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T-cell-dependent Antibody Responses and Immunopathology: A Comparative Study in Juvenile Cynomolgus Monkeys

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ABSTRACT

The objectives of this study were two-fold: to compare T-cell-dependent antibody responses (TDAR) to keyhole limpet hemocyanin (KLH) in juvenile and mature nonhuman primates (NHPs) and to compare findings of a retrospective analysis of organ microscopic evaluation data in infants of different origins. Based on NHP infant availability, it has become apparent that a better grasp of historical background data sets from different origins is crucial in utilizing this rare resource. This is to ensure adequate options to support research assessing the safety of the range of possible therapies for rare diseases that are growing rapidly, especially in therapeutic products such as gene-, cell- and oligonucleotide-based therapies. Given the high degree of genetic similarities between NHPs and humans, studies for these therapies are often performed in juvenile animals (~9- to 17-month-old) to predict better their safety and efficacy in the human pediatric population. The TDAR in vivo functional assay, with the use of KLH as the immunogen, was completed in Mainland Asia (MA, Cambodia [CA], and China [CH]) and Indonesian (IN) origin animals with analysis for IgG and IgM levels using ELISA. Analyses were conducted by measuring serial dilutions of each sample. Following primary and secondary KLH challenges, robust immune responses were generally evidenced by antibody production in all juvenile animals, with no notable differences between males and females and between different origins. A comparison with data from mature animals indicated the main difference to be in the incidence of response. In juveniles, mean IgG values (µg/mL) following the primary challenge were 2.2 (CH), 2.5 (CA), and 3.1 (IN) on Day 7; 29.4 (CH), 24 (CA), and 15.5 (IN) on Day 14; 45.2 (CH), 38 (CA), and 22.9 (IN) on Day 21. Mean IgM values (µg/mL) following the primary challenge were 95.2 (CH), 85.1 (CA), and 69.2 (IN) on Day 7; 88.1 (CH), 72.5 (CA), and 48.2 (IN) on Day 14; 42.6 (CH), 37.4 (CA), and 26.4 (IN) on Day 21. Background microscopic findings were limited to an increased incidence of mononuclear cell infiltration (7% in CB, 15% in CN, 20% in IN), extramedullary hematopoiesis (8% in CB, 23% in CN, 27% in IN), increase lymphocytes in lymphoid follicles (0% in CB, 15% in CN, 18% in IN), hepatocellular vacuolation (11% in CB, 10% in CN, 2% in IN), mineralization of adrenals (5% in CB, 13% in CN, 10% in IN), and occasional ectopic thymus (0% in CB, 9% in CN, 15% in IN).

MATERIALS AND METHODS

Animal use procedures were approved via protocol by the IACUC.

- **TDAR: Immunogen regimen and blood sample collection for IgG/IgM**
- Primary immunization—prior to KLH intramuscular injection, then7-day intervals to Day 28
  - Secondary immunization—prior to KLH intramuscular injection, then 7-day intervals to Day 57
- **IgG/IgM via ELISA**
  - Anti-KLH IgM/IgG ELISA—a solid phase enzyme-linked immunosorbent assay
- **Flow cytometry (peripheral blood lymphocytes)**
  - T-and B-cells and subsets
- **Macroscopic and Microscopic examination of tissues**

RESULTS—RESPONSES TO KLH IMMUNIZATION:

Antibody trends were similar between origins and age groups

Figure 1. Anti-KLH IgG titer by origin

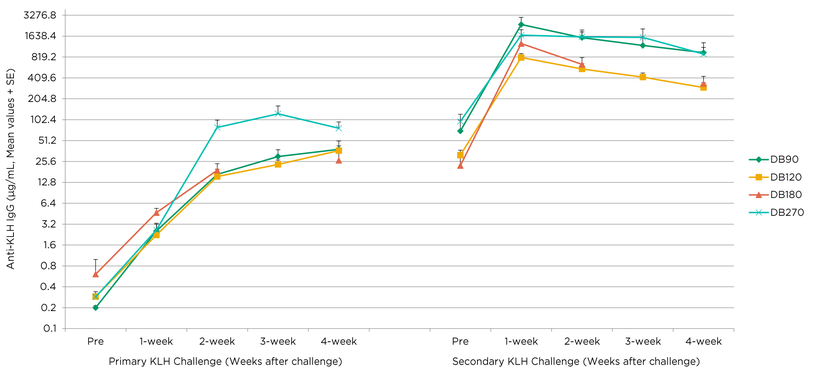


Figure 2. Anti-KLH IgG by age

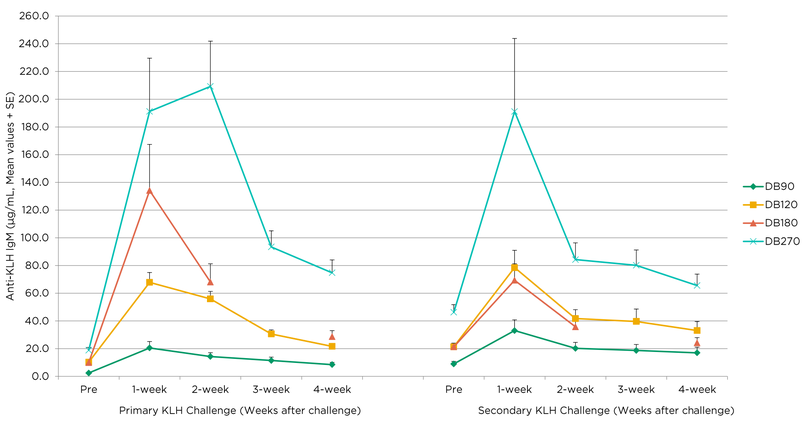
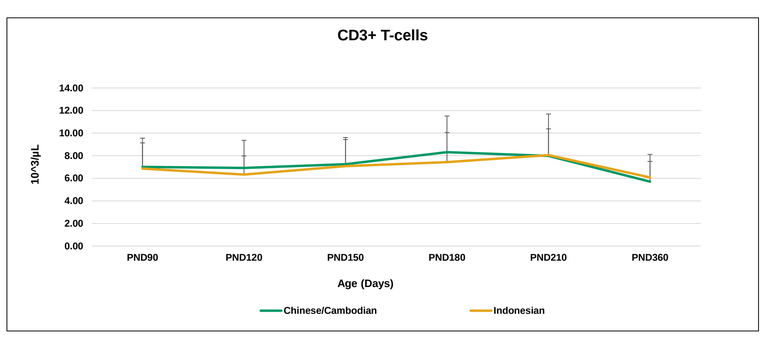


Figure 3. Anti-KLH IgG by age

Peripheral Blood Lymphocyte Populations:

Peripheral blood lymphocyte trends were similar between origins and age groups



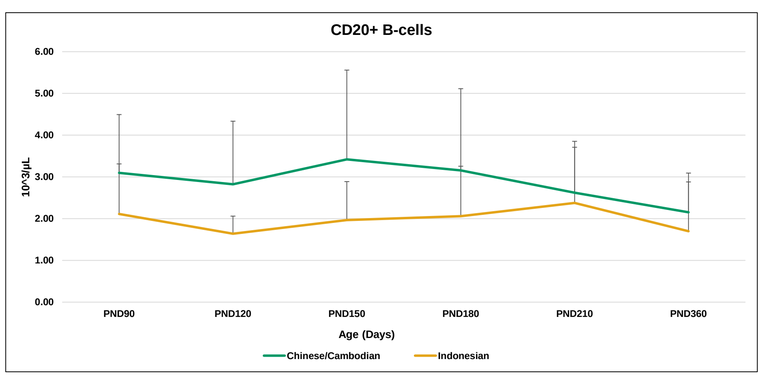


Figure 4. T-cells and B-cells - immunophenotyping by flow cytometry

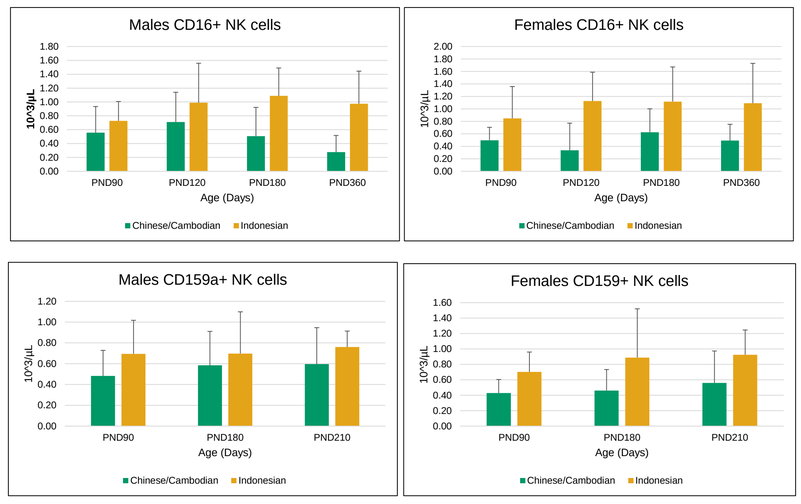


Figure 5. NK cells - immunophenotyping by flow cytometry

Background Microscopic Findings: There were minimal findings noted in control group animals

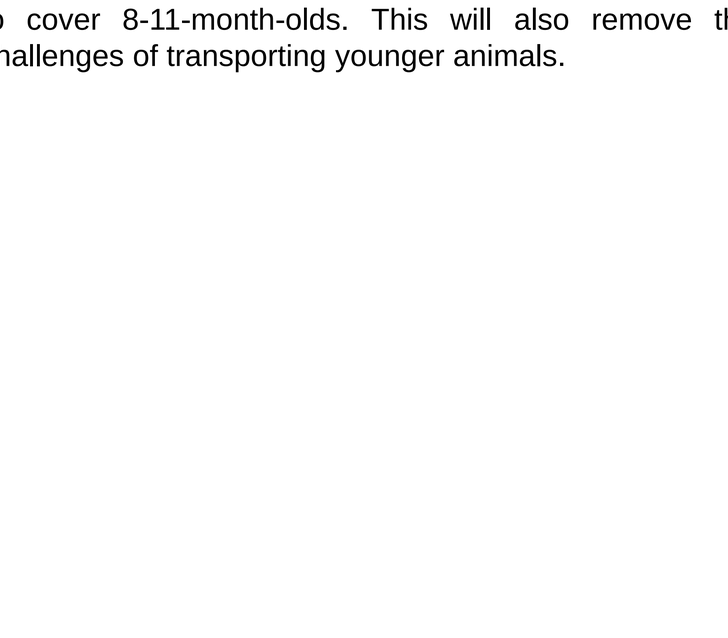


Figure 6. Summary of microscopic findings

CONCLUSIONS

Retrospective data review and comparison in NHPs aged 9-12 months of different origins and ages revealed unremarkable differences in immunological responses to KLH in peripheral blood lymphocyte populations and a few minor histological findings. Therefore, toxicology studies can be conducted in animals 12 months or older to cover 8-11-month-olds. This will also remove the challenges of transporting younger animals.