Immunoregulatory Responses in Trimellitic Anhydride Occupational Sensitization.

D. Ghosh Ms PhD

Post Doc Research Associate

Dept. of Internal Medicine (Immunology)

University of Cincinnati College of Medicine

Background:

- **The molecule**:

Trimellitic anhydride (TMA) is an organic acid anhydride widely used in plastic and paint industries.

- **The exposure pattern:**

Produced as powder, exposure occurs as dust and/or fume in the production phase or in packaging and logistic units. personal exposure ranges from <0.6 to 1900 ug/m^3

- **The Effects:**

Irritant effect: TMA is rapidly converted to trimellitic acid.

Sensitization: Potential sensitizing agent, demonstrated to elicit specific IgG and IgE-mediated immune responses, often leading to asthma.

- **Antigenicity:**

  Becomes antigenic only after conjugation with larger endogenous carrier proteins such as human serum albumin (HSA), often by binding to lysine residues (Zeiss et al 1977). Inhaled TMA hapten, deposited on mucosal surfaces, reacts rapidly to form stable antigenic trimellityl-protein complexes. Systemic induction of TMA-specific antibodies against trimellityl-protein complexes can occur upon re-exposure.

- **Disease development:**

  Rhinitis and asthma mediated by specific IgE responses to TMA-HSA complexes. Symptoms can occur within minutes after TMA re-exposure.

  In a cross-sectional study (Zeiss et al, 1992) in 474 employees, nearly 7% (n=32) developed immune disorders consisting of asthma/rhinitis (n=12), active LRSS (n=10), LRSS in remission (n=5), LPA (n=4), LAMS (n=1). One worker exposed to high levels of TMA fumes, developed PDA with TMA-HSA antibody levels of 16,000 ng/ml that decreased after removal from the workplace over six years to 1,000 ng/ml.

  LPA= Late Phase Asthma (Type I response) , LRSS= Late Respiratory Systemic Syndrome (Type III and IV responses); LAMS= Late Arthritic Myalgia Syndrome

In summery, a spectrum of diseases has been described within TMA-sensitized workers.

TMA-workers develop sensitization mediated by IgE, IgG or both (IgE+IgG).

But there are individuals working in the same environment without being sensitized.

The immunological basis of induction of sensitization / tolerance within TMA-workers remains to be elucidated.

- **Objective:** To investigate T-cell activation patterns in TMA exposed workers and whether altered TH1/TH2 balance is related to TMA sensitization.
- **Hypothesis:** Deficient Treg activation and subsequent altered TH1/TH2 balance will predict TMA sensitization in susceptible workers.

- **Specific Aims:** To accomplish the objectives and to test the hypotheses, the specific aims of the proposed study are:

1. To assess demographic characteristics and TMA-exposure levels of the workers who develop TMA-specific IgG and/or IgE immune responses
2. To identify T-cell activation patterns (TH1/TH2 balance and Treg activation) in TMA exposed/non-sensitized, exposed/IgG sensitized, exposed/IgG and IgE sensitized and non-exposed/non-sensitized workers.

- Preparation of a TMA-HSA conjugate (the skin test reagent) for rapid screening of sensitized individuals :
- This has been done following the method of Heuser et al. 1996. Briefly, TMA was dissolved in Carbonate Buffer and added drop-wise to 0.5% ice-cold HSA in stirring condition. The un-conjugated TMA was removed by dialyzing extensively against distilled water.

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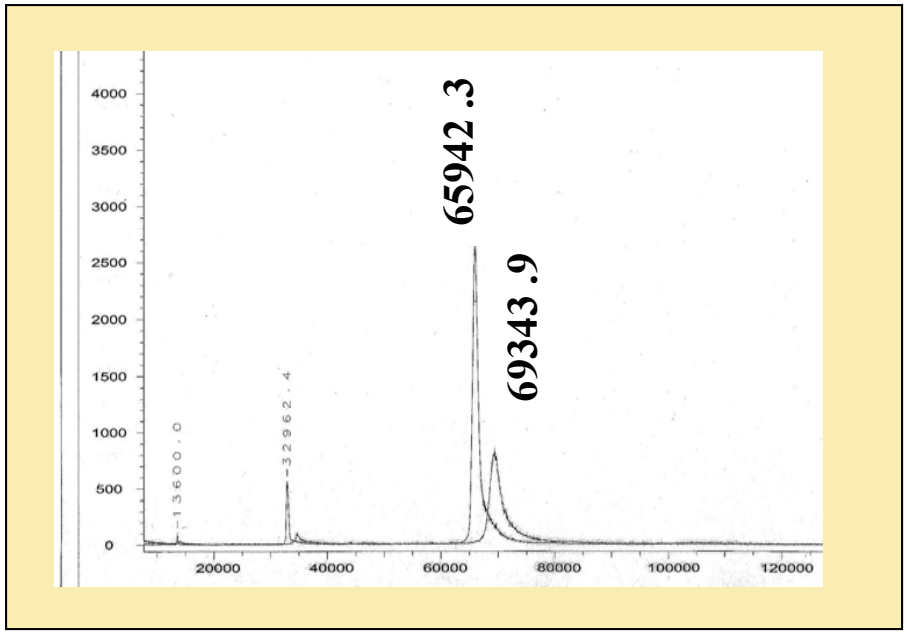


m/z

MALDI-TOF

Analysis of TMA-HSA conjugate

(69343.9 - 65942.3) /118 = 18.2

Molar Ratio

TMA: HSA = 1:18

Sensitivity, Specificity, and Positive Predictive Values (PPV) of IgE for Predicting SPT and IC Dilutions

Dilution	Dilution Level	Total Responses	IgE >		Sensitivity	Specificity	PPV*	p-value[b]
			N	%Total				
SPT	0	31	3	9.70%	88.90%	90.30%	72.70%	<0.001
	>0	9	8	89.90%				
SPT+ IC [a]	0	29	1	3.40%	90.90%	96.70%	90.90%	<0.001
	>0	11	10	90.90%				

PPV=positive predictive value; IC=intracutaneous

[a] SPT+ IC = 0 if SPT=0 and IC=0, otherwise (SPT+ IC) > 0.

[b] p-value <0.001 indicates a significant association between dilution category and IgE category.

Note: Specific IgE values that were entered as "< " were considered to be below the limit of detection (< LOD). Otherwise values were considered above the limit of detection (> LOD).

Correlation Coefficients (rho) and 95% Profile Confidence Limits (95%CL) Between Specific IGE and (i) Total IgE and (ii) SPT+ IC

	rho[a] (95% CL)	p-value[b]
Specific IgE vs. total IgE	0.24 (-0.15, 0.56)	P >.05
Specific IgE vs. SPT + IC	0.87 (0.79, 0.95)	P < .05

- [a] rho was obtained by assuming censored lognormal distributions for Specific IgE and SPT + IC values, and a lognormal distribution for total IgE values. Specific IgE values < LOD were censored. SPT+IC values =0 were censored.
- [b] Based on the p-value (p< .05), there is a significant correlation between Specific IgE and SPT+ IC, where the p-value is based on the inclusion of 0 in the 95% confidence interval for rho.

IgE-specific ELISA: Effect of serial dilution of sera of an IgE-sensitized worker indicates specificity

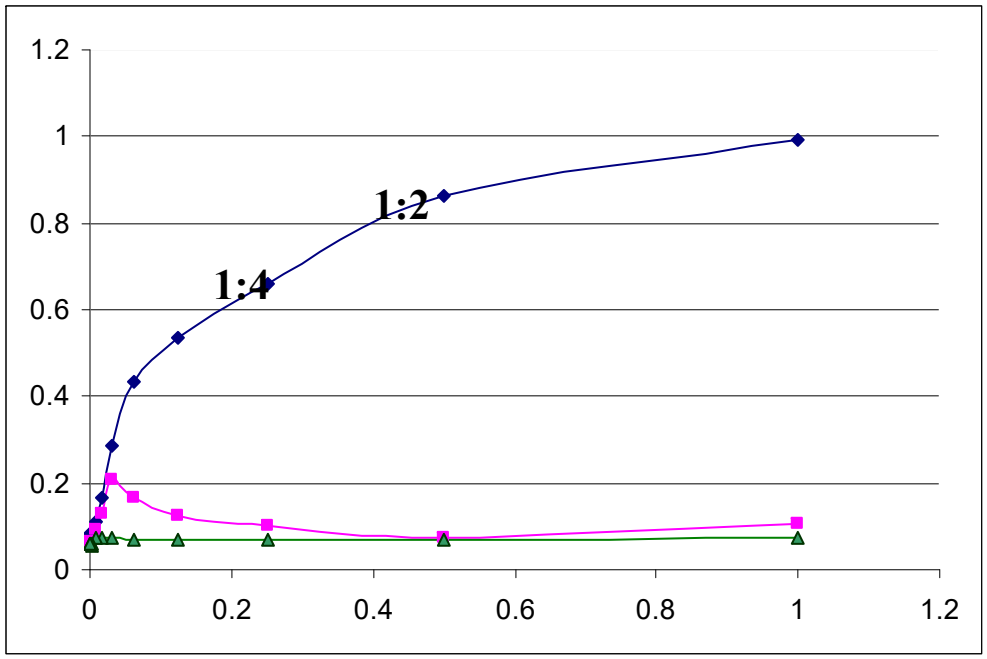


Plate coated with TMA-HSA or HSA as negative control

Incubated with sera from an IgE-sensitized worker.

Incubated with biotinylated mouse monoclonal anti human IgE

Detected with streptavidin-AP and pNPP as a substrate.

Serum dilution factor	1	0.5	0.25	0.125	0.06	0.03	0.02	0.008	0.004	0.002	0.001	0
TMA-HSA / IgE sensitized	0.99	0.86	0.66	0.537	0.43	0.29	0.16	0.113	0.072	0.073	0.082	0.058
TMA-HSA / non sensitized	0.105	0.08	0.101	0.126	0.17	0.21	0.13	0.092	0.061	0.059	0.063	0.056
HSA / IgE sensitized	0.075	0.07	0.07	0.071	0.07	0.08	0.08	0.072	0.061	0.057	0.062	0.062

Specific Aim – 1

TMA-exposure levels: Pumped filter/sorbent method. Air samples collected on TENAX sorbent are converted from TM Anhydride to TM Acid, which is then quantitated by HPLC attached to UV detector.

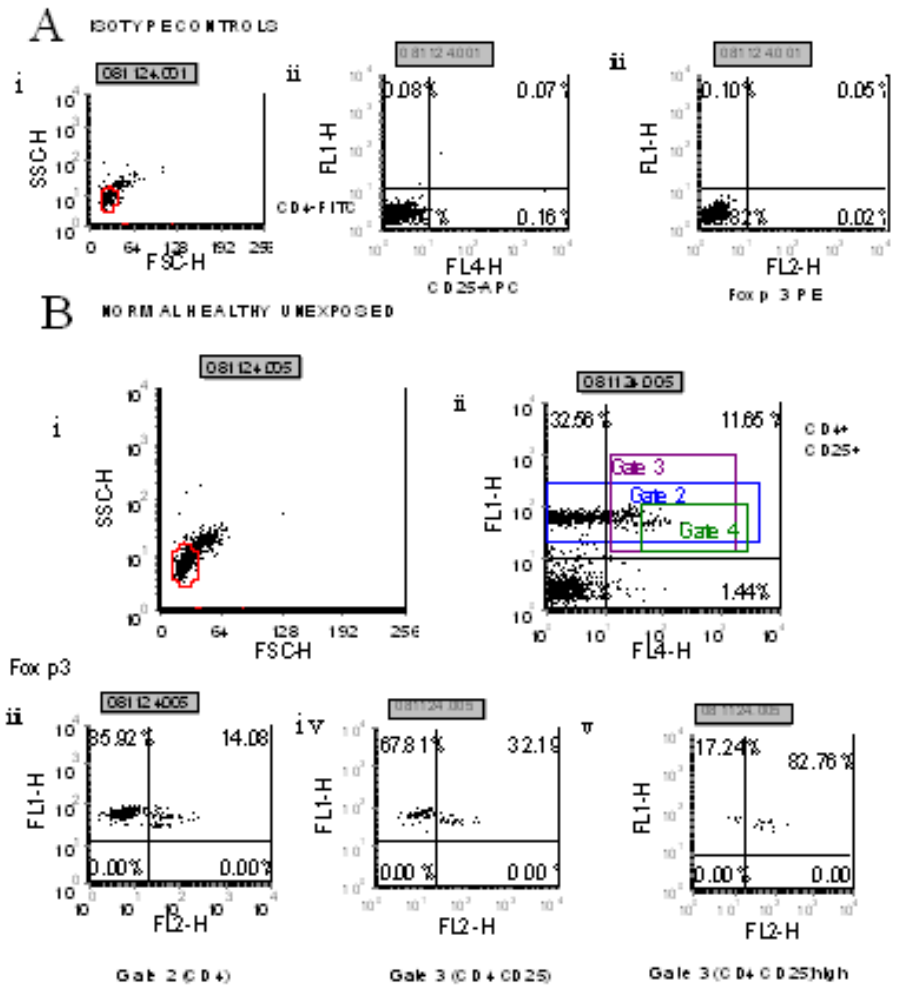
Study population: Age, gender, ethnicity, smoking history

Exposure and clinical data: spirometry data, duration of TMA exposure (in months), level of TMA exposure, total IgE, TMA specific skin testing, TMA specific IgG, IgG_4 and IgE.

Specific Aim - 2

To identify T-cell activation patterns (TH1/TH2 balance and Treg activation) in TMA exposed/non-sensitized, exposed/IgG sensitized, exposed/IgG and IgE sensitized and non-exposed/non-sensitized workers.

We have identified workers who are TMA exposed/non-sensitized (n=10), TMA exposed/IgG sensitized (n=10), TMA exposed/IgG and IgE sensitized (n=10) and TMA non-exposed/non-sensitized workers (n=10) for clinico-immunological study (specific aim-2).



Expected outcome: There will be a gradient of Treg cell activation across the spectrum of TMA non-sensitized (maximal Treg cell populations) to TMA sensitized workers (minimal Treg cell populations). The former group will be able to work safely.

Human Regulatory T-Cells were stained using ebioscience Treg staining kit. Briefly, PBMC isolated using Ficol and surface-stained by antiCD4 FITC antiCD25 APC cocktail. Cells are then permeabilized and stained for intracellar transcription factor Fox p3 using anti Fox p3 PE.

Gating: Gate2- CD4, Gate3-$CD4^{+}CD25^{+}$ and Gate4-$CD4^{+}CD25^{+hi}$

Sensitized workers show less number of $CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$ cells.

	CD4 CD25	CD4 CD25 Fox p3
Control	25.60	6.71
Subject 1	5.67	1.86
Subject 2	8.30	3.25
Subject 3	3.55	1.07
Subject 4	8.02	1.35

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