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T-CELL-DEPENDENT ANTIBODY RESPONSE (TDAR)

OVERVIEW

The immune system has been identified as a target organ for adverse effects caused by test material administration. Because of this, the T-Cell-Dependent Antibody Response (TDAR) assay is used to assess potential immunotoxicity and to determine the impact of a test material on the immune response at the preclinical stage of drug discovery. This assay is the gold-standard for studying these effects due to the requirement of multiple immune functions and cell types to generate an antibody response including antigen uptake and presentation, CD4 T cell help, B cell activation and antibody production.

Biomere has validated multiple TDAR models including non-human primates and rodent models.

TDAR ASSAY ANIMAL MODELS AVAILABLE

Non-Human Primates

- KLH-peptide immunization in cynomolgus macaques

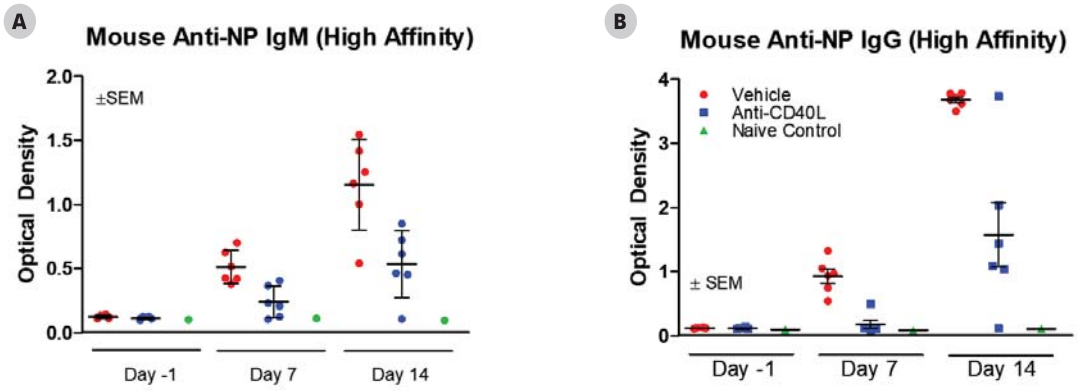
Rodents

- KLH-peptide immunization in C57BL/6 female mice
- OVA-peptide in alhydrogel immunizations in C57BL/6 female mice

TDAR ASSAY READOUTS

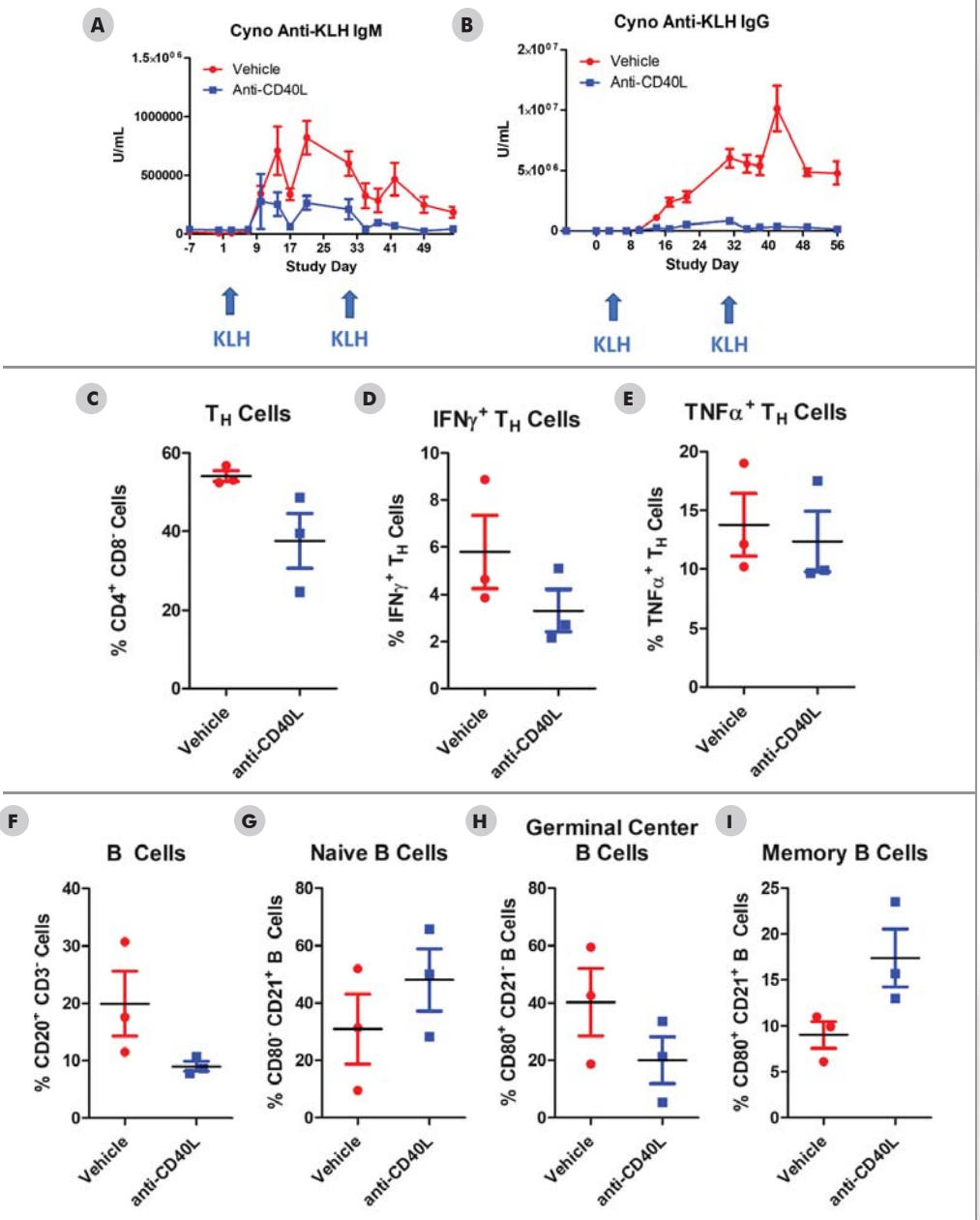
- IgG and IgM serum levels by ELISA
- Immune response analysis by flow cytometry
- Hematology analysis by complete blood count (CBC)

MOUSE T-CELL-DEPENDENT ANTIBODY RESPONSE (TDAR)



Female C57BL/6 mice from JAX were treated with either vehicle or anti-CD154 mAb intraperitoneally (IP) on Day -1. On Day 0, mice were given NP-KLH IP. Serum was collected from mice on Day -1, 7, and 14 and (A) anti-NP IgM and (B) anti-NP IgG ELISAs were performed.

NHP T-CELL-DEPENDENT ANTIBODY RESPONSE



Naïve male and female cynomolgus macaques of Chinese origin were treated with vehicle or anti-CD154 mAb IV on Day 0, 14, and 28. On Day 3 and 31, NHPs received KLH intramuscularly (IM). Serum was collected from animals twice weekly through Day 56 and (A) anti-KLH IgM and (B) anti-KLH IgG ELISAs were performed on serum samples. (C-I) Flow cytometry was performed on blood samples on Day 49. Frequency of (C) $CD4^+$ T_H cells, (D) $IFN\gamma^+$ TH cells, and (E) $TNF\alpha^+$ TH cells. Frequency of (F) $CD20^+$ B cells, (G) Naïve B Cells, (H) Germinal Center B Cells, and (I) Memory B Cells.

SUMMARY

Significant decreases were observed in IgM and IgG antibodies generated in both the mouse and NHPTDAR assays in immune suppressive treatment (α-CD40L) compared to vehicle control. A proportional decrease in germinal center B cells compared to other B cell groups, as well as a decrease in B cells as a total population in α-CD40L versus control were observed. Decreases in the proportion of $CD4^+$ T_H cells were also observed in α-CD40L treated versus control, with a decrease in the proportion of $IFN\gamma^+$ T_H cells. From these results, we can see α-CD40L was able to suppress the generation of antibodies against the target antigen and impacted the T_H cell and germinal center B cell response.