisms alone? In their examination of this question, researchers have observed a number of effects on tumor cell proliferation in all lines tested [22]. First, live BCG and, to a lesser but still significant extent, heat-inactivated BCG suppress tumor cell growth in a dose-dependent manner. It has been shown that BCG is not directly cytotoxic to tumor cells in vitro. In addition, the supernatant from heat-killed BCG does not have an effect on tumor cells. Second, researchers at the University of Edinburgh have observed that the interaction between tumor cells and BCG results in the enhanced ICAM-1 expression by the high grade, but not low grade, TCC cell lines (unpublished data). This effect cannot be explained by the autocrine stimulatory effects of TCC cell produced by IL-1α and may be due to direct effects of the interaction on the gene pool [21]. Third, BCG stimulation results in changes in the cytokine output by TCC cell lines (table 3).

Table 1. Surface phenotype of bladder cancer cells in vivo.

<table>
<thead>
<tr>
<th>Antigen Constitutive After BCG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I MHC (HLA-ABC)</td>
</tr>
<tr>
<td>Class II MHC (HLA-ABC)</td>
</tr>
<tr>
<td>ICAM-1</td>
</tr>
<tr>
<td>ICAM-2</td>
</tr>
</tbody>
</table>

**NOTE:** ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; +, expressed; −, not expressed; −/+ expressed in a minority of cases.

Table 2. Surface antigen expression by transitional cell carcinoma cell lines in vitro.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>RT4</th>
<th>UMUC3</th>
<th>RT112</th>
<th>5637</th>
<th>SD</th>
<th>EJ18</th>
<th>J82</th>
<th>MGHU1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor grade</td>
<td>G1</td>
<td>G1</td>
<td>G2</td>
<td>G2</td>
<td>G3</td>
<td>G3</td>
<td>G3</td>
<td>G3</td>
</tr>
<tr>
<td>MHC class I</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>MHC class II</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ICAM-1</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>ICAM-2</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**NOTE:** ICAM, intercellular adhesion molecule; MHC, major histocompatibility complex; ND, no data; +, expressed; −, not expressed.
Table 3. Constitutive secretion of cytokines by bladder cancer cell lines.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>RT4</th>
<th>UMU C3</th>
<th>RT112</th>
<th>MGH U1</th>
<th>EJ18</th>
<th>J82</th>
<th>SD</th>
<th>5637</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1α</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IL-6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>IL-8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>IL-40</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

NOTE: ND, no data; +, secretion.

Conclusion

This discussion suggests that the effects of BCG on the immune system, although many and varied, may act on tumor cells from 2 main directions, from either (1) the actions on the immune system that, from other studies, seem to require live BCG organisms or (2) the direct actions on tumor cells that may be activated with dead BCG. Therefore, we propose that both of these mechanisms are important for the antitumor response and that a deficiency in either may result in treatment failure. Enhanced tumor cell interactions with the immune system that, from other studies, seem to require live BCG, are therefore strongly suggested. Thus, we propose that the immune response within the bladder wall is crucial for the success of therapy.

REFERENCES