Immunotoxicological Assessment in Juvenile rats

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Abstract

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; however these need to be optimized for use in younger animals.

In this study we evaluated the primary and secondary T-dependant antibody response (TDAR) to the Keyhole limpet hemocyanin (KLH) in immunocompetent or immunosuppressed juvenile Sprague-Dawley rats.

Male and female juvenile rats were dosed by intraperitoneal injection for 42 consecutive days with 0, 2, 4 or 6 mg/kg/day Cyclophosphamide (CPA) starting from postnatal day (PND) 22. All rats were given both a primary immunization and a secondary challenge with KLH on Days 7 and 35 following the first CPA administration. Blood samples were collected from each animal on Days 7 (pre-KLH), 12, 28, 35 (pre-KLH) and 42. The anti-KLH Immunoglobulin (Ig) M response was evaluated from samples taken prior to the first KLH administration (Day 7) and on Day 12 whereas the anti-KLH IgG levels were measured prior to KLH immunization on Day 7 and on Days 28, 35 (primary response) and 42 (secondary response). Ig levels in serum samples were measured by ELISA.

A primary anti-KLH IgG response was measured in all the animals. Greater IgG levels were observed on Day 42 following the secondary challenge with KLH. CPA treatment at 6 mg/kg/day induced a significant inhibition of the anti-KLH IgG response at all tested occasions for both sexes. Statistically significant inhibition was achieved at a lower dose level (4 mg/kg/day) only for the secondary response in the females. This suggests that the secondary immunization provides a more sensitive assessment of immunosuppression by CPA. Anti-KLH IgM response was not detected in the vast majority of animals, thus, the effect of CPA administration on the IgM response could not be assessed.

While 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. The identification of a well-tolerated dose of cyclophosphamide which induced statistically significant immunosuppression will support future immunotoxicology studies in juvenile rats.

Introduction

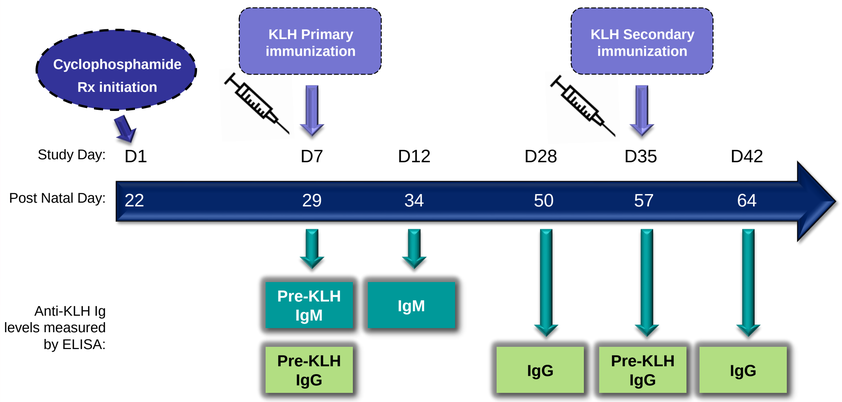
- The continuous emergence of novel immunomodulatory therapeutics has brought more attention in the past decades to the evaluation of potential adverse effects on the immune system and in particular for the developing immune system in the early life stages.
- A functional evaluation, such as the T-dependent 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 objective of this study was to evaluated the primary and secondary T-dependant antibody response KLH 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
•Secondary	35 days post CPA initiation
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)

Figure 1. Study Design Overview



Results

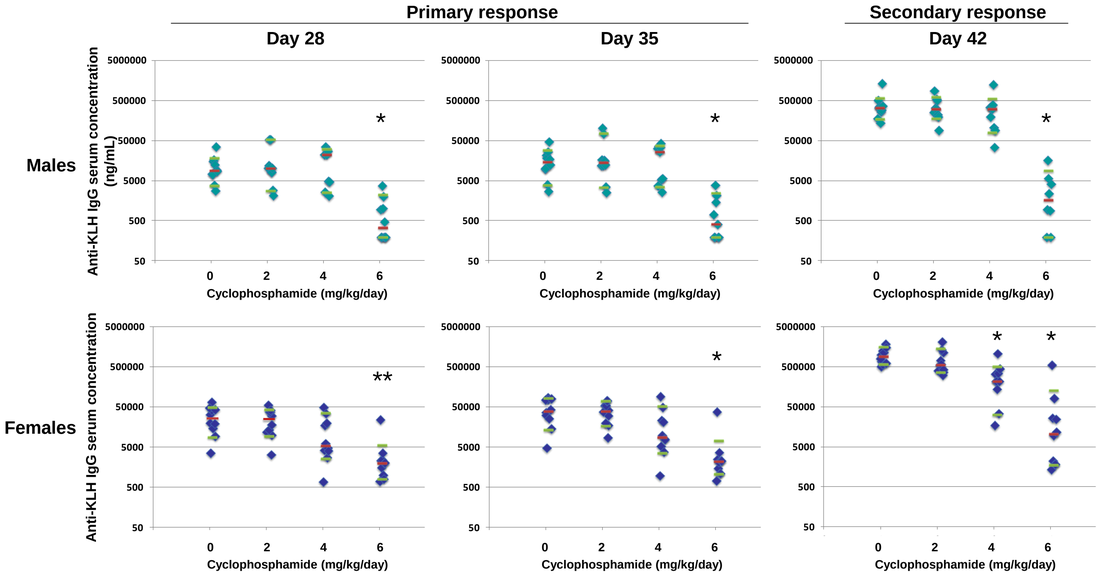


Figure 2. Primary and secondary anti-KLH IgG responses measured by ELISA. Individual values, median and 10th/90th percentile from males (top panel) and females (bottom panel) Sprague-Dawley rats are shown. Left-most graphs and middle graphs show the primary response obtained on Day 28 and 35, respectively. Right-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)).

Conclusions

- Uniform T-dependent antibody primary response was obtained from juvenile rats 21 days following a single KLH immunization.
- Secondary challenge produced a more robust antibody response than the primary challenge.
- Greater IgG response observed in the females compared to the males.
- No or low measurable IgM in all samples from Day 12.
- 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.