Immunoglobulin Levels in Rats Immunized with Keyhole Limpet Hemocyanin Following Diet Restriction and Comparison of Responses in Rats Immunized with Sheep Red Blood Cells

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1 ABSTRACT

During repeated dose toxicology studies in nonclinical species, low or no food consumption can contribute to toxicity. There is a paucity of data correlating restricted feed or low food consumption to immune responses. Common endpoints to measure immunotoxicity include clinical pathology, immunophenotyping of leukocytes, T cell dependent antibody response (TDAR), and histopathology. In the current study, we present TDAR response data following Keyhole Limpet Hemocyanin (KLH) administration in feed restricted rats, and compare immunological responses following sheep Red Blood Cells (sRBC) versus KLH administration to immunocompromised rats.

The first experiment included six cohorts of five rats each. Cohort 1 was fed ad libitum and administered de-ionized water (DI). Cohort 2 was fed ad libitum, administered DI, and immunized with KLH on study days 8 and 15. Cohort 3 was fed restricted diet (16 grams of Rodent LabDiet® 5002 pellets/day/animal). Cohort 4 was fed restricted diet and immunized with KLH. Cohort 5 was administered once daily oral administration of Cyclophosphamide monohydrate (CPS, immunosuppressant) for 5 days and immunized with KLH. Cohort 6 was CPS-treated positive control. In the second experiment 10 rats were immunized once with 1x10^8 sRBC by intravenous injection, followed by once daily oral administration of 5 rats each with either DI or CPS for 5 days. Experiment 1 serum samples were analyzed for anti-KLH IgG/IgM prior to and following KLH immunizations. Samples from experiment 2 collected at necropsy were analyzed for anti-sRBC IgG/IgM.

The anti-KLH IgM/IgG responses in experiment 1 were suppressed in CPS treated rats, compared to both DI and rats placed on restricted feeding. However, the restricted feed rats showed a more robust response compared to the DI rats with increased anti-KLH IgM in the restricted feed cohort (mean 7931 ng/mL on day 13, 19991 ng/mL on day 15) compared to the fed ad libitum cohort (mean 5714 ng/mL day 13, 9134 ng/mL day 15). The mean IgM and IgG concentrations in rats immunized with sRBC were 35364 and 2379 U/mL, respectively, as opposed to 1735 U/mL IgM and undetectable levels of IgG in rats immunized with sRBC and treated with CPS. The delay and/or suppression of the immune response correlated with lymphoid organ cell depletion and lower spleen weights. CPS+sRBC rats had lymphoid depletion in all four lymphoid organs examined, while KLH+CPS caused lymphoid depletion in spleen. This finding may have implications for the choice of immunogen when designing studies with a T-cell dependent antigen response arm in rats. In conclusion, feed restriction and in extension low food consumption in toxicology studies does not appear to interfere with a normal immune response.

2 MATERIALS AND METHODS

Forty Sprague Dawley rats (Crl: CD), 13-15 weeks of age, 290 grams average weight were used on this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee.

Experiment 1: Animals were divided into 5 cohorts, each with 5 animals as follows:
Cohort 1 – Fed ad libitum, administered deionized water (DI) by oral gavage
Cohort 2 – Fed ad libitum, administered DI, immunized by intramuscular injection of KLH (Thermo Fisher Scientific, Product No. NCI77600B)
Cohort 3 – Restricted diet
Cohort 4 – Restricted diet, immunized with KLH
Cohort 5 – Fed ad libitum, administered cyclophosphamide (Sigma, Cat. No. C-0768) at 15 mg.kg/day by oral gavage for 5 days, and immunized with KLH
Cohort 6 – Administered cyclophosphamide by oral gavage for 5 days
Experiment 2: Animals were divided into 2 cohorts, each with 5 animals as follows:
Cohort 1sRBC – Fed ad libitum, immunized once by intravenous injection of sRBC (Innovative Research, Cat. No. IC100-0210), administered DI for 5 days
Cohort 2sRBC – Fed ad libitum, immunized once with sRBC, administered cyclophosphamide for 5 days
Anti-KLH and anti-sRBC IgG/IgM analysis by ELISA (Life Diagnostics Cat. SRBCG-2 and SRBCM-2; in-house custom made for KLH**)**
Experiment 1: Analysis on serum samples collected prior to (anti-KLH IgG/IgM) and following (anti-KLH IgG) KLH immunizations on study days 11, 13, 15, 18, 20 and 22.
Experiment 2: anti-sRBC IgG/IgM analysis on serum samples collected once prior to necropsy

Other parameters evaluated, all experiments:
Body weights; food consumption; hematology (differential and leukocyte count); lymphoid organ weight and histology; Immunophenotyping (data presented previously) by flow cytometry [peripheral blood, bone marrow, spleen, and thymus for T lymphocytes (CD3) and subsets (CD4, CD8) and B cells (CD45RA)]. Tissues were gated using live, dead and pan leukocyte marker (CD45). Blood was gated on live cells and pan leukocyte marker.

3 RESULTS

As shown in Figure 1, anti-sRBC IgM showed a weak response while IgG was inhibited in cyclophosphamide treated rats compared to DI rats.

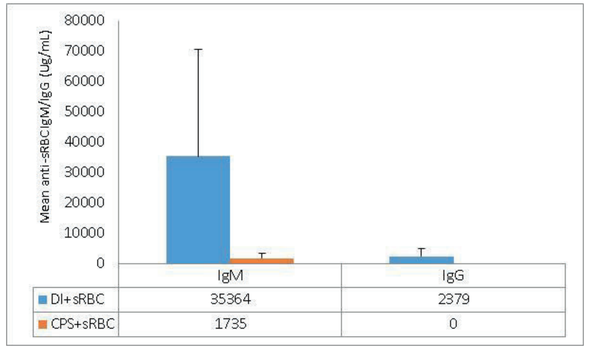

Figure 1. anti-sRBC IgM responses in rats

As shown in Figure 2, anti-KLH IgM response was inhibited on Day 11 in CPS rats, showed a weak recovery by Day 13 and had a stronger response by Day 15. Feed restricted rats had a more robust response on Days 13 and 15 compared to rats fed ad libitum.

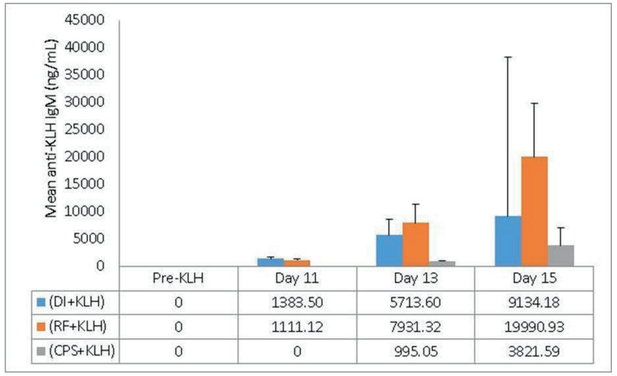

Figure 2. anti-KLH IgM responses in rats

4 RESULTS (Continued)

As shown in Figures 3a, a primary response was not apparent by Day 11, a single animal in the DI cohort had a response on Day 13, and 1 animal each in the DI and RF cohorts had a response on Day 15. Primary response detection in the CPS cohort was below limit of detection. In Figure 3b (secondary response) anti-KLH IgG response was inhibited on Days 18, 21 and 22 in CPS rats. Feed restricted rats showed a relatively more robust response compared to rats fed ad libitum.

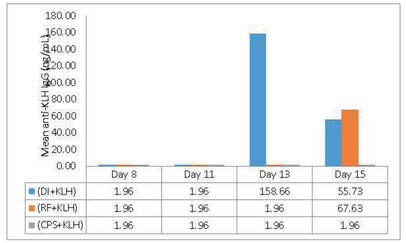

Figure 3a. anti-KLH IgG primary response

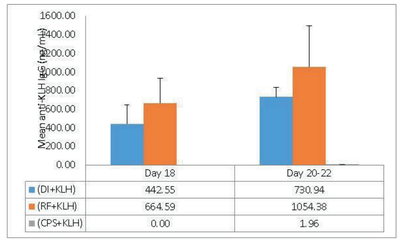

Figure 3b. anti-KLH IgG secondary response

Low splenic and thymus weights (Figure 4) were noted in CPS and sRBC (or KLH) groups but not in DI and sRBC or KLH groups. Histopathology (Table 1): lymphoid depletion in spleen (decreased number of lymphocytes, marginal zone lymphoid follicles), thymus (with atrophy of cortex that lacked a clear separation with the medulla), lymph nodes and bone marrow. Findings correlated with lower organ weights in CPS and sRBC (or KLH) groups.

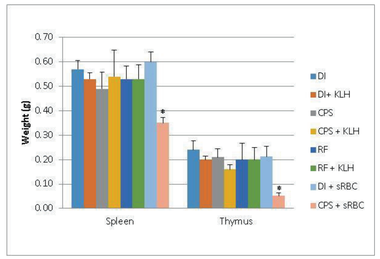

Figure 4. Organ weights changes

	CPS+sRBC	CPS	CPS + KLH
Spleen (N=5)			
Depletion, lymphoid	5	4	5
Mild	3	2	1
Moderate	2	2	4
Thymus (N=5)			
Depletion, lymphoid	5	0	0
Marked	5	0	0
LN, Mesenteric (N=5)			
Depletion, lymphoid	5	0	0
Mild	2	0	0
Moderate	3	0	0
LN, Axillary (N=5)			
Depletion, lymphoid	5	0	0
Mild	1	0	0
Moderate	4	0	0
Bone Marrow, Femur (N=5)			
Hypocellular	5	0	0
Mild	2	0	0
Moderate	3	0	0

Table 1. Histopathology findings

5 CONCLUSIONS

In rats fed ad libitum and administered DI, and in rats with feed restriction there was no effect on immunological responses following challenge with KLH, with the feed restricted rats showing overall more robust anti-KLH IgM and IgG responses compared to the rats fed ad libitum. These results add to previous findings in which rats treated with cyclophosphamide and sRBCs had lymphoid depletion in several lymphoid organs, while KLH +CPS caused lymphoid depletion in the spleen only. Overall, immunization with sRBC or KLH and food restriction is unlikely to cause effects that can interfere with immunotoxicology or other data interpretation in routinely monitored parameters in toxicology studies in rats.