Bladder cancer and Th1 chemokines

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Abstract

Bladder cancer arises from the epithelial lining of the urinary bladder, and it is known as transitional cell carcinoma (TCC). Tobacco smoking is the main known contributor to urinary bladder cancer. However thirty percent of bladder tumors probably result from occupational exposure in the workplace to carcinogens. Immunotherapy by intravesicular delivery of Bacillus Calmette–Guérin (BCG) is used to treat and prevent the recurrence of superficial bladder cancer. Successful BCG immunotherapy for bladder cancer is associated with proper induction of T helper (Th)1 immunity. In bladder cancer patients after intravesicular BCG, urine was found to contain high levels of IP-10, and Interferon (IFN)-γ. TCC and endothelial cell lines were able to secrete IP-10 in response to BCG or IFN stimulation in vitro. Furthermore intravesicular BCG induces a cytokine-rich urinary microenvironment that is inhibitory to human endothelial cells and it is anti-angiogenic by the induction of Th1 chemokines. Other studies suggest that therapeutic strategies involving Th1 induction and Th2 dampening may improve responses to immunotherapy. Further studies are needed to evaluate the IP-10 in circulation, and urine, as prognostic marker of bladder cancer patients, also in relation to BCG immunotherapy. Clin Ter 2017; 168(1):e59-63. doi: 10.7417/CT.2017.1984

Key words: bladder cancer, occupational bladder cancer, IP-10, Th1 chemokines

State of the Art

Bladder cancer arises from the epithelial lining of the urinary bladder (1), and it is known as transitional cell carcinoma (TCC) or more properly urothelial cell carcinoma (UCC). Five-year survival rate is around 70% (2). In 2012 bladder cancer caused 430,000 new cases (3) and 165,000 deaths (4).

Tobacco smoking is the main known contributor to urinary bladder cancer; in most populations, smoking is associated with over half of bladder cancer cases in men and one-third of cases among women (5), however these proportions have reduced over recent years since there are less smokers in Europe and North America (6). There is an almost linear relationship between smoking duration (in years), pack years and bladder cancer risk. A risk plateau at smoking about 15 cigarettes a day can be observed (meaning that those who smoke 15 cigarettes a day are approximately at the same risk as those smoking 30 cigarettes a day). Quitting smoking reduces the risk, however former smokers will most likely always be at a higher risk of bladder cancer compared to never smokers (6). Passive smoking has not been proven to be involved (7).

Thirty percent of bladder tumors probably results from occupational exposure in the workplace to carcinogens such as benzidine. 2-Naphthylamine, which is found in cigarette smoke, has also been shown to increase bladder cancer risk. Occupations at risk are bus drivers, rubber workers, motor mechanics, leather (including shoe) workers, blacksmiths, machine setters, and mechanics (8). Hairdressers are thought to be at risk as well because of their frequent exposure to permanent hair dyes (9-13).

Hematuria is the most common symptom in bladder cancer (macroscopic hematuria) or it may be detectable only by microscope (microscopic hematuria), and it occurs in approximately 85% of the patients. Other frequent symptoms include pain during urination, frequent urination, or feeling the need to urinate without being able to do it. Patients with advanced disease refer pelvic pain, or lower-extremity edema.

In addition to these major risk factors there are also numerous other modifiable factors that are less strongly (i.e. 10-20% risk increase) associated with bladder cancer, for example obesity (14).

The gold standard for diagnosing bladder cancer is biopsy obtained during cystoscopy (15). Cytology is not very sensitive (a negative result cannot reliably exclude bladder cancer) (16). There are newer non-invasive urine bound markers available as aids in the diagnosis of bladder cancer, including human complement factor H-related protein, high-molecular-weight carcinoembryonic antigen, and nuclear matrix protein 22 (17).
The treatment of bladder cancer depends on how deep the tumor invades into the bladder wall. Superficial tumors (those not entering the muscle layer) can be “shaved off” using an electrocautery device attached to a cystoscope, which in that case is called a resectoscope. The procedure is called transurethral resection of bladder tumor-TURBT. TURBT in case of muscle invasive cancer is insufficient for final treatment (18).

Immunotherapy by intravesicular delivery of Bacillus Calmette–Guérin (BCG) is also used to treat and prevent the recurrence of superficial tumors (19). BCG is a vaccine against tuberculosis that is prepared from attenuated (weakened) live bovine tuberculosis bacillus, Mycobacterium bovis, that has lost its virulence in humans. BCG immunotherapy is effective in up to 2/3 of the cases at this stage, and in randomized trials has been shown to be superior to standard chemotherapy (20). The mechanism by which BCG prevents recurrence is unknown, but the presence of bacteria in the bladder may trigger a localized immune reaction which clears residual cancer cells (21).

A combination of radiation and chemotherapy can also be used to treat invasive disease. It has not yet been determined how the effectiveness of this form of treatment compares to that of radical ablative surgery.

BCG prevents recurrence in the bladder by triggering a localized T helper (Th1) immune reaction which clears residual cancer cells (21). This suggests the importance of Th1 cytokines and chemokines in the pathogenesis of bladder cancer.

The objective of this narrative review is to evaluate the importance of interferon (IFN)-γ chemokines in bladder cancer. The presentation of data has been reported according to the International Narrative Systematic Assessment (INSA) tool (22).

**IFN-γ-induced protein 10 (IP-10) in inflammatory disorders**

IP-10 is a chemokine that can regulate inflammation at several levels: induction of integrin activation; mediation of chemotaxis of multiple cell types [including activated T cells, monocytes, and natural killer cells (23)]; induction of apoptosis of pancreatic beta cells; inhibition of the proliferation of both epithelial and endothelial cells (24, 25); induction of molecules, such as interleukin (IL)-8 and chemokine (C-X-C motif) ligand (CXCL)-5; and up-regulation of costimulatory cell surface molecules, such as CD54, CD80, and CD86, on monocytes.

IP-10 secretion depends on IFN-γ, which is itself mediated by the IL-12 cytokine family. Under this influence, IP-10 is secreted by multiple cell types, including T lymphocytes, monocytes, splenocytes, fibroblasts, keratinocytes, thyrocytes, preadipocytes, etc. The presence of high levels of IP-10 in peripheral liquids may be considered as a marker of host immune response, especially Th1 oriented T-cells.

Recruited Th1 lymphocytes can be responsible for enhanced IFN-γ and tumor necrosis factor (TNF)-α production, which in turn stimulates IP-10 secretion from a variety of the above mentioned cells, therefore creating an amplification feedback loop (26).

Circulating levels of IP-10 are increasing with age. Recent reports have shown an increase of serum/tissue expressions of IP-10 in organ specific autoimmune diseases (27), such as type 1 diabetes (T1D) (28), Graves’ disease (GD), or Graves’ ophthalmopathy (GO) (29-31), autoimmune thyroiditis (32-37), or systemic rheumatological disorders like rheumatoid arthritis (RA) (38), systemic sclerosis (SSc) (39-41), psoriasis or psoriatic arthritis (42-46), sarcoidosis (47, 48), HCV-related cryoglobulinemia (49-53), other HCV immune mediated disorders (54, 55), lupus (56, 57), and also in cancers (58-66).

**Bladder cancer and IP-10**

Intravesicular BCG induces a variety of cytokines into the urine of patients with superficial TCC of the bladder. A study (67) aimed to determine whether the potently antiangiogenic chemokine IP-10 and its inducing antiangiogenic cytokines, IFN-γ and IL-12, were increased during intravesicular BCG immunotherapy of bladder TCC.

Urine samples were evaluated from 8 patients before and after each weekly intravesicular BCG treatment and from 4 patients receiving maintenance BCG treatments.

In all cases after intravesicular BCG, patient urine was found to contain high levels of IP-10. Urinary IFN-γ and IL-12 levels also increased in similar patterns after intravesicular BCG. The peak weekly cytokine response per patient usually occurred between the fourth and sixth treatment for IFN-γ and IP-10. Human TCC and endothelial cell lines were able to secrete IP-10 in response to BCG or IFN stimulation in vitro.

This study demonstrated that IP-10 and its inducing cytokines are elevated in response to intravesicular BCG (67).

In another study (68) patients undergoing BCG treatment provided urine samples before and at peak cytokine production times after BCG instillation. Urinary IFN-γ, IP-10, TNF-α, and vascular endothelial growth factor were induced by BCG treatment. Post-BCG treatment urine became progressively inhibitory to endothelial cells during weekly treatment courses. Neutralizing antibodies to IP-10 greatly reduced this inhibitory effect. This study suggested that intravesicular BCG induces a cytokine-rich urinary microenvironment that is inhibitory to human endothelial cells and it is anti-angiogenetic (68).

Successful BCG immunotherapy for bladder cancer is associated with proper induction of Th1 immunity. Unfortunately, 30% to 40% of bladder tumors never respond to BCG. A study (69) evaluated if antagonistic Th2 chemokine production by bladder tumors might be a potential cause of BCG nonresponsiveness, in 9 clinical bladder tumor specimens and 7 human bladder cancer lines. Eight of 9 clinical specimens expressed IP-10 and 5 expressed macrophage derived chemokine (MDC). However, of 7 cancer lines only 1 low grade line (RT4) expressed IP-10 and MDC, and 1 high grade line (T24) expressed IP-10. Histological staining demonstrated MDC and IP-10 expression in human bladder tumors.

These results indicate that certain bladder tumors produce the Th2 chemokine MDC, which may antagonize the
local Th1 environment induced by BCG (69).

A further study (70) examined the signal mechanisms for inducing MDC and IP-10 in RT4 cells by lipopolysaccharide (LPS), IFN-γ and TNF-α. LPS did not induce RT4 cells to produce IP-10 and MDC. However, LPS plus IFN-γ synergized the productions of the two chemokines. IFN-γ up-regulated the expression of toll like receptor (TLR)-4, which is an LPS binding receptor.

This study shows that IFN-γ enhances LPS for the induction of MDC and IP-10 through up-regulation of TLR-4, and the signal pathways of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and activator protein (AP)-1/extracellular signal-regulated kinase (ERK) 1/2 (70).

In a further study (71) a profile of the gene expression changes that occur after BCG instillation in the bladders of healthy mice was produced and compared to the type of immune cells recruited into the bladder. BCG significantly upregulated genes for Th1 chemokines: Cxcl2, Cxcl9, Cxcl10, Cxcl11; and increased the expression of Th1/Th2 chemokines: regulated on activation, normal T cell expressed and secreted (RANTES), chemokine (C-C motif) ligand (CCL)6 and CCL7; Th1 polarizing cytokines: IL1β and TNF-α; and Fc-γ receptor I and inducible nitric oxide synthases (iNOS) as early as after four weekly instillations. Most of these genes remained highly expressed after 6 weeks (71).

The aim of a study (72) was to monitor changes in the expression of immune-related genes in the bladder after tumor implantation. Mice were orthotopically implanted with MB49-PSA cells (C57BL/6 mice) on day 1 and terminated on days 7, 14, 21, and 28. Another mouse model (MBT-2/C3H mice) was examined at day 7. Gene expression analysis was performed. Immune suppressive [IL13, IL1β, prostaglandin-endoperoxide synthase (PTGS)2, NOS2, IL10, Cytotoxic T-Lymphocyte Antigen 4 (CTLA4), and CCL22] and immune stimulatory genes [Colony Stimulating Factor 2 (CSF2), granzyme B (GZMB), IFNγ, IP-10, TNFα, CD80, IL12a, and IL6] and Angiotensin II Receptor Type 2 (AGTR2) were increased by day 7. The Authors suggested that therapeutic strategies involving Th1 induction and Th2 dampening may improve responses to immunotherapy (72).

The accumulation of high levels of adenosine in tumors activates A2A and A3 receptors on immune cells and inhibits their ability to suppress tumor growth. Deletion of adenosine A2A receptors (A2A-ARs) has been reported to activate antitumor T cells, stimulate dendritic cell (DC) function, and inhibit angiogenesis. A study (73) evaluated the effects of intermittent intratumor injection of a nonspecific adenosine receptor antagonist, aminophylline (AMO; theophylline ethylenediame) and of a selective A2A-AR antagonist, ATL801. AMO and ATL801 slowed the growth of MB49 bladder tumors in syngeneic mice and reduced by 85% metastasizes of breast cancer cells from mammary fat to lung. Based on experiments with A2A-AR(-/-) or adenosine A3-AR receptor(-/-) mice, the effect of AMO injection was unexpectedly attributed to A2A-AR and not to A3-AR blockade. AMO and ATL801 significantly increased tumor levels of IFN-γ and the IP-10, ligand for CXCR3. This was associated with an increase in activated tumor-infiltrating CXCR3(+) T cells and a decrease in endothelial cell precursors within tumors. Tumor growth inhibition by AMO or ATL801 was eliminated in CXCR3(-/-) mice and RAG1(-/-) mice that lack mature T cells. In RAG1(-/-) mice, A2A-AR deletion enhanced CD86 expression on CD11b(−) DCs. The data suggest that blockade of A2A-ARs enhances DC activation and CXCR3-dependent antitumor responses (73).

A last study (74) evaluated the impact of BCG on local production of chemokines attracting the desirable effector CD8(+) T cells (CTLs) and undesirable myeloid-derived suppressor cells (MDSCs) and regulatory T(reg) cells, and the ability of bladder cancer tissues to attract CTLs. Bladder cancer tissues spontaneously expressed high levels of the granulocyte/MDSC-attractant CXCL8 and Treg-attractant CCL22, but only marginal levels of the CTL-attracting chemokines: RANTES, monokine induced by interferon (MIG) and IP-10. Baseline IP-10 showed strong correlation with local expression of CTL markers. BCG selectively induced IP-10 production. The combination of IFN-α and a TLR3 ligand, poly-I:C (but not the combinations of BCG with IFN-α or BCG with poly-I:C), induced high levels of intra-tumoral production of IP-10 and promoted CTL attraction. This observation that the combination of BCG with (or replacement by) IFN-α and poly-I:C allows to reprogram bladder cancer tissues for enhanced CTL entry may provide for new methods of improving the effectiveness of immunotherapy of bladder cancer (74).

Discussion

Bladder cancer arises from the epithelial lining of the urinary bladder (1). Tobacco smoking is the main known contributor to urinary bladder cancer. However thirty percent of bladder tumors probably results from occupational exposure in the workplace to carcinogens. Immunotherapy by intravesicular delivery of BCG is used to treat and prevent the recurrence of superficial bladder cancer. Successful BCG immunotherapy for bladder cancer is associated with proper induction of Th1 immunity. In bladder cancer patients after intravesicular BCG, urine was found to contain high levels of IP-10, and IFN-γ. Human TCC and endothelial cell lines were able to secrete IP-10 in response to BCG or IFN stimulation in vitro. Furthermore intravesicular BCG induces a cytokine-rich urinary microenvironment that is inhibitory to human endothelial cells and it is anti-angiogenenic by the induction of Th1 chemokines. Other studies suggest that therapeutic strategies involving Th1 induction and Th2 dampening may improve responses to immunotherapy. In conclusion, further studies are needed to evaluate the IP-10 in circulation, and urine, as prognostic marker of bladder cancer patients, also in relation to BCG immunotherapy.

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