Immunotoxicological Assessment in Juvenile rats

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Abstract

The emergence of novel immunomodulatory therapeutics has brought more attention to the evaluation of potential adverse effects on the immune system, including the developing immune system. The T-dependent antibody response (TDAR) is considered as the main functional test to address immunotoxicity in non-clinical setting; however, assay designs need optimization for use in younger animals.
In this study, Keyhole Limpet Hemocyanin (KLH), selected as the T-cell-dependent antigen, was used to immunize juvenile rats. Cyclophosphamide (CPA) served as a positive control for immunotoxicity evaluation and demonstration of assay design appropriateness. Twenty-two days old rats were injected for 42 days with CPA at 0, 2, 4 or 6 mg/kg/day. Primary and secondary KLH immunizations were given on Days 7 and 35. Anti-KLH antibody response was evaluated by ELISA from serum samples collected prior and following each immunization. Complementarily, immunophenotyping was performed on terminal blood samples to evaluate immunotoxic effects on lymphocyte subsets.
An anti-KLH IgG response was detected following both immunizations, although the secondary challenge produced a more robust response. CPA treatment at 6 mg/kg/day significantly inhibited the anti-KLH response for both sexes on all occasions. Inhibition was also achieved at 4 mg/kg/day only for the secondary response, suggesting that the secondary immunization provides a more sensitive assessment of immunosuppression by CPA. Reduction in T lymphocytes was achieved with CPA at 2 mg/kg/day, and at 4 mg/kg/day for B lymphocytes and NK cells.
The identification of a well-tolerated dose of CPA which induced significant immunosuppression will support future immunotoxicology studies in juvenile rats.

Introduction

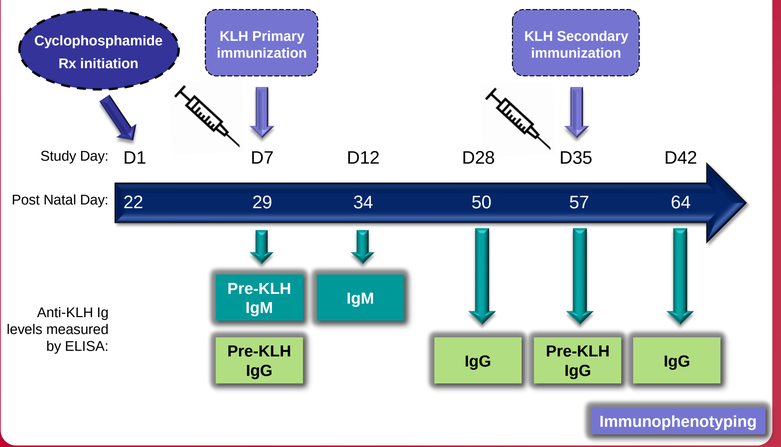
- The continuous emergence of novel immunomodulatory therapeutics has brought more attention in the past decades to the evaluation of potential adverse effects on the immune system and in particular for the developing immune system in the early life stages.
- A functional evaluation, such as the T-dependent antibody response is considered as most appropriate to address immunotoxicity in non-clinical setting[1].
- These assays need to be optimized for use in younger animals.

The objectives of this study were to evaluate the primary and secondary T-dependant anti-KLH antibody response and perform immunophenotypic analysis in immunocompetent or immunosuppressed juvenile Sprague-Dawley rats for subsequent use in regulatory immunotoxicity studies.

Experimental Design

Table 1. Experimental design	
Species	Sprague-Dawley rats
Sex	Males and Females
Age	22 days old at treatment initiation
No. of animals	10/ sex/ group
Rx with Cyclophosphamide	
•Route	Intraperitoneal
•Frequency	Daily
•Duration	42 Days
•Doses	0, 2, 4 or 6 mg/kg/day
Immunization with KLH	
•Primary	7 days post CPA initiation, 100 µg/animal
•Secondary	35 days post CPA initiation, 100 µg/animal
Blood collection	
•Anti-KLH IgM	Day 7 (pre-KLH) and Day 12
•Anti-KLH IgG	Day 7 (pre-KLH), Day 28, Day 35 (pre-KLH and Day 42 (termination)
•Immunophenotyping	Day 42 (termination)

Figure 1. Study Design Overview



Results

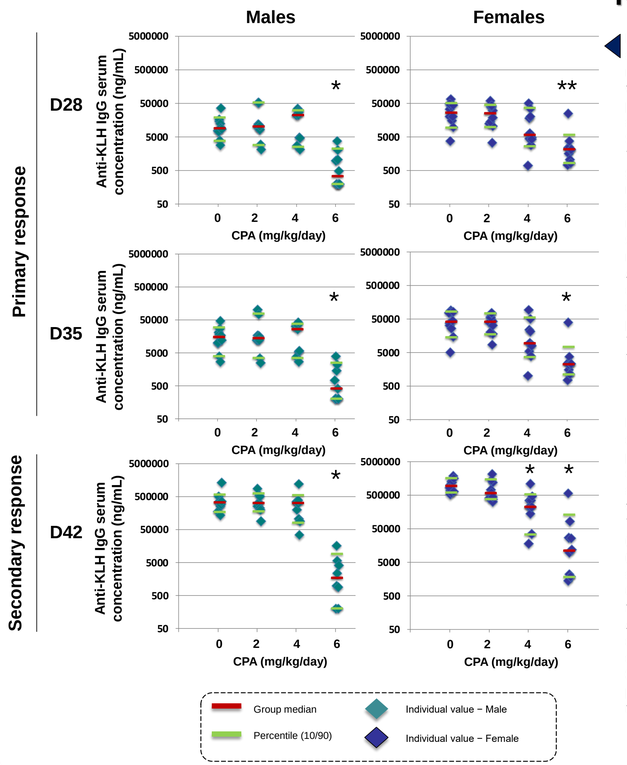
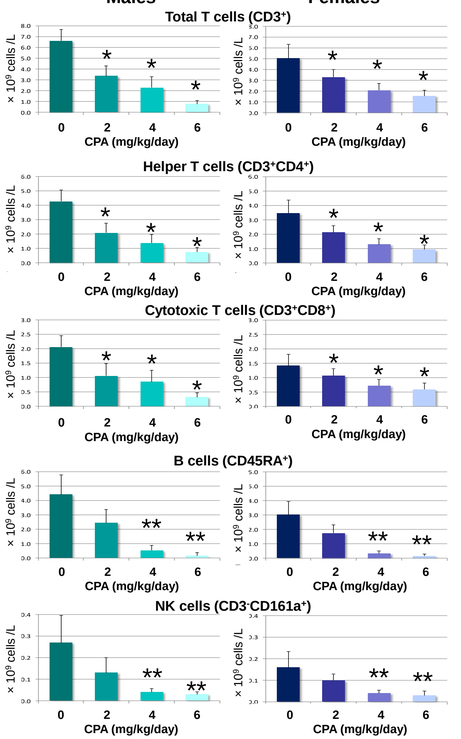


Figure 2. Primary and secondary anti-KLH IgG responses measured by ELISA. Individual values, median and 10th/90th percentile from males (left panel) and females (right panel) Sprague-Dawley rats are shown. Top-most graphs and middle graphs show the primary response obtained on Day 28 and 35, respectively. Bottom-most graphs show the secondary response as measured on Day 42.
Asterisks indicate significance compared with the same-day control group values (i.e.* p<0.05 (one-sided Dunn's test); ** p<0.05 (one-sided Dunnett's test)).

Figure 3. Absolute lymphocyte counts obtained from a dual platform method of flow cytometry and hematology analysis. The mean absolute value (× 10^9 cells/L) for the total T cells (CD3+), helper T cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), B cells (CD45RA+) and Natural Killer cells (CD3-CD161a+) from Day 42 is shown for each CPA treated group.
Asterisks indicate significance compared with the same-day control group values (i.e.* p < 0.05 (one-sided Dunn's test); ** p < 0.05 (one-sided Dunnett's test)).



Conclusions

- A uniform T-dependent antibody primary response was obtained from juvenile rats 21 days following a single KLH immunization.
- The secondary challenge produced a more robust antibody response than the primary challenge.
- No or low measurable IgM in all samples from Day 12.
- A reduction in T lymphocytes was achieved with CPA at 2 mg/kg/day, and at 4 mg/kg/day for B lymphocytes and NK cells
- A well-tolerated dose of cyclophosphamide which induced statistically significant immunosuppression was identified.
- The study design will support future immunotoxicology studies in juvenile rats.

Reference

1. Holsdapple, M. P. and O'Lone Raegan. 2012. Juvenile Immunotoxicology. *Toxicologic Pathology,* 40: 248-254.