TDAR and splenic lymphocyte subpopulation analysis in extended one-generation reproductive toxicity studies (OECD 443).


1 INTRODUCTION

OECD test guideline 443 provides a detailed description of the objectives and procedures of the Extended One-Generation Reproductive Toxicity Study (OECDRTS). The guideline describes three cohorts of F1 animals covering different developmental and reproductive endpoints. Among them, Cohort 3 is designed to assess the potential impact of chemical exposure on the developing immune system.

Over the last 3 years Citoxlab has performed a series of OECD 443 studies in Sprague-Dawley (SD) rats. Herein we share background control data on the TDAR and splenic lymphocyte subpopulation analysis in this species. TDAR is assessed by immunization of all Cohort 3 animals with KLH as a model antigen on Day 56 post-partum. Anti-KLH IgM concentrations in serum are measured before and 5 days after immunization as a quantitative validated ELISA (Ref: KLHM-2; Life Diagnostics). In which wells of a 96-well plate are coated with KLH antigens. Anti-KLH IgM present in the tested serum binds to the KLH coating and is revealed by a peroxidase conjugated anti-rat IgM for detection.

2 MATERIALS AND METHODS

On Day 56 (± 3) p.p., 0.2 ml of KLH solution at 1.5 mg/mL was administered to all Cohort 3 animals by the subcutaneous route (dorsal area, between the shoulders) using a sterile plastic syringe fitted with a single-use sterile needle. The KLH injections were performed before dosing.

The assay for the determination of anti-KLH IgM concentrations in rat serum is a quantitative validated ELISA (Ref: KLHM-2, Life Diagnostics) in which wells of a 96-well plate are coated with KLH antigens. Anti-KLH IgM present in the tested serum binds to the KLH coating and is revealed by a peroxidase conjugated anti-rat IgM for detection.

For investigation of pre- and post-natally induced immunotoxic effects, one half of the spleen from 10 male and 10 female Cohort 1A animals was used for splenic lymphocyte subpopulation analysis. The flow cytometry method was previously validated. Briefly, after necropsy, spleen tissues from animals were manually dissociated in a cell strainer using a piston syringe in order to obtain cellular suspensions. Debris were discarded from the cell suspension by successive washing/confinement steps in PBS + 1% FBS. Concentration and viability of each cell suspension were determined using a NucleoCounter (NC100, ChemoMetec). Then, 2x10⁶ cells were re-suspended at 10 x 10⁶ live cells/mL, in presence of Fc receptor blocking solution in order to obtain a Fc receptor blocking solution. Then, stained for immunophenotyping using a combination of six specific antibodies directed against membrane markers (CD3, CD4, CD8; CD45RA and CD69). After staining, red blood cells were lysed using a lysis/wash protocol (CAL-lys Wash Buffer). The following lymphocyte subpopulations were assessed by flow cytometry on a MACSQuant Analyzer 10 (Miltenyi Biotec) with the following panels:

3 RESULTS

Figure 1. Anti-KLH IgM concentration measurements before and 5 days after KLH immunization. Mean and SD of anti-KLH IgM (µg/mL) - results from 9 male and 10 female Sprague-Dawley rats from cohort 3 (control group) of an OECD 443 reproductive toxicology study. P.P = Post-Partum. KLH immunization performed on day 56 post-partum.

Figure 2. Representative example of plots for gating strategy of rat splenocyte subset immunophenotyping.

Figure 3. Inter-individual variability of rat splenocyte subset immunophenotyping. Mean and SD of percentages from related parental population - results from 9 male and 10 female Sprague-Dawley rats from cohort 1A (control group) of an OECD 443 reproductive toxicology study.

4 CONCLUSION

Before KLH immunization, samples displayed quantifiable concentrations of anti-KLH IgM. This result can be explained by the fact that rat serum may contain natural antibodies directed against shared epitopes, primarily polysaccharides (Fenco-Kent and Kawabata, 2005; Picotti et al., 2005; Korver et al., 1994). After KLH immunization, increased anti-KLH IgM concentration was observed when compared to levels before immunization.

In conclusion, these methods have been validated and successfully used at Citoxlab for assessment of the impact of chemical exposure on the developing immune system in the framework of OECD 443 studies.