Faculty Development Grant Spring/Summer 2025 - Report

Project Title:
Differential Gene Expression with Sequential Chorioamnionitis and Hyperoxia exposure to Neonatal Monocytes and Rat Model

Saurabh Gayen
Associate Professor
Chemistry and Physics Department

Introduction

Chorioamnionitis is a low grade infection of the placenta. Long term consequences of chorioamnionitis in the form of asthma has been reported.[1] Not much is known about the mechanism of long term immune changes caused by chorioamnionitis in the newborn. Development of a form of immunological memory in innate immunity cells, a phenomenon known as trained immunity has been shown by Netea et.al.[2] It is possible that the trained immunity due to exposure to chorioamnionitis may contribute to the long-term consequences of chorioamnionitis.

Oxygen toxicity in the newborn is also a well-known phenomenon, causing inflammation, including lung and eye disease. Additionally, in animal models with maternal chorioamnionitis, subsequent exposure to oxygen is shown to elicit an altered response. Paradoxically, response to oxygen after exposure to endotoxin was found to be associated with some form of oxygen tolerance. The exact mechanism of this change is not certain.[3] After exposure to chorioamnionitis, if the trained monocytes are subsequently exposed to hyperoxia, it can incite an exaggerated inflammatory response and contribute to the development of Bronchopulmonary Dysplasia (BPD) in premature infants' lung.

We carried out Bioinformatics analysis of the Microarray data files provided by Dr. Zubair Aghai's Lab at Thomas Jefferson University, Philadelphia. The experiment consists of four groups: Control-Normoxia, Control-Hyperoxia, Chorio-Normoxia and Chorio-Hyperoxia for human neonates as well as neonatal rat model.

CEL files (from microarray data) was first normalized and treatment groups were compared with control group using Transcriptome Analysis Console software 4.0 (TAC 4.0, Thermo Fisher Scientific, Waltham, MA). Student t-test was used for comparison of each treatment group with the control group. Genes with fold change ≥ 1.5 and $p \leq 0.05$ were identified as differentially expressed. We carried out Pathway analysis on differential gene list, using Ingenuity pathway analysis (IPA, QIAGEN Inc.., https://digitalinsights.qiagen.com/IPA)[4]. IPA is one of the advanced bioinformatics tools provided by Qiagen Inc. that is a web-based software application program for the analysis, integration, and interpretation of data derived from microarray, gene expression or other array based or sequencing methods. Using IPA we identified canonical pathways, networks, toxicological functions and upstream regulators. We used Morpheus software (Broad Institute, Cambridge, MA) for Cluster analysis (heat map) of differentially expressed genes.

While analyzing data we inquired in details following two aspects:

A. What kind of Gene expressions can be observed in Cord Blood Monocytes due to Postnatal Hyperoxia only?

B. How do the Gene expressions in Cord Blood Monocytes can be altered due to Sequential Exposure to Intrauterine Inflammation and Postnatal Hyperoxia?

Part A

Results:

Cord blood samples were exposed to normoxia (n=7) and hyperoxia (n=7). On RNA analysis, a total of 500 genes had altered expression, of which 171 were upregulated and 329 were downregulated. The top 10 altered genes with fold change are shown in Table 1.

Table 1: Top 10 altered genes with fold change in cord blood monocytes with hyperoxia exposure compared to normoxia

Up-regulated Genes	Fold Change	Down-regulated Genes	Fold Change
CDC42EP3	4.790	GBP5	-7.250
ASPH	4.450	TLR8	-6.680
DNAJB1	3.810	PECAM1	-6.520
TM4SF19	3.480	RNFT1	-4.930
CXCL8 2	2.960	FGL2	-4.830
TM4SF19-DYNLT2B	2.850	TUBA1A	-4.590
PLPP3	2.800	FPR3	-4.480
NRIP3	2.690	TLR6	-4.390
POLR3G	2.690	XPC	-4.330
GPNMB	2.610	CSF1R	-3.820

Top canonical pathways identified are shown in Table 2, which notably included SUMOylation of DNA which can modulate cellular damage response and function of repair proteins. Top molecular and cellular functions altered were cell-to-cell signaling and interaction and free radical scavenging. Physiological systems altered included organismal survival, tissue development, hematological system

development and function, immune cell trafficking and humoral immune response. Table 3 shows the pathological entities (diseases and disorders) correlating with genes altered.

Table 2: Top Canonical Pathways with hyperoxia in neonatal cord blood monocytes

Name	p-value
SUMOylation of DNA damage response and repair proteins	8.77E-05
Sirtuin Signaling Pathway	1.19E-04
FXR/RXR Activation	2.57E-04
Retinol Biosynthesis	4.36E-04
TREM1 Signaling	5.96E-04

Table 3: Diseases and Disorders

Name	P value
Cancer	7.55E-03 - 1.26E-48
Organismal Injury and Abnormalities	7.98E-03 - 1.26E-48
Endocrine System Disorders	5.49E-03 - 2.40E-32
Gastrointestinal Disease	7.47E-03 - 4.48E-32
Dermatological Diseases and Conditions	7.86E-03 - 6.80E-16

Conclusion(s)

Neonatal cord blood monocytes following in vitro exposure to hyperoxia compared to normoxia show altered gene expression with prominent effects on cellular injury and repair. The pathways identified may help enhance our understanding of long-term diseases associated with neonatal oxygen therapy and altered immune function.

Part B

A total of 14 cord blood and placental tissue samples were collected (7 histological chorioamnionitis and 7 control). 1,646 differentially expressed genes were identified after exposure to sequential chorioamnionitis and hyperoxia (1091 upregulated and 555 downregulated). Top upregulated genes included CLEC5A, FN1, KCNE1, DAB2, and RGCC and top downregulated genes included TMEM17B, IFIT1, USP18, IFIT2, and RGL1 (Table 4).

Table 4: Top 20 Upregulated and Downregulated genes in Corioamnionitis-Hyperoxia vs Control-Normoxia

Up-regulated Genes	Fold Change	Down-regulated Genes	Fold Change
CLEC5A	79.2	**TMEM176B**	-20.54
FN1	28.26	**IFIT1**	-10.59
KCNE1	11.65	**USP18**	-9.31
DAB2	10.15	**IFIT2**	-6.27
RGCC	9.78	**RGL1**	-6.11
GPRIN3	9.33	**PNPT1**	-5.61
SDC2	8.96	**HLA-DQB1**	-5.51
CCL24	8.94	**RGPD2; RGPD1**	-5.24
CENPW	8.86	**OAS3**	-4.49
CMYA5	8.57	**TNFSF10**	-4.26
PLIN2	8.05	**TMEM110-MUSTN1; TMEM110**	-4.13
HSPD1	7.99	**RSAD2**	-4.07
TREM1	7.94	**IL1RN**	-3.54
ST3GAL6	7.7	**PPP5C**	-3.5
VCAN	7.41	**IDO1**	-3.47
NEDD9	7.06	**TMEM154**	-3.47
ENTPD7	6.73	**JCHAIN**	-3.35
SLC22A5	6.31	**DDX11**	-3.34
GLA	6.02	**OTUD6B**	-3.21
KCNE1	5.96	**LMO2**	-2.98

IPA was performed on 1646 differentially expressed genes. Important Canonical pathways identified by IPA were neutrophil degranulation, protein ubiquitination, ferroptosis signaling, TCR signaling, and interleukin-1 family signaling (Table 5).

Table 5: Canonical Pathways associated with differentially expressed genes in Chorio-Hyperoxia group compared to Control-Normoxia

Ingenuity Canonical Pathways	-log(p-value)	z-score	Number of Molecules Involved
Neutrophil degranulation	9.98	5.812	74
Protein Ubiquitination Pathway	8.69	4.814	48
Class I MHC mediated antigen processing and presentation	7.87	5.252	58
Superpathway of Cholesterol Biosynthesis	7.85	2.309	13
Cholesterol biosynthesis	7.49	2.887	12
Ferroptosis Signaling Pathway	6.14	0.784	26
TCR signaling	5.67	2.2	25
Interleukin-1 family signaling	4.97	4.082	24
PTEN Regulation	4.75	3.71	26
Signaling by NOTCH4	4.31	3.153	17
Regulation of Apoptosis	4.22	3.051	13
Amino acids regulate mTORC1	4.04	3.606	13
TREM1 Signaling	3.9	3.873	15
Detoxification of Reactive Oxygen Species	3.75	3.162	10
NRF2-mediated Oxidative Stress Response	3.71	2.324	30
TNFR2 non-canonical NF-kB pathway	3.62	3.207	14
PPARα/RXRα Activation	3.3	-0.775	26
TP53 Regulates Metabolic Genes	2.92	2.84	15
ERK/MAPK Signaling	2.9	1.877	28
IL-10 Signaling	2.66	0.218	21
Sirtuin Signaling Pathway	2.45	-0.209	33
Macrophage Alternative Activation Signaling Pathway	2.38	2.294	23
Signaling by the B Cell Receptor (BCR)	2.37	2.985	22

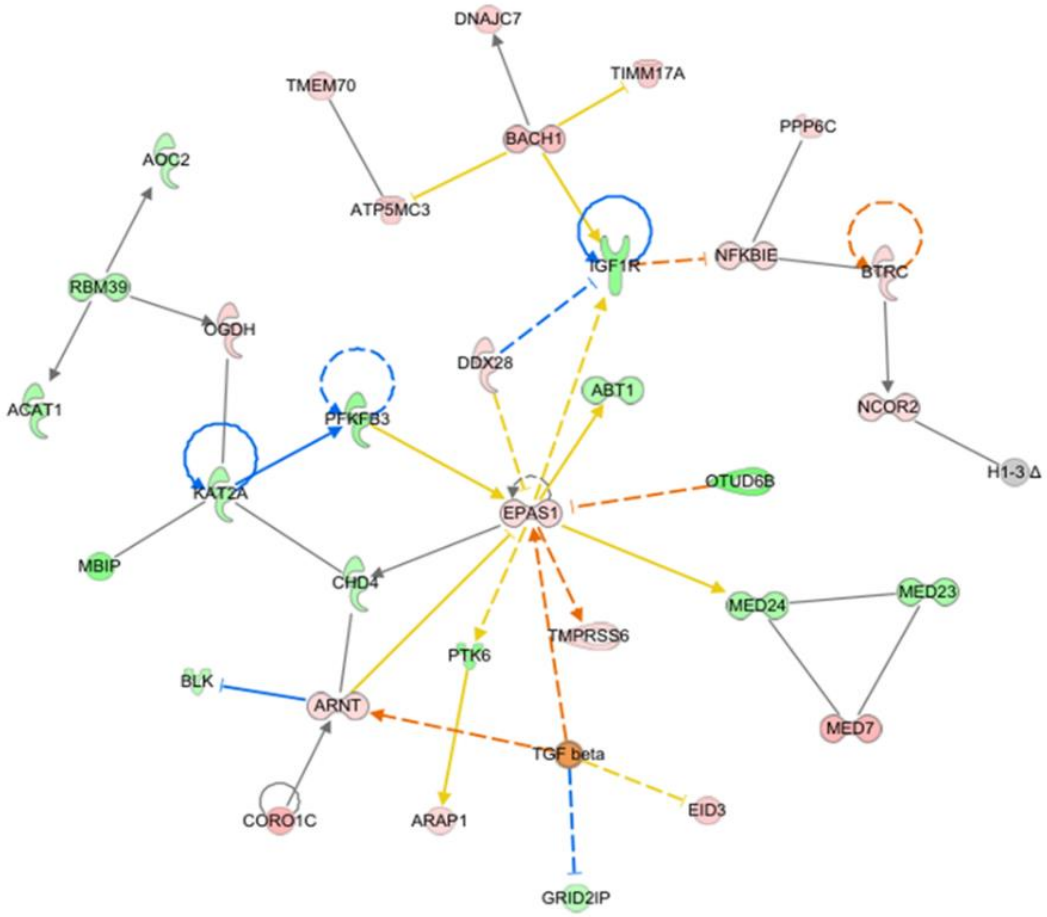
Important diseases and functions affected were cell death and survival, infectious diseases, cellular development, growth and proliferation, immunological disease, inflammatory disease and response (Table 6).

Table 6: Key diseases and functions modified with exposure to sequential chorioamnionitis and hyperoxia in newborns

Modified Disease and Functions	Number of Genes Involved	Range of p-value for genes involved
Organismal Injury and Abnormalities	1513	1.03E-43-2.43E-03
Cell Death and Survival	494	5.52E-14-2.43E-03
Infectious Diseases	358	3.34E-13-2.34E-03
Cellular Development	460	3.92E-13-2.34E-03
Cellular Growth and Proliferation	459	3.92E-13-2.25E-03
Immunological Disease	656	4.26E-13-2.34E-03
Inflammatory Disease	335	4.26E-13-2.34E-03
Cellular Function and Maintenance	478	1.28E-11-2.36E-03
Inflammatory Response	329	1.28E-11-2.34E-03
Cell-To-Cell Signaling and Interaction	270	2.86E-11-2.18E-03
Gene Expression	284	3.16E-11-2.35E-04
Neurological Disease	1057	4.14E-10-2.34E-03
Immune Cell Trafficking	78	4.94E-08-2.04E-03
Developmental Disorder	279	5.98E-08-2.15E-03
Cardiovascular System Development and Function	110	7.45E-07-2.18E-03
Respiratory Disease	474	8.15E-05-1.38E-03
Cell Signaling	147	1.11E-04-2.28E-03
Nervous System Development and Function	9	7.01E-04-7.01E-04
Cell-mediated Immune Response	21	7.54E-04-7.54E-04

IPA picked up important networks related to Cell-To-Cell Signaling and Interaction, Embryonic Development, Gene Expression, Cellular Assembly and Organization, DNA Replication, Recombination, and Repair, Gene Expression, Cellular Movement, Cell Cycle, Hereditary Disorder, Neurological Disease, Organismal Injury and Abnormalities, Immunological Disease, Inflammatory Disease, Organismal Injury and Abnormalities. One key network is shown in Figure 1.

Figure 1: Gene Expression, Cell to Cell signaling and Embryonic Development



Conclusion(s)

Sequential exposure to intrauterine inflammation and postnatal hyperoxia leads to differential gene expression in cord blood monocytes, many of which regulate immune and inflammation pathways. Our data may enhance understanding of key genes and pathways involved in sequential exposure to intrauterine inflammation and postnatal hyperoxia.

References:

1. Getahun D, Strickland D, Zeiger RS, et al. Effect of chorioamnionitis on early childhood asthma. Arch Pediatr Adolesc Med. 2010. doi:10.1001/archpediatrics.2009.238
2. Netea MG, Quintin J, Van Der Meer JWM. Trained immunity: A memory for innate host defense. Cell Host Microbe. 2011;9(5):355-361. doi:10.1016/j.chom.2011.04.006
3. Jobe AH, Kallapur SG. Long term consequences of oxygen therapy in the neonatal period. Semin Fetal Neonatal Med. 2010;15(4):230–235. doi:10.1016/j.siny.2010.03.007
4. Kramer A, Green J, Pollard J Jr., Tugendreich S. Causal analysis approaches in ingenuity pathway analysis. Bioinformatics. 2014; 30:523–30. doi: 10.1093/bioinformatics/btt703

Based on these analyses two abstracts are accepted for poster presentations in 97th Pediatric Academic societies Meeting (PAS 2026) at Boston from April 24-27, 2026

Dr. Saurabh Gayen is a Co-author for the following abstracts:

1. Abstract ID: 2277017

Abstract Title: Gene Expression Signatures in Cord Blood Monocytes After Exposure to Postnatal Hyperoxia

2. Abstract ID: 2277053

Abstract Title: Sequential Exposure to Intrauterine Inflammation and Postnatal Hyperoxia Alters Gene Expression in Cord Blood Monocytes