

Optimizing Cancer Treatments to Induce an Acute Immune Response: Radiation Abscopal Effects, PAMPs, and DAMPs

Charles M. Ludgate

Abstract

Clinical results indicate improved survival in poorly differentiated prostate cancer patients following a treatment schedule that maximizes hormone therapy prior to radiation. This may be because of a systemic immune response, called an abscopal effect. A literature review showed an association between acute infection and abscopal cancer remission. This led to the theory that, in the presence of endogenous cancer-specific antigens exposed by cancer necrosis, an innate immune response can adapt to respond to those antigens via a cross-talk mechanism. This theory was validated in an animal model. An acute innate immune T-cell response was stimulated using cluster vaccination with Poly(I:C). In the presence of exogenous cancer-specific antigens, this immune response became adaptive, creating an abscopal effect that resulted in cancer resolution. These concepts may be of clinical value, improving outcomes by inducing systemic abscopal effects. *Clin Cancer Res*; 18(17); 4522–5. ©2012 AACR.

Introduction

In the 1990s, the British Columbia Cancer Agency had a waiting list for treatment of prostate cancer patients of up to 1 year, which had developed over time through a shortage of treatment facilities. As a holding measure, all prostate cancer patients were given hormone therapy while waiting for radiation; specifically, they were given androgen withdrawal therapy, which caused initial remission of prostate cancer. Hormone therapy was discontinued at the completion of radiation. Overall cancer outcomes were unchanged, but in subset analysis, patients with poorly differentiated cancer (Gleason 8–10) had better outcomes than expected (1–4). The benefit was independent of the duration of hormone treatment but was seen in those whose prostate-specific antigen (PSA) indicated full remission before radiation started and only in poorly differentiated cancers. These patients had a significant decrease in death from prostate cancer. A randomized multicenter study carried out across Canada confirmed these results, with no difference in outcome or survival in the overall group but improved outcomes and survival for the patients with the worst prognosis (5). Poorly differentiated prostate cancers (Gleason 8–10) tend to spread early, and death from prostate cancer is because of metastatic rather than local disease. Our data suggested that

hormone therapy prior to radiation of the primary tumor site might produce systemic changes that "prime the system"; local radiation might then induce systemic control of cancer at the metastasized sites as well as the primary site. This phenomenon has been reported as the "abscopal effect"—the regression of distant disease after localized treatment (6). Abscopal effects are most often attributed to activation of antitumor immunity, which, unlike site-specific radiation therapy, can have broader systemic effects.

We turned to our immunology colleagues to provide an underlying testable concept and a means of measuring and validating antitumor immune responses in this setting. One of the earliest examples of antitumor immunity was documented by Coley in the 1890s. In 1891, he injected a large inoperable recurrent sarcoma in a man's neck with a live culture of *Staphylococcus pyogenes*. This resulted in an infection with prolonged high fever, from which the patient nearly died, but which resulted in complete regression of the tumor over a severe 2-week illness. Coley then treated further patients who had inoperable cancers by the injection of live staphylococcal broth and later killed bacteria, into their cancers. Some of these patients developed systemic signs of infection with prolonged fever, and several of these patients showed abscopal regression of metastatic disease (7). We now know that the immune system comprises 2 distinct but highly interactive arms known as adaptive and innate. Cross-talk may result in presentation of weak cancer antigens, normally insufficient to create an adaptive immune response, to CD4 and CD8 lymphocytes activated and multiplied by the innate response to bacterial infection. This would result in a systemic T-cell immune response to cancer and cause an abscopal systemic response.

As bacterial and viral infections progress, they release increasing amounts of pathogenic material. The recognition of these pathogen-associated molecular patterns (PAMP),

Author's Affiliation: Department of Radiation Oncology, Vancouver Island Centre, BC Cancer Agency, Victoria, British Columbia, Canada

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Corresponding Author: Charles M. Ludgate, FRCPC, Radiation Oncology, Vancouver Island Cancer Centre, 2410 Lee Avenue, Victoria, BC, Canada V8R 6V5. Phone: 215-440-9300; Fax: 215-440-9354; E-mail: cludgate@bccancer.bc.ca

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Translational Relevance

Clinical observations, validated in an animal model in the laboratory, have yielded conceptual foundations for new clinical treatments of cancer. Unexpectedly positive outcomes from an unusual hormone and radiation schedule led to the theory that the treatment triggered an acute adaptive immune response. To validate this concept, an acute innate immune response was stimulated in an animal model using cluster vaccination with Poly(I:C). In the presence of exogenous cancer-specific antigens, this immune response adapted itself to create an abscopal effect that resulted in cancer resolution. These concepts may be translated back to the clinic to improve treatment outcomes. This article reviews historic evidence of abscopal effects in cancer and discusses the relationship between infection and cancer regression as it relates to immune signaling. It then proposes ways to amplify standard cancer treatments to induce systemic abscopal effects.

such as bacterial RNA, by the innate Toll-like receptors (TLR) results in a robust amplification of CD4 and CD8 T cells. This stimulation of innate TLRs provides a nonspecific warning to the immune system of the presence of invading pathogens and elicits appropriate nonspecific innate immune response. In Coley's case, staphylococcal RNA and other PAMPs initially stimulate an innate response, and then the adaptive response was triggered. Mechanisms of adaptive immunity are precisely targeted and focused on specific antigens. These antigens are produced as the acute phase of infection progresses to necrosis and inflammation, with cytokine and chemokine release that attracts dendritic and other antigen-presenting cells. These cells engulf and present antigens from the causative pathogen to the T cells, driving an adaptive acute immune response. CD4 and CD8 T cells expressing receptors specific for the staphylococcus pyogenes bacteria expand and fight the infection (8).

Cross-presentation of cancer antigen in the uncommitted stage could explain Coley's phenomenon (9, 10). Infection caused by *Staphylococcus* organisms releases bacterial antigens, but also, by causing cancer necrosis, the release of cancer-specific antigens. The sequence of alarm or "danger" signals (11) followed by cross-talk regulates both the quality and the intensity of adaptive immune responses (12, 13). Once evoked, this type of antitumor immunity, particularly CD8 immunity, can have rapid and dramatic effects on tumor regression and cause a systemic abscopal response.

Related research shows that it is possible to emulate the timing and nature of an acute infection through daily injections of PAMPs; the resulting robust T-cell response is that of an acute infection. It is also possible to induce cross-talk with cancer antigen to create a systemic response. For example, when animals with human papilloma virus (HPV) E7-positive cancer are given HPV vaccine targeting the E7

proteins admixed with the TLR3 agonist Poly(I:C) or the TLR9 agonist CpG DNA and then delivered as daily subcutaneous injections over 4 successive days, an environment similar to what would be experienced during an authentic acute infection develops. The ensuing vaccine-specific CD8 immune response is highly robust and results in cure of cancer in all animals (14, 15). The cross-recognition and presentation of cancer antigen has been shown to boost the effector CD8 T-cell expansion (16). Unfortunately, in many tumor settings, and particularly in prostate cancer, well-defined and highly reliable tumor antigens that can be targeted with synthetic vaccines are often nonexistent, although antigen discovery efforts are under way around the world to hopefully define future vaccine candidates.

We hypothesize that the clinical response that we have previously observed and attributed to abscopal effects are the result of immunogenic prostate cancer antigens that are released by the tumor necrosis caused by radiation therapy. The effect is seen when hormone therapy has been maximized, as reflected in the PSA level (17). Androgen-withdrawal hormone therapy is known to shut down gene expression (18) and change the cellular and membrane environment (19), which may change tolerance and enable cancer cell recognition by the immune system.

Death of cancer cells by medical therapy causes the release of endogenous danger signals known as damage-associated molecular patterns (DAMP; refs. 20, 21). These DAMPs augment the presentation of tumor antigens released from necrotic tumor cells (20, 22), ultimately inducing the immune system to attack cancer and thereby mimicking an acute infection. DAMPs and PAMPs may share some commonality of expression, and it may be possible to evoke an acute anticancer immune response using an "endogenous" vaccine approach. The response to DAMPs produced by sterile necrosis caused by conventional treatment could be amplified by local delivery of PAMP compounds to emulate the Coley phenomenon. In our prostate cancer therapy, we used a sequence of hormone therapy to change the molecular environment before causing cancer necrosis and inflammation with radiation. Radiation death of poorly differentiated cancer cells is usually by necrosis, while well-differentiated cancer cells die by apoptosis, without necrosis or signaling (23). This difference may explain the lack of benefit in our overall series and focuses the need for immune-based treatment strategies capable of causing necrotic cancer damage that will induce inflammation and DAMP signaling. That cancer abscopal effects can occur without an exogenous foreign danger signal (PAMP) has been shown in studies of radiation with amplification of the dendritic cell recognition pathway (6), or by using TLR agonist along with tumor-specific antigen (15). Therefore, while all 3 components of the acute infection danger signal, inflammation, necrosis, and PAMPs, may not be required to invoke a systemic abscopal response, the sequencing and intensity of those used may be critical.

We anticipate that it may be beneficial to design conventional treatments that maximize immune signaling from endogenous vaccines produced by necrotic tumor cell death

and to amplify the response to the DAMPs produced locally and systemically. Daily injections of TLR3/9 agonists either by intratumoral or peritumoral administration in the days immediately following radiation of a primary tumor will result in cross-presentation of tumor antigens released from the radiation-damaged tumor (21). Amplification of the systemic recognition of DAMPs could be achieved by increasing dendritic cells with such drugs as FLT.3 Ligand (6), by activated dendritic cell infusion, or by reducing CD8 T-cell inhibition through CTLA4 antagonist therapy (24). Immunologic correlates of this abscopal effect have been documented in a patient with metastatic melanoma treated with the CTLA4 antagonist ipilimumab and palliative paraspinal radiation. This treatment resulted in regression of hilar nodal and splenic lesions not treated by radiation, with changes in CD4 T cells and antibody titers suggesting a systemic immune response (25). Similar systemic amplification of focused localized radiation followed by interleukin-2 has resulted in metastatic cancer resolution in renal cell cancer and melanoma patients with an increase in CD4 and memory cells (26). The degree and balance of amplification necessary will depend on the innate immunogenicity of the individual cancer; the toxicity may be minimized by local treatment at the cancer site (20). A successful sequence would achieve antitumor immunity

that is both potent and long-lived and will have the desired abscopal effect on metastatic tumor sites. The sequence, amount, and timing of events will be critical to initiate an acute infectious type of response to cancer, and it is antigen that has been able to evade immune surveillance by inducing central and peripheral tolerance. This strategy opens the way for novel cancer therapies amplifying endogenous vaccines created by conventional cancer treatment and a framework for future cancer therapies in which cancer antigens are known.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: C.M. Ludgate

Development of methodology: C.M. Ludgate

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C.M. Ludgate

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C.M. Ludgate

Writing, review, and/or revision of the manuscript: C.M. Ludgate

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.M. Ludgate

Study supervision: C.M. Ludgate

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