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Review

Cancer immunotherapy in veterinary medicine: Current options and new developments

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ABSTRACT

Excitement in the field of tumor immunotherapy is being driven by several remarkable breakthroughs in recent years. This review will cover recent advances in cancer immunotherapy, including the use of T cell checkpoint inhibitors, engineered T cells, cancer vaccines, and anti-B cell and T cell antibodies. Inhibition of T cell checkpoint molecules such as PD-1 and CTLA-4 using monoclonal antibodies has achieved notable success against advanced tumors in humans, including melanoma, renal cell carcinoma, and non-small cell lung cancer. Therapy with engineered T cells has also demonstrated remarkable tumor control and regression in human trials. Autologous cancer vaccines have recently demonstrated impressive prolongation of disease-free intervals and survival times in dogs with lymphoma. In addition, caninized monoclonal antibodies targeting CD20 and CD52 just recently received either full (CD20) or conditional (CD52) licensing by the United States Department of Agriculture for clinical use in the treatment of canine B-cell and T-cell lymphomas, respectively. Thus, immunotherapy for cancer is rapidly moving to the forefront of cancer treatment options in veterinary medicine as well as human medicine.

Introduction

The future of immunotherapy for cancer has never been brighter, with excitement in the field being driven by with several remarkable breakthroughs in recent years (Forde et al., 2013; Raval et al., 2014; Sanlorenzo et al., 2014). First, inhibition of T cell checkpoint molecules such as PD-1 and CTLA-4 using monoclonal antibodies has achieved notable success against advanced tumors in humans, including melanoma, renal cell carcinoma, and non-small cell lung cancer (Topalian et al., 2012; Wu et al., 2012; Ott et al., 2013; Quezada and Peggs, 2013; Perez-Gracia et al., 2014) Moreover, many of the responding patients have achieved durable tumor remissions, leading to speculation that this type of therapy may render some types of cancer manageable as chronic diseases (Topalian et al., 2012).

The second major breakthrough in tumor immunotherapy involves the ex vivo engineering of T cells to target them to specific tumor antigens. Impressive tumor responses observed following adoptive transfer of tumor-specific T cells have stimulated tremendous excitement in the field (Aldrich et al., 2010; McCormack et al., 2013; Riches and Gribben, 2013; O'Connor and Wilson-Robles, 2014; Raval et al., 2014). The general approach to generating tumor specific T cells has been to expand large numbers of autologous T cells ex vivo, then to transfect the cells with tumor specific T cell receptors or attach tumor-antigen specific antibodies to the T cells, and to then re-infuse the modified autologous T cells back into the host (Muller and Kontermann, 2010). Dramatic control and in some cases cures of previously refractory tumors have been achieved using these approaches (Restifo et al., 2012; Stroncek et al., 2012). Many of these new advances may soon find application in veterinary medicine, particularly as the costs of certain technologies such as monoclonal antibody production have declined substantially in recent years. Therefore, it is likely that immunotherapy will become an important 'fourth arm' of the cancer therapy arsenal for treatment of companion animals.

The third major advancement in tumor immunotherapy is the development of engineered monoclonal antibodies to tumor or immune cell antigens, which directly target the cells for destruction (Maleki et al., 2013; Miller et al., 2013; Sliwkowski and Mellman, 2013; Zigler et al., 2013; Zhou et al., 2014). Tumor destruction by targeted monoclonal antibodies involves both immune and non-immune mechanisms. The number of new antibody targets and the overall complexity of the field are however beyond the scope of the present review. Nonetheless, it should be noted that several canine lymphoma antibodies have undergone evaluation in phase I and II clinical trials and impressive results following administration of a canine anti-CD20 antibody in combination with chemotherapy in dogs with B-cell lymphoma were reported recently at the 2014 annual meeting of the Veterinary Cancer Society[1]. Accordingly, a monoclonal canine anti-CD20 antibody has been fully approved by

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[1] Ogilvie, G; Veterinary Cancer Society Proceedings, October 2014; http://finance.yahoo.com/news/aratana-therapeutics-announces-presentation-clinical-132500219.html.

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the United States Department of Agriculture (USDA) for clinical usage in dogs with B-cell lymphoma, and is currently being commercialized in the United States and Canada.

Cancer immunotherapy: Tumor specific versus non-specific immunity

Tumor growth can be controlled by multiple immune mechanisms, and these mechanisms can be divided into tumor non-specific and tumor antigen specific responses. Non-specific tumor destruction is mediated by the innate immune system and is independent of T cells, whereas tumor specific immunity is mediated primarily by both T and B cells.

Non-specific tumor immunity is most often elicited by administration of broadly activating compounds that target pattern-recognition receptors, such as Toll-like receptors (TLR) and Nod-like receptors (NLR). Examples of non-specific immunotherapeutics used to treat cancer in companion animals include liposomal muramyl tripeptide (MTP), topical imiquimod (a TLR7 agonist), and Immunocidin (see below) (Andersen et al., 2013). The immune effector cells involved in non-specific tumor immunity include primarily activated natural killer (NK) cells and activated monocytes and macrophages. Tumor cells may be eliminated both by direct lysis and by the effects of interferon- γ (IFN-γ) produced by NK cells. Because non-specific immune therapeutics often fail to elicit effective memory T cell responses, they must be repeatedly administered frequently (e.g., weekly) in order to generate sustained tumor control.

In contrast, tumor-specific immunity is mediated by effector and memory T cells, especially CD8 T cells, and to a lesser degree by B cells. Effector T cell responses have historically been generated by cancer vaccines, but that strategy is now changing with the advent of checkpoint molecule blockade and adoptive transfer of engineered T cells. Nonetheless, currently the most efficient and cost-effective means of generating tumor specific immunity involves tumor vaccines. Tumor vaccines are a form of active cancer immunotherapy in which patients are repeatedly immunized with tumor-derived antigens aimed at eliciting de novo anti-tumor T cell responses, and/or amplifying pre-existing anti-tumor immune responses (Buonaguro et al., 2011). Tumor vaccines can be prepared using antigens derived from either allogeneic or autologous tumor cells (obtained fresh via patient-derived tumors, or by propagation of cell lines in vitro), whole tumor lysates, purified tumor peptides, or in some cases through the use of defined tumor antigens such as tyrosinase (Grosenbaugh et al., 2011). Additionally, tumor vaccines can be gene-based, in which DNA constructs encoding tumor antigens are administered to patients. These constructs can be delivered either naked or within vectors (e.g., viruses) and are intended to be taken up locally by antigen presenting cells (APCs), with the antigens subsequently synthesized and presented to naïve T cells (Pol et al., 2014). Tumor vaccines elicit both effector and memory T cells, which can in theory control tumor metastases over long periods of time (months to years). However, the availability of well-characterized tumor-specific antigens is currently a major impediment to the widespread application of tumor vaccines in veterinary medicine. Moreover, checkpoint molecule blockade and engineered T cells may ultimately replace the need for specific cancer vaccines, given the results of a recent clinical trial combining anti-CTLA4 therapy with a peptide (gp100) vaccine in human patients with metastatic melanoma (Hodi et al., 2010).

Non-specific tumor immunotherapy: Manipulation of innate immunity

Until recently, most tumor immunotherapy in veterinary medicine relied on administration of non-specific immunotherapeutics, or the so-called biologic response modifiers. A number of early studies in canine cancer were done using MTP (an NLR agonist) infused intravenously (IV) as a liposomal formulation (MacEwen, 1977, 1985; Kurzman et al., 1995; Vail et al., 1995; MacEwen et al., 1999). Other studies utilized intratumoral administration of live BCG or *Corynebacterium parvum* (Parodi et al., 1983; MacEwen et al., 1986; Klein et al., 1991; Klein, 2003; Mukaratirwa et al., 2009). More recently, topical administration of the TLR-7 agonist imiquimod was utilized in combination with an autologous tumor cell vaccine in dogs with invasive meningioma (Andersen et al., 2013). Additionally, the use of imiquimod as a monotherapy for cats with multicentric squamous cell carcinoma in situ has been previously reported (Gill et al., 2008). The immunotherapy field is currently moving toward therapeutics that target more specific innate immune cell populations such as NK cells or monocytes. Nonetheless, there is still a need for effective non-specific immune therapeutics that can be used in conjunction with chemotherapy or radiation therapy or combined with tumor vaccines or other T cell targeted therapies.

Macrophage and monocyte activators

Activated macrophages and monocytes can kill tumor cells directly, or can indirectly control their growth by producing cytokines such as tumor necrosis factor-α (TNF-α). However, macrophages and monocytes, unless activated in a sustained manner, may also paradoxically stimulate cancer growth and angiogenesis and promote the development of tumor metastases (Ruffell et al., 2012). Indeed, the net overall impact of macrophages in tumors is primarily immune suppressive. Therefore, to avoid paradoxically worsening tumor immunity, therapeutics that target macrophages in cancer patients need to be potent but short-lived immune activators, and should be administered repeatedly over a relatively short period of time (weeks). The NLR agonist MTP (administered as a liposomal formulation known as L-MTP-PE) is one of the most thoroughly evaluated macrophage targeted immune therapeutics in veterinary medicine, with demonstrated anti-metastatic activity in canine osteosarcoma (Kurzman et al., 1995). At present however L-MTP-PE (Mifamurtide) is only available for use in Europe. Macrophages in tumors can also be activated by direct injection of fungal (e.g., acemannan) or mycobacterial cell wall (e.g., immunocidin) extracts directly into tumors (Harris et al., 1991; Zhang and Tizard, 1996).

NK cell targeted therapy

Natural killer cells are the primary antitumor effector cells for most non-specific immune therapeutics. Activated NK cells can kill tumor cells through direct cytotoxicity and importantly, NK cells also release large amounts of IFN-γ, a cytokine that exerts both direct and indirect anti-tumor activities. At present, products designed to specifically activate NK cells are not available in veterinary medicine. However, our laboratory has previously investigated the effectiveness of cationic liposome–DNA complexes (CLDC), which are particularly effective activators of NK cells, in clinical studies in dogs with cancer (Dow et al., 1999, 2005). For example, we found that IV administration of CLDC stimulated NK cell activation and significantly controlled the growth of osteosarcoma metastases in dogs with metastatic osteosarcoma (Dow et al., 2005). In addition, CLDC immunotherapy significantly inhibited tumor angiogenesis in dogs with soft tissue sarcoma (Kamstock et al., 2006). This compound is currently being evaluated for use as a veterinary immunotherapeutic in Europe.

NK cells can also be activated using recombinant cytokines. For example, both IFN-α and IFN-γ synergize to expand and activate NK cell populations in lung and other tissues to generate enhanced anti-tumor activity. In addition, interleukin-12 (IL-12) potently activates NK cells and triggers IFN-γ release. As such, a number of different