Introduction

Limited nonclinical immunotoxicity data are available in the dog, although this is a major non-rodent species in regulatory safety studies.

The aim of the present study was to measure primary anti-KLH IgM and IgG responses in Beagle dogs treated with the reference immunosuppressive drugs cyclosporine and cyclophosphamide, using a dog-specific sandwich ELISA assay jointly developed in-house by CIToxLAB France and Atlanbio.

Materials and methods

Treatments

Groups of 3 male and 3 female Beagle dogs were treated orally for 4 weeks with 2 mg/kg cyclophosphamide daily, or the same volume of drinking water daily. The animals were given one single IM injection of 3 mg KLH on day +11. Blood samples were collected pre-test and on days +18 and +23 to measure anti-KLH IgM, and pre-test and on days +23 and +28 to measure anti-KLH IgG levels using a dog-specific sandwich ELISA assay using a peroxidase-conjugated anti-dog IgM or IgG.

Dog-specific sandwich ELISA development and validation

Three dogs were immunized with 5 mg KLH intramuscularly and their sera obtained 7 and 12 days later were pooled. Specific anti-KLH polyclonal IgM and IgG solutions were prepared from pooled serum samples by KLH-coupled NHS sepharose chromatography and then protein A affinity chromatography. Anti-KLH antibodies were characterized by 2-D gel electrophoresis and quantified for serum samples by KLH-coupled NHS sepharose chromatography and then protein A affinity chromatography. Anti-KLH antibodies were characterized by 2-D gel electrophoresis and quantified for serum samples by KLH-coupled NHS sepharose chromatography and then protein A affinity chromatography. Anti-KLH antibodies were characterized by 2-D gel electrophoresis and quantified for serum samples by KLH-coupled NHS sepharose chromatography and then protein A affinity chromatography. 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