Modulation of Innate Cytokines by TLR Agonists to Achieve Tolerance.

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Asthma is a chronic, reversible airway disorder, which predisposes individuals to recurrent episodes of breathlessness and wheezing.

- The World Health Organization estimates that 300 million people currently suffer from asthma worldwide.

- Asthma is the most common chronic disease among children.
Allergenic Stimuli of Asthmatic Responses.

- House dust mite
- Pollens
- Cockroach allergens
- Animal danders
- Fungi - *Aspergillus fumigatus*

- Sensitization to *A. fumigatus* affects up to 10% of all asthmatics and is correlated with incidence and severity. (O’Hallaren et al 1991; Newson et al 2000; Denning et al 2006).

- Anti-fungal therapy reverses asthmatic features in patients with severe asthma with fungal sensitization (SAFS). (Denning et al 2009).

- Periods of increased asthma hospitalizations have been linked to high airborne conidia or spore counts. (Denning et al 2006).
Role of toll-like receptors in the pathogenesis of fungal asthma: regulatory or amplifying?

Extra-cellular TLRs that recognize fungus:
- TLR2, TLR4, **TLR6**.

Only TLR6 is induced following allergic sensitization to Aspergillus in mice (Kurup et al., 2005)
The role of TLR6 during clinical asthma.

- Three studies indicated that single nucleotide polymorphisms in TLR6 might have a role in the pathogenesis and/or diagnosis of asthma, particularly in children.  (Tantisara et al. *Genes Immun.* 2004; Puthothu and Heinzmann *Allergy* 2006; Hoffjan et al. *BMC Med Genet.* 2005).

- Protective effects of TLR6 polymorphisms in asthma were associated with increased expression and greater mononuclear cell generation of Th1-type cytokines. A TLR6 polymorphism leading to defective TLR6 expression was associated with increased incidence of asthma.  (Kormann et al. *J. Allergy Clin. Immunology* 2008).

**Objective:** to determine whether TLR6 expression protects from the development and maintenance of fungal asthma.
Is TLR6 expressed in asthmatic lung?
Role of TLR6 in Fungal Asthma.

Intratracheal injection of $5 \times 10^6$ A. fumigatus spores or conidia into A. fumigatus-sensitized TLR6$^{+/+}$ and TLR6$^{-/-}$ mice

Analysis of physiologic and histologic indices of allergic airway disease.
A. fumigatus-sensitized mice

A. fumigatus conidia

Time after conidia

Airway Hyperresponsiveness (mmH2O/ml/sec)

Baseline 0 7 15 30

WT TLR6-/-

* **
Exacerbation of airway inflammation in TLR6$^{-/-}$ mice compared with TLR6$^{+/+}$ mice.

30 days

TLR6$^{-/-}$
Muc5AC $\uparrow$
Gob5 $\uparrow$
Lung IL-23 levels during the course of fungal asthma.
IL-23

Thymocytes

Naive CD4+ T cell

Th1

Th2

Treg

IL-12

IL-23

STAT1

STAT4

T-bet

IL-17

IL-17F

IL-6

TNF-α

IL-23R

IL-27

IL-12Rβ1

IL-12Rβ2

IL-23R

gp130

WSX1

STAT6

GATA-3

IL-4R

IL-5

IL-10

IL-13

TGF-β1

TGF-β3

FoxP3

CD25

Cytokine-receptor homology domain

Fibronectin-like domain

Immunoglobulin-like domain
PAMPs drive IL-23 expression by dendritic cells *in vitro.*

from asthmatic mice Day 15

DC → RNA-TAQMAN

6h

PAMPs

Dendritic cell

Fold increase in IL-23 (above medium)

WT

TLR6–/–

LPS

Zymosan

PAM2

PolyIC

Dectin-1 ligand

TLR6 ligand

* ND
Dectin-1 activation drives IL-23 and Th17 anti-fungal responses.
TLR6 is necessary for dectin-1 expression during fungal asthma.

Day 15 post-conidia
TLR6 deficient DCs express less Dectin-1.

Lung LN DCs from Asthmatic mice

Flow cytometry

CD80

CD86

CD40

Dectin-1

WT

TLR6−/−
Dectin-1 expression is required for IL-23 generation and Th17 differentiation.

DCs and T cells from asthmatic mice

WT or TLR6-/- WT only

Asp Ag 48 h

Anti-Dectin-1

WT

Medium (+ Ig)

TLR6-/-

Antigen

Anti-dectin1

CD4

IL-17

WT

TLR6-/-

IL-23 pg/ml

0 500 1000 1500

Antigen

Anti-dectin1

- + + +

- - + +

ND ND

Dectin-1 expression is required for IL-23 generation and Th17 differentiation.
Decreased Th17 in TLR6-/- mice during chronic fungal asthma.

Lung draining lymph nodes

% of CD3+CD4+IL-17+ T cells

Days after Conidia

WT
TLR6-/-
TLR6−/− mice lack T regulatory cells during fungal asthma.

Day 30 post-conidia
TLR6 is a regulatory TLR in the pathogenesis of fungal asthma possibly through the generation of Th17 and T regulatory cells.