Modulation of dendritic cell differentiation and function by human milk oligosaccharides

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1. HMOs are unique and diverse in structures, and are highly enriched in human milk (~ 10 mg/mL).

2. Although infants cannot digest them, HMOs are thought to act in the intestine of infants to prevent infections and nurture some beneficial commensal bacteria (i.e. B. infantis).

3. Measurable (~100 µg/mL) amounts of HMOs are also found in the plasma and urine of breastfed infants.

4. HMOs have been shown to exhibit anti-inflammatory function in a fetal intestinal culture system.

5. Some of the HMO sugars (e.g. sialyl(a2,3)lactose and Lacto-N-fucopentaose III) are suggested to modulate immune response in mouse models.
HYPOTHESIS

Human Milk Oligosaccharides (HMOs) may directly modulate the developing immune system and immune response in infants, which would help reduce the risk of inflammatory diseases in the absence of fully developed tolerance.
SPECIFIC AIMS

Aim 1: Study the effect of HMOs in dendritic cell (DC) differentiation.

Aim 2: Elucidate the mechanism of anti-inflammatory function in DC maturation.

Why DCs?
- DCs are professional antigen presenting cells.
- By bridging innate and adoptive immune system, DCs orchestrate immune response.
- DCs express various types of lectin receptors that modulate their functions.
Dendritic cells are key players in intestinal immune regulation

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Experimental Design

Cord blood
Adult blood

Lymphocytes

Magnetic beads isolation

CD14+ Monocytes

CD34+ HSC

CD1c DC

Differentiation

GM-CSF
IL-4

+/- HMOs

SCF, Flt3L
GM-CSF
IL-4

Maturation

+/- HMOs

+/- LPS, poly IC (dsRNA)

• Maturation markers by FACS
• Cytokine production by Luminex
• Gene expression by qRT-PCR
Fractionation of HMOs to test the role of terminal sugars in anti-inflammatory functions

DC-SIGN

Siglec

Fucosyl enriched HMO (HMO-A)

Sialyl enriched HMO (HMO-B)

Modulation of dendritic cell differentiation by HMOs
HMOs promote DC differentiation in a CD34+ HSC culture system

HMOA: fucosyl enriched
HMOB: sialyl enriched

Lymphocyte gate

% of CD11c+ cells in total cells

control  HMO  HMOA  HMOB

HMOA: fucosyl enriched
HMOB: sialyl enriched
Physiological concentrations of HMOs could promote *in vitro* dendritic hematopoiesis.
Sialyl HMO down regulate HLA-DR expression in CD11c+ DCs

CD11c+ gate

HMOB
HMOA
HMO
Control
(Isotype)

HMOA: fucosyl enriched
HMOB: sialyl enriched
Sialyl HMO promote expression of Clec9a and Clec12a (inhibitory receptors for dead cell response)

CD1c+ DC gate

HMOB: sialyl enriched
HMOA: fucosyl enriched

HMO: sialylated HMO
HMOA: fucosylated HMO
Control
Isotype

HMOA: fucosyl enriched
HMOB: sialyl enriched
Dendritic cells differentiated in the presence of HMOs show reduced cytokine production profile in response to LPS.
HMOs may promote differentiation of low inflammatory DCs

- HMOs promote in vitro differentiation of CD11c+ DCs in CD34+ HSC culture.
- Dendritic cells differentiated in the presence of HMOs show a low inflammatory profile.
Modulation of dendritic cell maturation by HMOs
HMOs reduce inflammatory cytokine production in a dose dependent manner.
HMOs regulate pro-inflammatory cytokine production from CD1c DCs in a manner dependent on sialyl HMO.
HMOs regulate type-I IFN production from CD1c DCs in a manner dependent on sialyl HMO

HMOA: sialyl-reduced
CD1cDCs down regulate expression of maturation markers after exposure to HMOs

**HLA-DR**

**CD80**

**CD83**

**HMO (-)**

**HMO (+)**

**Isotype**

**MFI**

**Maturation markers**

![Graph showing expression levels of HLA-DR, CD80, and CD83 in HMO (-) and HMO (+) conditions.](image)
Sialyl HMO inhibits LPS-induced NFkB target gene expression in monocyte derived DCs

LPS

Control

HMOA: Fucosyl enriched
HMOB: Sialyl enriched
Multiple signaling pathways are induced by HMOs: the sum is anti-inflammatory
MAP3K14 (NIK) and CHUCK (IKKA) are key players in non-canonical NFκB pathway
MAP3K14 and CHUCK gene knockdown in monocyte derived DCs abrogate HMO-mediated attenuation of TNF production in response to LPS.
MAP3K14 and CHUCK gene knockdown in monocyte derived DCs abrogate HMO-mediated attenuation of IFN-beta production in response to poly IC.
Sialyl HMO and non-canonical NFkB pathway are critical mechanisms for anti-inflammatory function

- HMOs attenuate NFkB pathway gene expression and pro-inflammatory cytokine production in monocyte-derived DCs and blood-DCs.
- Sialyl HMO plays a role in anti-inflammatory function.
- NIK and IKKa in non-canonical NFkB pathway play critical roles in some anti-inflammatory functions.
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