Toxicogenomic Investigation into False Positive Responses in the Local Lymph Node Assay (LLNA)

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Background

The mouse LLNA is the preferred assay for identifying skin sensitization potential of industrial materials and is a critical endpoint for defining their safe handling and use. However, recent publications have identified several chemistries that yield false positive responses in the LLNA compared to guinea pig or human assays.



2-Way ANOVA Linear Contrasts for Differentially **Expressed Genes**

2 Way ANOVA linear contrasts, 3-Class coding: 0 = Control, 1 = Sensitizers, 2 = Presumptive False **Positives** - Probes with FDR < 0.05 AND FC > ± 1.5

Key Regions of interest

Genes similarly regulated by sensitizers and FPs - B Genes differentially regulated between sensitizers and FPs – E, D and G



<u>Hypothesis:</u> Genome-wide gene expression at the level of the lymph node is indicative of the mechanism of immune response (antigen-specific or non-immune specific) and can be used to discriminate between true sensitizers and false positives

False Positive Criteria:

- Clear positive in the LLNA with consistent dose-response
- Clear negative in guinea pig maximization tests
- Non-sensitizer in human tests or based on human experiences

Study Design



The Day 4 and 6 time point exhibited robust changes in gene expression with differential responses between sensitizers and false positives. The Day 10 time point was not active and was not examined further.

Sensitizers and Presumptive False Positives Regulate Distinct Sets of Genes





Gene lists from Regions D and E were examined to identify false positive-specific genes. Key functional categories represented included- Acute inflammatory response and Innate defense response

Local Lymph Node Assay

Stimulation Index and Irritation Potential

				Average Erythema Score					% Δ Ear Thickness			
	#	Dose Group	SI	Day 2	Day 3	Day 4	Day 6	Day 10	Day 3	Day 4	Day 6	Day 10
	1	Untreated	NA	0.0	0.0	0.0	0.0	0.0	4.2	2.0	0.7	1.5
Controls	2	DMSO	1.0	0.0	0.0	0.0	0.0	0.0	6.2	8.2	6.5	3.3
	3	Acetone	1.0	0.0	0.0	0.0	0.0	0.0	-2.0	3.6	0.1	-1.0
	4	DNCB 0.1%	18.9	0.0	0.0	0.0	0.0	0.0	-2.8	5.5	-1.1	-6.4
	5	HCA 25%	5.3	1.0	1.7	2.0	0.6	0.0	19.1	25.4	21.7	21.0
	6	lso 10%	5.1	0.0	0.0	0.0	0.0	0.0	-0.3	9.5	3.3	2.3
	7	PPD 1%	5.4	0.0	0.0	0.0	0.0	0.0	1.9	7.4	2.4	0.2
Sensitizers	8	HQ 0.25%	9.3	0.0	0.0	0.0	0.0	0.0	0.7	4.1	3.9	-1.7
	9	MDBGN 20%	3.7	0.9	1.9	1.0	0.9	0.0	44.6	67.2	34.8	4.1
	10	TDI 0.04%	11.8	0.0	0.0	0.0	0.0	0.0	-0.1	1.6	1.5	4.0
	11	TMA 0.65%	10.4	0.0	0.0	0.0	0.0	0.0	1.3	6.2	0.5	1.5
	12	AHCP 0.7%	7.6	0.0	0.0	0.0	0.0	0.0	0.6	13.6	4.4	5.3
	13	Oleic Acid 50%	7.2	0.9	2.0	2.0	2.0	0.0	41.4	69.3	99.2	33.0
	14	Maleic 11.5%	3.3	0.7	1.0	1.0	0.9	1.2	28.0	60.4	92.0	48.1
Presumptive	15	SLS 25%	2.4	0.1	0.3	0.0	0.8	0.0	20.3	49.3	<mark>8</mark> 2.5	42.4
False Positives	16	TGME 20%	6.9	0.0	1.1	1.0	1.9	0.0	49.0	63.5	97.0	8.2
	17	Siloxane 45%	20.6	0.0	0.0	0.0	0.0	0.0	65.0	120.5	152.5	-2.6
	18	DPP 35%	12.6	0.2	1.0	2.0	1.0	0.0	17.1	35.9	19.7	8.4
	19	HDE 30%	20.4	0.0	0.3	0.0	1.0	0.4	11.3	25.6	17.8	22.6

Developing Hypothesis & Classifier Development



□ Similar distribution of SI responses across sensitizers and presumptive false positives □ More robust irritation (average erythema score > 0, % change in ear thickness \ge 25%) observed with presumptive false positives compared to sensitizers □ Sensitizers, <u>HCA & MDBGN</u>, also appear to be irritating □ SLS, included as a classic LLNA false positive and skin irritant

False Positives

vxane rGME laleic Oleic

0.656

0.878

0.857

1.000

0.950

0.930

0.833

0.975

The optimal day 6 classifier gene list included 44 and 30 genes for sensitizers and false positives, respectively, with 19

The 55 total genes displayed functional relevance to sensitization and irritation responses

Conclusions

- □ Toxicogenomic responses may have the potential to enhance the LLNA by identifying genes that discriminate between chemical classes
- □ Functional analyses of the gene expression responses have indicated that:
 - □ Sensitizers induce a gene expression response consistent with an antigen-mediated T-cell response
 - □ False Positives induce a gene expression response consistent with a non-immune specific proinflammatory response
- □ These data have been used to develop statistical classification models capable of accurately predicting skin sensitizing chemicals based on the gene expression signatures
- □ These models are being further evaluated on a distinct list of sensitizers and false positives (6 sensitizers and 6 false positives) to independently assess model performance