Identification of Immunologic and genetic biomarkers for TMA sensitization

Debajyoti Ghosh PhD
Research Scientist
Internal Medicine, Division of Immunology
University of Cincinnati College of Medicine

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Funding, lab support and mentorship

- **Funding**:

Supported by the National Institute for Occupational Safety and Health Pilot Research Project Training Program of the University of Cincinnati Education and Research Center Grant #**T42/0H008432-09**.

- **Lab-support and mentorship**:

Dr. Jonathan Bernstein MD (lab director)
Department of Internal Medicine, UC College of Medicine

Dr. Ian Lewkowich PhD
Div. of Immunobiology
Cincinnati Children's Hospital and Medical Center

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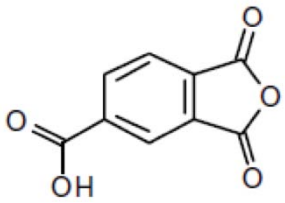
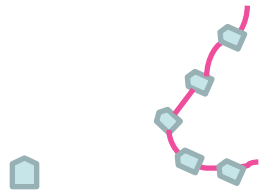
Trimellitic Anhydride (TMA)

- Hardening agent used in preparing plastics and paints.
- 100,000 metric tons produced annually worldwide (65,000 metric tons/year in the U.S.).
- An estimated 20,000 workers in the US are currently exposed to trimellitic anhydride.



TMA:$C_9H_4O_5$
MW:192.13

- Free TMA can be converted to TMLA. It is an irritant (can't induce antibody response).
- Numerous TMA molecules can irreversibly bind to a large carrier protein (e.g. HSA) to make a complete antigen. This can cause allergenic antibody responses leading to occupational asthma.
- TMA is a unique molecule responsible for producing both irritant and allergnic responses.



TMA **TMA-HSA**

Ref. http://www.inchem.org/documents (accessed Oct., 2015)
http://www.cdc.gov/niosh/docs/1970/78121_21.html (accessed Oct, 2015)

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Irritant *versus* allergenic response

- A reversible inflammatory effect on living tissue by chemical action at the site of contact. Not antibody-mediated.
- Non-specific reaction, can be caused by any irritant. Irritants are usually low molecular weight chemicals.
- No latency period
- Temporary and reversible. But sometimes can cause 'irritant-induced occupational asthma'.

- Usually caused by antibody-mediated systemic reactions. IgE is the key molecule.
- Very specific, mediated by Ag-Ab interaction. Usually high molecular weigh protein/glycoproteins.
- Has latency period (sensitization and effector phase)
- A wide range of occupational disorders ranging from airway obstruction to asthma.

- **There is a paucity of information regarding biomarkers that can discriminate irritant response from immunologic responses to TMA exposure.**
- **This would be helpful for distinguishing two divergent responses in TMA-exposed factory workers.**

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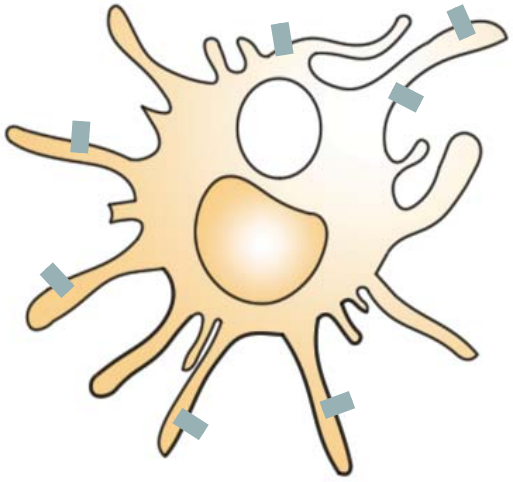
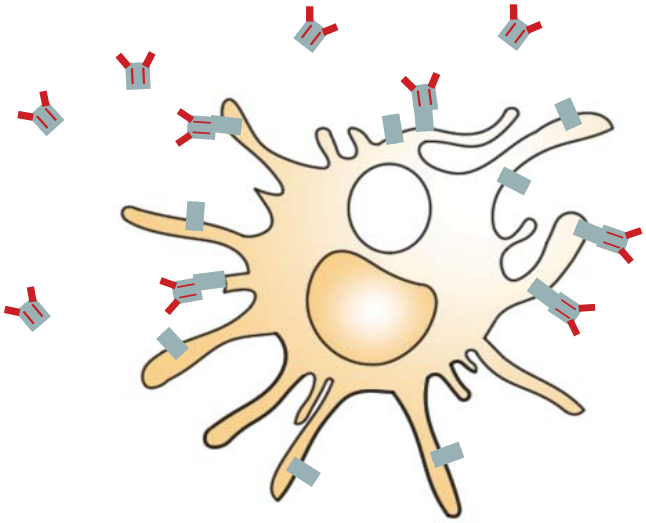
Dendritic Cell: A sensitive model for allergenicity assessment. Specifically pDCs are very sensitive to allergens and irritants. They also express high affinity IgE receptor FcER1



DCs express FcER1



They can be sensitized by IgE

- Plasmacytoid DCs are a DC sub-population characterized by CD123+ CD11- surface expression pattern.
- They can express FCER1 (high affinity receptor for IgE)
- They are very responsive to allergen and irritant exposure

Ref. Ayehunie et al (2009) Toxicology 264:1-9

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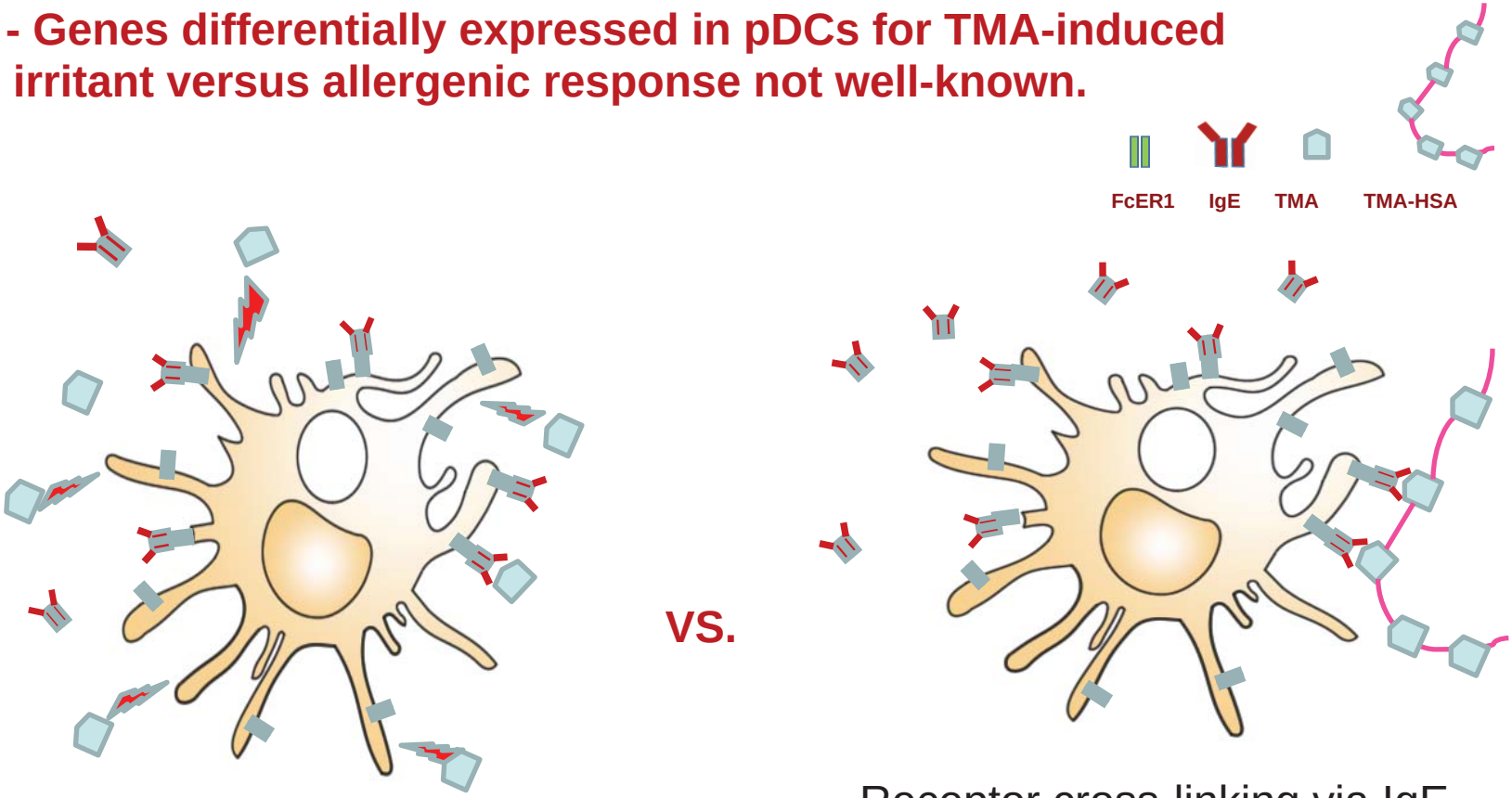
- **pDCs are very sensitive to environmental irritants and allergens and drive subsequent immune reactions.**
  - Genes differentially expressed in pDCs for TMA-induced irritant versus allergenic response not well-known.



Irritant response

VS.

Receptor cross-linking via IgE
IgE-mediated Immune response

We wanted to use pDCs to distinguish.

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Human FcER1^{+} Plasmacytoid Dendritic Cells:

- Human pDCs were obtained from Matteck Inc.
- These cells are differentiated from CD34+ umbilical cord blood cells of a single heathy donor
- They are clean and devoid of any autologous IgE which permits sensitization using serum samples from TMA exposed workers.
- Certified to be CD123+ CD11- and FcER1+
- Doubled-checked using ImageSteamX (Ames imaging flow-cytometry, Flow Core, CCHMC)

Starting material: Homogeneous population of dendritic cells expressing receptor for Immunoglobulin E **.**

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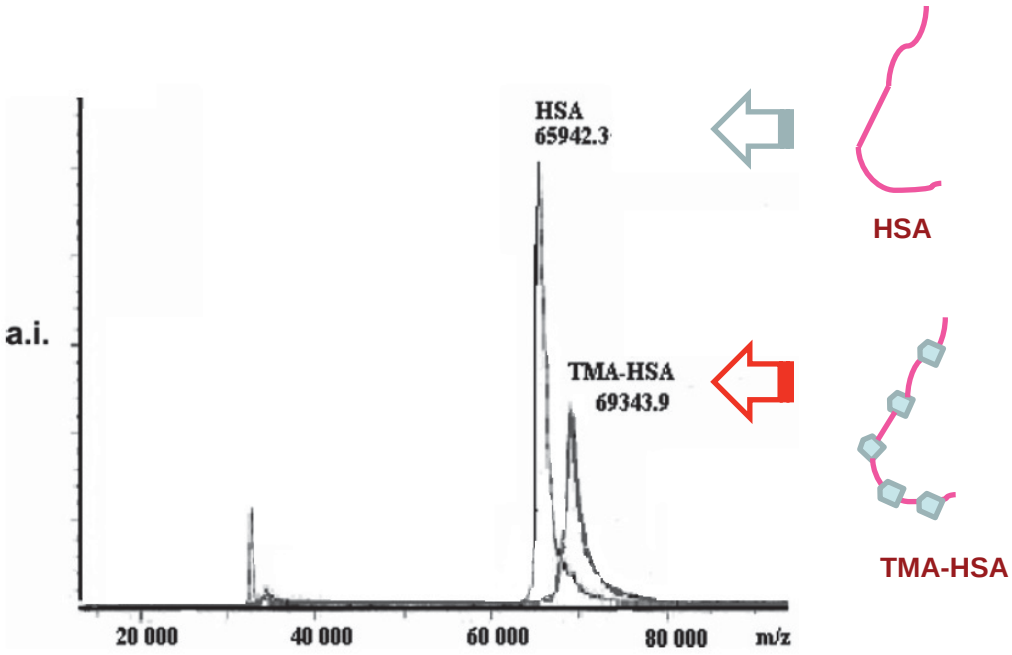
TMA and well-characterized TMA-HSA conjugate:



Analysis of conjugation product using MALDI-TOF

- **Synthesis**: TMA-HSA conjugate has been produced by conjugating TMA with HSA following published procedure (Bernstein, Ghosh et al).
- **Product Analysis**: The shift in molecular weight (69343.9 – 65942.3 = 4301.6 kD) indicates conjugate formation.
- **Epitope density**: The number of TMA molecules (molecular weight = 192) bound to one molecule of HSA molecule was estimated to be 18.
- **Clean up**: (a) Extensive dialysis to get rid of unbound TMA (b) Check for LPS contamination (c) Pass through a column to get rid of LPS.

Ref. Bernstein JA, Ghosh D et al.
Journal of Occupational & Environmental Medicine: October 2011 – V. 53 (10) 1122–1127

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TMA-specific serum IgE :

- Our lab director Dr. Bernstein is the medical supervisor of the occupational Immuno-surveillance program at Flint Hill Recourses, USA.
- We have access to serum samples obtained from TMA-exposed factory workers who developed IgE antibodies against TMA.
- TMA-specific serum IgE titer was commercially determined (Viracore-IBT, Inc. USA), using Immunocap 4000 machine (Pharmecia, Sweden).

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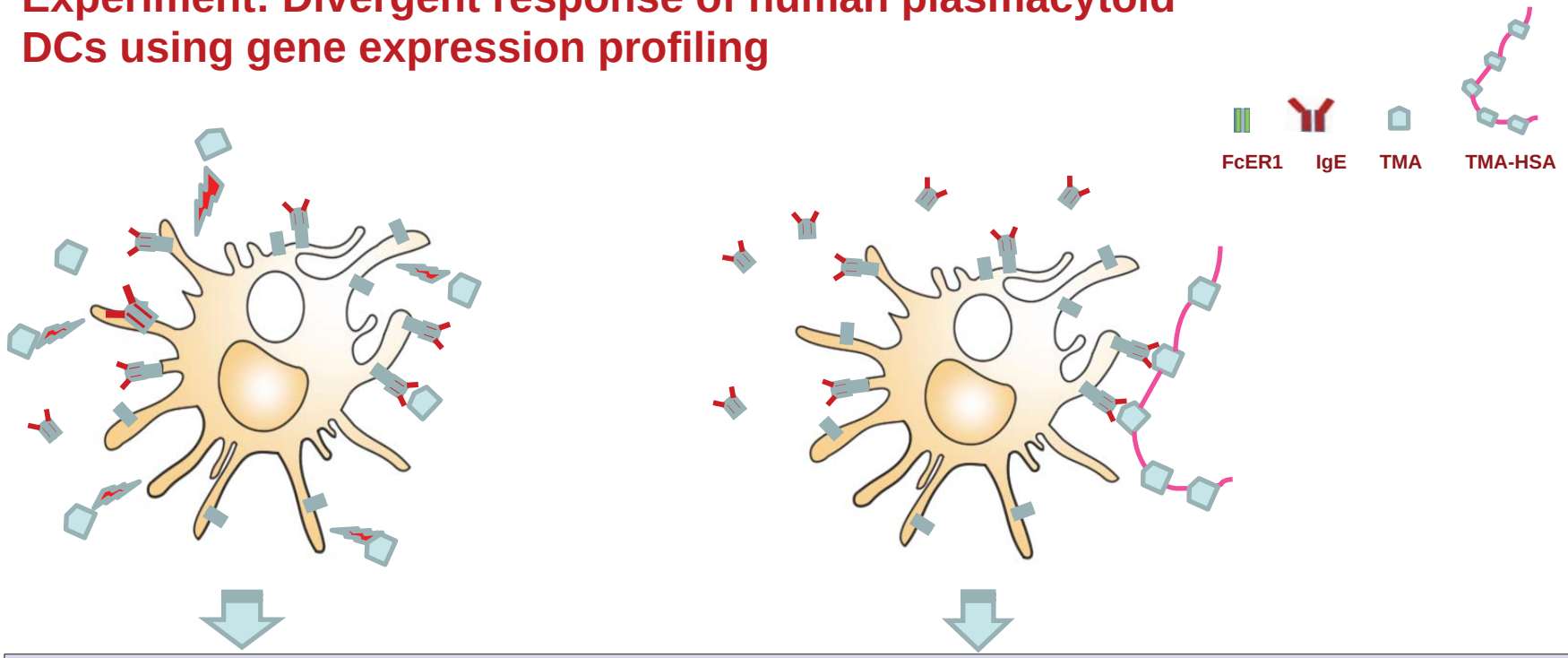
Experiment: Divergent response of human plasmacytoid DCs using gene expression profiling



Transcriptome analysis by RNAseq to identify differentially expressed genes

Irritant response (free TMA)

IgE-mediated Immune response (TMA-HSA)

RNAseq was done using UC Environmental Health Genomics and sequencing core facility.

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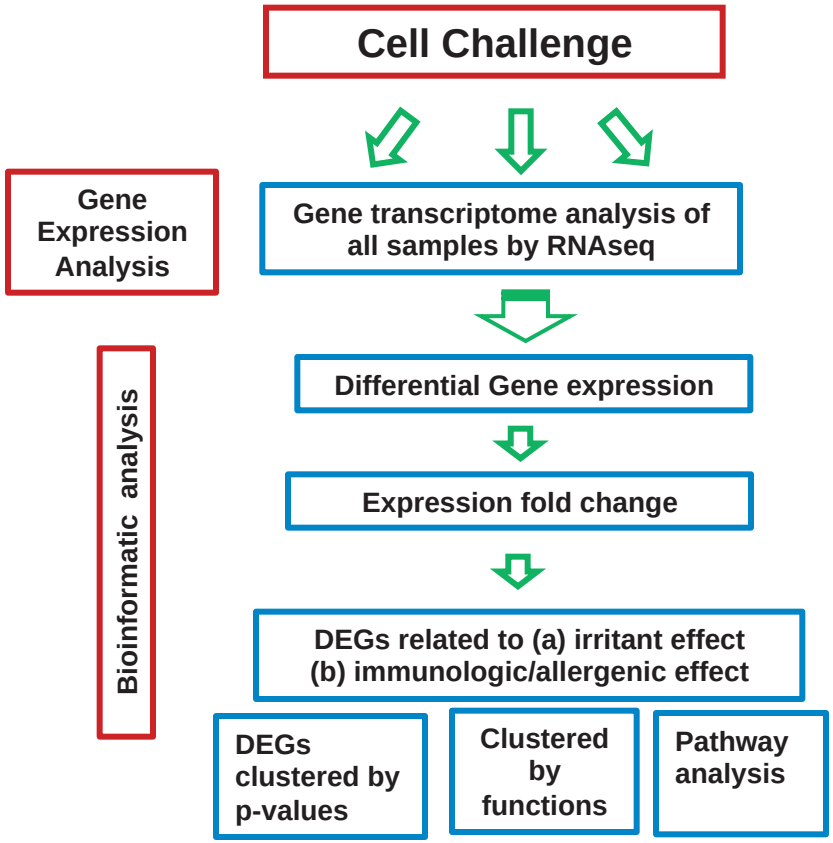
Methods: Brief outline



- Human plasmacytoid DC (pDC) expressing FcER1+ were sensitized with TMA-IgE sensitized worker sera at a 1:20 dilution, washed and exposed to either (a) HSA; (b) free TMA or; (c) an LPS-free TMA-HSA conjugate. Cells were harvested and analyzed by RNA sequencing.

- Unexposed DCs or DCs incubated with non-TMA exposed control sera served as additional controls. Differentially expressed genes (DEGs; adjusted p-values <0.05) were used for bioinformatic analyses.

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Results and Conclusion: Differentially regulated genes and pathways

- **Free TMA exposure:** Associated DEGs involved innate immunity and cell migration pathways including IL23A, IL15, epiregulin and endothelin.
- **TMA-HSA exposure:** Associated DEGs involved humoral immune response pathways including SORBS1, TNFSF13B, CD300LB and LYN tyrosine kinase.
- **Pathway analyses:** Over-representation of IFNg and Granzyme B pathway genes was observed for the free TMA exposure.

  Humoral antibody and inflammatory immune pathway genes were over-represented in TMA-HSA group.

  Specific cytokines (IL1, IL6) and chemokines (CCL4, CCL20) were upregulated in both groups.

Gene expression of pDCs exposed to free TMA is distinctly different from pDCs exposed to TMA-HSA.

*Top genes being further validated by real-time quantitative PCR.

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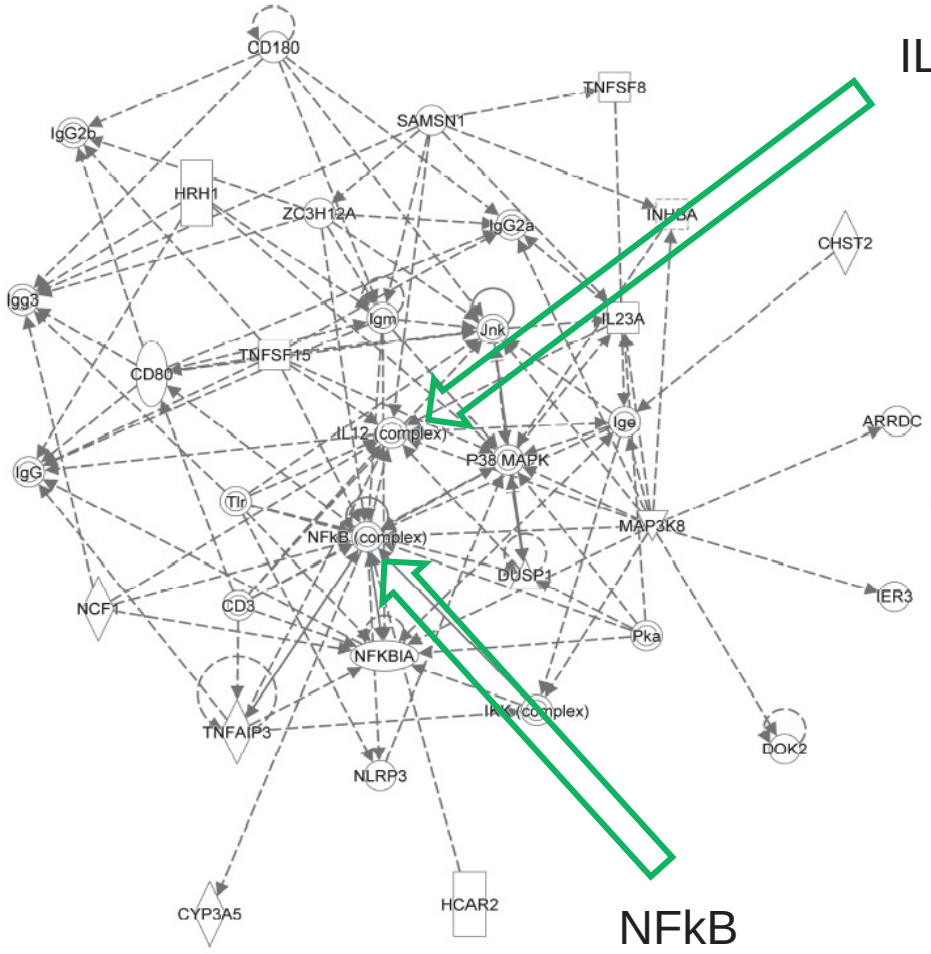
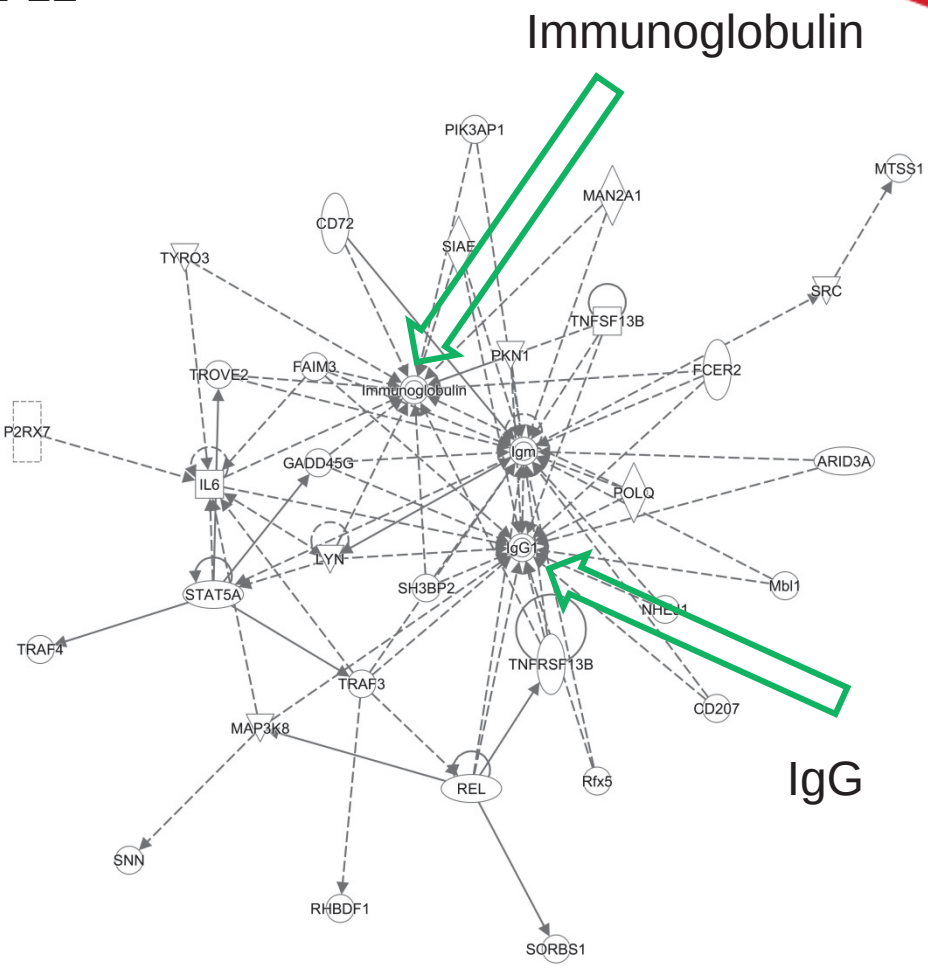
Major gene networks





TMA-exposure: cell-mediated immune-inflammatory response

TMA-HSA exposure: Antibody-mediated humoral response

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R2P application and public health relevance:

In summary, we have identified relevant genes and pathways differentially expressed between free TMA exposure and TMA-HSA allergen exposure to plasmacytoid Dendritic cells, which is a key cell type to sense environmental allergens and irritants.

- This study strongly suggests there are divergent mechanistic pathways for irritant vs. antibody-mediated immunologic responses for TMA exposure.

- This is an important starting point to determine if these DEG are useful biomarkers for differentiating workers at risk for TMA- induced irritant or allergenic responses who have continuous or intermittent exposures.
  - Contribute to better diagnosis and management of occupation health hazard caused by TMA exposure.

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Publication/presentation and potential for future funding

- Results have been submitted to American Academy of Allergy Asthma and Immunology (AAAAI) for oral presentation in the 2016 annual meeting, Los Angeles.
- Manuscript to be submitted.

- **Plan for obtaining extramural funding**: A research proposal focusing on cytokine immune responses in exposed TMA factory workers was submitted to NIOSH. The present results will be added as preliminary data to strengthen the resubmitted proposal.

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Acknowledgement:

- TMA factory workers of Flint Hill Resources

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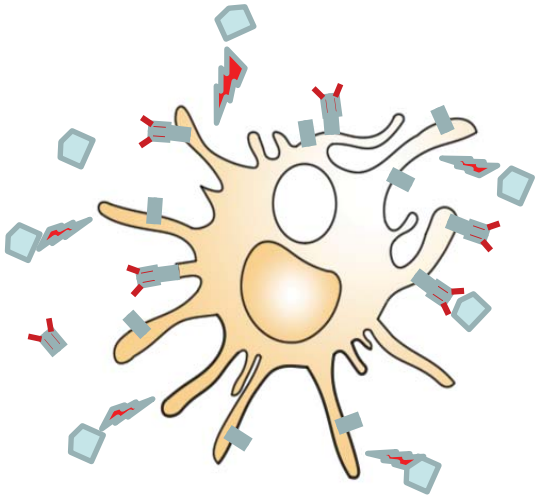
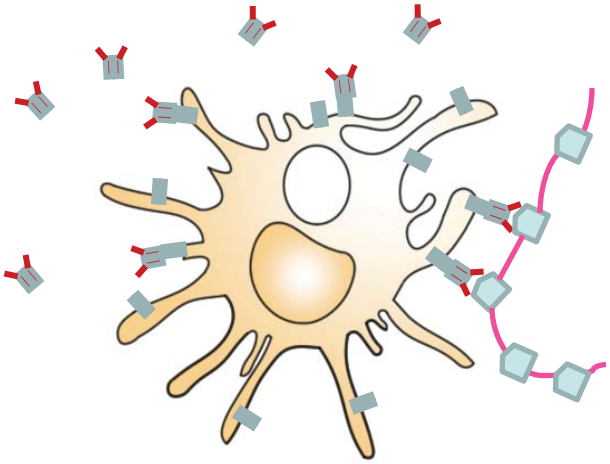


Thank you !



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University of Cincinnati
16th Annual
Pilot Research Project
Symposium
October 8-9, 2015

Hosted by: The University of Cincinnati Education and Research Center
Supported by: The National Institute for Occupational Safety and Health. (NIOSH) Grant #: T42-OH008432

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Produced by Kurt Roberts Department of Environmental Health