Role of IL-10 in Urinary Bladder Carcinoma and Bacillus Calmette-Guerin Immunotherapy

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Abstract: Problem statement: Bladder cancer is a common urologic cancer and intravesical Mycobacterium bovis Bacillus Calmette-Guerin (BCG) is the mainstay in the treatment of superficial bladder cancer. However, the current BCG therapy is not desirable with respect to its efficacy and side effects. Interleukin (IL)-10, a T helper type (Th) 2 cytokine, plays an important regulatory role in bladder cancer immunosurveillance and BCG immunotherapy. Therefore, blocking IL-10 activity could be beneficial for bladder cancer patients undergoing BCG therapy. Approach: Treatment with intravesical BCG in combination with systemic IL-10 monoclonal antibody (mAb) specific for IL-10 neutralization or IL-10 receptor (IL-10R) blockage has been evaluated in preclinical bladder cancer models. Results: Addition of anti-IL-10 neutralizing mAb or anti-IL-10R1 mAb enhances BCG induction of Th1 immune responses and anti-bladder cancer immunity. Conclusion/Recommendations: BCG immunotherapy of bladder cancer can be enhanced by addition of IL-10 blocking mAb. Future studies should aim to explore the mechanisms underlying the induction of enhanced antitumor immunity by BCG combination therapy and develop therapeutic regimens for clinical evaluation of the safety and efficacy of BCG combination therapy.

Key words: IL-10, bladder cancer, BCG, immunotherapy

INTRODUCTION

Urinary bladder carcinoma is the second most common urologic neoplasm in the United States, with an estimated 73,510 new cases and 14,880 deaths in 2012 (Siegel et al., 2012). At the time of diagnosis, 20-25% of cases are muscle invasive (stage T2 or higher) and are typically treated with surgical resection (Williams et al., 2010). The remainders are Nonmuscle Invasive Bladder Cancer (NMIBC) including tumors confined to the epithelial mucosa (Ta), tumors invading the Lamina Propria (T1) and Carcinoma In Situ (CIS). Transurethral Resection of Bladder Tumor (TURBT) is the primary treatment for Ta and T1 lesions. Intravesical therapy is used as adjuvant treatment to prevent recurrence and progression of the disease after TURBT and is also the treatment of choice for CIS. Intravesical instillation of BCG, a widely used vaccine against tuberculosis, is currently the gold standard therapy for NMIBC. BCG therapy results in 50-60% effectiveness against small residual tumors and a 70-75% complete response rate for CIS. Unfortunately, a high percentage of patients fail initial BCG therapy and 40-50% of BCG responders develop recurrent tumors within the first 5 years (Williams et al., 2010). In addition, up to 90% of patients experience various side effects and rarely life-threatening complications such as sepsis.

Cytokines are important immunomodulators that can be classified into two major categories, i.e., Th1 cytokines that drive cellular immunity and Th2 cytokines that promote humoral immunity (Fearon and Locksley, 1996). The Th1/Th2 cytokine balance reflects the type of immune responses that occur in the immune system. Studies have revealed a crucial role of Th1 immune response in cancer immunosurveillance including bladder cancer (Mocellin et al., 2001; Brandau and Suttmann, 2007; Sheu et al., 2008). Studies have also shown the dominance of regulatory T (Treg) cells and Th1 inhibitory cytokines (e.g., TGF-β and IL-10) in bladder cancer (Loskog et al., 2007). Consistent with human studies, animal studies have also revealed that Th1 (e.g., IFN-γ and IL-12) but not Th2 (e.g., IL-10) cytokines are required for local tumor surveillance (Riemensberger et al., 2002) and that bladder cancer is dominated by IL-10 which inhibit Th1 immune responses at the tumor site (Halak et al., 1999; Yang and Lattime, 2003). It has been known that the therapeutic effect of intravesical BCG is associated with its ability to reverse the disproportion of Th1/Th2
cytokines, suggesting a crucial role for a Th1 immune response in the intrinsic and immunotherapeutic control of bladder cancer. In this review article we discuss the role of IL-10 in bladder cancer and BCG immunotherapy as well as our progress in BCG combination therapy for bladder cancer. Future direction of study and prospective use of BCG in combination with IL-10 blocking agents are also addressed.

**IL-10 and biological activity:** IL-10 is a pleiotropic cytokine that functions as a positive or negative mediator in innate and adaptive immunity under different circumstances (Mocellin et al., 2003; 2005). IL-10 is produced by numerous cell types including T cells (Th1, Th2 and Treg) (Fiorentino et al., 1991; Cenci et al., 1993), thus inhibiting inflammatory and tumoricidal activities of these cells.

Although IL-10 was initially named a cytokine synthesis inhibitory factor and is classified as a Th2 cytokine (Moore et al., 2001), recent studies revealed that Th1 cells have an ability to produce IL-10 for the feedback control of their own activities (O’Garra and Vieira, 2007; Trinchieri, 2007). In addition to suppressive effects, IL-10 also exerts some stimulatory effects, favoring immune-mediated rejection of cancer that has been evidenced in a large body of animal studies (Zheng et al., 1996; Berman et al., 1996; Kundu et al., 1996; Kundu and Fulton, 1997; Fujii et al., 2001; Segal et al., 2002; Tanikawa et al., 2012). IL-10 can promote Cytotoxic T Lymphocyte (CTL) differentiation and expansion (MacNeil et al., 1990; Chen and Zlotnik, 1991; Groux et al., 1998; Rowbottom et al., 1999; Fujii et al., 2001), enhance NK cell cytolytic activity (Zheng et al., 1996; Kundu et al., 1996; Kundu and Fulton, 1997), induce DC antigen uptake (Morel et al., 1993; Willems et al., 2001), increase leukocyte IFN-γ production (Tilg et al., 2002) and inhibit Treg cell development (Tanikawa et al., 2012). In addition, IL-10 can also inhibit cancer development and progression through its regulatory effects on inflammatory cytokine production, as inflammation is often associated with increased tumor angiogenesis and invasiveness (Mocellin et al., 2003). Thus, IL-10 plays a dual role in tumor-associated immune responses, either promoting antitumor immune responses or mediating tumor escape from immune surveillance. The biological activities of IL-10 appear to be context dependent and vary in different cancer model systems.

**IL-10 in bladder cancer:** IL-10 functions as a Th1 inhibitory cytokine in bladder cancer. Studies have revealed that patients with bladder cancer develop a Th2 dominant status with a deficient Th1 immune response. Increased levels of IL-10 and other Th2 cytokines (e.g., IL-4, IL-5 and IL-6), along with decreased levels of Th1 cytokines (e.g., IFN-γ and IL-2), have been observed in the serum of bladder cancer patients (Agarwal et al., 2010; Satyam et al., 2011). This circulating cytokine profile seems to correlate well with the grade and severity of bladder cancer. Peripheral CD4+ T cells from bladder cancer patients have also been observed to express increased IL-10 and other Th2 cytokines, along with decreased Th1 cytokines (Agarwal et al., 2006). Consistent with the systemic cytokine profiles, increased concentrations of
IL-10 and other Th2 cytokines (e.g., IL-13) have been observed in the urine of bladder cancer patients (Margel et al., 2011). A recent study suggested that urinary IL-6/IL-10 ratio might be useful as a prognostic marker of recurrence in patients with intermediate risk superficial bladder cancer (Cai et al., 2007). To support this local Th2 dominant status, IL-10 has been detected in bladder cancer specimens at both protein and mRNA levels (Cardillo et al., 2000; Loskog et al., 2007).

All animal studies consistently support the Th2 cytokine dominance observed in human bladder cancer. Studies have demonstrated that IFN-γ and IL-12 but not IL-10 are required for local tumor surveillance in a syngeneic mouse model of bladder cancer (Riemensberger et al., 2002). Mice genetically deficient in IFN-γ (IFN-γ-/-) or IL-12 (IL-2-/-) died much earlier than wild-type mice after tumor implantation; however, this intrinsic antitumor response was not altered in IL-10−/− mice. Studies have also shown that a marked polarization exists towards the expression of Th2 cytokines, including IL-10, during progression of bladder cancer in a syngeneic mouse model (Tham et al., 2011). Studies have also revealed that MB49 cells, a widely-used mouse bladder cancer cell line (Chen et al., 2009), can induce IL-10 production by infiltrating immune cells, resulting in inhibition of specific antitumor immune responses (Halak et al., 1999). This inhibition has been attributed to DC dysfunction for T cell stimulation resulting from tumor-induced IL-10 production (Yang and Lattime, 2003). Studies have also revealed the ability of macrophages to inhibit bladder cancer cell growth in vitro; however, this macrophage mediates growth arrest can be reversed by the addition of IL-10 (Dufresne et al., 2011), suggesting that IL-10 is inhibitory to macrophage tumoricidal activity in vivo. All these findings suggest that therapeutic strategies for Th1 induction and Th2 dampening may be effective in the treatment of bladder cancer.

There are limited studies on IL-10 gene polymorphisms in bladder cancer. IL-10 functions in a highly complex and coordinated manner and its gene variations may lead to altered production and activity of this cytokine, thus affecting the susceptibility to bladder cancer. A recent study revealed that two single nucleotide polymorphisms of IL-10 gene at G-1082A and C-819T positions are associated with increased bladder cancer risk and may be useful as a molecular marker of bladder cancer (Ahirwar et al., 2009). Similarly, another study showed that IL-10 gene polymorphisms (-1082GG and -1082GCC/GCC) are potential risk factors for bladder cancer progression and may even alter the effect of BCG immunotherapy (Basturk et al., 2006). These observations warrant further investigation on genetic markers for bladder cancer diagnosis, prognosis and treatment.

**IL-10 in BCG treatment of bladder cancer:** The effect of BCG immunotherapy on bladder cancer depends on the proper induction of Th1 immune responses. Intravesical instillation of BCG induces a complex inflammatory cascade in the bladder mucosa, reflecting activation of multiple types of immune cells and bladder tissue cells (Ratliff, 1991; Brandau and Suttmann, 2007; Alexandroff et al., 2010). Following BCG instillation, various leukocyte types infiltrate into the bladder wall including neutrophils, monocytes/macrophages, lymphocytes, NK cells and DC (Bohle et al., 1990a; Prescott et al., 1992; Saban et al., 2007). These infiltrating leukocytes, together with activated urothelial cells, produce numerous pro-inflammatory cytokines and chemokines. Subsequently, a transient massive amount of cytokines and chemokines (Bohle et al., 1990b; Boer et al., 1991a; De Reijke et al., 1996; Taniguchi et al., 1999; Saint et al., 2002; Nadler et al., 2003; Luo et al., 2007), together with a variety of leukocyte types (Boer et al., 1991a-c; Simons et al., 2008), can be detected in voided urine. The urine of mice treated with intravesical BCG also exhibits increased concentrations of cytokines and chemokines (Saban et al., 2007). In addition to local immune induction, intravesical BCG also results in systemic immune responses as manifested by increased levels of cytokines and chemokines in the serum (Elsasser-Beile et al., 2000; Magno et al., 2002).

It has been noted that the development of a dominant Th1 cytokine profile (e.g., IFN-γ, IL-2 and IL-12) is associated with the therapeutic effect of BCG, whereas the presence of a high level of Th2 cytokines (e.g., IL-10) is associated with BCG failure (De Reijke et al., 1996; Saint et al., 2002; Nadler et al., 2003). The kinetic analysis of urinary cytokines indicates that the production of IFN-γ, IL-2 and IL-12 precedes the production of IL-10 in patients undergoing BCG therapy (Nadler et al., 2003). A tendency toward higher ratios of IFN-γ vs. IL-10 has been observed for BCG responders (Saint et al., 2001; 2002; Nadler et al., 2003). IL-10, as a major Th2 cytokine, appears to play an important inhibitory role in BCG immunotherapeutic control of bladder cancer. To support this, it has been reported that intravesical BCG induces markedly increased local immune responses in IL-10−/− mice, coinciding with increased therapeutic efficacy compared to wild-type mice (Riemensberger et al., 2002). In line with this, we have observed that both IL-10−/− mice and mice treated with anti-IL-10 neutralizing
mAb develop enhanced BCG induced anti-bladder cancer immunity in a syngeneic mouse orthotopic tumor model (Nadler et al., 2003). We have also demonstrated that blocking IL-10 signal transduction by anti-IL-10R1 mAb that binds to IL-10R1 enhances BCG-induced anti-bladder cancer immunity in a similar mouse model of bladder cancer (Bockholt et al., 2012). In addition to in vivo studies, we have observed the inhibitory effect of IL-10 on BCG induced macrophage cytotoxicity against bladder cancer cells in vitro through antibody neutralization of IL-10 for wild-type macrophage cultures or use of IL-10+/− macrophages in assays (Luo et al., 2010).

Blocking IL-10 enhances BCG-induced Th1 immune responses and anti-bladder cancer immunity: Bladder cancer is dominated by a Th2 polarized immunopathologic response. Intravesical BCG therapy can shift the Th2 environment toward a Th1 milieu, a phenomenon linked to the therapeutic effect of BCG on treating bladder cancer. Although BCG therapy has long been proven to be effective for treating bladder cancer, 30-40% of patients fail BCG therapy and 40-50% of BCG responders develop recurrent tumors (Williams et al., 2010). Therefore, the current BCG therapy is not desirable with respect to its efficacy. Since evidence supports the Th1 immune response to be essential in BCG-mediated bladder cancer destruction, studies have focused on enhancing the BCG induction of Th1 immune responses. During the past 2 decades, we and others have developed a strategy to combine BCG with Th1-stimulating cytokines (e.g., IFN-α) to enhance BCG induction of Th1 immune responses (Luo et al., 1999; O’Donnell et al., 1999; O'Donnell and Boehle, 2006). In addition, we have also combined BCG with IL-10 blocking agents, either at the protein or receptor level, to enhance BCG induction of Th1 immune responses. We have observed that blocking IL-10 enhances BCG-induced Th1 immune responses and anti-bladder cancer immunity (Nadler et al., 2003; Bockholt et al., 2012).

Our early studies showed that absence of IL-10 abrogated either by systemic anti-IL-10 neutralizing mAb or the use of IL-10−/− mice resulted in enhanced delayed-type hypersensitivity (DTH) responses that were associated with increased mononuclear cell infiltration and Th1 cytokine production (e.g., IFN-γ) in the BCG-treated bladders (Nadler et al., 2003). Under the condition of enhanced DTH responses, a significant enhancement in BCG-induced anti-bladder cancer immunity was observed in a syngeneic mouse orthotopic tumor model (Nadler et al., 2003), suggesting that blocking IL-10 production and/or activity may have therapeutic values for BCG immunotherapy of bladder cancer.

In addition to IL-10 neutralization, we recently evaluated the effect of IL-10 blockage at the receptor level on BCG induction of Th1 immune responses and anti-bladder cancer immunity (Bockholt et al., 2012). Mice treated with intravesical BCG plus systemic anti-IL-10R1 mAb showed significantly increased IFN-γ mRNA and protein in the bladder and urine, respectively, in a dose-dependent manner. Accordingly, under the bladder cancer cell implantation condition, mice treated with BCG in combination with anti-IL-10R1 mAb showed substantially improved tumor-free and survival rates compared to control mice. Studies further revealed that the combination therapy with a reduced dose (1/3 full-dose) of BCG could significantly prevent bladder cancer metastasis to the lung during an extended experimental period (no metastasis in mice treated with combination therapy vs. 36-53% of incidence in control mice) (our unpublished observations). These observations suggest that anti-IL-10R1 mAb could serve as an effective agent for treating bladder cancer, particularly for high-risk patients, when combined with BCG.

CONCLUSION

Bladder cancer is a common urologic malignancy dominated by a Th2 polarized immunopathologic response. Intravesical BCG can shift the Th2 environment toward a Th1 milieu, leading to effective anti-bladder cancer immunity in the majority of patients. However, the current BCG therapy is associated with high disease recurrence and progression as well as a lack of therapeutic response in some patients. In addition, BCG-associated side effects are common and occasionally life-threatening. The efficacy of BCG can be improved by combination with Th1-stimulating agents, such as Th1 cytokines and Th2 blocking agents. Since IL-10 plays an important regulatory role in bladder cancer immunosurveillance and BCG immunotherapy, blocking IL-10 activity could enhance BCG induction of Th1 immunity and therapeutic control of bladder cancer. This assumption has been proven to be true in our animal studies. We combined BCG with anti-IL-10 neutralizing mAb or anti-IL-10R1 mAb to treat bladder cancer in a syngeneic mouse orthotopic tumor model and found that these combination therapies were more effective than BCG monotherapy. We further observed that a 1/3 full-dose of BCG in combination with anti-IL-10R1 mAb effectively prevented bladder cancer metastasis to the lung. These observations suggest that BCG could be
used at a reduced dose when combined with an IL-10 blocking agent to minimize BCG side effects while maintaining BCG efficacy. Future studies should aim to investigate the mechanisms through which BCG combination therapy induces enhanced anti-bladder cancer immunity and develop therapeutic regimens to evaluate the safety and efficacy of BCG combination therapy in clinical settings.

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