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Historical Background Data in Juvenile Cynomolgus Monkeys: Comparative Immunotoxicology and Pathology of Different Origins

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ABSTRACT

As the range of possible therapies for rare diseases grows at a rapid rate, advanced therapeutic products such as gene-, cell- and oligonucleotide-based therapies have shown enormous potential, particularly in the treatment of inherited diseases. As such, pediatric patients, in particular, may benefit enormously from these novel therapies. Given the high degree of genetic similarities between nonhuman primates (NHPs) and humans, along with the ability to access anatomic sites for product administration, biodistribution, or analysis, studies for these therapies are often performed in young-aged animals (9- to 14-month-old) to better predict their safety and efficacy in the human pediatric population. This has resulted in limited availability of these young animals, compounded by the overall limited supply of NHPs. It is, therefore, essential to explore alternatives such as the use NHPs of underutilized origins, such as Indonesian animals.

INTRODUCTION

To address the paucity of historical data on juvenile animals of Mainland Asia (MA: Cambodian [CB] and Chinese [CN]) and Indonesian (IN)-origins, a retrospective evaluation of immunology and pathology data was conducted. The T-cell-Dependent Antibody Response (TDAR*) in vivo* functional assay, characterizing the immune response to Keyhole Limpet Hemocyanin (KLH), was completed in MA and IN origins with analysis for IgG and IgM levels. Following primary KLH immunization, a robust immune response was evidenced by antibody production.

MATERIAL AND METHODS

Animals and Animal Care

- Species: Cynomolgus monkeys (*Macaca fascicularis*, purpose-bred; male and female)
- Origin: China, Cambodia, and Indonesia
- Age: up to 7 months of age
- Housing: Infants were co-housed with maternal animals in cages that comply with the Animal Welfare Act and recommendations set forth in "The Guide for the Care and Use of Laboratory Animals".
- Environmental conditions: Controlled temperature, humidity, ventilation, and lighting (12-hour light and dark cycles).
- Handling of animals: Use of a procedure cage.
- Diet and feeding: Infants suckled for the duration of various studies. Occasionally, throughout the study, the infants could have consumed biscuits and treats provided for the maternal animals.
- Dose administration route for KLH (Keyhole Limpet Hemocyanin): subcutaneous injections in the shaved interscapular area of the back once on DBs 120 (initial immunization), 150 (1st boost), and 180 (2nd boost).

List of Abbreviations

- DB: Day of Birth
- Ig: Immunoglobulin
- KLH: Hemocyanin from Keyhole Limpets
- SST: Serum Separator Tube
- ART: Ambient Room Temperature
- Gr: Grams; Kg; Kilograms

MATERIAL AND METHODS (CONT.)

T-Cell-Dependent Antibody Response (TDAR)

- Total immunoglobulin determination (IgA, IgM, and IgG) on DBs 120 (pre-dose with KLH) and 187. Blood samples (up to 0.6 mL) were collected into SSTs from 5 juvenile animals. Serum (approximately 0.3 mL) was obtained, transferred to cryovials, and stored in a freezer set to maintain -80C. Quantitative total immunoglobuin levels for anti-KLH IgG and IgM were determined using an Olympus automated analyzer.

Anti-KLH (IgG and IgM) Antibody Analysis

- Blood (approximately 0.5 to 0.5 mL) was collected at select time points on DBs 120 and 150 (following the initial immunization and the 1st boost). Samples were deposited into additive-free tubes. Samples were allowed to stabilize at ART for approximately 30 to 80 minutes. Serum was obtained and divided into three aliquots. Serum samples were analyzed by a validated ELISA method using the following equipment: SPECTRAmax®PLUS 384 spectrophotometer, SOFTmax®PRO application software, and SkanStacker 300.

Histopathology

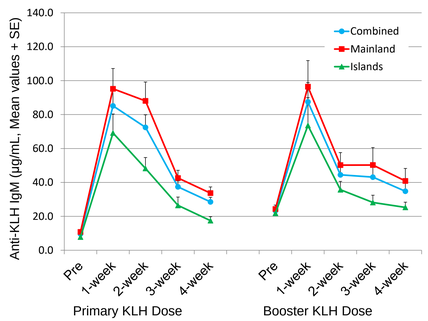
- Animals were fasted overnight (not to exceed 24 hours) prior to termination. Following appropriate blood collection, animals scheduled for necropsy were sedated, weighed, and euthanized by an overdose of euthanasia solution. Animals were subjected to a complete gross examination and tissue collection. Tissues were, subsequently, fixed in appropriate fixative and histopathologically evaluated by a board-certified veterinary pathologist.

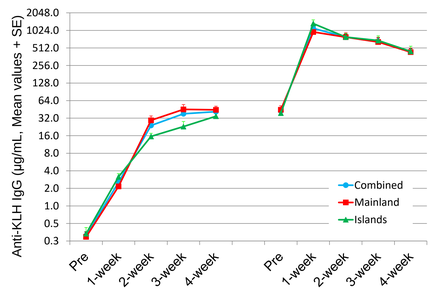
RESULTS

Differences of Anti-KLH IgM and IgG Responses by Animal Origin

- The data are shown in Figure 1 and Table 1.
- IgM elevated rapidly following the primary and booster doses, then equally sharply dropped on subsequent days. The highest IgM in IN (69.2 and 73.6 µg/mL after the primary and booster doses, respectively) was 24-27% lower than MA (95.2 and 96.4 µg/mL).
- IgG elevated gradually after the primary dose but rapidly after the booster dose. The highest IgG after the primary dose in IN (34.5 µg/mL) was 24% lower than MA (45.2 µg/mL). After the booster dose, the highest IgG in IN (1339.7 µg/mL) was 39% higher than MA (941.7 µg/mL).

Figure 1. Anti-KLH IgM and IgG responses in infant NHPs compared by animal origin





RESULTS (CONT.)

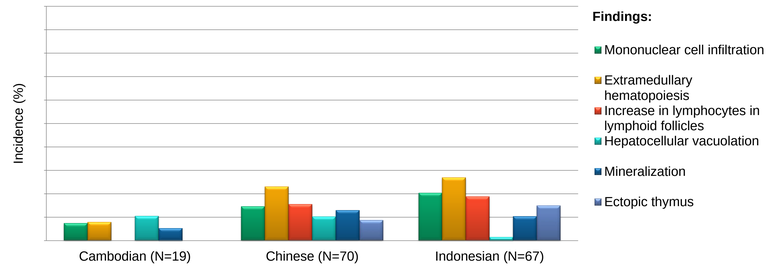
Table 1. Anti-KLH IgM and IgG responses in infant NHPs – the peak values compared to pre-immunization values (by origin)

IgM				
	Pre	Peak		
Origin	(µg/mL)	µg/mL	Week after challenge	x-fold change from pre
Primary KLH Dose				
MA	10.8	95.2	1 week	8.8
IN	7.8	69.2	1 week	8.9
All	9.6	85.1	1 week	8.9
Booster KLH Dose				
MA	24.3	96.4	1 week	4
IN	21.8	73.6	1 week	3.4
All	23.3	87.5	1 week	3.8

IgG				
	Pre	Peak		
Origin	(µg/mL)	µg/mL	Week after challenge	x-fold change from pre
Primary KLH Dose				
MA	0.3	45.2	3 weeks	150.7
IN	0.3	34.5	4 weeks	115
All	0.3	41.3	4 weeks	137.7
Booster KLH Dose				
MA	44.6	961.7	1 week	21.6
IN	39	1339.7	1 week	34.4
All	42.4	1109.7	1 week	26.2

Comparison of Incidence of Select Histopathology Findings by Animal Origin

Figure 2. Percent Incidence of Histopathology Findings by Animal Origin



- Comparison of select histopathology findings is included in Figure 2.
- Histopathology 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).

CONCLUSION

Retrospective review of the data indicates that there were no remarkable differences in antibody response to exogenous antigen (KLH) between animals of different origins. These result are consistent with previously reported results from general toxicology studies using older (1.6 to 7 years old) cynomolgus monkeys[1].

In conclusion, juvenile studies can be successfully conducted using either CB, CN, or IN origin animals without concern for differences in critical immunological response or notable (or non-adverse) microscopic pathology findings.

REFERENCES

1. Lebrec, H., Cowan, L., Lagrou, M., Krejsa, C., Neradilek, M.B., Polissar N.L., Black, L., Bussiere, J., 2011. An inter-laboratory retrospective analysis of immunotoxicological endpoints in nonhuman primates: T-cell-dependent antibody responses, *J. Immunotoxicol.* 8:238-250.