Allergens as triggers of pattern recognition receptors

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Allergic disorders are CD4+ Th2 Cell Driven

**Allergens**

- Th1
  - Resistance
- Treg
  - Tolerance
- Th2
  - ASTHMA

**Epithelium**

- DC
  - IL-12
  - TGF-β
  - TSLP
  - IL-33
  - IL-25

**Mast cell**

- IgE

**EOS**

- IL-13
  - IL-4
  - IL-5

**B**

- AHR

- Y Y
Allergy/Asthma: A Central Conundrum

- Why do specific proteins out of a universe of antigens act as aeroallergens in susceptible hosts (in “susceptible” environments)?

  - Why do such (innocuous) diverse proteins tend to generate effector T cell responses? **Adjuvanticity**

  - Why are effector T cell responses to these proteins tend to be Th2-polarized? **Allergenicity**
Allergens are derived from diverse sources which have diverse molecular structures

- Allergens derive from a variety of environmental sources such as:
  - plants (trees, grasses)
  - fungi (Alternaria alternata)
  - arthropods (mites, cockroaches)
  - mammals (cats, dogs, cows)

- As they are derived from complex living organisms they serve a broad range of functions in their respective hosts, from structural to enzymatic.
The common house dust mite contains many enzymatically active molecules

- several cysteine proteases (Der p 1, Der p 3)
- serine proteases (Der p 6, Der p 9)
- chitinases (Der p 15, Der p 18)
- lipid-binding molecules (Der p 2)
- structural molecules--tropomyosin (Der p 10)
Allergens may uniquely induce innate immune pathways

• There is currently no compelling evidence for common structural characteristics among the diverse T and B cell epitopes.

• Other factors such as the size, glycosylation status, resistance to proteolysis, and enzymatic activity, have been suggested to play an important role in allergenicity.

• However, none of these factors have been consistently linked with allergenic potential.

• Recent studies suggest that allergens are linked by their ability to stimulate innate immune pathways.
Steps in the initiation of allergic inflammation

Signal 1
- DC
- MHC
- TCR
- CD80
- CD86
- CTLA4
- Naïve T Cell
- IL-33, IL-25
- TSLP
- TGF-B
- IL-6
- TGFB
- IL-12

Signal 2
- PAMP
- Cytokines

Signal 3
- Recruitment
- Activation

Allergy
- Asthma

Tolerance
- Infections
- Severe Asthma

Intracellular Pathogens
Pathogen-associated molecular patterns (PAMPs) are recognized by pattern recognition receptors (PRR).

- **Inborn**
- **Germ-line encoded**
- **Genetically determined**
- **Passed onto progeny**

Cytokines

**PAMPs (signal 3)**

Invariant structures shared by classes of pathogens
# Human Pattern Recognition Receptors

<table>
<thead>
<tr>
<th>Types</th>
<th>Recognizes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toll-like receptors (membrane bound)</td>
<td>TLR1-10, CD14</td>
</tr>
<tr>
<td>C-type lectin receptors</td>
<td>Dectin 1, 2, collectins, SP-A and SP-D</td>
</tr>
<tr>
<td>NOD-like receptors</td>
<td>NOD1,2, cryopyrin</td>
</tr>
<tr>
<td>RIG-I receptors</td>
<td></td>
</tr>
<tr>
<td>Anti-microbial peptides (secreted)</td>
<td>Defensins, cathelicidin</td>
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</tbody>
</table>
Toll-like Receptors and Agonists

- **TLR2/1**, **TLR2/6**, **TLR3**, **TLR4**, **TLR5**, **TLR7**, **TLR8** (Hu), **TLR9** (Hu), **TLR10**, **TLR11** (mouse), **TLR12/13** (mouse)

**Agonists**:
- **PAM3CysK4**
- **19 kDa Mtb lipoprotein**
- **AraLAM**
- **Lipoarabinomannan**
- **LTA**
- **Zymosan**
- **MALP-2**
- **Enterobacterial LPS**
- **VSV-G**
- **MMTV-G**
- **chlamydial HSP60**
- **Flagellin**
- **dsRNA**
- **ssRNA**
- **CpG DNA**
- **T. Gondii prophilin**
- **UPEC?**
LPS doesn’t bind TLR4 directly.
CD14 and MD-2 act as a bridge between LPS and TLR4.
TLR4 Signaling Pathways

Proinflammatory cytokines
Type I and II IFN’s
Structural Comparison of a Homology Model of MD-2 with the Crystal Structure of Der p 2

Der p 2 belongs to MD-2-like lipid binding family

Allergens - House dust mites

- Dust mites are major source of aeroallergens for patients with allergic asthma
  - highest rates of skin test positivity among allergens in atopic patients

- Dust mites contain many allergenic peptides
  - Der p 1 (cysteine protease)
  - Der p 2, Der f 2 (“major group II allergens”)
• Is there functional homology between MD-2 and Der p 2?
Der p 2 reconstitutes TLR4 signaling


MD-2 deficient HEK293 cells

Der p 2 binds LPS and CD14

10ng/ml E.coli LPS

IL-8 (pg/ml)

TLR4 + +
CD14 + +

E.V
Der p 2-HA
All sequenced MD-2 contain a conserved tyrosine (Tyr 102) at a site functionally important for TLR4 signaling

<table>
<thead>
<tr>
<th>Species</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td>Human MD-2</td>
<td>PRKEVICRGSDDDDYSFCRALKGETVNTTISFSFKGKYSKGYKCYYKCVV 135</td>
</tr>
<tr>
<td>Chimpanzee MD-2</td>
<td>PRKEVICRGSDDDDYSFCRALKGETVNTTISFSFKGKYSKGYKCYYKCVV 135</td>
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<tr>
<td>Macaca MD-2</td>
<td>PRKEVICRGSDDDDYSFCRALKGETVNTTTVSFSFKGKYSKGYKCYYKCVV 135</td>
</tr>
<tr>
<td>Murine MD-2</td>
<td>PRKEVLCHGHDDDDYSFCRALKGETVNTSIPFSFEGILFPKGYRCVA 135</td>
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<tr>
<td>Rat MD-2</td>
<td>PRKEIVCHGYDDDDYSFCRALKEAVNTAIPFSFDIGLFPKGYRCVA 135</td>
</tr>
<tr>
<td>Hamster MD-2</td>
<td>PTRKEICHGYDDNYSFCKALKEVNTVVPFSFKGILFPKGYRCVA 135</td>
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<tr>
<td>Rabbit MD-2</td>
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<td>Pig MD-2</td>
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<td>Human MD-1</td>
<td>LNFSYPICEAALPFSFCGRRKGEQIYYAGFVNNPEFTIPQGEYQVVL 142</td>
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<td>Murine MD-1</td>
<td>LNFSYPICEAEADLPFSFCGRRKGEQIYYAGFVNNPGLDVPQGEYQVLL 142</td>
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<td>Pig MD-1</td>
<td>LNFSYPICEALPFSFCGRRKGEQIYYAGFVNNPGLDVPQGEYQVLL 142</td>
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<tr>
<td>Chicken MD-1</td>
<td>LSYSETLCGPGLSKLIFCGKKGKGEHLYYEGPITLGKEIPQGDYTITA 137</td>
</tr>
</tbody>
</table>

**Der p 2**

82 PGIDPNACH-----YMCKPLVKGQQYDIKYTWNVP-KIAPKSE-NVVV 122

**tyrosine**
Mutation of Y91A impairs the ability of Der p 2 to modulate TLR4 signaling

![Graph showing IL-8 production in response to Der p 2-HA and Y91A Der p 2-HA treatments.](image-url)
**TLR4-dependent induction of experimental allergic asthma by Der p 2**

**BAL Cell Number**

**Total IgE (ng/ml)**

**W.T.** (LPS)  
**W.T.** (rDer p 2/LPS)  
**TLR4-KO** (LPS)  
**TLR4-KO** (rDer p 2/LPS)

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**Histological images**

WT/Der p2  
TLR4--/ Der p2

**Trompette, Nature, 2009**
Der p 2 activates TLR4 signaling

• Der p 2 binds LPS

• Der p 2 interacts directly with the TLR4 complex, facilitating LPS signaling

• Der p 2 drives Th2 inflammation in the airway in a TLR4-dependent fashion by mimicking MD-2

• Der p 2 can drive airway Th2 inflammation in the absence of MD-2

• These findings are consistent with other studies demonstrating a role for TLR4 signaling in type-2-mediated inflammation (Hammad, 2009; Tan, 2009)
Is this phenomenon generalizable?

• Numerous other ML family members are allergens

• A large number of defined major allergens are lipid-binding proteins

• Der f 2 also binds LPS (Ichikawa S, Genes Cells, 14:1055, 2009).

• Der p 7 binds to the lipoprotein polymixin B (Mueller, JACI, 125:909-917, 2010).
Why do we respond to and mount a Th2 immune response to Der p 2?

Der p 2 likely serves a host defense role in the mite.

It is unlikely that mammalian host responses are intentionally directed against the harmless mite.

Likely a case of mistaken identity-host response likely evolved to protect against bacterial infection.

The question remains as to why TLR4 signaling can drive both Th1 and Th2 immune responses?
TLR4 Signaling Drives Th2 Immune Responses Through Induction of TSLP

Hammad, 2009; Tan, 2009
Other PAMPs contained in HDM

- Chitin
- β-glucans — Carbohydrate moieties
C-type lectin receptor (CLRs)

- Carbohydrate recognition domain (CRD)
- Ligands are not present in mammalian cells
- Calcium dependent binding
- No signaling domains - couple with stimulatory (ITAMs) & inhibitory (ITIMs)
Carbohydrate recognition in Th2 responses

- Glucans are a diverse class of naturally occurring glucose polymers, which can be short or long, branched or unbranched, exist as α or β isomers, and be soluble or particulate.

- Native but not deglycosylated Ara h 1, a peanut glycoallergen has been shown to activate human monocyte derived DC and induce IL-4 and IL-13-secreting Th2 cells (Shreffler et al, 2006).
Dust mite-induced CCL20 is β–glycan dependent

Der p 1 and Der p 2 Don’t Induce CCL20

Dectin-1-2, β-glucan receptors, signaling pathways

Derived from Brown, G Nat Immunol 2006
Dust mite & Aspergillus allergens are recognized by Dectin-2, release of LTC4

Activation of the lectin pathways of complement activation

Like other PRR, the complement system can be activated by hard-wired PRRS such as the mannose binding lectin (MBL) that has evolved to recognize danger “motifs”.

Consistent with a role for lectins in driving Th2 immune responses, blockade of the mannose receptor, an endocytic C-type lectin receptor, significantly reduced Der p 1 uptake by DCs (Royer, 2010).

Fed d 1 is a ligand of the mannose receptor and MR deficient mice have attenuated allergic responses to Fed d 1 (Emara, 2011).
Allergens induce complement factor 3 gene expression in AEC

Der p 1 containing proteases cleave C3 into its active fragment, C3a.

SNPs in C3 are associated with asthma

C3 leads to preferential uptake of Ag by mDCs
Protease Containing Allergens Can Activate Several Innate Immune Cells Leading to Type 2 Immunity

- Papain
- OxL
- ROS
- TLR4
- TRIF
- Epithelium
- TSLP
- mDC
- TSLPR
- Basophil
- CCL7
- Naïve CD4+ T Cell
- Th2

Danger Associated Molecular Patterns

Alum-NLR (Eisenbarth, 2008)

ATP-(Idzko, 2007)

Uric acid-(Kool, 2011)
The danger sensor TFF2 drives Th2 immunity to allergens
Blockade of IL-33 in TFF2-treated macrophages abrogates $T_h^2$

Figure: MΦ + OVA-specific CD4+ T cell (OTII) +

Th2 cytokines GATA-3, Tbet

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-13 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>GATA-3 (Relative mRNA expression)</th>
<th>Tbet</th>
</tr>
</thead>
<tbody>
<tr>
<td>TFF2 + OVA</td>
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<td></td>
</tr>
<tr>
<td>TFF2</td>
<td>n.d.</td>
<td>n.d.</td>
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</tr>
<tr>
<td>OVA</td>
<td>n.d.</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MED</td>
<td>n.d.</td>
<td>n.d.</td>
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Wills-Karp, JEM, 2012
Allergens are recognized by and activate type 2 immune responses through a constellation of PRRs.
CONCLUSIONS

- Allergens possess the ability to induce a variety of innate immune pathways (particularly in the mucosal epithelium) which override the otherwise tolerogenic environment.

- Much more needs to be learned about the full spectrum of innate immune pathways activated by different classes of allergens.

- Genetic differences in innate immune pathways may underlie susceptibility to allergic diseases.

- Modulation of these pathways holds promise for screening for sensitizing abilities and the development of therapeutics aimed at preventing allergic sensitization.
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Uric acid activates several innate immune pathways resulting in IL-1β