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The immune response to disialoganglioside GD3 vaccination in normal dogs: A melanoma surface antigen vaccine

R.J. Milner [a,*], M. Salute [a], C. Crawford [a], J.R. Abbot [b], J. Farese [a]

[a] Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, United States
[b] Department of Pathobiology, College of Veterinary Medicine, University of Florida, United States

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Abstract

As a result of its metastatic potential, canine malignant melanoma like its human counterpart like its human counter part, has a poor response to conventional treatment protocols. This prompted us to investigate the possibility of enhancing the immune response against the melanoma cell surface antigen, disialoganglioside GD3. Initially a flow cytometric study was designed in which the incidence of GD3 on the cell surface, recognized by the monoclonal antibody Mel-1 (R24), was established in canine melanoma cell lines. Results from the flow cytometry found GD3 to be highly expressed (94.2%) in six out of seven canine melanoma cell lines. Since it was thus potentially a good target, a study in which normal dogs were vaccinated intradermally with a vaccine containing GD3 plus adjuvants was designed. The adjuvant included CpG oligodeoxynucleotide (CpG-ODN) sequences and RIBI-adjuvant, which are known to target toll-like receptors (TLR) of the innate immune system. From a cohort of 10 dogs, 4 were vaccinated 3 times, at 4 weekly intervals with GD3 plus adjuvant, and 4 received only RIBI-adjuvant, and 2 phosphate buffered saline. Caliper measurements were collected to assess skin reaction at the vaccination site and sera assayed for IgM and IgG antibodies against GD3 and cell-mediated cytotoxicity against a melanoma cell line. Results from the study found significant differences ($P < 0.05$) in the vaccine site reactions, IgM/IgG levels and cell-mediated cytotoxicity in the vaccinated versus unvaccinated dogs. The addition of CpG-ODN sequences and increasing GD3 concentration in the vaccine increased the inflammation response at the injection site. GD3 IgG and IgM antibodies in vaccinated dogs showed increasing titers over time and achieved significance at weeks 9 and 12, respectively. Cell-mediated cytotoxicity was only detected in peripheral blood mononuclear cells from vaccinated dogs. In conclusion, by combining the tumor antigen GD3 (a known weak self-antigen) and an adjuvant, tolerance was overcome by an innate and adaptive immune response in this population of normal dogs.

Keywords: Ganglioside GD3; C-type lectins; Melanoma; Vaccine; Dogs; Cytotoxicity; Humoral response

1. Introduction

Malignant melanoma is well recognized as a fatal disease with metastases to the regional lymph node, lungs, and brain in both animals and man. Because of poor treatment results and the relentless progression of the disease there is currently extensive research being conducted to find methods to improve survival. These include nonspecific immunomodulation such as α-INF or specific targeting of melanoma tumor antigens in the form of cancer vaccines (Chapman et al., 2004; Kruth, 1998). Selection of potential tumor antigens for vaccines requires careful consideration of the antigen's expression in normal and melanoma

* Corresponding author at: Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, PO Box 100126, Gainesville, FL 32610-0126, United States.
Tel.: +1 352 392 4700x5258; fax: +1 352 392 6125.
E-mail address: milnerr@mail.vetmed.ufl.edu (R.J. Milner).

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tissue, its potential immunogenicity, and its potential to trigger autoimmune disease. A number of melanoma tumor antigens have been characterized and are considered potential vaccine targets, these include disialoganglioside GD3, tyrosinase, gp100 and others (Bergman et al., 2003; Maguire, 1993; Sulaimon et al., 2002; Zhang et al., 1997). The cell surface disialoganglioside (GD3) appears promising as its expression in melanoma cells is at a much higher level than normal cells of neuroectodermal origin (Chapman et al., 2004; He et al., 1989; Nakakuma et al., 1992; Ravindranath et al., 1996; Urmacher et al., 1989). The immunogenicity of GD3 as a vaccine antigen is known for humans, guinea pigs, and mice (Chapman, 2003; Chapman et al., 2004; Helling et al., 1993, 1994; Houghton et al., 1989; Nasi et al., 1997; Ragupathi et al., 2000; Ravindranath et al., 1997; Ritter et al., 1995; Takahashi et al., 1999). In addition, immune response to the GD3 in the form of increased IgM antibodies has been associated with improved survival (Takahashi et al., 1999). While GD3 is regarded as an autoantigen due to its expression in normal tissue, no significant side effects were associated with vaccine studies (Chapman, 2003; Chapman et al., 2004; Helling et al., 1993, 1994; Houghton et al., 1989; Nasi et al., 1997; Ragupathi et al., 2000; Ravindranath et al., 1997; Ritter et al., 1995; Takahashi et al., 1999). Disialoganglioside GD3 has been identified in normal canine and melanoma tissue using the mouse monoclonal antibody Mel-1 (R24) (Bianco et al., 2003; Helfand et al., 1994a,b, 1996, 1999; Soergel et al., 1999; Sulaimon et al., 2002). However, no reports (to the author's knowledge) were found in the literature quantifying GD3 staining in canine melanoma cells and by inference its applicability as a tumor antigen. In addition, no known reports of GD3's immunogenicity were found for the dog. Thus, our long-term goal was to develop a cancer vaccine containing GD3 that could be used in dogs with malignant melanoma and to develop methods to monitor the immune response. As a prelude to deciding on GD3 as the vaccine antigen, a study was designed to quantify the surface expression of GD3 on melanoma cell lines using flow cytometry. Based on these study results and prior reports in the literature, a vaccine was then prepared using GD3 as the antigen. Normal dogs were then vaccinated in a controlled study and their response to the vaccine was monitored. In turn, these results have allowed the investigators to progress to a clinical trial in dogs with melanoma. The results from flow cytometry study and the normal dog trial are reported in this article.

2. Materials and methods

2.1. Identification of GD3 surface antigen in seven canine melanoma cell lines using flow cytometric methods

Seven previously described (Bianco et al., 2003; Dow et al., 1998; Koenig et al., 2002) canine melanoma cell lines (Jenny, Bear, Shadow, CML-2, Scooter, CMGD-5, CMGD-2) and one human melanoma cell line (CRL-1619, ATCC, Manassas, VA, USA) were trypsinized from T75 flasks and counted using trypan blue exclusion dye (Mediatech, Herndon, VA, USA). Live cells (1×10^6) were placed in Falcon #2052 tubes (BD, Franklin Lakes, NJ, USA) and spun down at $250 \times g$. Supernatant was removed and cell pellet mixed with 1 ml PBS wash solution (PBS with 0.5% BSA and 0.1% sodium azide). Samples were then centrifuged, supernatant removed, and cells washed. Primary antibodies Mel-1 (R24) (Signet Lab, Dedham, MA, USA) or negative isotype control (Southern Biotech, Birmingham, AL, USA) at 1–10 mg/ml final concentration, depending on antibody, was added to samples that were then incubated on ice in the dark for 30 min. Cells were again washed in PBS and supernatant removed. Samples were blocked with normal goat serum, washed and then incubated with 2 μg of detection antibody (goat anti-mouse IgG F(ab′)2 conjugated to Alexa Flour 488® 2 mg/ml [Molecular Probes, Eugene, OR, USA]) for 30 min on ice in the dark. Cells were washed and resuspended in 1 ml of PBS wash solution. Samples were read at 10,000 events/tube on FACScan (BD, Franklin Lakes, NJ, USA). Immunofluorescence microscopy (Olympus BX50, 1.3 Mega pixel monochrome, cooled CCD Q-Imaging Retiga 1300B camera) was used to establish the staining distribution of Mel-1 (R24) on the cell surface.

2.2. Animal studies

The animal study was approved by the Institutional Animal Care and Use Committee of the University of Florida. Ten dogs of mixed decent from the University of Florida Animal Care Services were enrolled and housed in approved kenneling. Before dogs were enrolled they were certified free of disease and were not on any medication. The prospective study was designed as a randomly assigned nonblinded control study. A detailed vaccination protocol is given in Table 1. A weekly physical exam was done on all animals and blood was collected from the jugular vein for complete blood counts (CBC) and peripheral blood