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Immune response following intravesical bacillus Calmette-Guerin instillations in superficial bladder cancer: a review

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Abstract Local immunotherapy with bacillus Calmette-Guerin (BCG) can prevent recurrences and progression of superficial bladder cancer, but the antitumoral mechanism of BCG is still unclear. The first event seems to be binding of BCG to urothelial cells via fibronectin, and processing of mycobacterial antigens by antigen-presenting cells. Experimental data suggest that bacterial antigens can also be processed by urothelial cells. CD4 lymphocytes subsequently recognize antigenic peptides presented by HLA class II molecules. The most common profile of urinary cytokines is interleukin-2 and interferon-gamma, suggesting the predominant involvement of the Th1 lymphocyte subpopulation. Natural killer cells, lymphocyte-activated killer cells, BCG-activated killer cells and macrophages are able to kill bladder tumor cells in vitro, but there is no evidence that a major histocompatibility complex (MHC)-restricted specific T cytotoxic response is involved in BCG antitumor activity.

Key words Bladder neoplasm · BCG vaccine · Immune response

Introduction

Bacillus Calmette-Guerin (BCG) is an attenuated strain of *Mycobacterium bovis* used as a vaccine for the prevention of tuberculosis. Intravesical BCG has proven effective in superficial bladder carcinoma prophylaxis, with a significant reduction in progression and cancer-related death [27]. BCG is also an effective treatment of carcinoma in situ [28]. An immune response against both *Mycobacterium bovis* and the tumor may underlie the mechanism of action of BCG in patients with bladder cancer. Several immunological aspects have been studied following intravesical BCG instillations, including immune cellular infiltrates in the bladder wall [8, 19, 41, 55], cytotoxic effector cells [7, 33, 35, 43, 59, 60], major histocompatibility complexes (MHC), adhesion molecule expression on urothelial cells [30, 47, 52, 54, 57] and urinary cytokines [5, 9, 16, 22, 48, 51], but none is clearly involved in BCG antitumor activity. We review aspects of the immune response to BCG treatment and their potential contribution to tumor eradication.

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Immune response to mycobacteria

During mycobacterial infection, macrophages process mycobacterial antigens and release cytokines: interleukins (IL-1, IL-6, IL-8, IL-10, IL-12), tumor necrosis factor-alpha (TNF-α) and interferons (IFN-α, IFN-γ). Mycobacterial antigens are presented at the cell surface via MHC class II molecules to CD4 T lymphocytes which are then activated. Mycobacterial infection preferentially induces a Th1-like cytokine profile, composed of IL-2 and IFN-γ [23]. Immune cells involved in the lysis of infected target cells can be antigen-specific α/β T lymphocytes (CD4 or CD8 T cells), γ/δ T lymphocytes, or nonspecific effector cells such as natural killer cells and macrophages [31, 34, 44].

The *Mycobacterium tuberculosis* antigens that trigger a protective immune response are not fully characterized. There are known mycobacterial surface antigens of 12, 14, 19, 37, 65 and 71 kDa. Secretory antigens have also been identified (10 kDa, 19 kDa, 30 kDa, 38 kDa antigens and the 85 kDa complex) [3]. Zlotta et al. have demonstrated that peripheral blood lymphocytes (PBL) derived from patients treated with BCG show a significantly increased proliferation response to antigen 85 (Ag 85) in vitro [66].

Immune response to tumor antigens

The description of tumor rejection antigens (TRA) represented a major advance in the field of antitumor cytotoxicity mechanisms. Some of these antigens have recently been identified by Boon's group [10]. They are presented at the cell surface by HLA class I molecules and recognized by MHC-restricted specific CD8 cytotoxic T lymphocytes (CTL). Genes coding for these antigens have been cloned and termed *MAGE* genes [62]. These genes are not expressed in normal tissues, except for the testis. We have recently shown that *MAGE* genes are expressed by bladder tumors [46].

BCG treatment can enhance MHC class I and II molecule expression on urothelial cells [47, 56]. Upregulation of class I molecules on urothelial cells is particularly interesting, as CD8 T cells might recognize tumor antigens on urothelial cells. This is supported by the observation that MHC class I molecule expression on urothelial cells correlates with the clinical response to BCG [57] and that CD8 T lymphocytes are present in the bladder wall after BCG treatment. The possible impact of cytotoxic lymphocytes on bladder tumor cells after endovesical BCG treatment has not been fully elucidated.

Antigen presentation

Exogenous antigens are usually presented to T cells via MHC class II molecules, while endogenous antigens are presented via MHC class I molecules. MHC class II molecules are expressed only by antigen-presenting cells (APC) composed of macrophages, B lymphocytes, dendritic cells and Langerhans cells while MHC class I molecules are expressed by almost all cell types. APC process and present exogenous antigens, which are broken down into small fragments in lysosomes and are then linked to MHC class II molecules before migrating to the cell surface for recognition by CD4 T helper lymphocytes [2]. Endogenous antigens are processed in the cytoplasm, where they are cleaved into peptides and are transported into the endoplasmic reticulum before linking to MHC class I molecules. The MHC class I–peptide complex moves to the cell surface, where it is recognized by CD8 cytotoxic T lymphocytes [6, 11].

The role of macrophages in antigen presentation to CD4 T lymphocytes seems to be crucial in BCG-induced antitumor cytotoxicity. Recently, Thanhauser et al. [61] showed that depletion of peripheral blood mononuclear cells of either monocytes or CD4 T cells abolished BCG-induced antitumor cytotoxic activity.

It has also been suggested that, in particular conditions, cells other than macrophages can process and present antigens to T lymphocytes. For example, activated urothelial tumor cells expressing MHC class II molecules can present microbial antigens to CD4 T cells. Lattime et al. [39] demonstrated in a murine bladder tumor model that urothelial cells were able to present BCG antigens to specific CD4 T cells in MHC class II restricted fashion. Tumor urothelial cells, like APC cells, can also release cytokines such as IL-1 and IL-6 and this release is modulated by BCG [21, 25]. Moreover, expression of MHC class I and II molecules on urothelial cells is enhanced after BCG treatment, increasing the potential capacity for antigen presentation [47, 56]. Internalization of BCG by urothelial tumor cells has been demonstrated in vitro [4, 38] and BCG phagocytosis by tumor cells has been shown in other tumors such as sarcomas [18].

BCG binds to urothelial cells, and fibronectin seems to be the principal ligand of BCG on epithelial cells [1, 52, 54]. Adhesion to fibronectin is necessary for the antitumoral activity of BCG, and enhanced binding to fibronectin can augment the antitumor activity of BCG [29, 52]. The binding site is located on the carboxy-terminal chain of fibronectin [13]. Glycosaminoglycans could provide another binding site [58].

Cytotoxic effector cells

αβ T lymphocytes

Evidence of T cell involvement in the BCG-mediated antitumor response was first provided by Ratliff et al. [50, 53], who demonstrated that athymic mice could not eradicate tumor after BCG treatment, and that depletion of CD4 or CD8 T cells in mice abrogates BCG antitumor activity. Several authors have also observed that both CD4 and CD8 T cells are present in bladder wall infiltrates after BCG treatment, and that the CD4 phenotype is predominant [8, 19, 41].

CD4 T lymphocytes

CD4 T helper lymphocytes are composed of two subpopulations – Th1 and Th2 – defined by the cytokines they produce. Th1 cells produce IL-2, TNF-β and IFN-γ and are mainly involved in cellular immunity. Th2 cells produce IL-4, IL-5 and IL-10, have a predominant role in humoral immunity and can inhibit the Th1 pathway. T cell activation requires two signals. The first is T cell receptor binding to the antigen via MHC. The second is T cell binding to costimulatory molecules expressed on APC cells, such as intercellular adhesion molecule-1 (ICAM-1), lymphocyte function associated antigen-3 (LFA-3), B7-1 and B7-2. Recently it has been suggested that B7-1 activates Th1 cells, while B7-2 activates Th2 cells [36]. We have recently found that B7-1 is enhanced on urothelial cells after BCG treatment [56], like ICAM-1 [30]. IL-2 and IFN-γ are usually detected in urine after BCG injection but IL-4 is not. Cytokine mRNA expression has been studied in mice by McAveney et al. [42]: intravesical MB49 tumor growth was associated with IFN-γ and IL-4 expression. BCG enhanced IFN-γ expression and reduced IL-4 expression [42], leading to a

dominant Th1 cytokine pattern. This latter point is controversial, however, as IL-10 has also been detected in patients with bladder tumors and in the urine of BCG-treated patients [30, 40]. It would be interesting to determine whether BCG failures correspond to the emergence of Th2 cell effector activity.

CD8 T lymphocytes

MHC class I-restricted CD8 T cells are specifically activated in mycobacterial infections. They are able to lyse infected cells and to produce IFN-γ [34]. Normally the CD8 T cell subset predominates in the bladder wall, but BCG treatment reverses the CD4/CD8 ratio [8, 19, 41, 55]. There is no experimental evidence for tumor-specific cytotoxic activity for these CD8 T cells.

γδ T cells

γδ T cells are cytotoxic lymphocytes. Few express CD4 or CD8 surface molecules, and antigen recognition is not restricted to MHC molecules. γδ T cells can recognize mycobacterial and viral proteins, including heat-shock proteins and superantigens [24, 31]. γδ T cells can produce cytokines such as IL-2, IL-4 and IFN-γ. Using an immunohistochemical technique, we compared γδ T cell expression before and various times after BCG treatment. γδ T cell expression was enhanced 3 weeks after BCG instillation and was maximal at 3 months [56]. Recently, Wang et al. [65] showed that γδ T cells from healthy individuals, activated by mycobacteria in vitro, were able to lyse bladder tumor cells in MHC-unrestricted fashion.

NK, LAK, BAK cells

Three nonspecific lymphocyte subsets have been implicated in cytotoxicity against bladder tumors following BCG treatment: natural killer (NK) cells, lymphokine-activated killer (LAK) cells and BCG-activated killer (BAK) cells. NK cells are a distinct subset of lymphocytes that can be distinguished from T lymphocytes, B cells and monocytic cells. NK cells do not express CD3 antigen or the alpha, beta, gamma or delta chains of the T cell receptor. They are phenotypically $CD3^{-}$, $CD2^{\pm}$, $CD8^{\pm}$, $CD16^{\pm}$, $CD56^{+}$ lymphocytes and mediate MHC-unrestricted killing and antibody-dependent cell-mediated cytotoxicity (ADCC). NK cells are the main precursor of LAK cells. NK and LAK cells are able to lyse bladder tumor cells after BCG stimulation in vitro [35, 59]. Böhle tested the capacity of PBL to lyse bladder cell lines in various experimental conditions [7, 60]. BCG alone, cytokines (IL-2, TNF-α, TNF-β, IFN-γ) and unstimulated PBL (NK) were unable to kill tumoral cells, whereas LAK cells and PBL incubated with viable BCG (BAK cells) showed significant lysis of four bladder tumor cell lines. NK cells activated by BCG in vitro exhibit significant lysis, not only of bladder tumor cell lines but also of fresh tumor cells [33, 43]. Shemtov et al. [59] have shown that LAK-mediated cytotoxicity after BCG treatment involved tumor cell apoptosis rather than necrosis. However, the in vivo activity of NK and LAK cells against bladder tumor cells has not been demonstrated. Intravesical BCG treatment is not able to induce significant LAK cytotoxicity against the T24 bladder cell line [26]. In an immunohistochemical study we observed very few NK cells in the bladder wall 3 weeks after BCG [56].

Macrophages

A role of macrophages in cytotoxicity against bladder tumor following BCG treatment cannot be excluded [20]. Several studies have shown cytotoxic activity of monocytes and macrophages against bladder tumor cell lines after in vitro stimulation with BCG [14, 37, 49]. The induction of nitric oxide synthetase activity in macrophages has also been documented after BCG treatment [32].

Cytokines

Immunocompetent cells communicate with each other via cytokines, which can have growth, immunomodulatory and cytotoxic activities. Many authors have shown that cytokines are present in the urine of bladder cancer patients after BCG treatment [5, 9, 15, 22, 30, 48, 51], but their cellular origin is not clear. Immunocompetent cells and urothelial cells can produce cytokines. Macrophages can release IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-α and IFN-γ [12]. Th1 cells release IL-2 and IFN-γ whereas Th2 cells release IL-4, IL-5 and IL-10. α/β CD8 T cells produce IL-2 and IFN-γ. γδ T cells can produce IL-2, IL-4 and IFN-γ. NK and LAK cells can release TNF-α and IFN-γ. Normal urothelial cells can produce IL-6 and granulocyte-macrophage colony stimulating factor (GMCSF) [64]. Urothelial tumor cells can produce IL-1, IL-6, IL-10 and TNF-α [17, 21, 25, 40].

Jackson recently studied the kinetics of certain cytokines [30]. IL-1, IL-6, IL-8, IL-10 were detected after the first BCG instillation. Other cytokines such as IL-2, TNF-α and IFN-γ were detected later during BCG treatment. One can speculate that macrophages and urothelial cells are responsible for the initial production of cytokines, while cytokines such as IL-2 and IFN-γ are produced by activated T cells. IL-4 was not detected in urine, supporting the previously described recruitment of Th1 T cells. Recently much attention has been given to IL-12, a cytokine secreted by macrophages and capable of inducing a strong Th1 response [45]. Wagner et al. [63] have shown that in vitro BCG stimulation of PBL and mononuclear cells was able to induce a strong production of IL-12.

Conclusion

Several lines of evidence suggest that BCG attachment to urothelial cells and presentation of BCG antigens to T helper cells are required to trigger effective antitumoral activity. The cytokines mainly involved in the antitumoral effect of BCG seem to be preferentially released by Th1 cells. However, the mechanism of BCG-mediated tumor killing remains unclear. In vitro studies have provided evidence that nonspecific cytolytic mechanisms are involved (NK, LAK, BAK cells) but the functional role of CD4, CD8 and γδ T cells in the bladder wall remains to be established. The first hypothesis is that these lymphocytes are directed against mycobacterial antigens, leading to the eradication of infected tumor cells. The second hypothesis is that they recognize tumor rejection antigens at the cell surface, leading to specific cell lysis. Further experiments, including cloning and characterization of the lymphocytes involved in vivo, are necessary to answer this question. A better understanding of the specificity of killing activity will no doubt have an impact on our management of immunological prophylaxis.

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