T-CELL DEPENDENT ANTIBODY RESPONSES IN THE RAT: FORMS AND SOURCES OF KEYHOLE LIMPET HEMOCYANIN (KLH) MATTER

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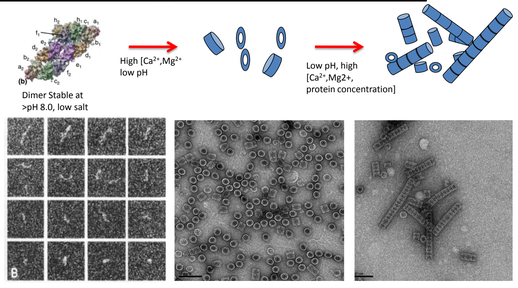
Abstract

The T-cell dependent antibody response (TDAR) is a functional assay used in immunopharmacology and immunotoxicology to assess the ability to mount an antibody (IgM and/or IgG) response to immunization. Keyhole limpet hemocyanin (KLH) is extensively used as immunogen of choice both in nonclinical and clinical settings. Native KLH is comprised of high molecular weight (HMW) assemblies (>600-800 kDa) of KLH subunit dimers. It is not known how the different forms (HMW vs. subunit) and manufacturing processes (commercial sources) may impact the nature of anti-KLH immune responses (e.g., magnitude and inter-animal variability). Anti-KLH IgM and IgG responses were studied in female Sprague-Dawley rats immunized on days 1 and 22 with 100 mg of HMW KLH from two different sources or subunit KLHs from three different sources without co-injection with any adjuvant. Dose analysis and biophysical characterization of KLH formulations were conducted. Anti-KLH IgM and IgG responses were measured on days 1, 8, 15, 22, 29, 36, and 43 using a proprietary indirect electrochemiluminescence immunoassay. The two HMW KLH preparations showed a greater number of subvisible particles (2-150 µm size range) than the three subunit KLHs. All HMW KLHs and all subunit KLHs were equivalent on SEC (hydrodynamic volume), PAGE (size and charge) and SDS-PAGE (molecular radius). Robust primary and secondary anti-KLH IgG and IgM responses were detected for both sources of HMW KLH. The subunit KLH immunizations resulted in lower IgG and IgM responses, with the exception of Stellar Biotechnologies subunit KLH which produced a robust secondary response. Inter-animal variability for IgM and IgG responses was lower with HMW KLH than with subunit KLHs. In conclusion, different forms and sources of KLH are associated with different magnitudes and inter-animal variabilities in IgM and IgG responses, a critical finding to take into consideration when designing TDAR studies for proper immunotoxicology or immunopharmacology testing.

Introduction

- TDAR = T-cell Dependent Antibody Response, immunotoxicology functional assay
- KLH = large glycoprotein complex extensively used as TDAR antigen in pharmaceutical industry
  - High Molecular Weight (HMW, 4 - 8 MDa) assemblies of KLH Subunit dimers (>600 - 800 kDa)
- Unknown impact of different KLH forms (HMW vs Subunit) and manufacturing processes (commercial sources) on TDAR performance

Electron Microscopy of Subunit and HMW KLH



Dimeric subunit KLH | Decamer and didecamer | Multidecamer

Assembly is controlled by cation- & salt-concentration and pH; accelerated by electrohydrodynamic mixing

Sohngen et al. Eur. J. Biochemistry 248: 602-614, 1997

Objectives

- Overall aim: optimize TDAR assay
- Specific study objectives: evaluate antibody response to different forms and sources of KLH using an Indirect Immunoassay
  - Magnitude of anti-KLH IgM and IgG responses
  - Inter-animal variability of anti-KLH IgM and IgG responses

Test Articles

Identification	Grade	Physical Form and concentration	Formulation Procedure
Stellar Biotechnologies HMW KLH	GMP	Solution in D-PBS 1 mg/mL	No dilution or preparation required
Stellar Biotechnologies KLH Subunit	GMP	Solution in water 20 mg/mL	Dilute in water for injection
Biosyn KLH Subunit (VACMUNE®)	GMP	Solution in water 20 mg/mL	Dilute in water for injection
Pierce KLH Subunit (Imject®)	Research	Solution in water 20 mg/mL	Dilute in water for injection
Pierce HMW KLH (Imject®)	Research	Lyophilized in PBS 20 mg units	Reconstitute in water for injection and dilute in PBS

All test articles administered without co-injection of an adjuvant

Study Design

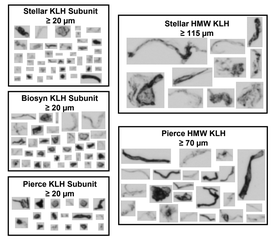
Group	No Animals [a]	Route / Frequency	Test Article	Dose (ug)	Dose vol. (uL)	Dose conc. (mg/mL)
1	10	SC / D1&22	Stellar Biotechnologies HMW KLH	100	100	1
2	10	SC / D1&22	Stellar Biotechnologies KLH Subunit	100	100	1
3	10	SC / D1&22	Biosyn KLH Subunit (VACMUNE®)	100	100	1
4	10	SC / D1&22	Pierce Imject® KLH Subunit	100	100	1
5	10	SC / D1&22	Pierce Imject® HMW KLH	100	100	1

[a] Female Sprague-Dawley rats, approximately 11 weeks of age at first dose, were cared for in accordance to the *Guide for the Care and Use of Laboratory Animals*, 8th Edition (2011). Animals were pair-housed at an AAALAC, Intl- accredited facility in non-sterile ventilated micro-isolator housing on corn cob bedding. All research protocols were approved by the Amgen Inc. (Seattle, Washington) Institutional Animal Care and Use Committee. Animals had *ad libitum* access to pelleted feed (2020X Teklad; Harlan Laboratories Inc., Madison Wisconsin) and water (reverse osmosis-purified) via automatic watering system or water bottle. Animals were maintained on a 12:12 hr light: dark cycle in rooms at 72°F ± 5°F and 30% to 70% relative humidity and had access to enrichment opportunities (enrichment tubes and Nylabones®). All animals were determined specific pathogen free.

Biophysical and Biochemical Characterization of KLH

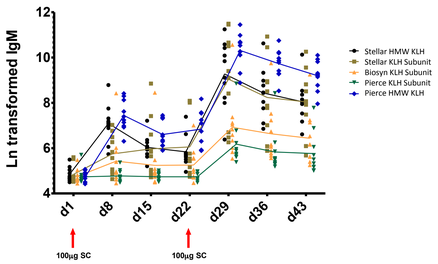
- Largest number and size of aggregates in HMW KLHs

KLH samples	Particle numbers / mL
	2-150 µm
Stellar HMW KLH	46633 ± 1506
Stellar KLH Subunit	2181 ± 53
Biosyn KLH Subunit	2983 ± 243
Pierce KLH Subunit	1921 ± 145
Pierce HMW KLH	26629 ± 594



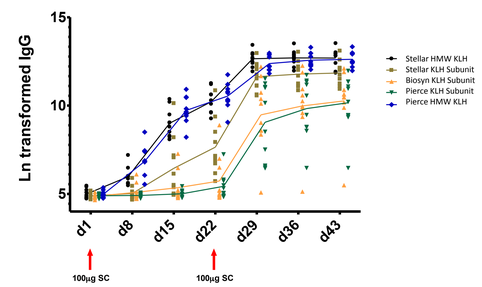
- All test articles are equivalent for biochemical analysis
  - ➢ KLH1 and KLH2 isoforms present

Anti-KLH IgM response



- ➢ Greater response with HMW KLHs and Stellar KLH Subunit (after d22 for the latter)
- ➢ Greater response with Stellar KLH Subunit as compared to other KLH subunits
- ➢ Detection of primary and secondary IgM responses

Anti-KLH IgG response



- ➢ Greater response with HMW KLHs overall
- ➢ Greater response with Stellar KLH Subunit as compared to other subunits

Inter-animal variability for primary and secondary IgM and IgG AUCs

Anti-KLH AUC %CV		Pierce HMW KLH	Stellar HMW KLH	Stellar KLH subunit	Biosyn KLH subunit	Pierce KLH subunit
Primary response	IgM	56	79	167	138	23
	IgG	85	81	273	105	30
Secondary response	IgM	194	194	194	194	194
	IgG	44	50	81	582	406

Similar

Subunit more variable

Very variable

Low CV but very low responses

Conclusions

- KLH is a valuable neoantigen for TDAR
- KLH form and source impact magnitude and variability of antibody responses
- Next steps
  - Understanding sensitivity of response to different forms of KLH to immunomodulation
  - Harmonization of nonclinical / clinical reagents for translational purposes