The continuous emergence of novel immunomodulatory therapies 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-Dependent antibody response (TDAR) to the Keyhole limpet hemocyanin (KLH) in immunocompetent juvenile Sprague-Dawley rats. Male and female juvenile rats were dosed by intraperitoneal injection for 42 consecutive days with 2, 4, 6 or 8 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 sex on Day 7, 35 and 42. The primary 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). IgG levels in serum samples were measured by ELISA. A primary anti-KLH IgG response was measured in all animals. Greater IgG levels were observed on Day 35 following the secondary challenge with KLH as compared to the primary response. The secondary response was statistically significant in males at the lower dose level of 4 mg/kg/day. The secondary response was not statistically significant in females, which induced statistically significant anti-KLH IgG response at all tested occasions for both sexes. Statistically significant inhibition was achieved at a lower dose level (2 mg/kg/day) only for the secondary response in the females. This suggests that the secondary immunization provides a more sensitive assessment of immunotoxicity by CPA. Anti-KLH IgG response was not detected in the vast majority of animals, thus, the effect of CPA administration on the IgG response could not be assessed. While a uniform T-dependent antibody primary response was observed from juvenile rats 21 days following a single CPA administration the secondary response was only observed at 42 days post Pascal immunization.

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.
- A well-tolerated dose of cyclophosphamide which induced statistically significant immunosuppression was identified.
- The study design will support future immunotoxicology studies in juvenile rats.

Abstract

The objective of this study was to evaluate the primary and secondary T-dependent antibody response (TDAR) in immunocompetent or immunosuppressed juvenile Sprague-Dawley rats for subsequent use in regulatory immunotoxicity studies.

The continuous emergence of novel immunomodulatory therapies 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.

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