Veterinary Immunology and Immunopathology 137 (2010) 235–242

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Research paper

A selective high affinity ligand (SHAL) designed to bind to an over-expressed human antigen on non-Hodgkin's lymphoma also binds to canine B-cell lymphomas

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ARTICLE INFO

Article history:
Received 30 March 2010
Received in revised form 19 May 2010
Accepted 25 May 2010

Keywords:
Canine
Lymphoma
HLA
Lym-1
Non-Hodgkin's

ABSTRACT

Therapies using antibodies directed against cell surface proteins have improved survival for human patients with non-Hodgkin's lymphoma (NHL). It is possible that similar immunotherapeutic approaches may also benefit canine NHL patients. Unfortunately, variability between human and canine epitopes often limits the usefulness of such therapies in pet dogs. The Lym-1 antibody recognizes a unique epitope on HLA-DR10 that is expressed on the majority of human B-cell malignancies. The Lym-1 antibody has now been observed to bind to dog lymphocytes and B-cell NHL. Sequence comparisons and computer modeling of a human and three canine DRB1 proteins identified several orthologs of human HLA-DR10 expressed by dog lymphocytes. Immuno-staining confirmed the presence of proteins containing the Lym-1 epitope on dog lymphocytes and B-cell NHL. In addition, a selective high affinity ligand (SHAL) SH-7139 designed to bind within the Lym-1 epitope of HLA-DR10 was also observed to bind to canine B-cell NHL tissue. This SHAL, which is selectively cytotoxic to cells expressing HLA-DR10 and has been shown to cure mice bearing human B-cell lymphoma xenografts, may prove useful in treating B-cell malignancies in pet dogs.

1. Introduction

Human B-cell lymphocytes express a number of cell surface proteins that continue to be produced, and are often over-expressed (as much as 10-fold), on the surface of malignancies derived from these lymphocytes. CD19 (Hekman et al., 1991; Scheuermann and Racila, 1995), CD20 (Perkins et al., 2003), CD22 (Perkins et al., 2003), CD37 (Zhao et al., 2007), CD79a (Pilozzi et al., 1998) and the MHC-II complex protein HLA-DR (Epstein et al., 1987; Rose et al., 1996) are only a few examples of the receptors that have been studied extensively as potential markers for diagnosis or therapeutic targeting of lymphoma.

The retention of these abundant cell surface proteins on various forms of B-cell lymphoma and leukemia has led to the development of a variety of antibody-based immunotherapies that are currently in use or are being evaluated for treating human non-Hodgkin's lymphoma (NHL) and other B-cell lymphomas. Antibodies to CD19 have been coupled to toxins and have shown efficacy in treating acute lymphocytic leukemia and NHL both *in vitro* and *in vivo* (Scheuermann and Racila, 1995). Rituximab/Rituxan (Genentech/Roche), a monoclonal antibody that targets CD20, has been widely used for more than a decade, either

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doi:10.1016/j.vetimm.2010.05.014

alone or in combination with chemotherapy, for treating human NHL (Fu et al., 2008; Perkins et al., 2003). Smaller immuno-pharmaceuticals containing the variable regions of CD37 have been used to induce apoptosis in B-cell lymphoma cell lines and chronic lymphocytic leukemia cells (Zhao et al., 2007). The Lym-1 antibody targets an epitope located on the beta-subunit of HLA-DR10, a leukocyte antigen that is expressed on >95% of human B-cell malignancies (White et al., 2001). Although therapy with the Lym-1 antibody initially appeared promising *in vitro*, therapy in human patients with advanced disease was found to be of limited efficacy due to poor extravascular penetration of the antibody (Hu et al., 1989). Despite this limitation, clinical responses and improved survival were documented when the Lym-1 antibody was tagged with a short-lived radionuclide and used in radio-immunotherapy studies to treat human patients with NHL (DeNardo et al., 1997; O'Donnell et al., 1999).

In order to overcome the limited penetration and help mitigate the development of human anti-mouse antibodies (HAMA's), small molecule targeting agents called selective high affinity ligands (SHALs) have been designed to bind to the same HLA-DR epitope recognized by the Lym-1 antibody (Balhorn et al., 2007). These compounds, which have high (nM to pM) affinities for HLA-DR10, are small molecules with molecular weights below 3000 Da. One of these compounds, SH-7139, has been shown to bind selectively and exhibit cytotoxicity *in vitro* only to tumor cells expressing HLA-DR10 (DeNardo et al., 2009). Mouse xenograft studies confirmed that SH-7139 is also cytotoxic to B-cell tumors *in vivo*. In fact, significant (69%) cure rates were achieved using only nanogram doses of SH-7139 (DeNardo et al., 2009).

Characterization of allelic variation in the canine MHC complex class II DRB1 genes has led to the identification of eleven distinct DRB1 alleles (Francino et al., 1997). The proteins produced by these genes are orthologs of the human MHC complex class II DRB1 genes that produce the HLA-DR proteins. In this study we show that several of the canine MHC complex class II alleles identified by Francino et al. (1997) have a structural region that is nearly identical to the epitope of the human MHC complex class II protein HLA-DR10 targeted by the Lym-1 antibody and the SHAL SH-7139. To test the possibility that SH-7139 might be useful for treating canine B-cell NHL, a series of canine NHL biopsies were tested to determine if the Lym-1 epitope was present and whether SHAL SH-7139 would bind to canine NHL cells.

2. Materials and methods

2.1. SHAL SH-7139 synthesis and purification

The tridentate SHAL SH-7139 was synthesized and purified as described previously (DeNardo et al., 2009). Synthesis was performed on a chlorotrityl-chloride resin using Fmoc solid phase chemistry to conjugate PEG monomer units and selected ligands to the alpha and epsilon amines of an N-terminal lysine. The dabsylvaline (Dv) ligand was attached to the terminal amine of a PEG spacer conjugated to the alpha amine of the 2nd lysine residue and a 4-[4-(4-chlorobenzyl)piperazino]-3-nitrobenzenecarboxylic acid (Cb) ligand was attached to the terminal amine of a PEG conjugated to the alpha amine of the 3rd lysine. The 3-(2-([3-chloro-5-trifluoromethyl)-2-pyridinyl]oxy)-anilino-3-oxopropanionic acid (Ct) ligand was then linked to the epsilon amine of the 3rd lysine. The DOTA (1,4,7,10-tetraazacyclododecane-N,N,N,N-tetraacetic acid) and biotinylated derivatives were prepared by attaching a biotin or DOTA chelate (1,4,7,10-tetraazacyclododecane-N,N,N,N-tetraacetic acid) to the epsilon-amino group of the first lysine. The reactions were monitored by analytical high performance liquid chromatography (HPLC), and the DOTA (or biotinylated) derivatives were purified using reverse phase, high performance liquid chromatography (RP-HPLC). Analytic electrospray ionization-mass spectrometry (Agilent 1100 instrument, Waters Symmetry C18 column) was used to confirm the elemental and mass composition of the SHALs. The observed molecular weight was within 0.07% of the theoretical molecular weight.

2.2. Sequence and structure comparison of HLA-DR10 and Cafa-DRB1*11

The predicted 81 amino acid segments of the beta-subunit of the canine MHC-II proteins Cafa-DRB1*11, Cafa-DRB1*Dw1 and Cafa-DRB1*Dw8 were obtained from the transcript sequences of the amplified second exon of Cafa-DRB1 (UniProtKB/TrEMBL Q30426 (Q30426_CANFA)) reported by Francino et al. (1997). These sequences were then aligned with the amino acid sequence of the same exon of the HLA-DR10 beta-subunit to compare the dog sequences with a human ortholog. Using the homology model of HLA-DR10 developed previously (Balhorn et al., 2007), the Cafa-DRB1*Dw1 amino acid residues were then mapped onto the HLA-DR10 model and colored to identify amino acids that are identical (yellow), similar (orange), or different (teal) using the molecular visualization program Pymol (www.pymol.org).

2.3. Tissue biopsies

Canine NHL samples were obtained from archived tissues stored within the UC Davis Comparative Cancer Center. Tissues were originally collected using routine biopsy procedures performed on client-owned pet dogs with NHL that were presented to the UC Davis Veterinary Medical Teaching Hospital. Owner consent was obtained prior to collection of any patient tissues. Formalin fixed, paraffin embedded samples from seven canine patients were sectioned for staining with the Lym-1 antibody and the SHAL SH-7139. H&E stained sections were used to identify tumor type. Immunophenotyping was performed using monoclonal mouse anti-canine CD3 and CD21 antibodies to determine T- and B-cell immunophenotypes, respectively. Normal lymph nodes were obtained from dogs that did not have NHL, but were recently euthanized and presented for necropsy. Owner consent was also obtained prior to collection of necropsy tissues.