

Abstract

Keyhole Limpet Hemocyanin (KLH) is a heavily glycosylated protein that can exist as 8MDa cylinders composed of 20 copies (dodecamer) of a 400kDa subunit, or as separate subunits. KLH is a well-established immune stimulant, hapten carrier and immunotherapy vaccine component. However, literature reports cite dramatically different immune responses to different preparations of KLH.

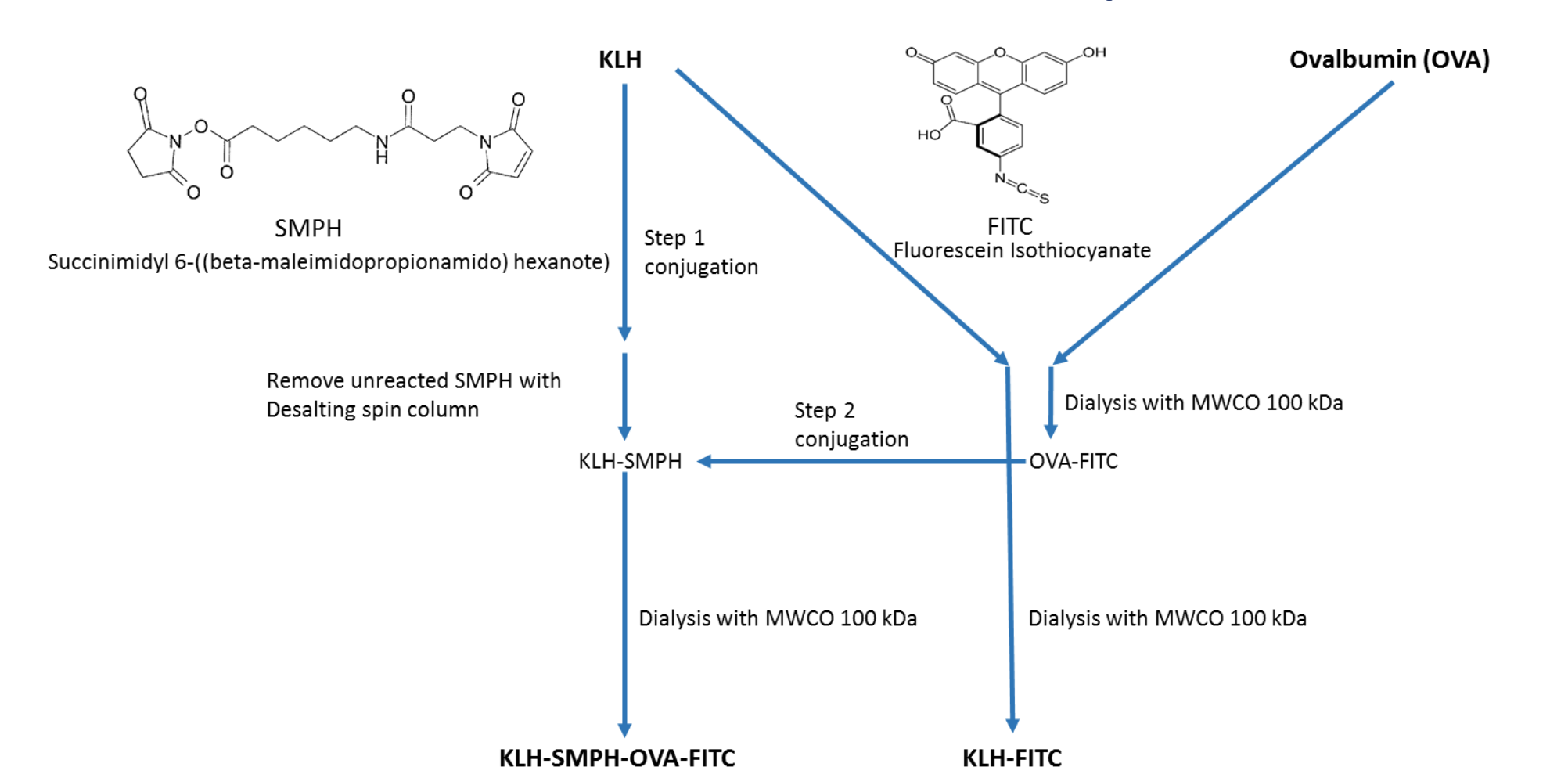
We are developing functional assays to investigate the immunogenicity of different forms and preparations of KLH with the goal of improving the predictive outcome of its use as vaccine carrier or diagnostic agent for both research and clinical studies.

Dendritic cells (DCs) derived from mouse bone marrow mononuclear cells, human peripheral blood mononuclear cells, mouse DC line JAWSII, as well as human leukemia lines THP-1 and KG-1, were treated with different forms of KLH. To compare the efficiency of DCs to stimulate KLH-specific adaptive immune responses, KLH treated primary mouse DCs or JAWSII cells were used to activate T cells extracted from spleens of mice that were immunized with KLH. DC antigen uptake efficiency, DC maturation, T cell proliferation and cytokine secretion were compared. Ovalbumin was conjugated to different forms of KLH. DCs derived from mouse bone marrow mononuclear cells were treated with the KLH-Ovalbumin conjugates for antigen uptake efficiency and ovalbumin-specific T cell activation studies.

We found that dodecameric KLH and subunit KLH have significantly different properties. We report here the results of these studies which suggest the need for methods to identify appropriate forms of KLH when used as hapten carrier or immunogen.

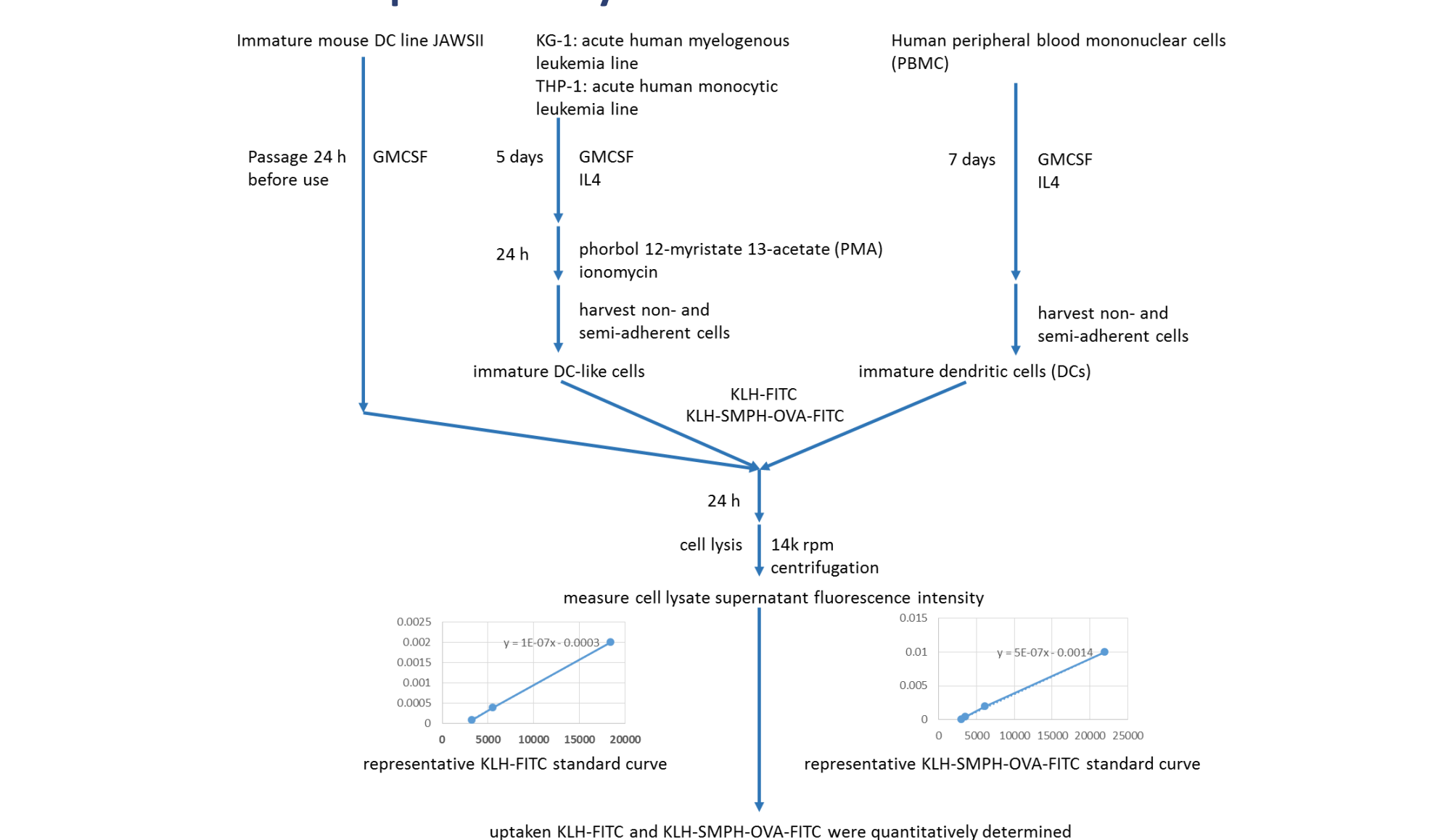
Methods

KLH-FITC and KLH-SMPH-OVA-FITC Preparation



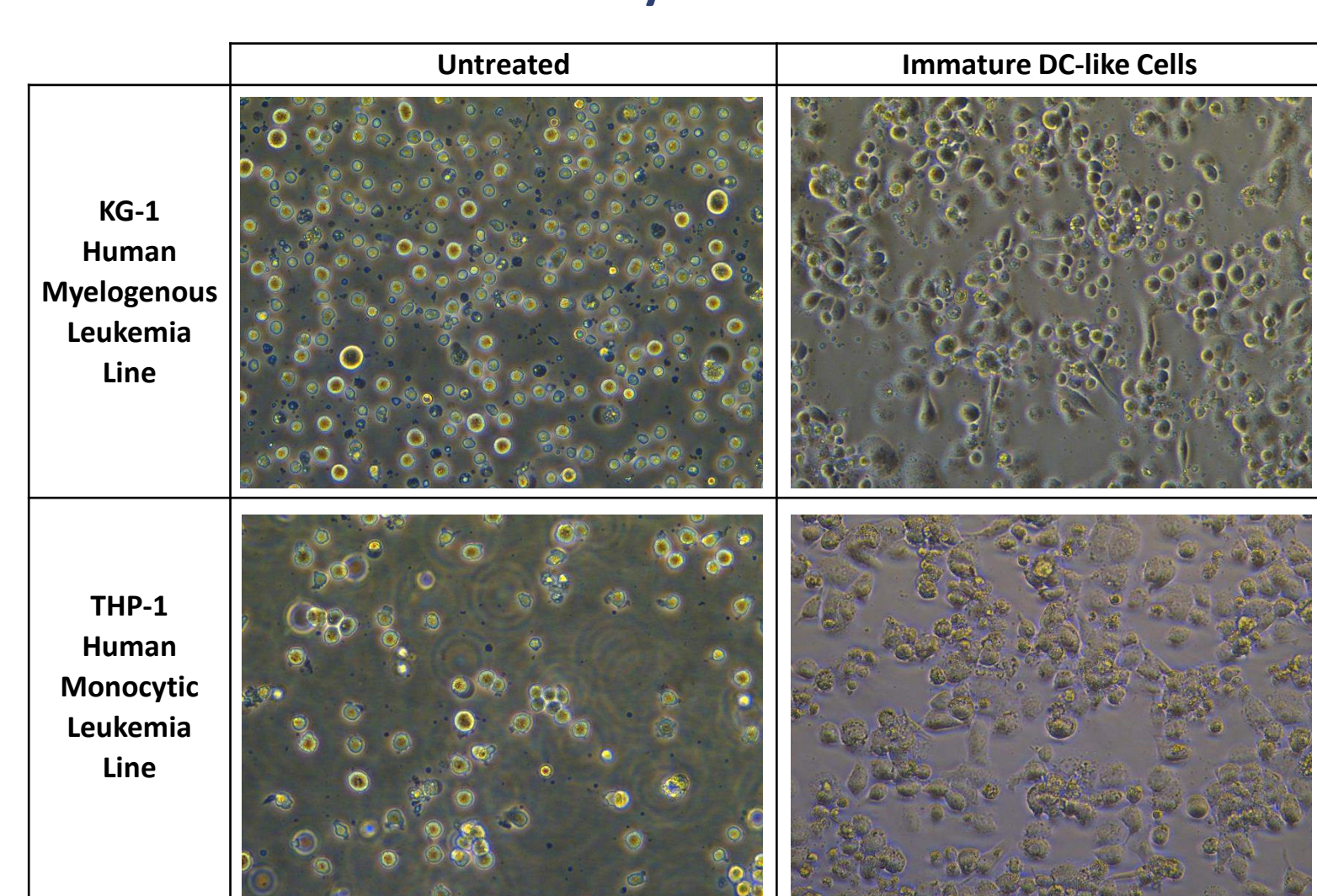
KLH-FITC was prepared by reacting KLH directly with FITC/dimethyl sulfoxide (DMSO) solution. Model antigen ovalbumin (OVA) was conjugated to KLH using heterobifunctional linker SMPH through two-steps procedure. Final concentration of the conjugates was determined by Bradford protein assay.

Dendritic Cell Uptake Assay with KLH-FITC and KLH-SMPH-OVA-FITC



Immature murine DC line JAWSII cells and immature DCs or DC-like cells derived from human PBMC and leukemia lines KG-1 and THP-1 were pulsed with KLH-FITC and KLH-SMPH-OVA-FITC for 24 h. The amount of KLH-FITC and KLH-SMPH-OVA-FITC taken up by cells was determined by comparing the fluorescence intensity of KLH conjugates in cell lysate supernatant to standard curves built with known concentrations of the conjugates.

Dendritic-like Cells Successfully Induced from KG-1 and THP-1 Cells

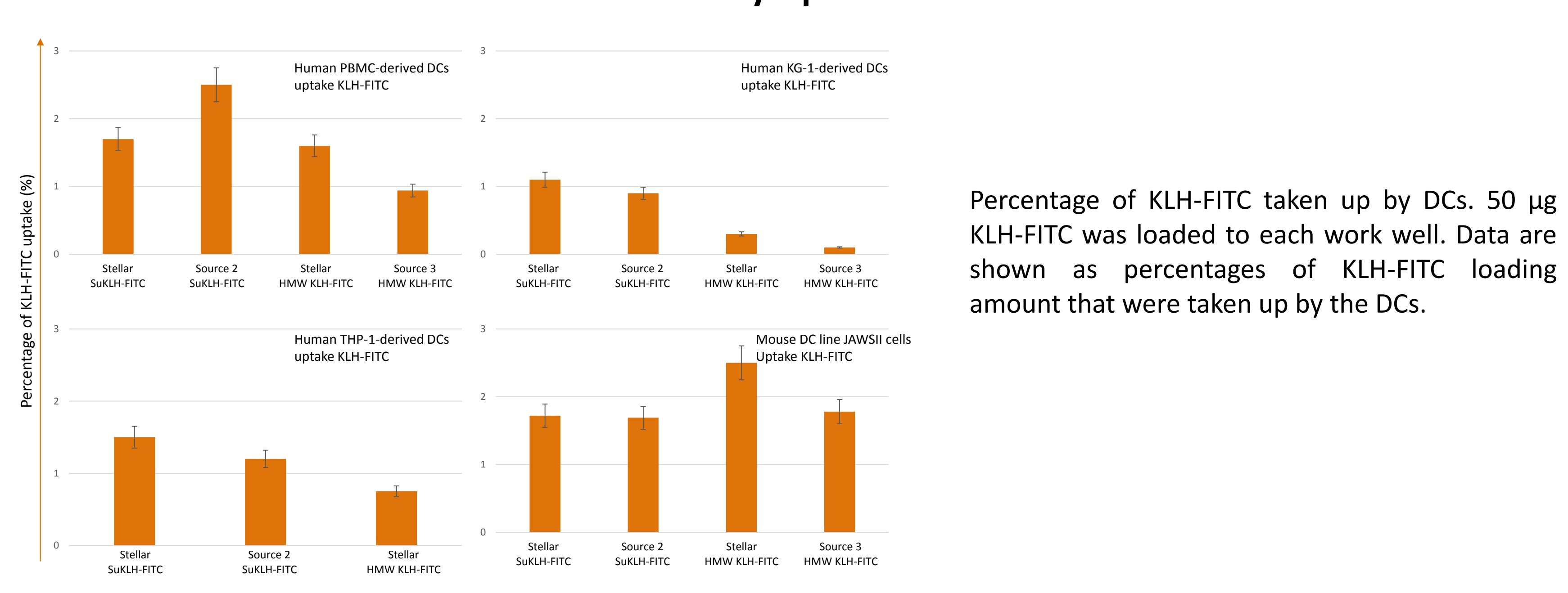


Images of human leukemia cell lines KG-1 and THP-1 before & after DC-like cell generation. KG-1 and THP-1 cells were treated with GM-CSF and IL4 for 5 days & were further induced into DC-like cells with PMA and Ionomycin.

Results

Four different KLH (from three commercial sources) were evaluated – Stellar Biotechnologies' Subunit KLH (Stellar SuKLH), Stellar Biotechnologies' HMW KLH (Stellar HMW KLH), Source 2 Subunit KLH (Source 2 SuKLH), and Source 3 HMW KLH. All experiments were performed three times in triplets. Standard errors are shown.

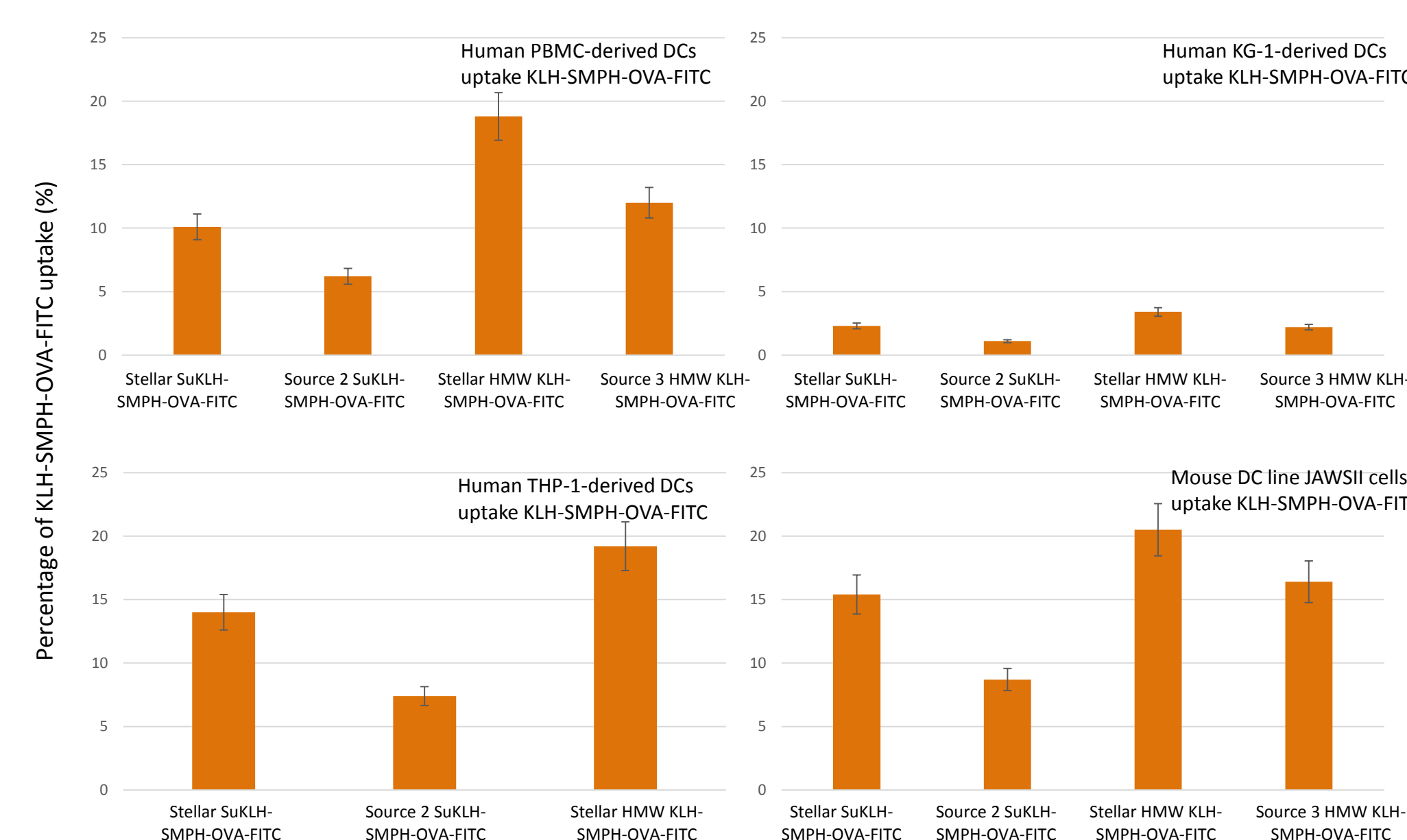
Dendritic Cells Can Efficiently Uptake Stellar SuKLH and HMW KLH



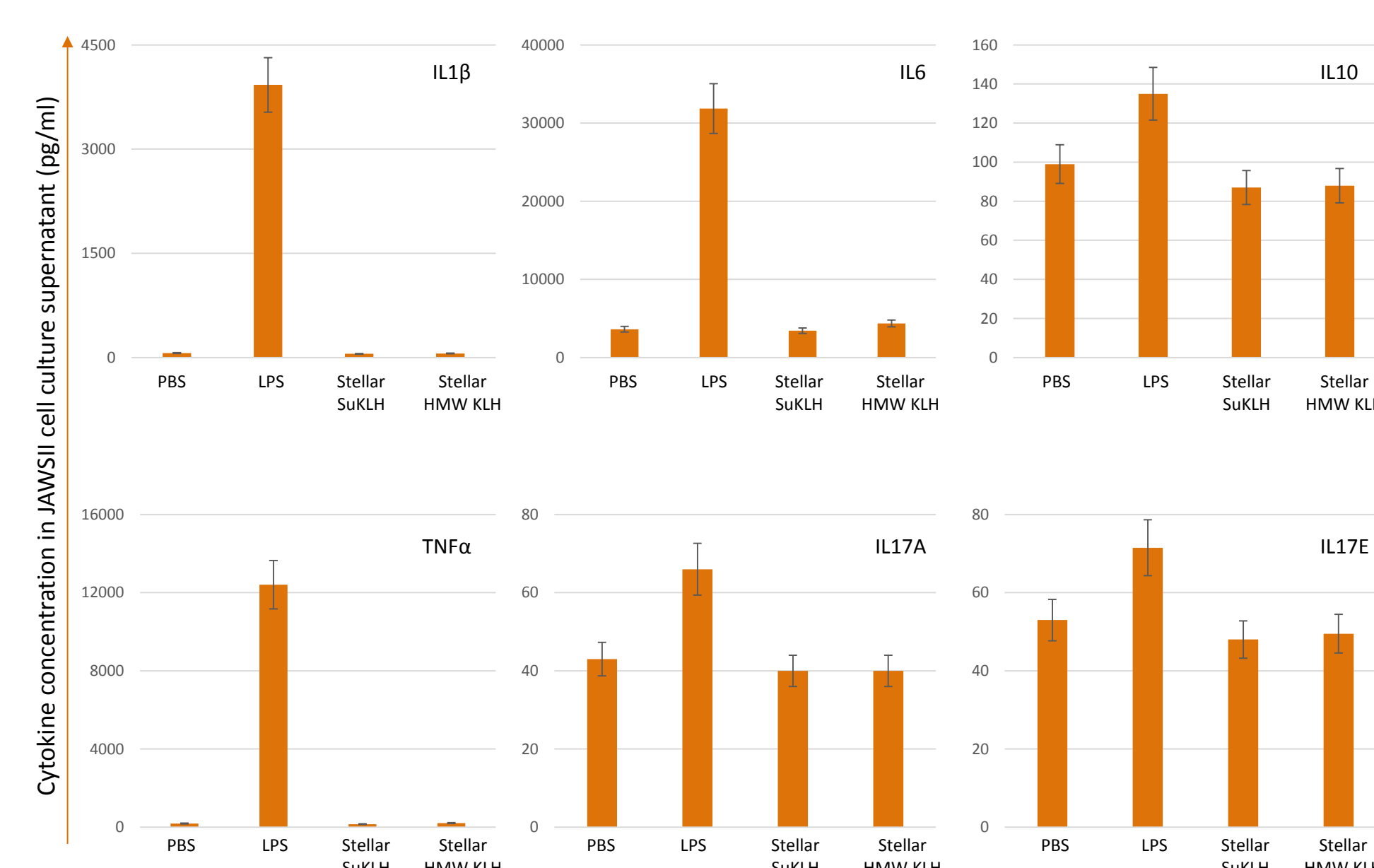
Percentage of KLH-FITC taken up by DCs. 50 µg KLH-FITC was loaded to each work well. Data are shown as percentages of KLH-FITC loading amount that were taken up by the DCs.

Dendritic Cells Can Efficiently Uptake Antigen Conjugated with Stellar SuKLH & HMW KLH

Percentage of KLH-SMPH-OVA-FITC taken up by DCs. 50 µg KLH SMPH-OVA-FITC was loaded to each work well. Data are shown as percentages of KLH SMPH-OVA-FITC loading amount that were taken up by the DCs.



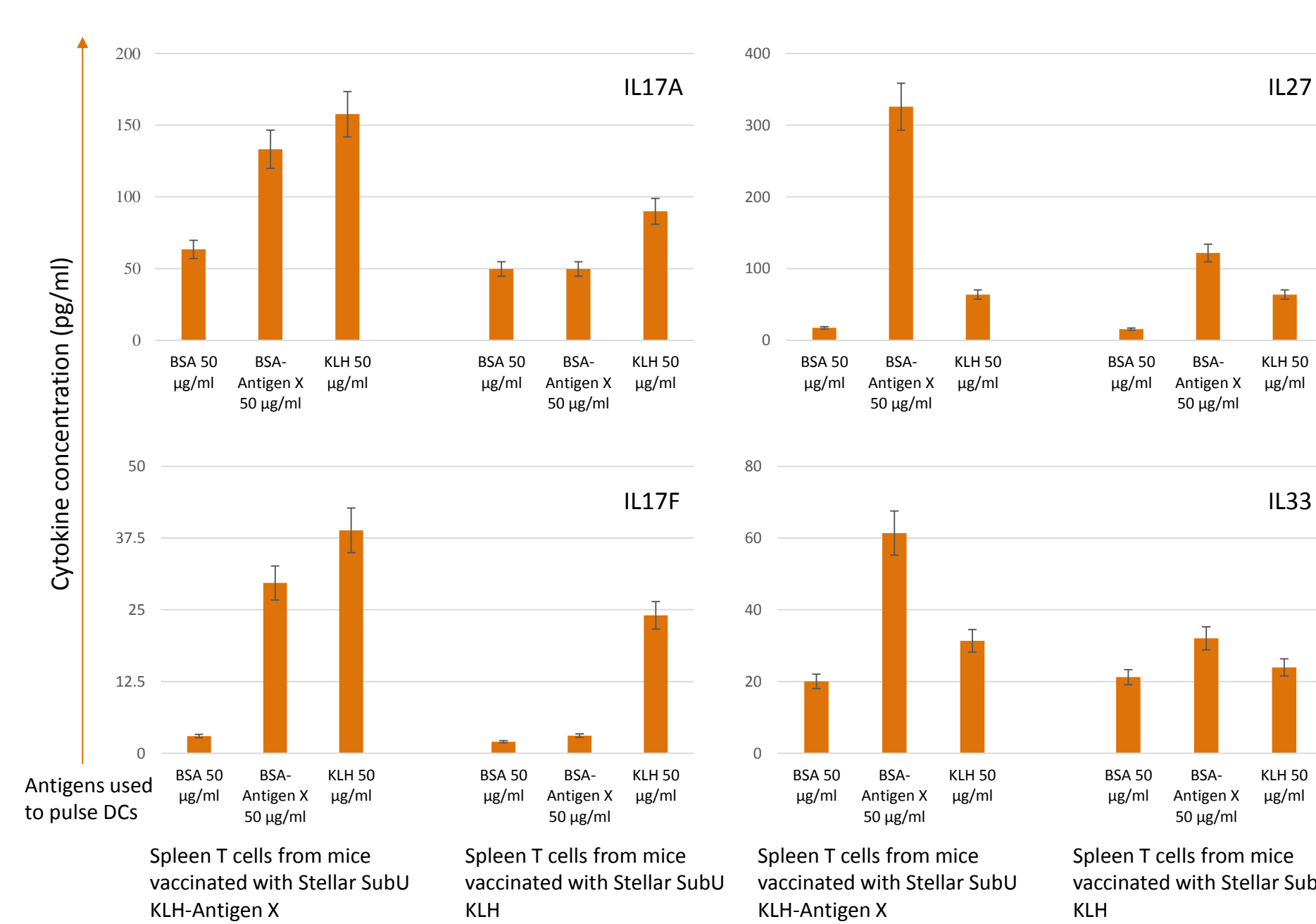
Stellar Subunit KLH Induces Mild Secretion of Inflammatory Cytokines by Dendritic Cells



Cytokine secretion by murine DC line JAWSII cells after KLH and LPS treatment. JAWSII cells were pulsed with PBS, 1 µg/mL LPS, or 50 µg/mL KLH for 24 h at 37°C and 5% CO₂. Cytokine amount in cell culture supernatant was determined with Milliplex Map kit using a mouse Th17 magnetic panel on a Luminex Multiplexing instrument with MAGPIX system.

Stellar Subunit KLH is A Good Carrier for Carbohydrate Antigen

SuKLH was conjugated with a carbohydrate antigen (Antigen X). CD4 T cells extracted from spleens of mice vaccinated with SuKLH-Antigen X were reacted with DCs generated from bone marrow harvested from immune history-free mice. DCs were pulsed with BSA (negative control), BSA-Antigen X conjugate and KLH respectively for 24 h before mixing with CD4 T cells. Cytokine amount in cell culture supernatant was determined with Milliplex Map kit using a mouse Th17 magnetic panel on Luminex Multiplexing instrument with MAGPIX system.



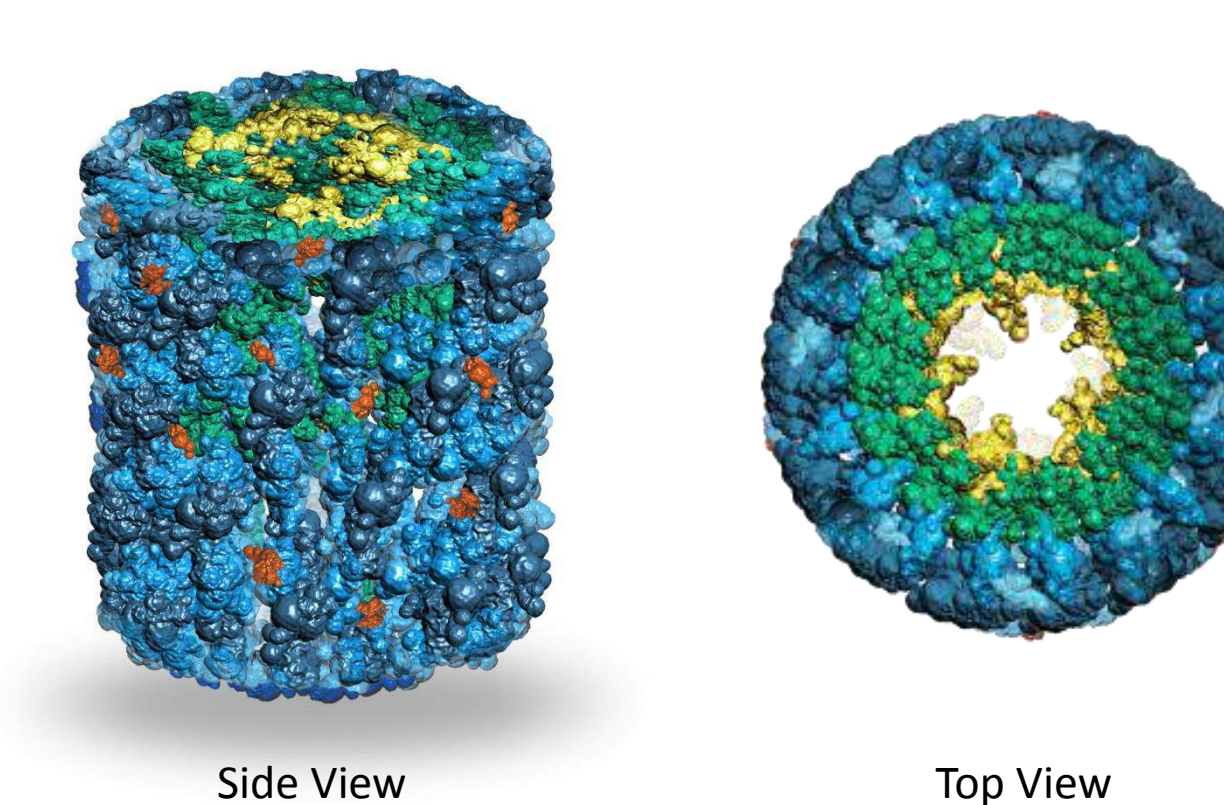
Summary

- Both subunit KLH (suKLH) and high molecular weight KLH (HMW KLH) can be easily conjugated with antigens, suggesting that both have the potential for being good antigen carriers.
- All types of DCs (mouse DC line, human leukemia line-derived DC-like cells and human PBMC-derived DC) uptake antigen conjugates built with Stellar suKLH and HMW KLH more efficiently than that built with KLH from other sources. It is worthy to mention that only limited lots of KLH samples from other sources were tested.
- When compared to LPS, KLH only induced mild inflammatory cytokine release, demonstrating the safety of KLH as a potential vaccine carrier.
- Besides its well accepted role as a good protein antigen carrier for vaccine development, Stellar suKLH might be a good carrier for carbohydrate antigens for vaccine development.

About KLH

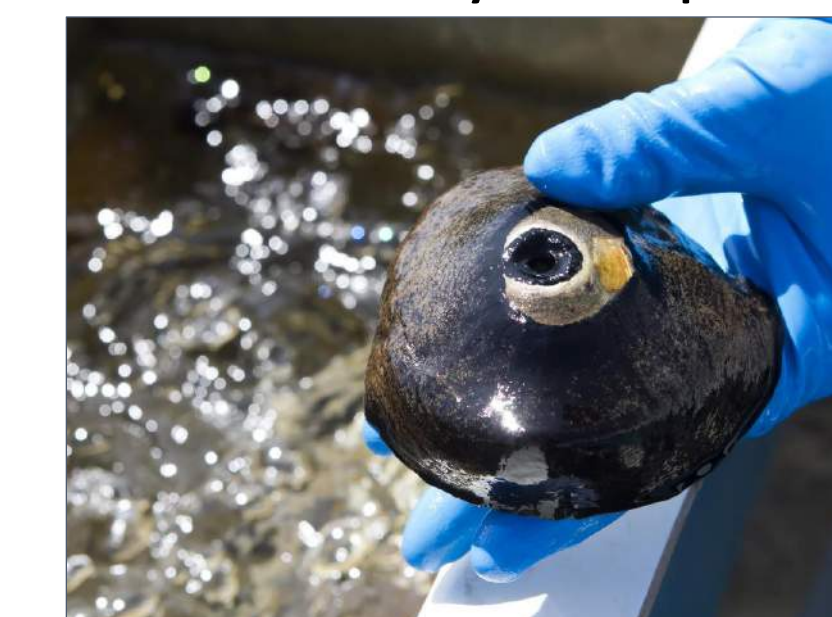
Keyhole Limpet Hemocyanin (KLH) exists as cylinder-shape dodecamer (20-mer) (side and top view below), which can dissociate into monomers (KLH subunits or suKLH). The subunit isoforms (approx. 360-400 kDa monomeric molecular weight) are each composed of 7 or 8 functional units. This complex molecular structure can be used to generate multiple product configurations.

HMW KLH Structure



The Giant Keyhole Limpet (*M. crenulata*) is a scarce marine mollusk and the sole source for KLH protein. It naturally lives in the rocky shallows only along a limited stretch of Pacific Ocean coastline. Stellar Biotechnologies is the leader in sustainable manufacture of KLH.

Giant Keyhole Limpet



KLH Product in Vials



Contacts & Resources

Stellar Biotechnologies, Inc.
332 East Scott Street
Port Hueneme, CA USA 93041
(805) 488-2800
www.stellarbiotech.com

Stellar
BIOTECHNOLOGIES
Powering and Improving Immunotherapy

Contact:
Catherine Brisson, Ph.D.
Chief Operating Officer
cbrisson@stellarbiotech.com

KLH Site™
www.KLHsite.org

Presented at: Immunology 2015,
Annual Meeting of The American Association of Immunologists,
May 8-12, 2015, New Orleans, LA
© 2015 Stellar Biotechnologies, Inc.