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Seminars in Oncology

Immunotherapeutic Strategies for High-Risk Bladder Cancer

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Transitional cell carcinoma (TCC), which is the pathological diagnosis for the majority of bladder cancers, is a solid tumor entity that is responsive to immunotherapy as evidenced by a substantial cure rate documented with the use of intravesical bacillus Calmette-Guérin (BCG) therapy in selected patients with high-grade superficial disease. The nonspecific immune modulation that occurs as a result of BCG therapy is not well understood; however, the success of BCG therapy provides a basis for the exploration of mechanisms related to immune responses and the development of novel immunotherapeutic agents for the treatment of high-risk disease. In this review, we discuss the complexity of the immune system and therapies that are considered capable of manipulating it to potentially benefit patients with bladder cancer.
Semin Oncol 34:165-172

Immunotherapy has been an appealing concept for the treatment of cancer for more than a century. The exquisite specificity with which we can direct the human immune system against infectious organisms is the same result we wish to obtain when targeting cancer. Recognizing the strength of the immune response against infectious organisms and the fact that certain patients with cancer experience tumor regression when they contract acute bacterial infections, Dr William Coley, in the 1890s, injected live bacteria into a patient with advanced cancer. Subsequently, Dr Coley went on to develop a safer, more effective mixture of bacteria for the treatment of cancer.[1] Later preclinical studies demonstrated tumor regression when mice were injected with bacillus Calmette-Guérin (BCG) prior to transplantation of tumor cells compared with mice that did not receive BCG treatment.[2] These preliminary observations formed the conceptual foundation used to develop the first standard clinical practice using nonspecific immunotherapy: intravesical administration of BCG for the treatment of bladder cancer. Although the precise mechanism of action of BCG therapy is not fully understood, the success of intravesical BCG in the treatment of superficial bladder cancer has opened the door for further investigations of other immunotherapeutic agents in the treatment of high-risk bladder cancer.

Innate and Adaptive Immunity

The immune system is finely orchestrated by a myriad of cell types and mediators that are required to be tolerant of self but responsive to alterations perceived as non-self. The first line of defense against non-self is a nonspecific response by cells such as macrophages, neutrophils, dendritic cells (DCs), and natural killer (NK) cells, which are part of the innate immune system. The innate immune response is then followed by coordination of antigen-specific recognition via lymphocytes, which are part of the adaptive immune system and that provide long-term memory. These two distinct compartments, innate and adaptive, communicate via sophisticated networks that allow for an activated immune response when an appropriate "danger" signal is perceived and regulation of the response so as to prevent continuous proliferation of immune cells. This complex system has evolved to simultaneously provide tolerance to self, eliminate pathogens, and control an activated immune response in order to prevent self-damage. In order to harness the power of this biological machinery for the treatment of cancer, it is necessary to understand some of the basic principles that govern this system. Rational design of immunotherapeutic agents is an ongoing process that targets some of these principles for the generation of tumor-specific immune responses.

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Supported in part by the UTMDACC Bladder Cancer SPORE Career Development Award 5P50 CA091846 04 (PP-CDP4), Physician Scientist Program Award and Institutional Research Grant (P. Sharma).
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0093-7754/07/$-see front matter
doi:10.1053/j.seminoncol.2006.12.004

The primary mechanism for an immediate response to infection or cellular injury is via innate immunity. The innate immune system uses germline-encoded or pathogen recognition receptors (PRRs) to identify pathogen-associated molecular patterns (PAMPs) on microbes.[3] Receptor signaling leads to production of cytokines, proteases, reactive oxygen species, and other inflammatory mediators in order to recruit leukocytes to the area of injury. This inflammatory response, when limited and controlled, provides for host responses, including endothelial and fibroblast responses, to initiate a first-line defense against injury and repair of damage. In situations of chronic inflammation, due to disruptions of innate and/or adaptive immune cells, tissue destruction, and eventual DNA damage, may lead to disease states, including cancer.

PRRs include toll-like receptors (TLRs), which are widely expressed on innate immune cells and consist of 13 receptors known to date. Although all TLRs are structurally similar and recognize conserved molecular patterns on microbes, it has been demonstrated that signaling via specific TLRs can induce distinct responses. For example, viral and intracellular bacterial DNA will induce signaling via TLR9, which leads to interferon (IFN)-α production, favoring a Th1 profile, as well as enhanced cytotoxic T-lymphocyte (CTL) responses.[4] Conversely, stimulation of TLR2 by acylated outer membrane lipoproteins of gram-positive bacteria can result in interleukin (IL)-10 production, which favors a bias towards a Th2 profile or regulatory T-cell responses.[5]

While bacterial cells express TLR ligands, it should be noted that tumor cells do not. Therefore, appropriate adjuvants, which provide TLR signaling via the innate immune system, can be used with immunotherapeutic agents to enhance immune responses. Potent stimulators of the innate immune system may provide an effective immune response against tumor cells. This mechanism may be the most likely way in which intravesical BCG exerts its influence in the treatment of superficial bladder cancer.

Although the exact mechanism of action of intravesical BCG in the treatment of bladder cancer is poorly understood, recent evidence continues to corroborate an immunological mechanism. BCG plays a significant role in the maturation of DCs by signaling through different TLRs, as indicated by its upregulation of the DC maturation marker CD83[6] and secretion of inflammatory cytokines such as IL-12, IFN-γ, and tumor necrosis factor (TNF)-α. A variety of cytokines has been detected in the urine of patients treated with intravesical BCG, and BCG causes an influx of granulocytes and mononuclear cells into the bladder wall.[7-14] Although the significance of these infiltrating cells remains controversial, we recently studied intratumoral lymphocytes in urothelial carcinoma and determined that the presence of $CD8^+$ T cells correlated with better disease outcome in patients with more advanced disease.[15] Improved clinical outcomes have been noted in association with the presence of intratumoral T cells in ovarian,[16] esophageal,[17] and colorectal carcinomas.[18] It is possible that activation of the innate immune system by intravesical BCG leads to stimulation of an antigen-specific adaptive response, associated with T-cell infiltration, that allows for eradication of tumor cells. The identification of the tumor antigen(s) that these T cells recognize may provide a potential vaccine target for the development of specific immunotherapy for bladder cancer.

Activation of the innate immune system is crucial for establishing an effective adaptive immune response. Activated antigen-presenting cells (APCs) from the innate immune system, such as DCs, migrate to lymphoid organs, such as lymph nodes, and present antigens to adaptive immune cells, such as T cells. Similarly, inflammatory cytokines produced by innate immune cells recruit adaptive immune cells, including B and T cells, thereby propagating a more specific immune response.

Adaptive immune cells, such as B and T cells, in contrast to innate immune cells, express somatically generated antigen-specific receptors, which are formed as a result of random gene rearrangements to allow for a diverse repertoire. These somatically rearranged receptors allow for specific recognition of a vast array of antigens. B-cell recognition of intact antigen via immunoglobulin receptor leads to proliferation of the B cell and secretion of neutralizing antibodies against the antigen. The clonal expansion of an antigen-specific B cell subsides after elimination of the antigen, and a resultant pool of memory B cells will exist to provide for a rapid antibody response against subsequent exposure to the antigen. Similarly, T cells recognize antigens via a specific T-cell receptor (TCR). However, unlike B cells, T cells require processed antigen to be presented in the context of the major histocompatibility complex (MHC).

Classical MHC molecules comprise class I and class II proteins, which enable each individual's immune system to distinguish self from non-self. TCRs of $CD8^+$ T cells (cytotoxic T cells) recognize antigen in the context of class I MHC, and TCRs of $CD4^+$ T cells (helper T cells) recognize antigen in the context of class II MHC. The initiation of self-tolerance occurs early in development as a process of positive and negative selection when T cells interact with thymic APCs that express self-MHC molecules bearing self-antigens. The avidity between TCRs and MHC plus self-peptides determines the fate of T cells, such that high- and low-avidity interactions lead to negative selection of T cells, while intermediate-avidity binding allows for positive selection. Therefore, circulating T cells in cancer patients are already selected for their ability to ignore self-antigens, which are the majority of tumor antigens. However, in certain patients, there are tumor-infiltrating lymphocytes that tend to correlate with improved outcomes, thus indicating the possibility of manipulating the immune system to favor tumor-reactive T cells. Furthermore, apart from TCR engagement of MHC plus antigen (signal 1), additional signals and costimulatory molecules (signal 2) are necessary to attenuate the T-cell response and create an activated T cell. Upon T-cell activation, there are also "co-inhibitory" signals generated to regulate the T-cell response and limit T-cell proliferation. The interplay of these signals provides opportunities to target T-cell pathways in an effort to induce anti-tumor immunity.