Molecular Mechanisms of Ventilator-Induced Lung Injury

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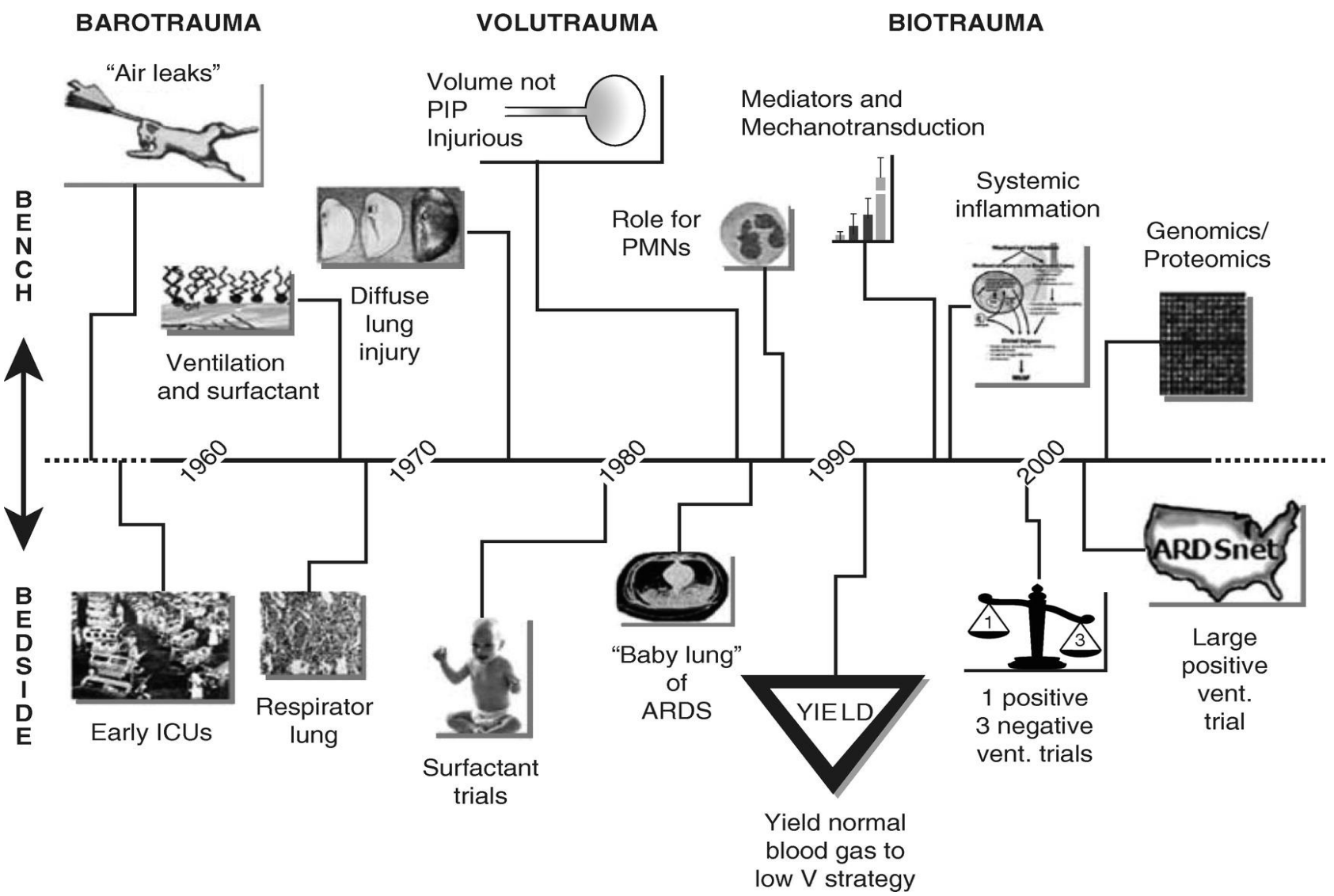
Definition

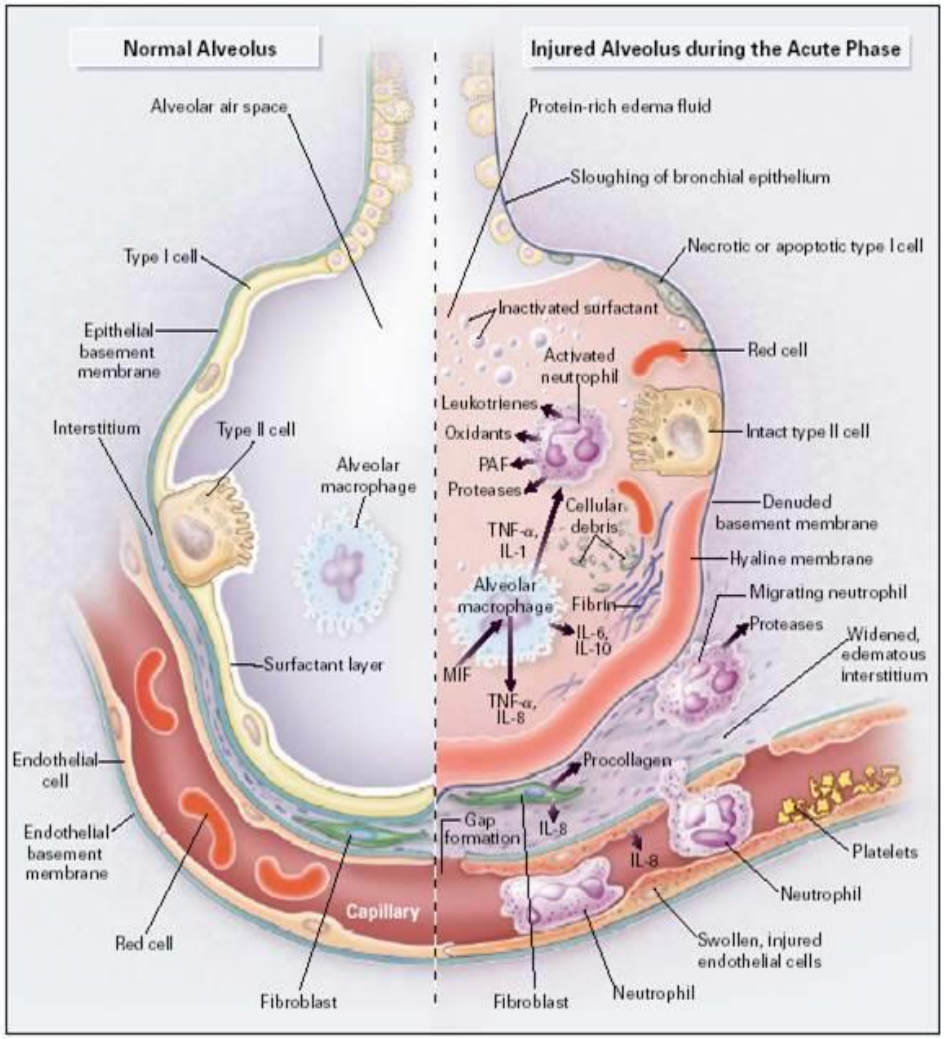
- Ventilator-induced lung injury (VILI)
- Ventilator-associated lung injury (VALI)-a causative relationship between lung injury and the mechanical ventilator cannot be proven.

Timeline of bench to bedside research on VILI



***AJRCCM**. 2015; 191(10):1106–1115*

Acute respiratory distress syndrome (ARDS)



Direct Lung Injury	Indirect Lung Injury
Common causes	**Common causes**
Pneumonia	Sepsis
Aspiration of gastric contents	Severe trauma with shock and multiple transfusions
Less common causes	**Less common causes**
Pulmonary contusion	Cardiopulmonary bypass
Fat emboli	Drug overdose
Near-drowning	Acute pancreatitis
Inhalational injury	Transfusions of blood products
Reperfusion pulmonary edema after lung transplantation or pulmonary embolectomy	

N Engl J Med. *2000;342(18):1334-1349*

The New England
Journal of Medicine

VOLUME 342 MAY 4, 2000 NUMBER 18

VENTILATION WITH LOWER TIDAL VOLUMES AS COMPARED WITH TRADITIONAL TIDAL VOLUMES FOR ACUTE LUNG INJURY AND THE ACUTE RESPIRATORY DISTRESS SYNDROME

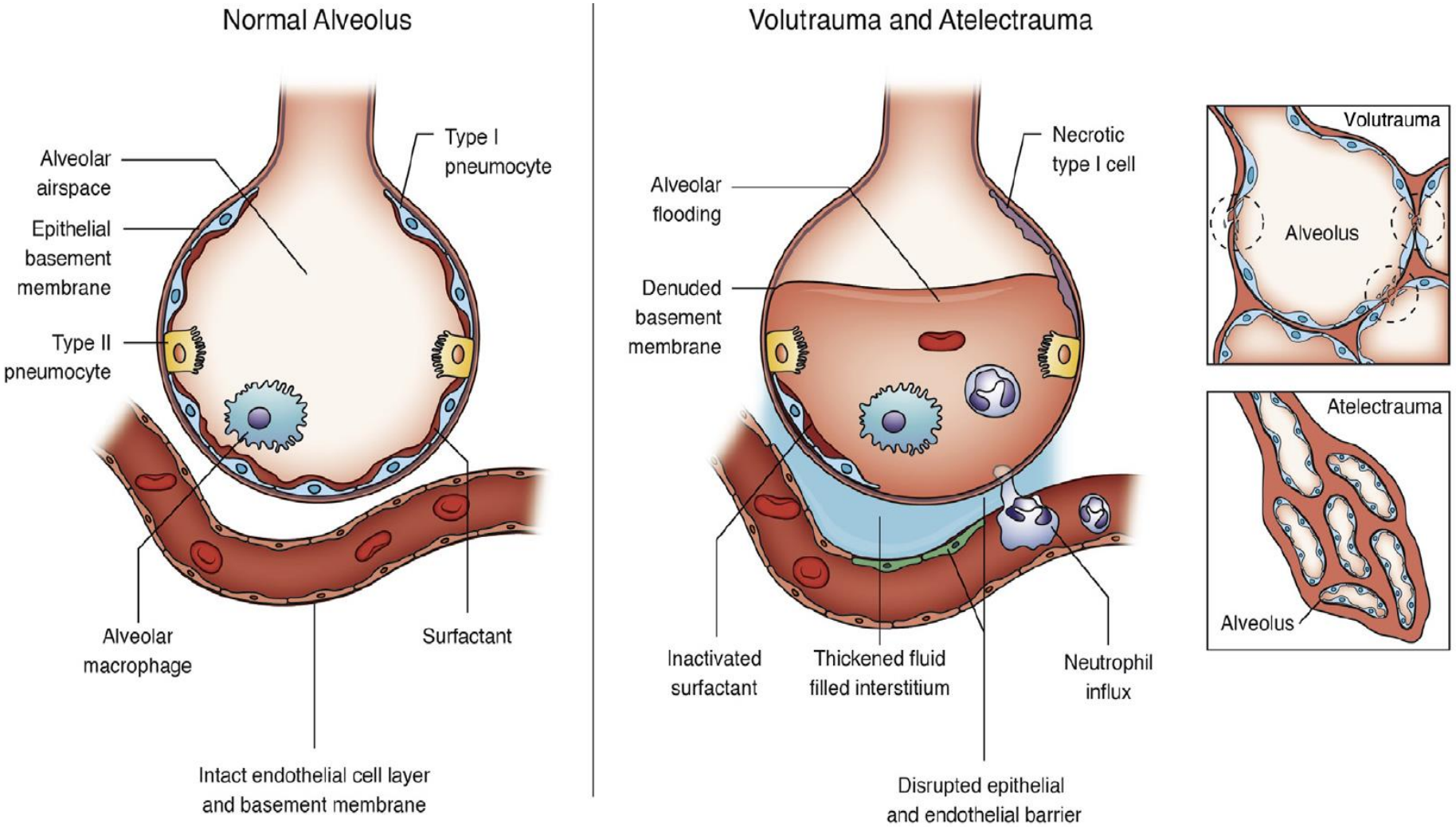
THE ACUTE RESPIRATORY DISTRESS SYNDROME NETWORK*

- The results showed a relative risk reduction of mortality by 22% in patients ventilated with the lower tidal volume.
- This indicates that mortality attributable to ventilator-induced lung injury (VILI) is at least 9 to 10%.

Mechanisms

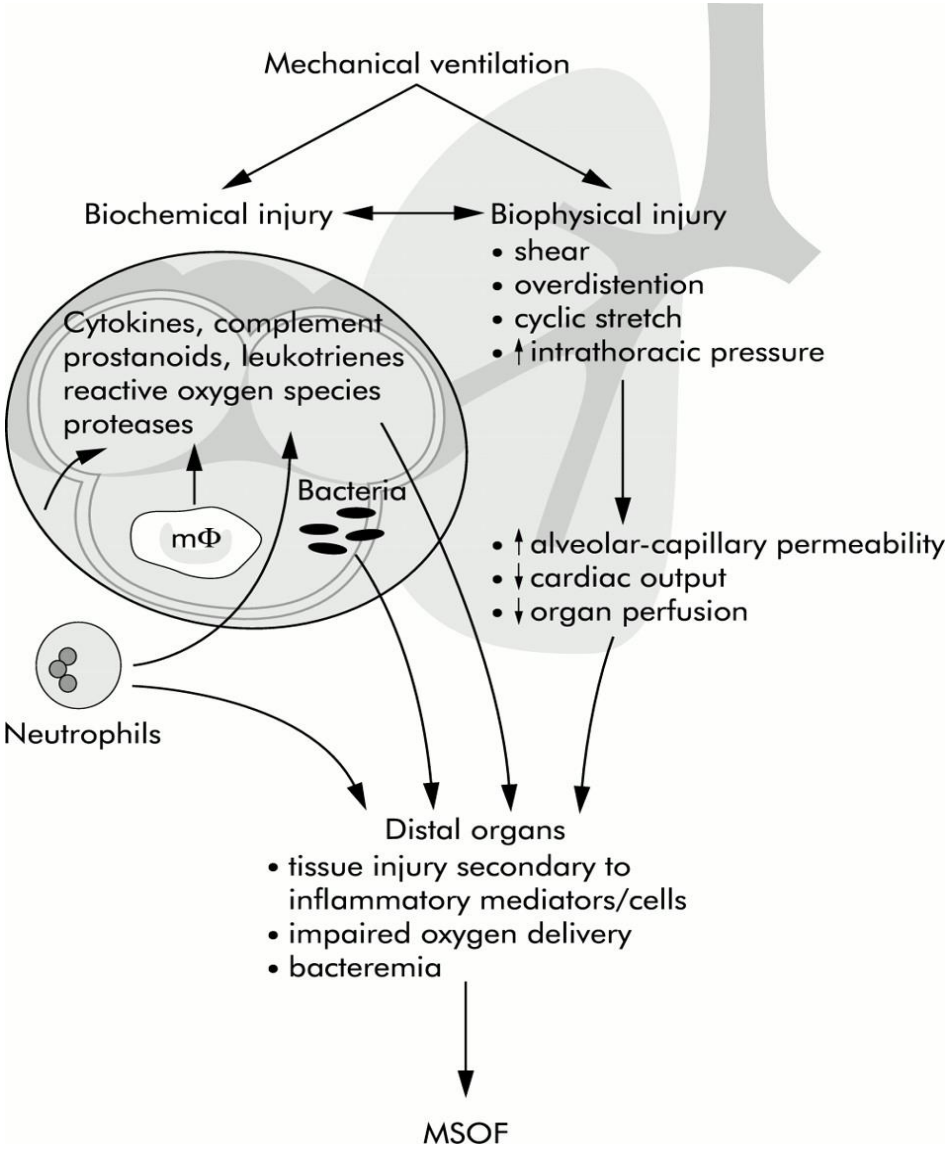
- Alveolar overdistension (volutrauma)
- Atelectrauma
- Biotrauma

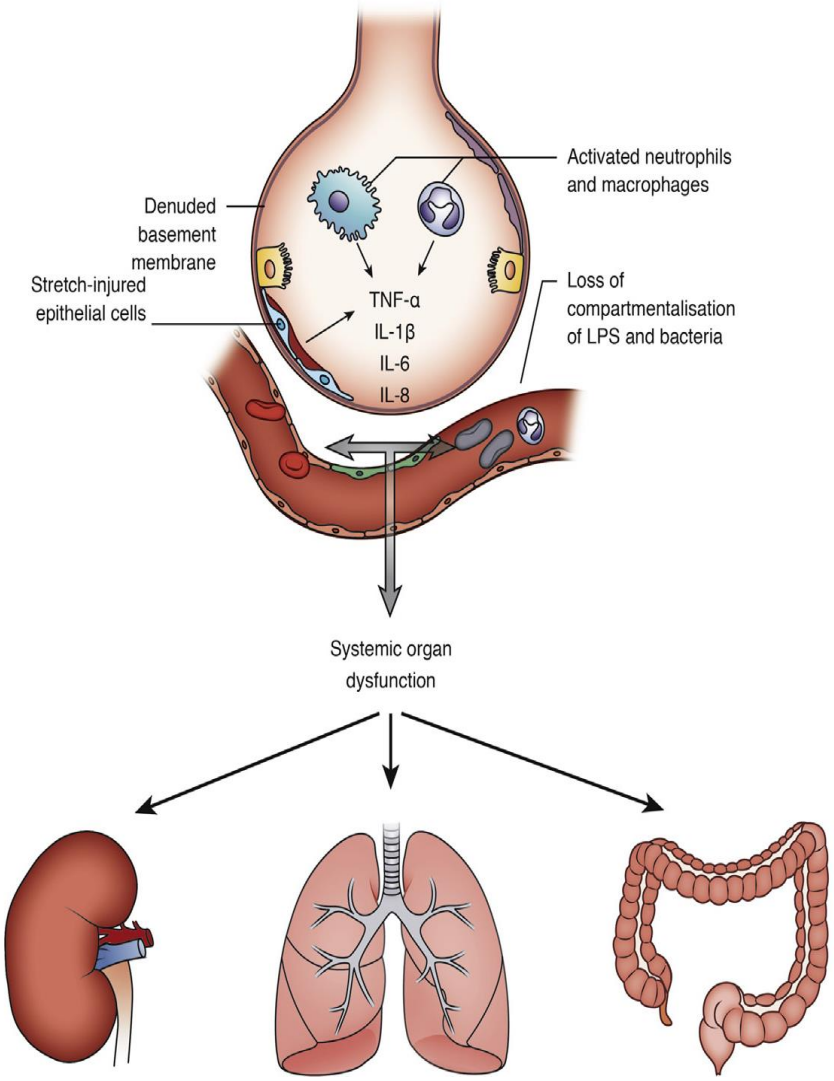
Volutrauma and Atelectrauma



Chest *2016; 150(5):1109-1117*

Biotrauma





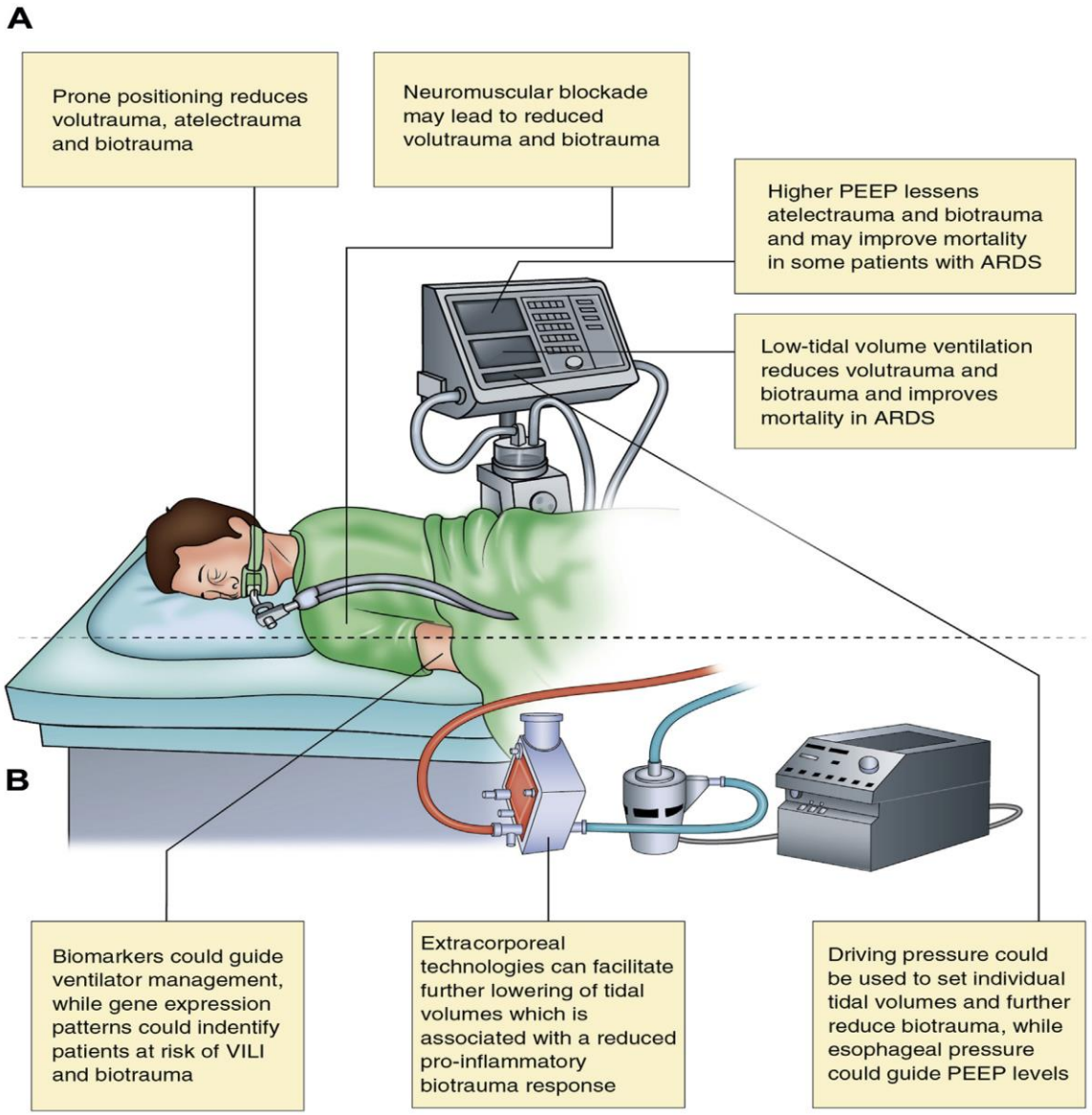
Chest *2016; 150(5):1109-1117*
Thorax *2002;57:635-642*

How to treat or prevent VILI?

- Lung Protective Ventilation (Precision Ventilation)
- Prone Positioning
- Biomarkers and Pharmacologic Strategies (ARDS heterogeneity)

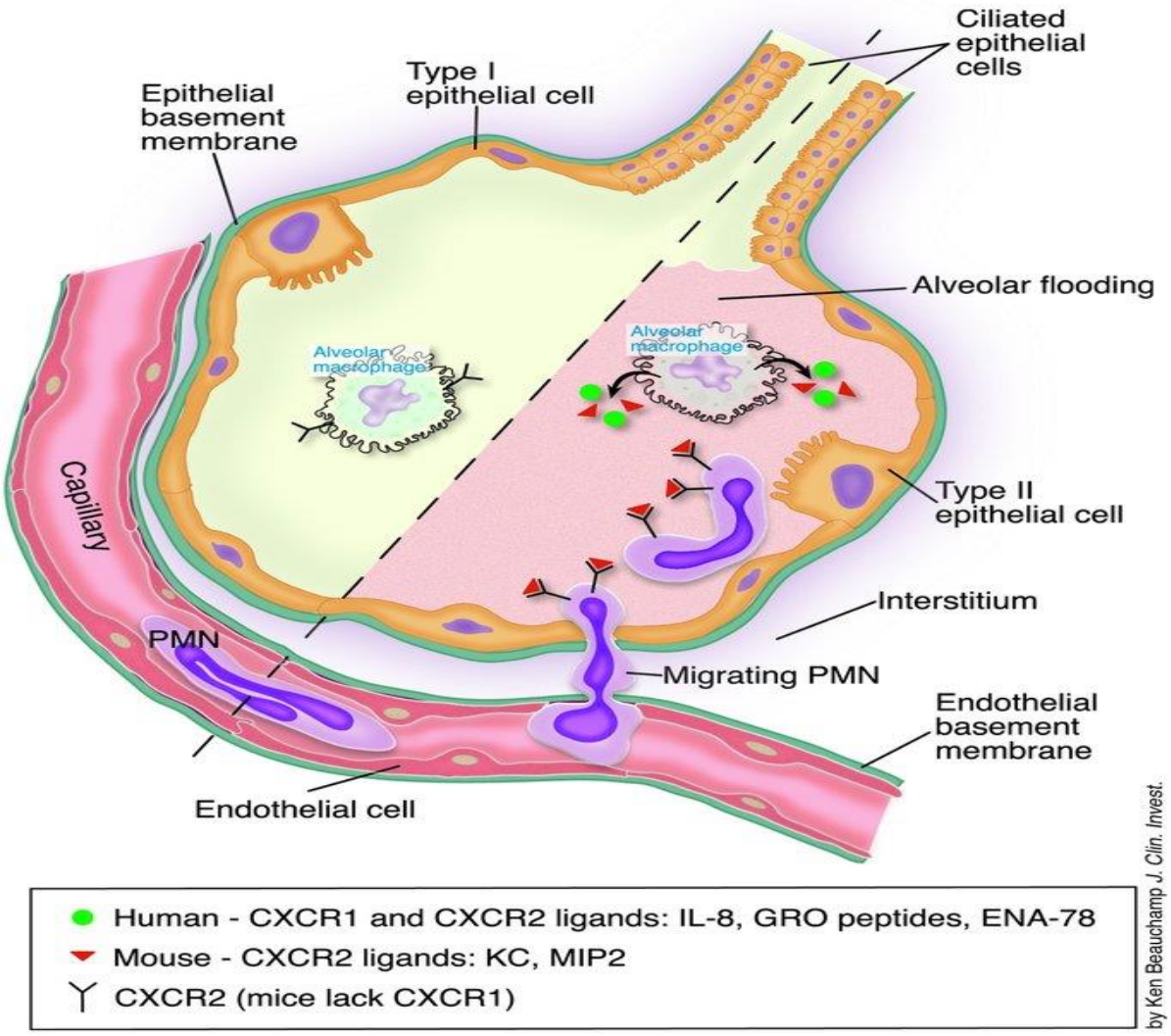
CHEST 2016; 150(5):1109-1117

Current Clinical Practice



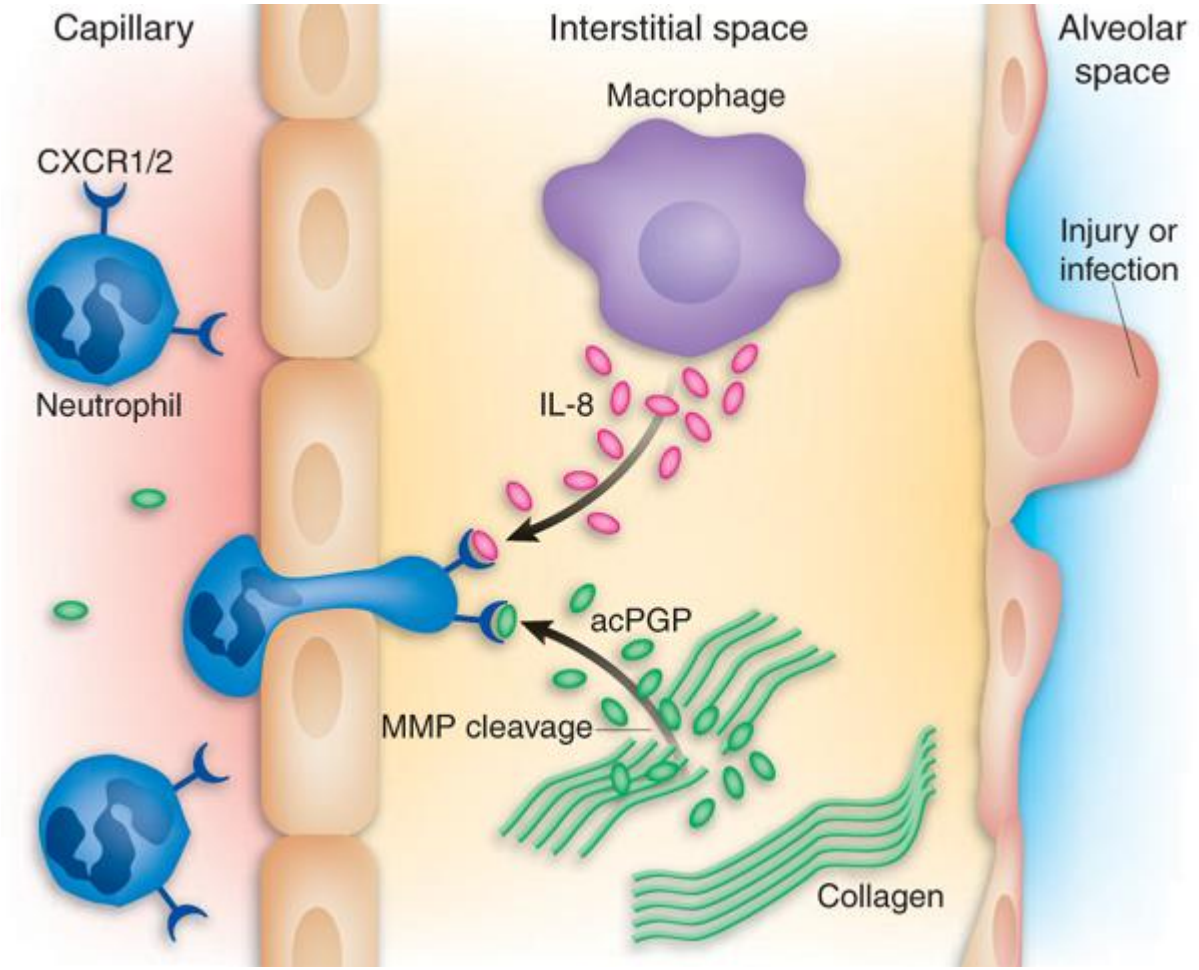
***Chest** 2016; 150(5):1109-1117*

Mechanisms of alveolar macrophage in VILI



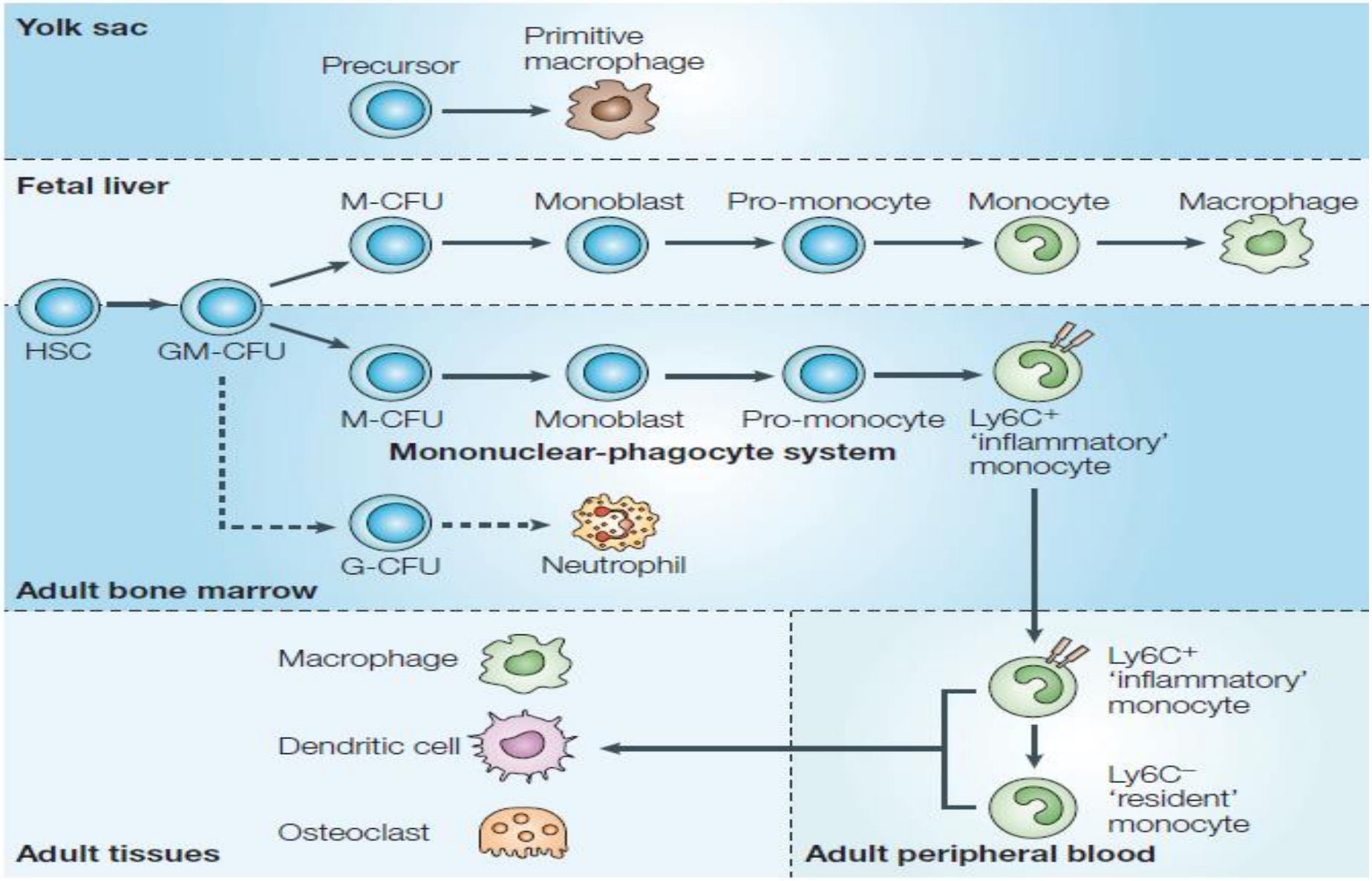
J Clin Invest. 2002;110(11):1603-1605.
Am J Physiol Lung Cell Mol Physiol 2006; 291: L1191–L1198, 2006.

Mechanisms of pulmonary neutrophil sequestration in VILI



Nature Medicine. 2006; 12, 280 - 281
Am J Physiol Lung Cell Mol Physiol 2004; 287: L902–L910
J Clin Invest. 2002;110(11):1603-1605.

Box 1 | **The mononuclear-phagocyte system**



Nat Rev Immunol 2005;5:953-964.

Table 1 | **Phenotype of the two best-characterized monocyte subsets in various mammals***

Antigen	Human $CD14^{hi}CD16^{-}$ 'inflammatory' monocytes	Human $CD14^{+}CD16^{+}$ 'resident' monocytes	Mouse $CCR2^{+}CX_3CR1^{low}$ 'inflammatory' monocytes	Mouse $CCR2^{-}CX_3CR1^{hi}$ 'resident' monocytes	Rat $CD43^{low}$ 'inflammatory' monocytes	Rat $CD43^{hi}$ 'resident' monocytes	Pig $CD163^{-}$ 'inflammatory' monocytes	Pig $CD163^{+}$ 'resident' monocytes
Chemokine receptors								
CCR1	+	–	ND	ND	ND	ND	ND	ND
CCR2‡	+	–	+	–	+	–	ND	ND
CCR4	+	–	ND	ND	ND	ND	ND	ND
CCR5	–	+	ND	ND	ND	ND	ND	ND
CCR7	+	–	ND	ND	+	–	ND	ND
CXCR1	+	–	ND	ND	ND	ND	ND	ND
CXCR2	+	–	ND	ND	ND	ND	ND	ND
CXCR4	+	++	ND	ND	ND	ND	ND	ND
CX_3CR1‡	+	++	+	++	–	+	ND	ND
Other receptors								
CD4	+	+	ND	ND	+/–	++	ND	ND
CD11a	ND	ND	+	++	ND	ND	+	++
CD11b	++	++	++	++	++	++	ND	ND
CD11c‡	++	+++	–	+	–	+	+	++
CD14	+++	+	ND	ND	ND	ND	++	+
CD31	+++	+++	++	+	ND	ND	ND	ND
CD32	+++	+	ND	ND	+++	+	ND	ND
CD33	+++	+	ND	ND	ND	ND	ND	ND
CD43	ND	ND	–	+	–	+	ND	ND
CD49b	ND	ND	+	–	ND	ND	ND	ND
CD62L‡	++	–	+	–	+	–	ND	ND
CD80	ND	ND	ND	ND	ND	ND	+	++
CD86	+	++	ND	ND	ND	ND	+	++
CD115	++	++	++	++	ND	ND	ND	ND
CD116	++	++	++§	++§	ND	ND	ND	ND
CD200R	ND	ND	ND	ND	+	–	ND	ND
F4/80	ND	ND	+	+	ND	ND	ND	ND
Ly6C	ND	ND	+	–	ND	ND	ND	ND
7/4	ND	ND	+	–	ND	ND	ND	ND
MHC class II	+	++	–	–	ND	ND	+	++

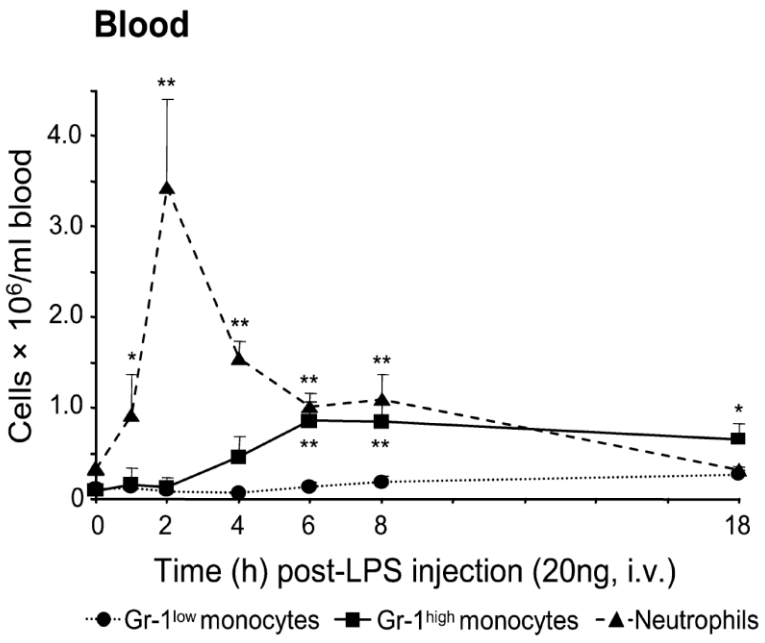
Nat Rev Immunol 2005;5:953–964.

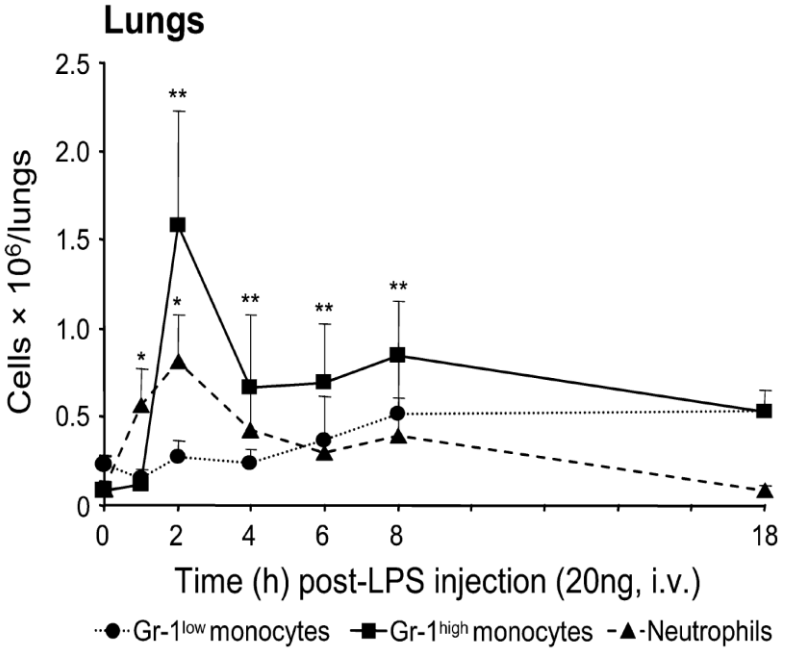
THE JOURNAL OF IMMUNOLOGY

Mobilization and Margination of Bone Marrow Gr-1 ^{high} Monocytes during Subclinical Endotoxemia Predisposes the Lungs toward Acute Injury

Kieran P. O'Dea, Michael R. Wilson, Justina O. Dokpesi, Kenji Wakabayashi, Louise Tatton, Nico van Rooijen and Masao Takata

J Immunol 2009;182;1155-1166



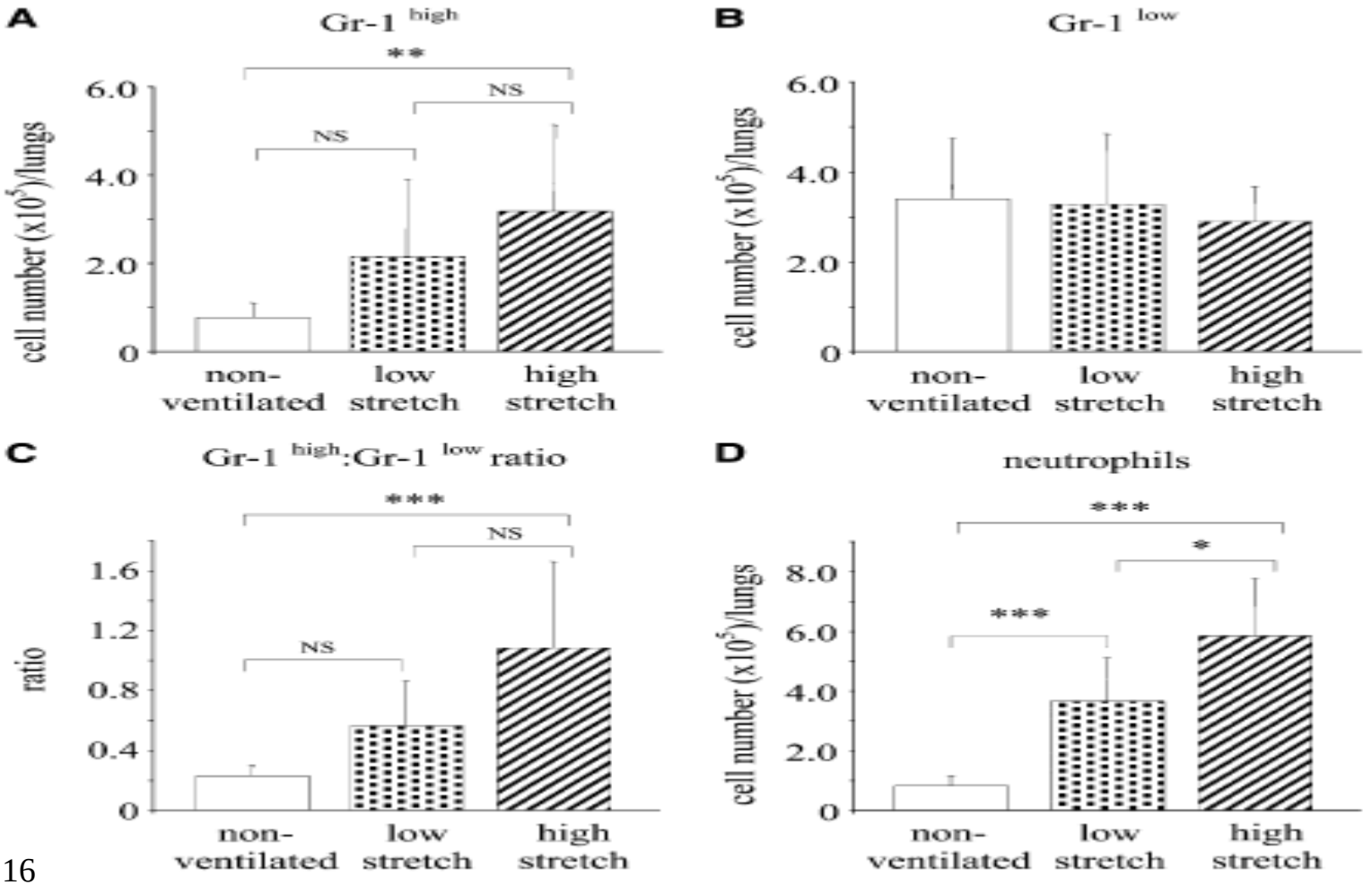


Role of Lung-marginated Monocytes in an *In Vivo* Mouse Model of Ventilator-induced Lung Injury

Michael R. Wilson[1], Kieran P. O'Dea[1], Da Zhang[1], Alexander D. Shearman[1], Nico van Rooijen[2], and Masao Takata[1]

[1]Department of Anaesthetics, Pain Medicine and Intensive Care, Faculty of Medicine, Imperial College London, Chelsea and Westminster Hospital, London, United Kingdom; and [2]Vrije Universiteit, Vrije Universiteit Medisch Centrum, Department of Molecular Cell Biology, Faculty of Medicine, Amsterdam, The Netherlands

Am J Respir Crit Care Med Vol 179. pp 914–922, 2009

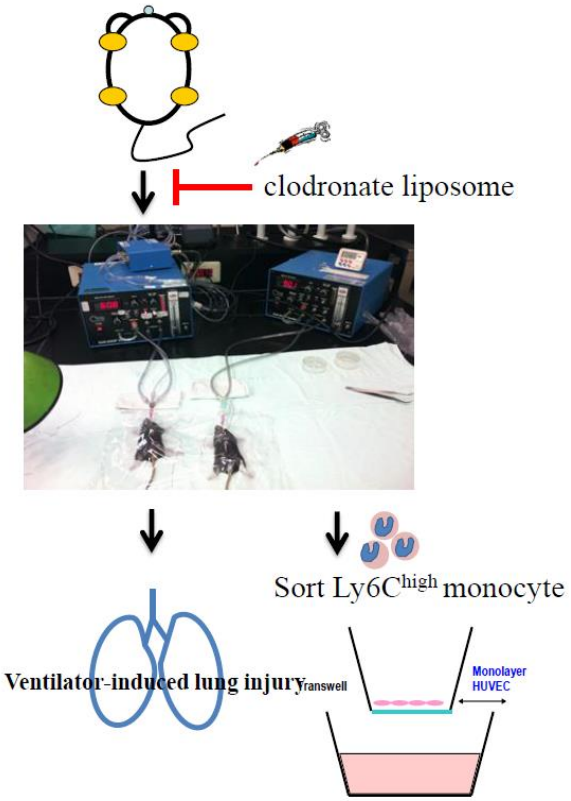


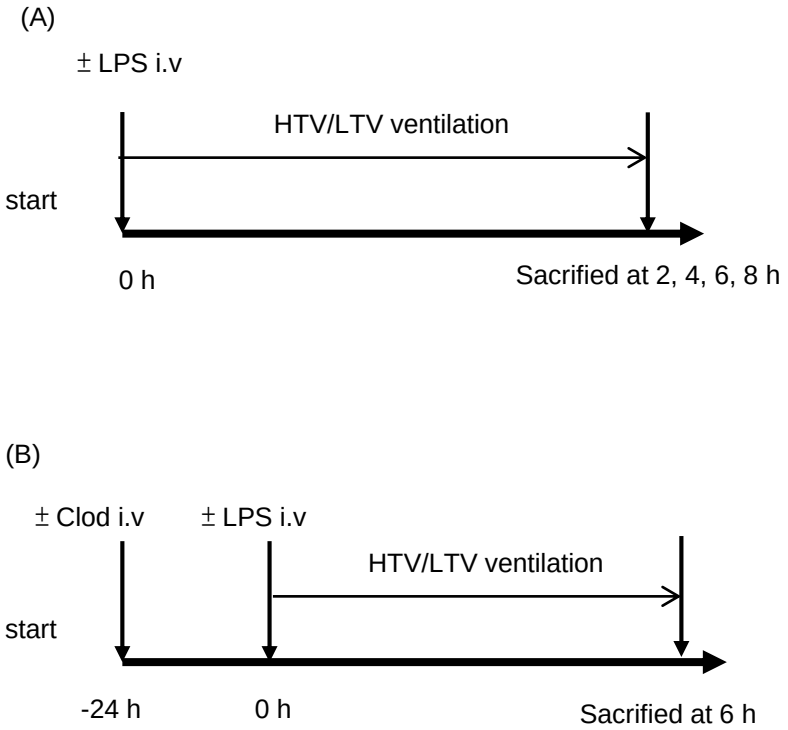
RESEARCH ARTICLE

VEGF Production by Ly6C^{+high} Monocytes Contributes to Ventilator-Induced Lung Injury

Chung-Sheng Shi[1,2☯], Tzu-Hsiung Huang[3☯], Chin-Kuo Lin[4], Jhy-Ming Li[1], Mei-Hsin Chen[1], Mei-Ling Tsai[5], Chih-Ching Chang[6]*

PLoS ONE *2016 11(10): e0165317.*

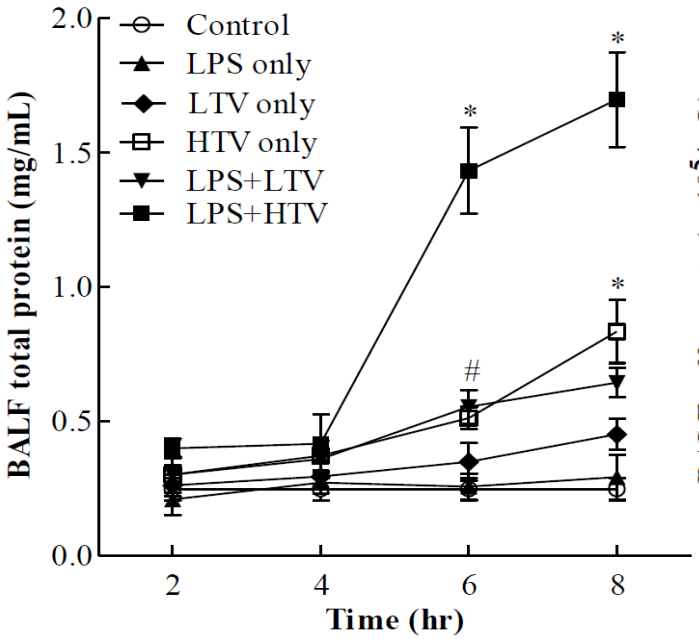




To establish an murine model of ventilator-induced lung injury

***PLoS ONE** 2016 11(10): e0165317.*

(A)



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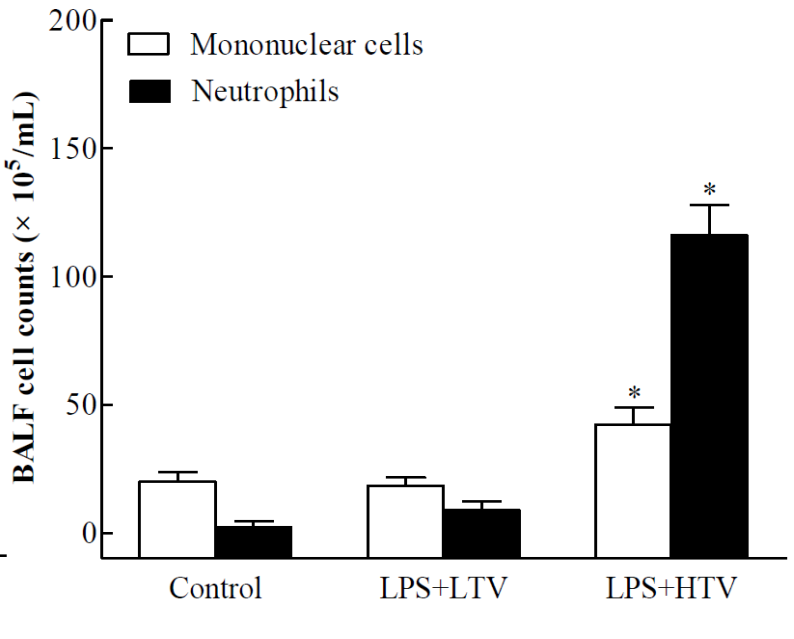

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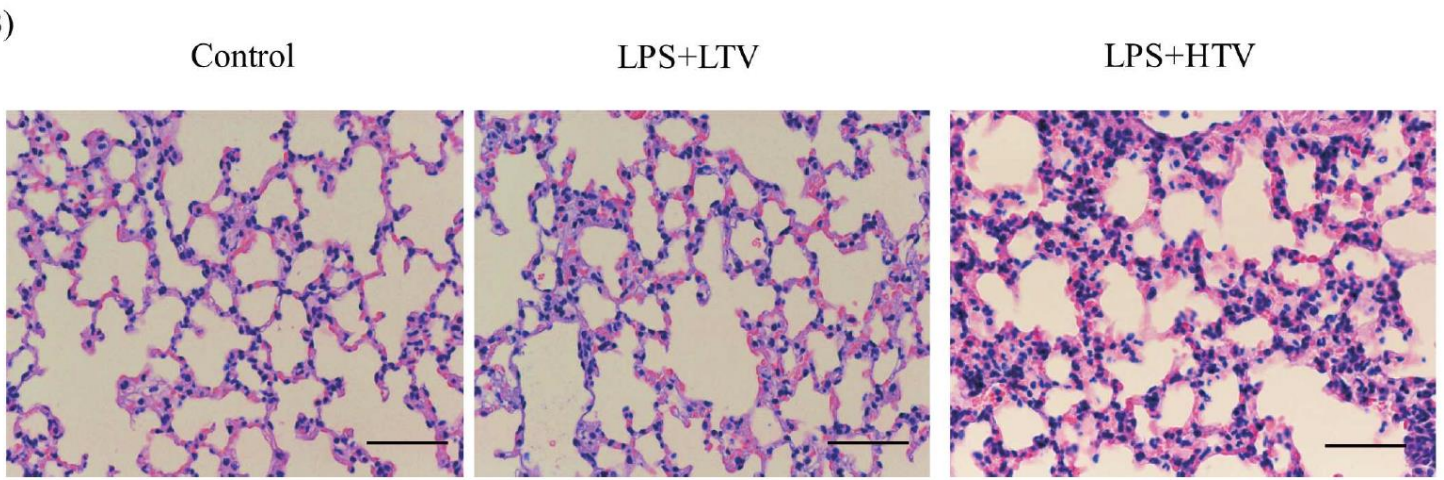
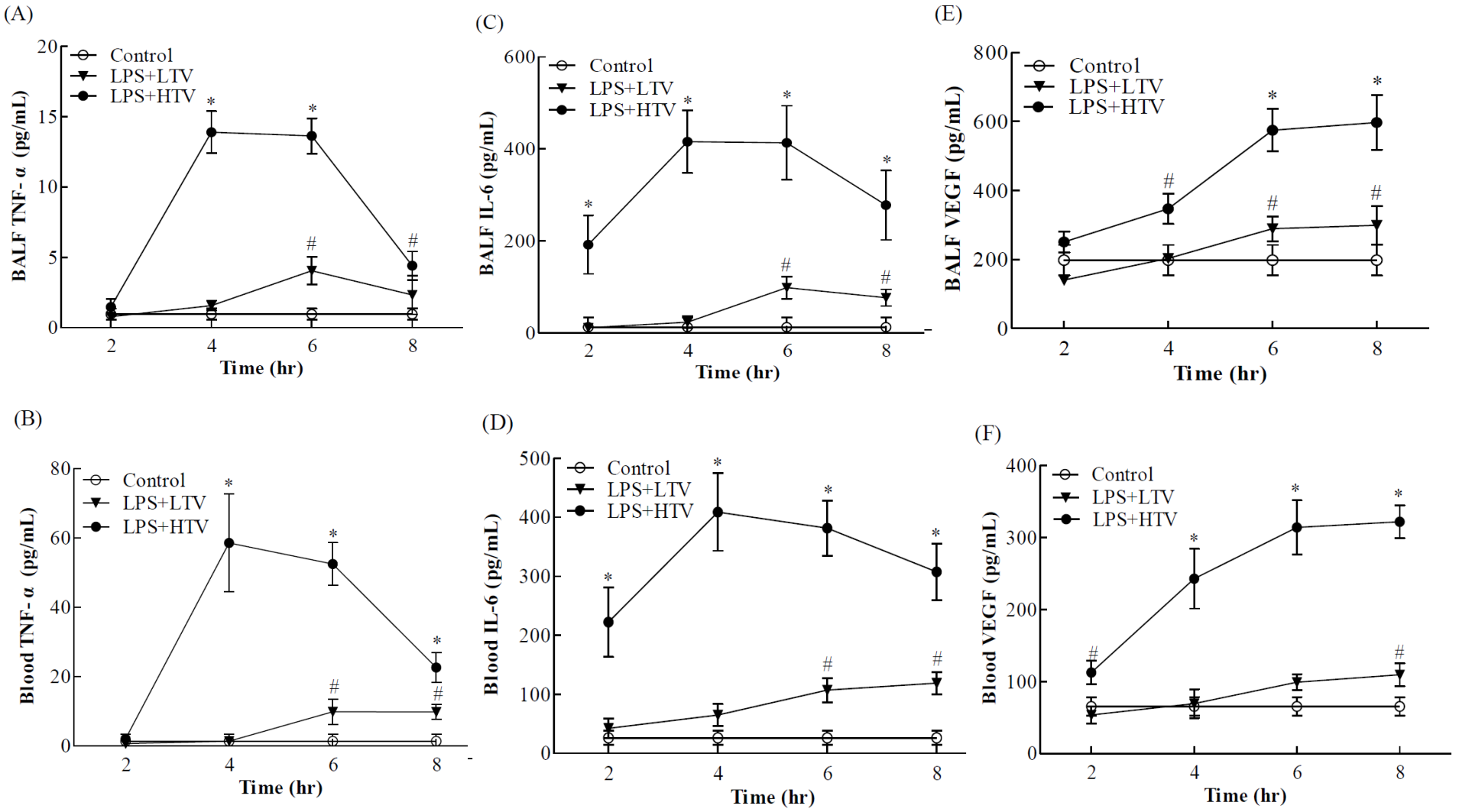
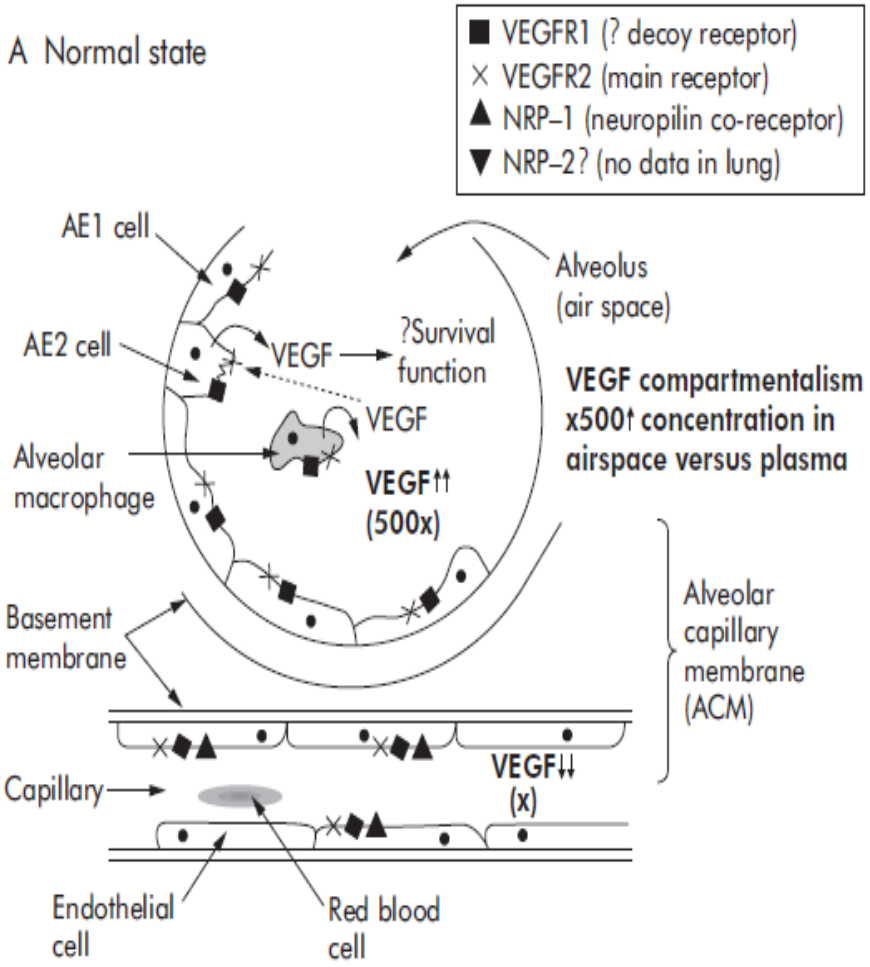


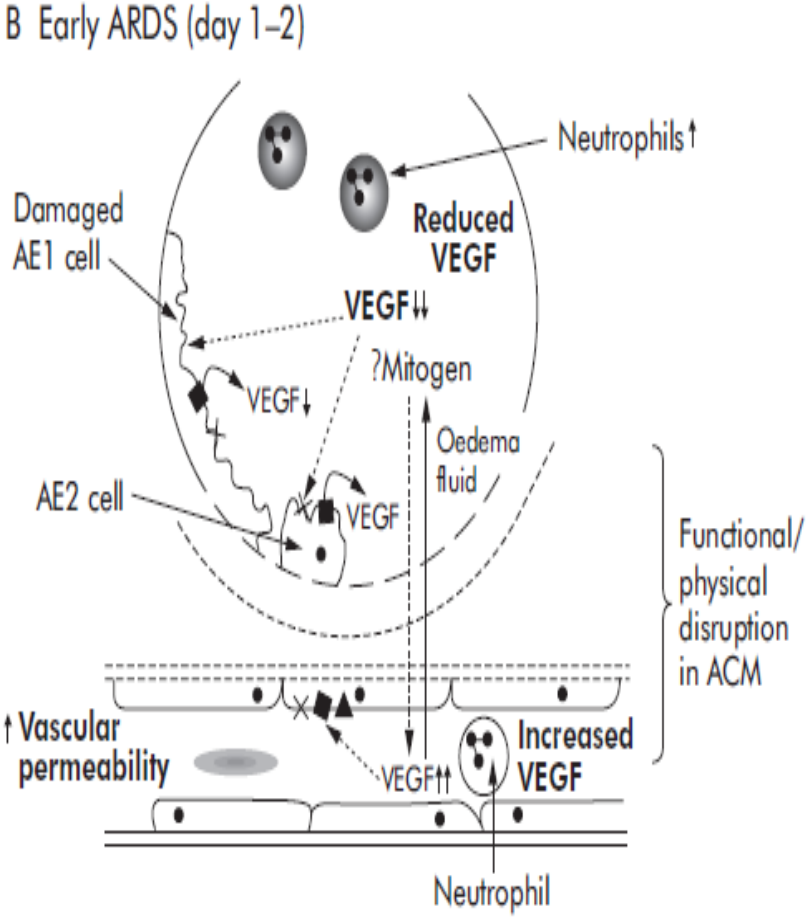
To determine time dependent production of TNF-α, IL-6, and VEGF in VILI



PLoS ONE 2016 11(10): e0165317.

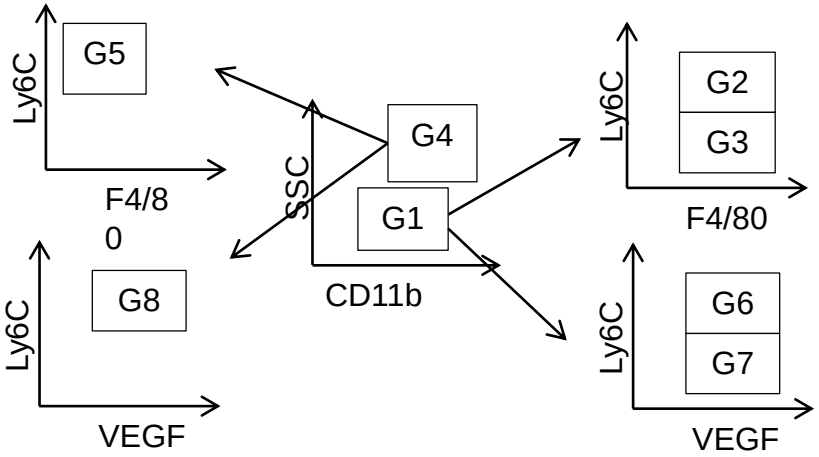
Vascular endothelial growth factor (VEGF)





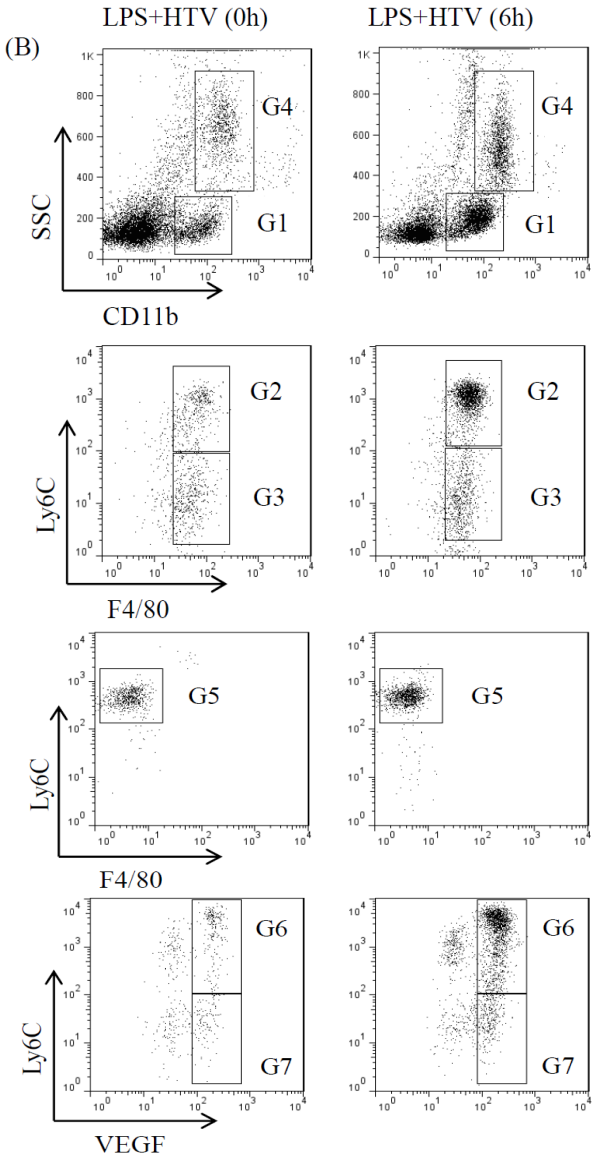
Thorax *2006;61:621-626*

Gating strategy in flow cytometry



G1: monocyte (SSC^{low} CD11b+)
G2: $Ly6C^{+high}$ monocyte (SSC^{low} CD11b+F4/80+ $Ly6C^{+high}$)
G3: $Ly6C^{+low}$ monocyte (SSC^{low} CD11b+F4/80+ $Ly6C^{+low}$)
G4: favor PMN (SSC^{high} and CD11b+)
G5: Neutrophil (SSC^{high} CD11b+F4/80- $Ly6C^{+int}$)
G6: VEGF expression of $Ly6C^{+high}$ monocyte (SSC^{low} CD11b+$Ly6C^{+high}$ VEGF+)
G7: VEGF expression of $Ly6C^{+low}$ monocyte (SSC^{low} CD11b+$Ly6C^{+low}$ VEGF+)
G8: VEGF expression of : neutrophil (SSC^{high} CD11b+$Ly6C^{+int}$ VEGF+)

To determine time course for the recruitment of Ly6C^{+high}, Ly6C^{+low} monocytes, and neutrophils in VILI.



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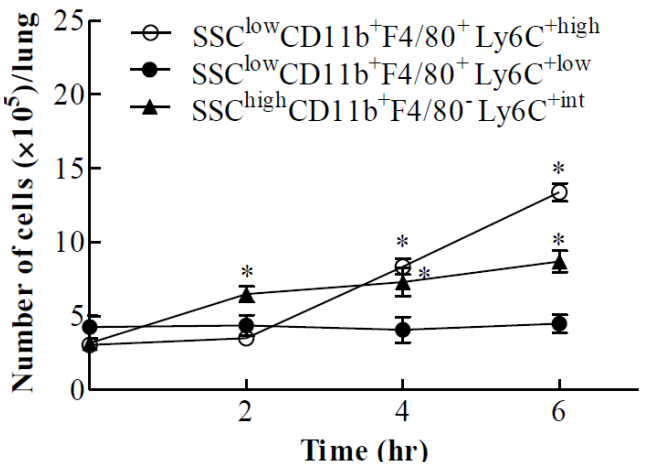

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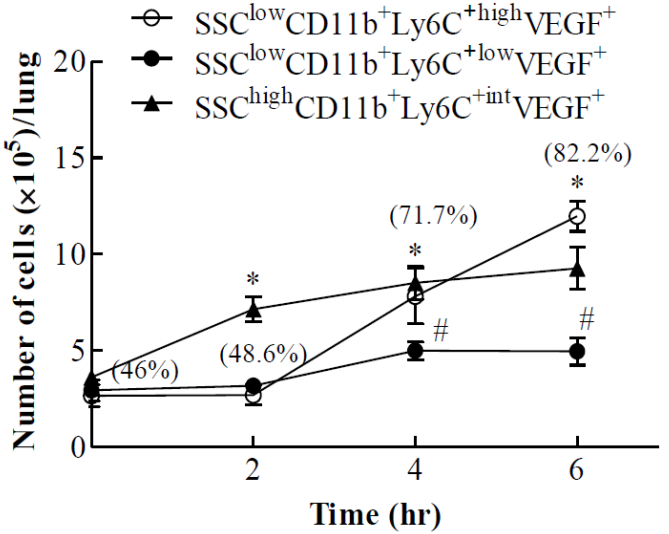
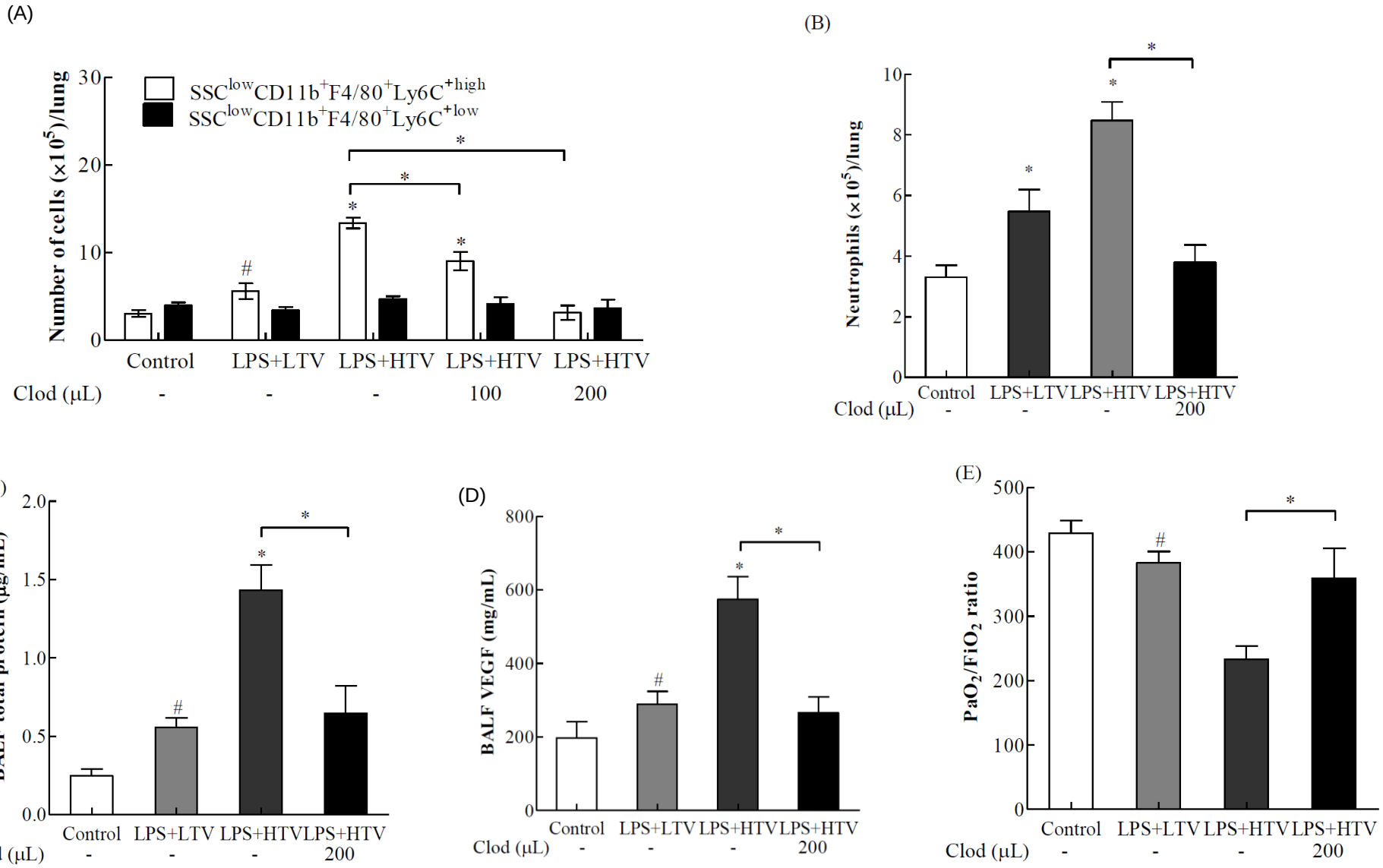


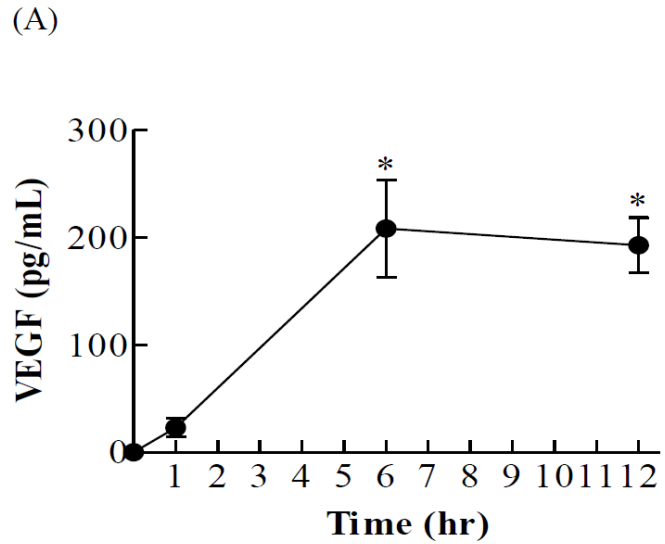
***PLoS ONE** 2016 11(10): e0165317.*

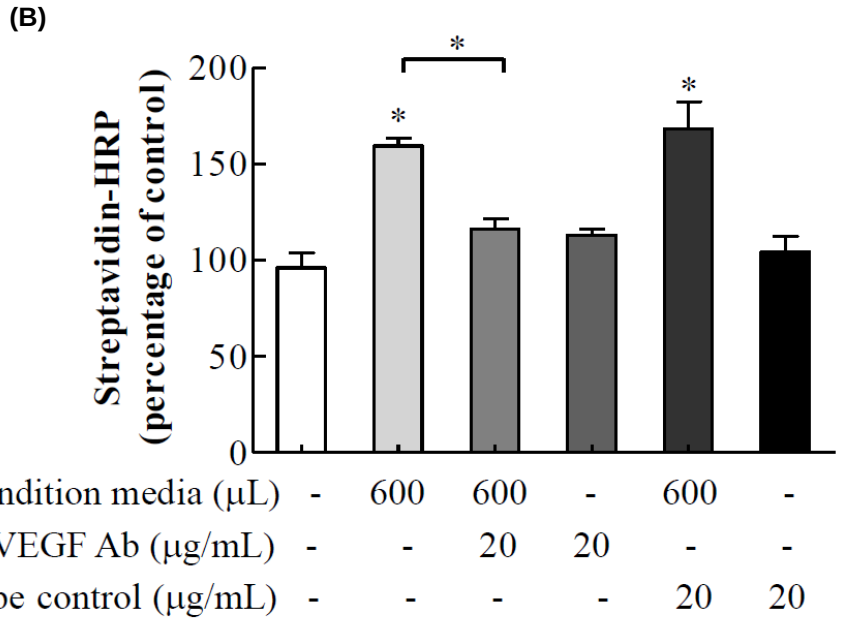
Depleted Ly6C^{+high} monocytes attenuated VILI

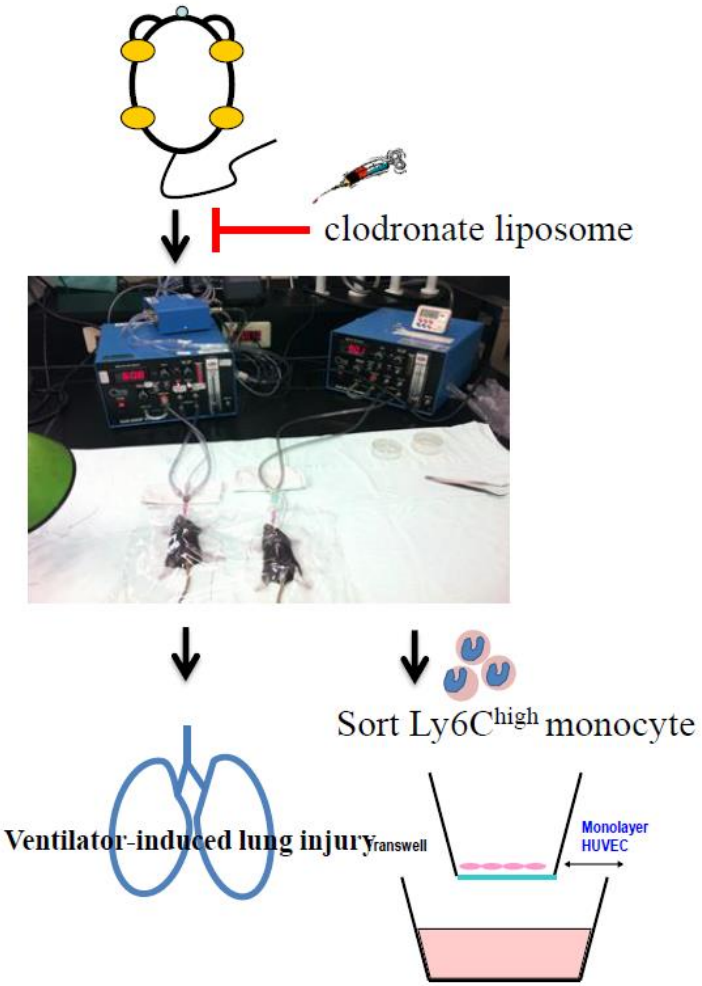


***PLoS ONE** 2016 11(10): e0165317.*

Sorted Ly6C^{+high} monocytes are capable of secreting VEGF, leading to increased permeability in endothelial cells.







PLoS ONE 2016 11(10): e0165317.

Int. J. Mol. Sci. 2019, 20, 1771

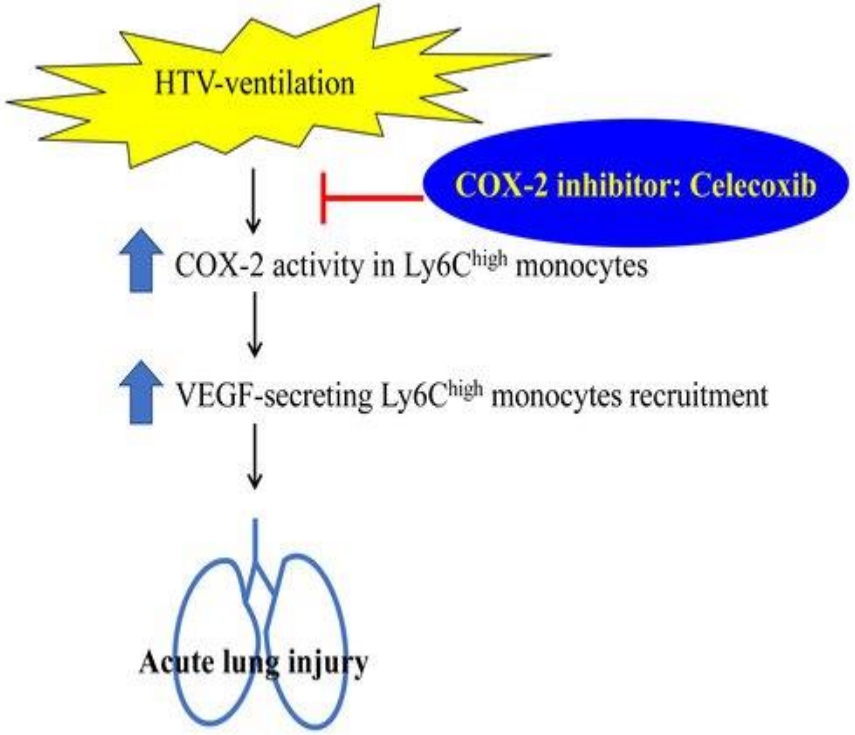
International Journal of *Molecular Sciences*

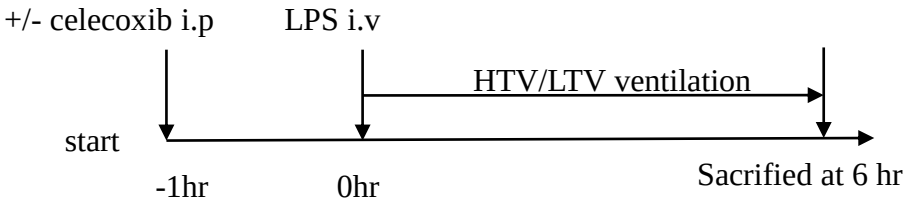
MDPI

Article

Cyclooxygenase-2 Activity Regulates Recruitment of VEGF-Secreting Ly6C^{high} Monocytes in Ventilator-Induced Lung Injury

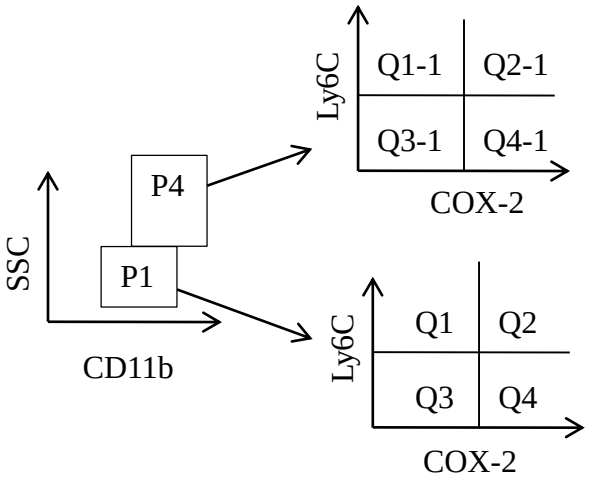
Tzu-Hsiung Huang [1,2], Pin-Hui Fang [3], Jhy-Ming Li [2,4], Huan-Yuan Ling [1], Chieh-Mo Lin [2,5,6,*] and Chung-Sheng Shi [2,4,7,*]





COX-2-expressing Ly6C^{high} monocytes recruited into the lung during VILI.

(A)



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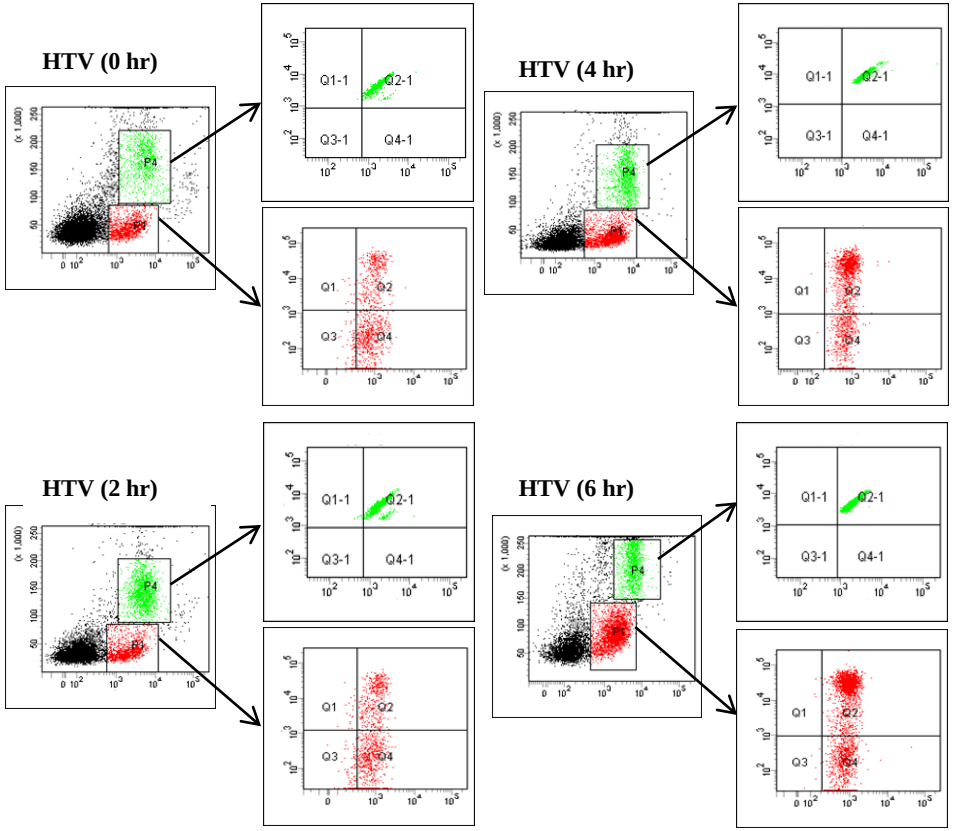

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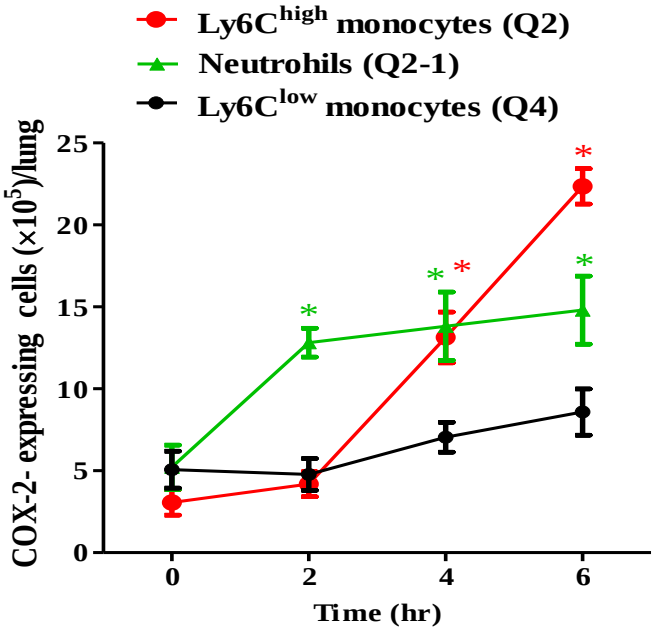
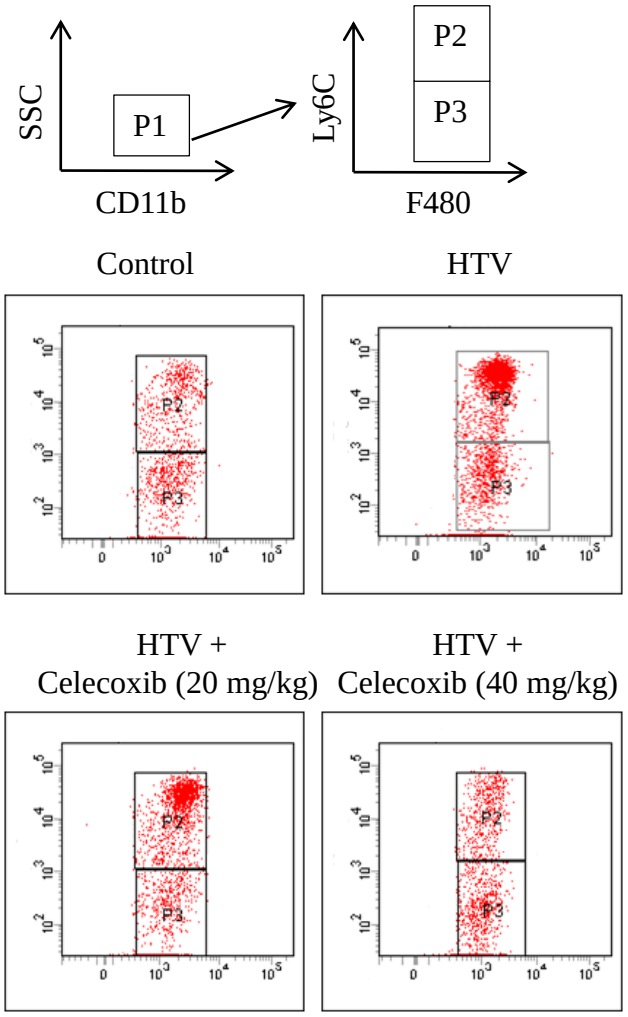


Celecoxib significantly diminished the recruitment of Ly6C^{high}, but not Ly6C^{low}, monocytes in VILI.

(A)



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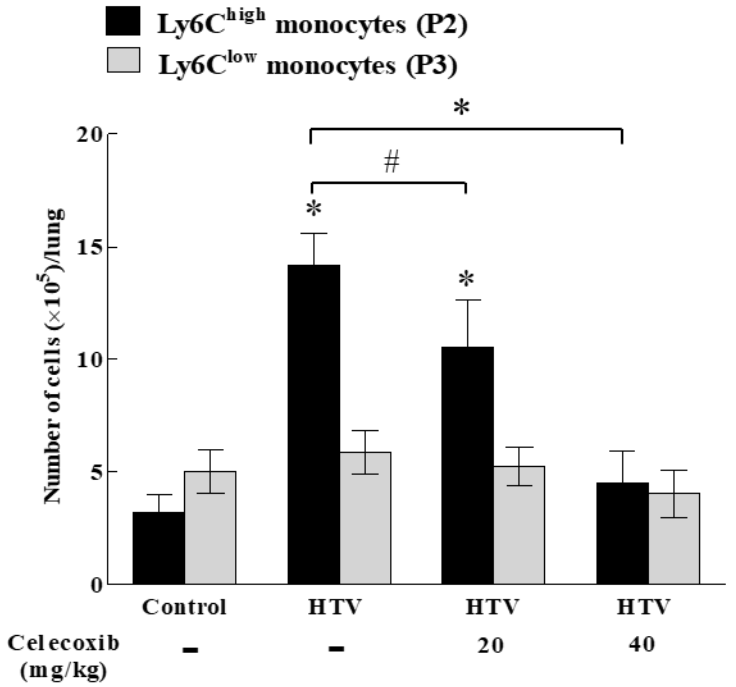
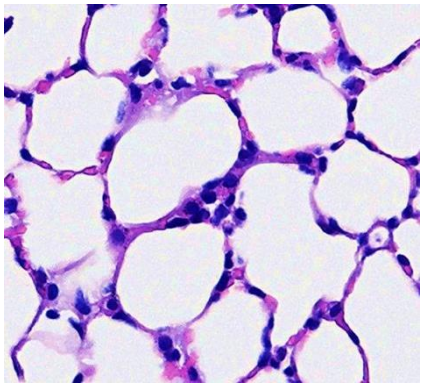


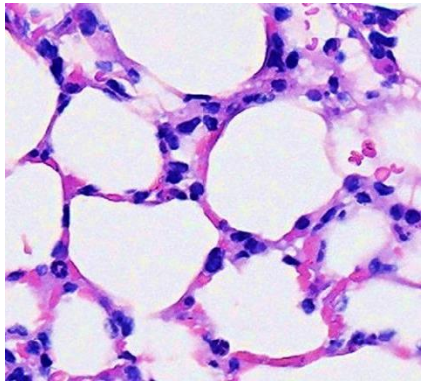
Celecoxib attenuated ventilator-induced lung injury

(A)

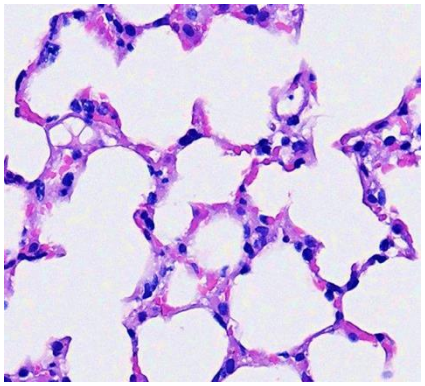
Control



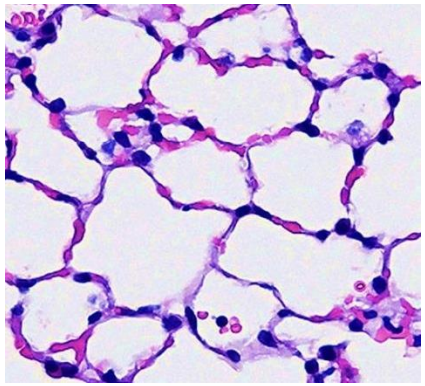
HTV



HTV +
Celecoxib (20 mg/kg)



HTV +
Celecoxib (40 mg/kg)



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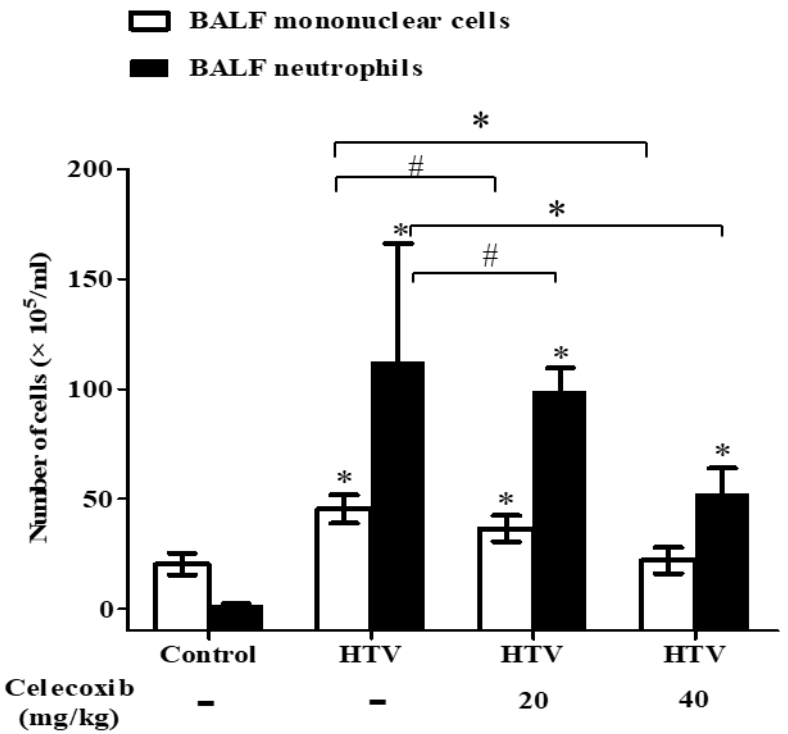
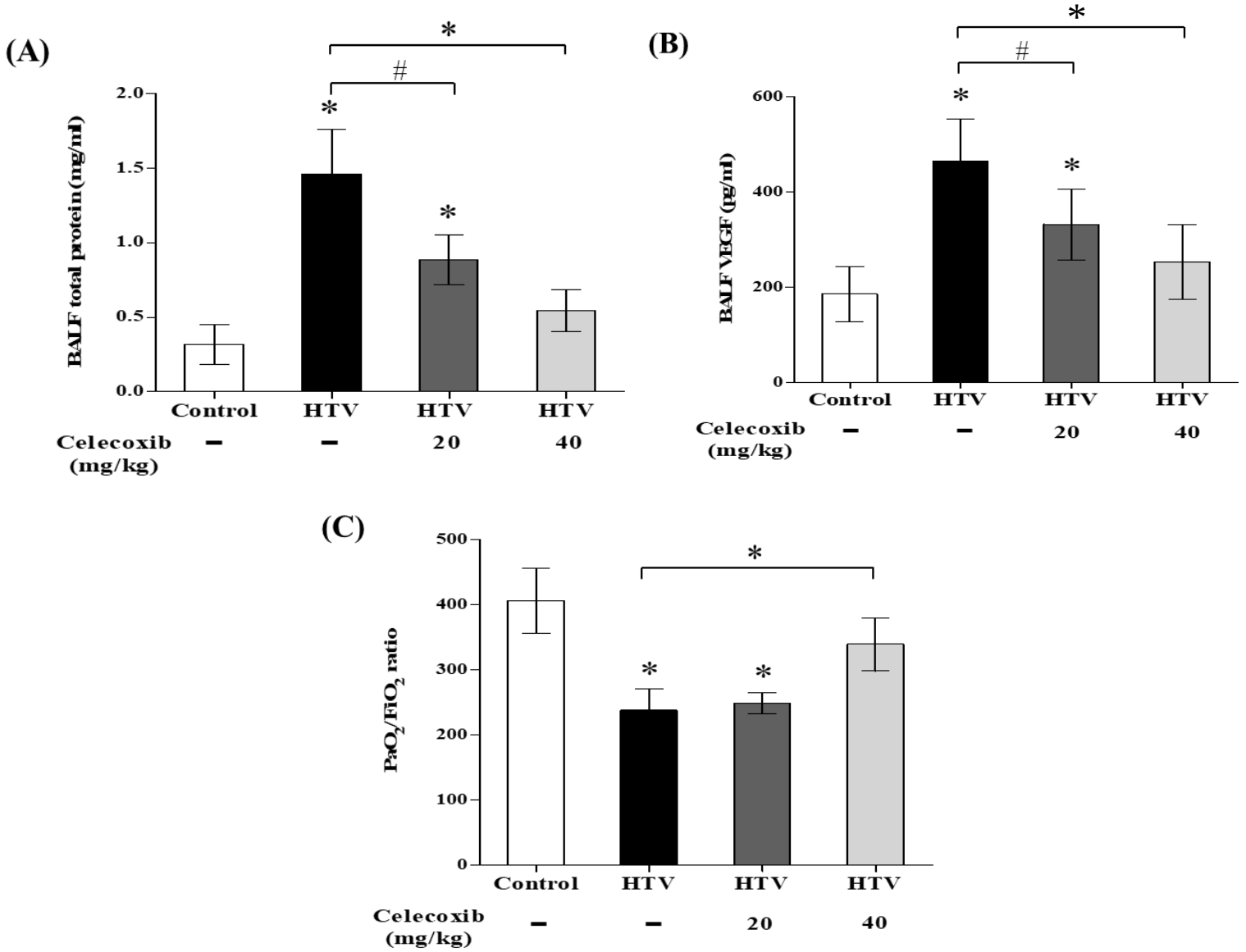
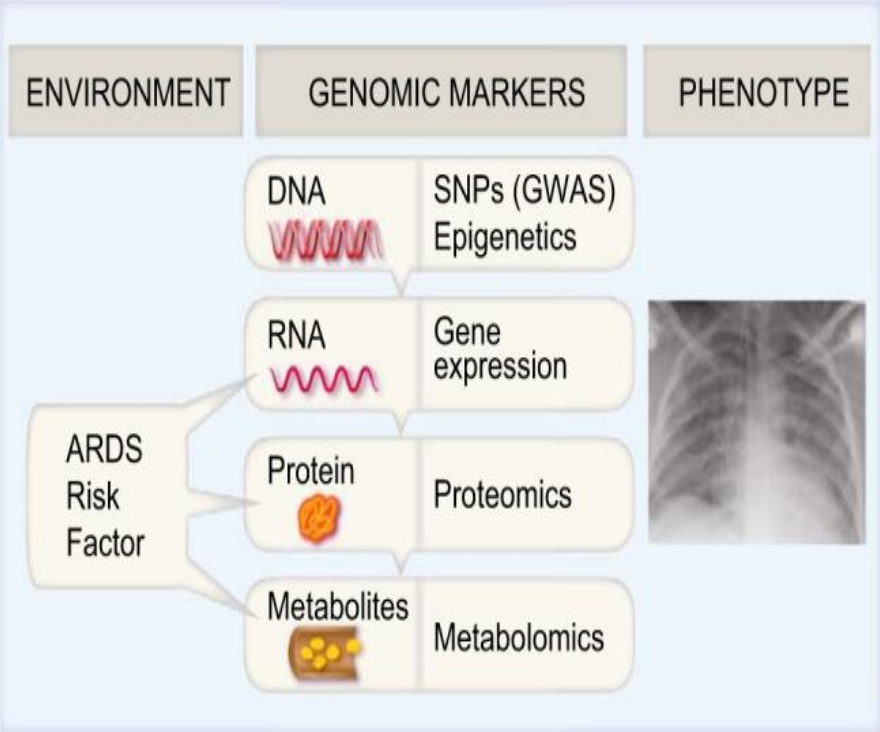


Celecoxib significantly reduced pulmonary-vasculature permeability and improved pulmonary oxygenation in VILI.



Genomic markers



1. ARDS is a heterogeneous syndrome, with a diverse set of risk factors, etiologies, and outcomes.
2. Therefore, personalized treatment necessitates first understanding the underlying physiology for better risk stratification.
3. The National Heart Lung and Blood Institute has also emphasized on the use of biological methodology and translational model to pave new directions in addressing ARDS heterogeneity and its resolution.
4. Systematically integrate processes by high throughput and high content analytical platform.

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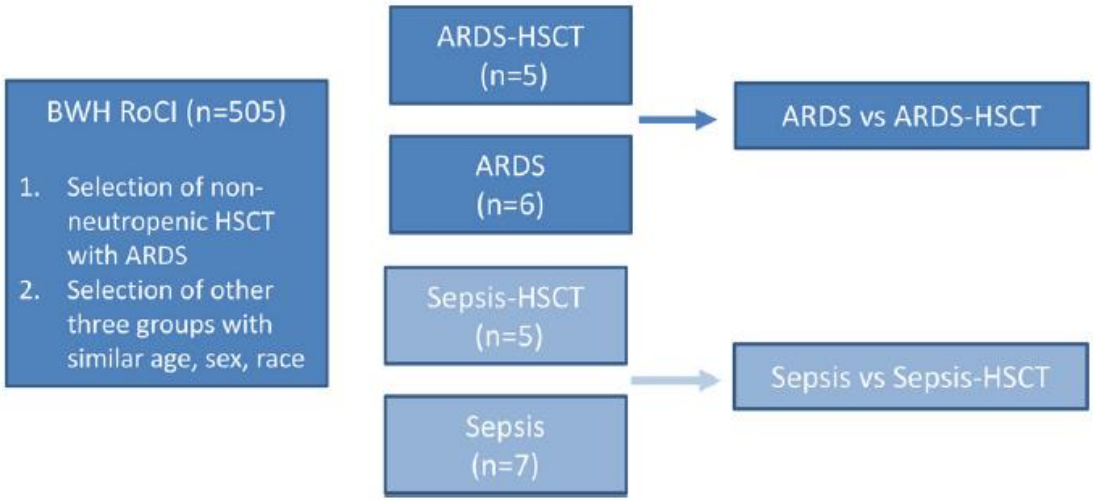
Respiratory Research

RESEARCH Open Access

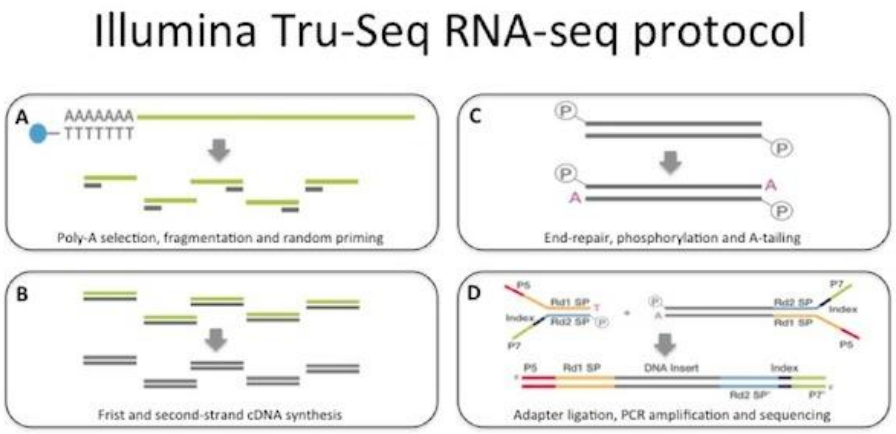
Whole blood RNA sequencing reveals a unique transcriptomic profile in patients with ARDS following hematopoietic stem cell transplantation

Joshua A. Englert[1*†], Michael H. Cho[2,3†], Andrew E. Lamb[2], Maya Shumyatcher[4], Diana Barragan-Bradford[3], Maria C. Basil[3], Angelica Higuera[3], Colleen Isabelle[3], Mayra Pinilla Vera[3], Paul B. Dieffenbach[3], Laura E. Fredenburgh[3], Joyce B. Kang[5], Ami S. Bhatt[5], Joseph H. Antin[6], Vincent T. Ho[6], Robert J. Soiffer[6], Judie A. Howrylak[7], Blanca E. Himes[4†] and Rebecca M. Baron[3†]



Method

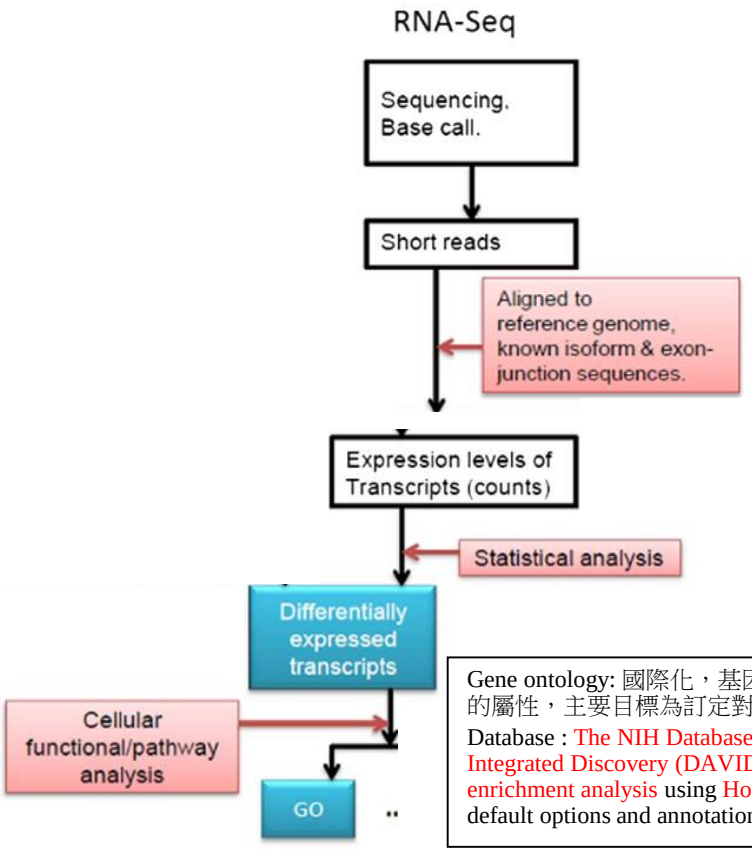
- Whole blood samples were used for gene expression profiling.
- RNA was extracted. The cDNA library were prep with primers: IFI44L (Hs00915292_m1), OAS2 (Hs00942643_m1), OAS3 (Hs00196324_m1), SPATS2L (Hs91916364_m1).
- RNASeq libraries were prepared with 100 ng–1 µg total RNA using Illumina TruSeq RNA-Seq v2 kit (Illumina, San Diego,CA). Sequencing of 75 base pair, paired-end reads was performed with an Illumina HiSeq 2500 instrument.



Library prep begins from 100ng-1ug of Total RNA which is poly-A selected (A) with magnetic beads. Double-stranded cDNA (B) is phosphorylated and A-tailed (C) ready for adapter ligation. The library is PCR amplified (D) ready for clustering and sequencing.

Illumina HiSeq 2500
next-generation sequencing (NGS) platforms

mRNA-seq data analysis

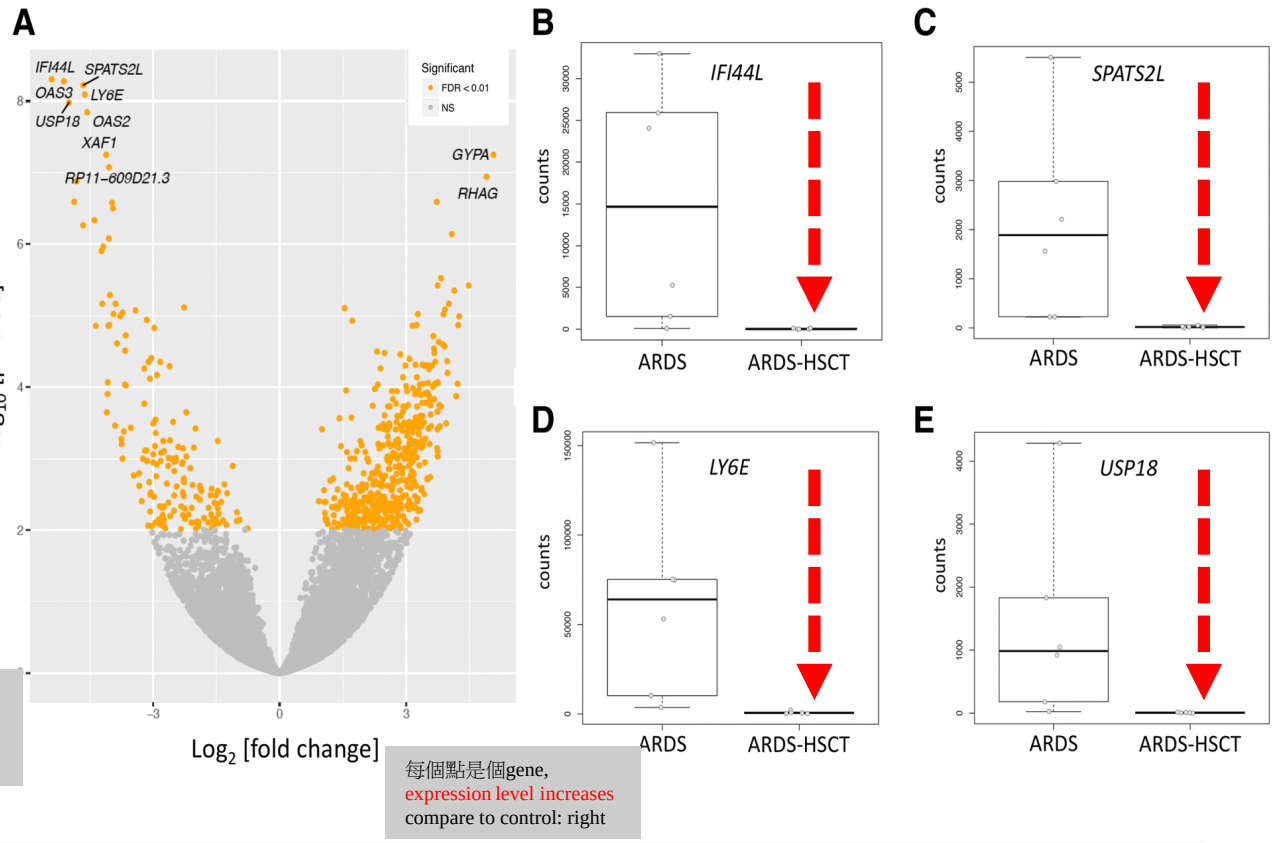


Reads were aligned to the human genome build 38 with STAR v2.4.0.

Gene ontology: 國際化，基因功能分類體系，描述基因及基因產物的屬性，主要目標為訂定對於所有基因功能之分類標準

Database : The NIH Database for Annotation,Visualization and Integrated Discovery (DAVID) gene ontological(GO) category enrichment analysis using Homo sapiens (Human) as background, and default options and annotation categories.

Fig 1.



RNA-seq 中，火山圖 (Volcano Plot) 顯示了兩個重要指標：
(1)Fold-Change
(2) 校正後得 *p* value，利用 *t-test*分析出兩樣本間顯著差異表現的基因後，

以 $\log_2$(Fold-Change) 為橫座標，以 *t-test* 顯著性檢驗 *p*值的負對數 $\log_{10}$(*padj*) 為縱座標

每個點是個gene,
expression level decreasses
compare to control: left

每個點是個gene,
expression level increases
compare to control: right

Top differentially expressed genes upregulated in ARDS vs. ARDS-HSCT included immune response and interferon signaling genes (e.g. IFI44L, OAS3, LY6E, OAS2, USP18)

Table 2 Top 10 most differentially expressed transcripts from ARDS vs ARDS-HSCT

Gene	feature_name	log2FoldChange	Adjusted P
IFI44L	ENSG00000137959	−5.4	5.0E-09
OAS3	ENSG00000111331	−5.1	5.3E-09
SPATS2L	ENSG00000196141	−4.6	6.0E-09
LY6E	ENSG00000160932	−4.6	8.1E-09
USP18	ENSG00000184979	−5.0	1.1E-08
OAS2	ENSG00000111335	−4.6	1.4E-08
XAF1	ENSG00000132530	−4.1	5.7E-08
GYPA	ENSG00000170180	5.1	5.7E-08
RP11-609D21.3	ENSG00000279296	−4.0	8.4E-08
RHAG	ENSG00000112077	4.9	1.2E-07

1.Top differentially expressed genes upregulated in ARDS vs. ARDS-HSCT **included immune response and interferon signaling genes** (e.g. IFI44L, OAS3, LY6E, OAS2, USP18)

2. These genes were not differentially expressed in sepsis vs sepsis-HSCT.

Table 3 Top results from DAVID Functional Annotation Clustering

Cluster	Enrichment score	Representative category	Count	Adjusted *P*-value
1	11.05	Antiviral defense	30	3.9 × 10–19
2	5.51	Response to virus	17	1.3 × 10–5
3	3.84	Heme biosynthesis	7	2.8 × 10–5
4	3.29	Hereditary hemolytic anemia	9	4.2 × 1–05
5	3	Herpes simplex infection	16	1.8 × 10–2

Gene ontological category enrichment analysis with all 687 differentially expressed genes found that immune response- and interferon signaling-related pathways were statistically overrepresented

SCIENTIFIC REPORTS

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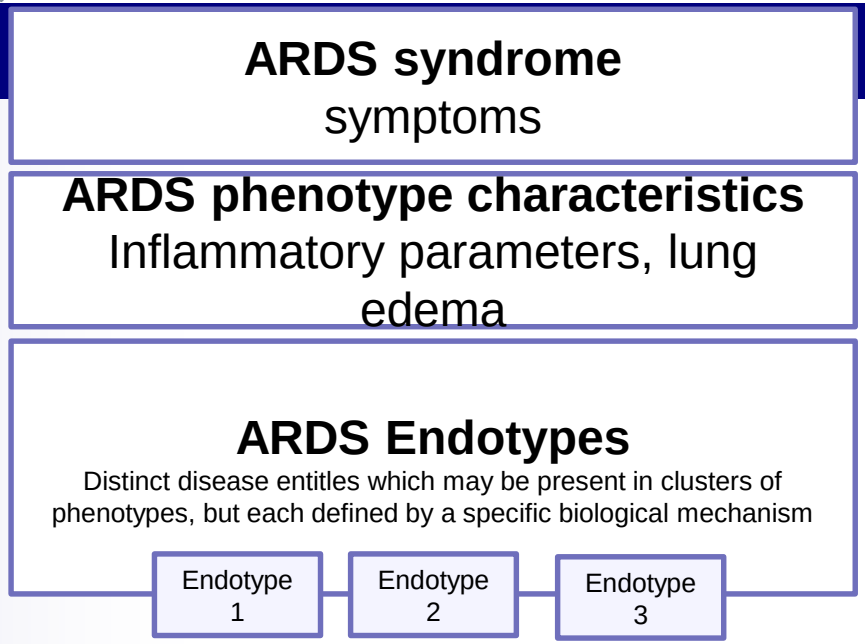
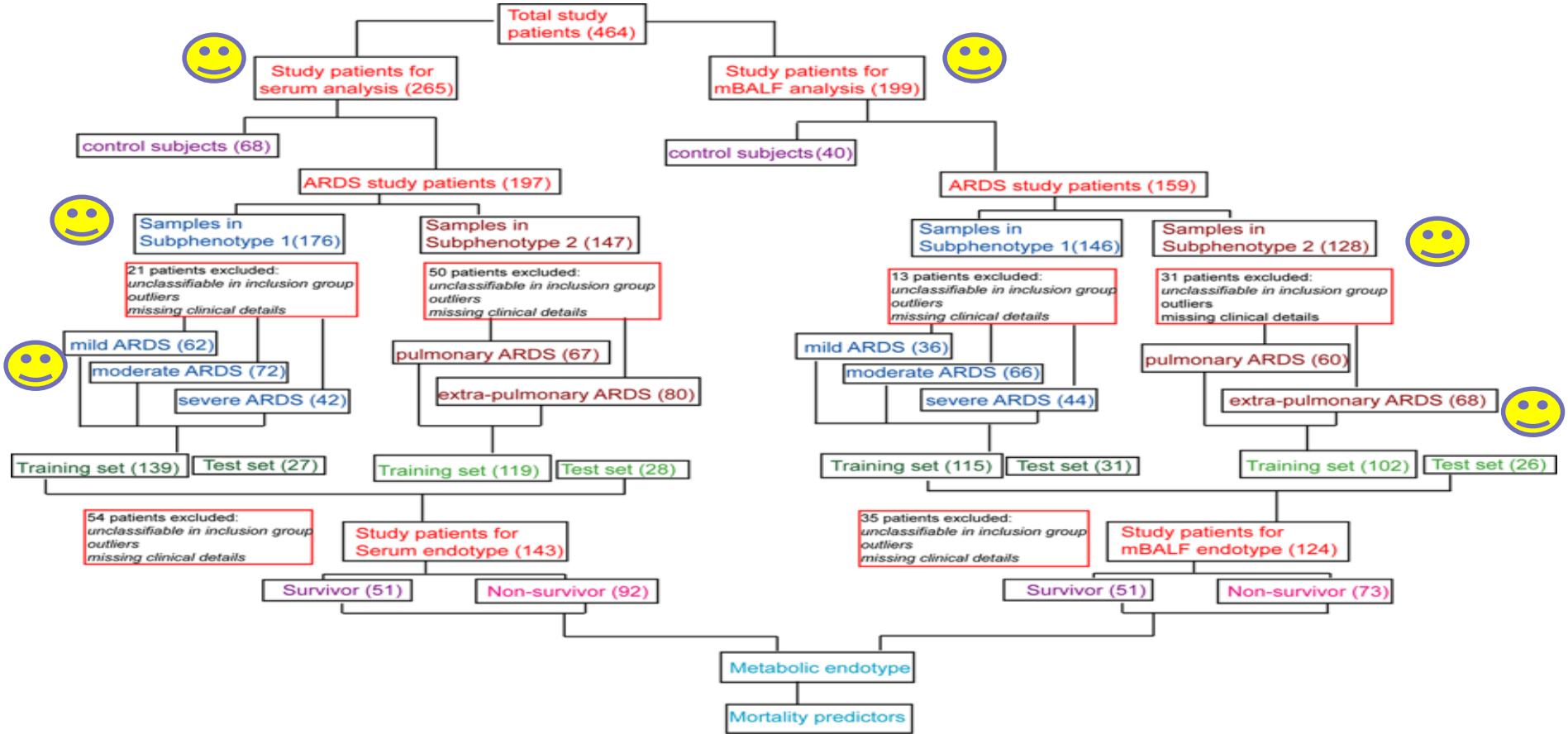
Distinct Metabolic Endotype Mirroring Acute Respiratory Distress Syndrome (ARDS) Subphenotype and its Heterogeneous Biology

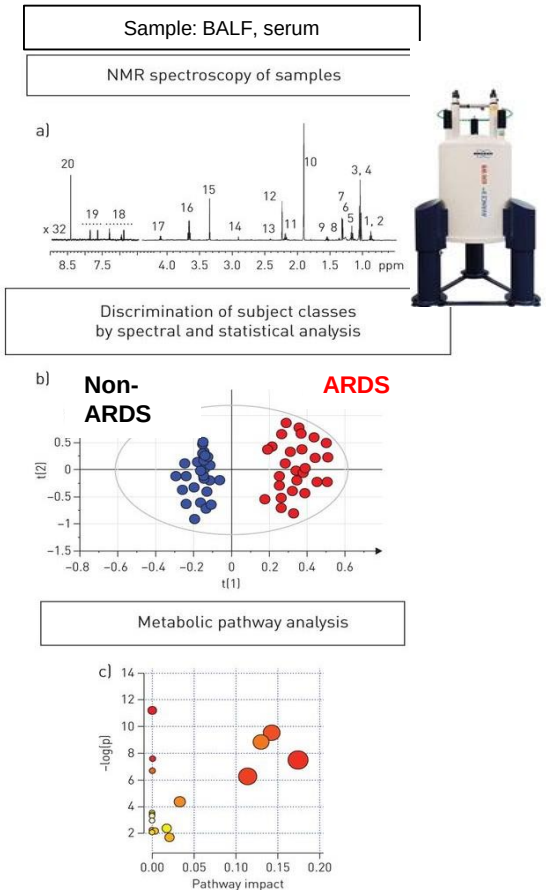
Akhila Viswan[1,2], Pralay Ghosh[3], Devendra Gupta[4], Afzal Azim[3] & Neeraj Sinha[1]



Aim: To identify endotype that best stratify ARDS phenotype with predictive power in determining favorable outcome and accurate and sensitive enough when associated with clinical variable.



Method



Target metabolites
NMR is employed

Relative Quantification

The amount of each metabolite is reported as relative area.

Statistical Analysis

Heat Map and PCA are provided.

* Only for 3 or more samples

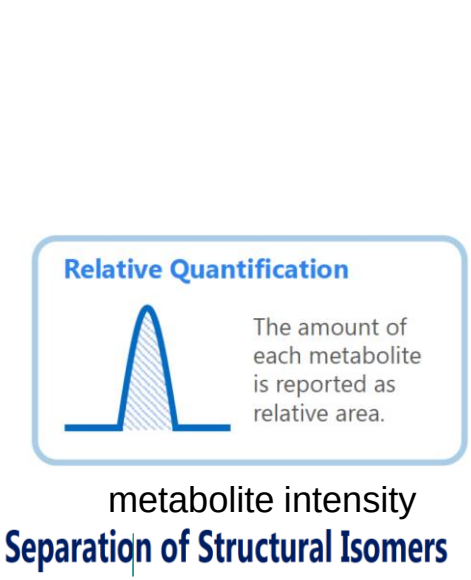
Pathway Mapping

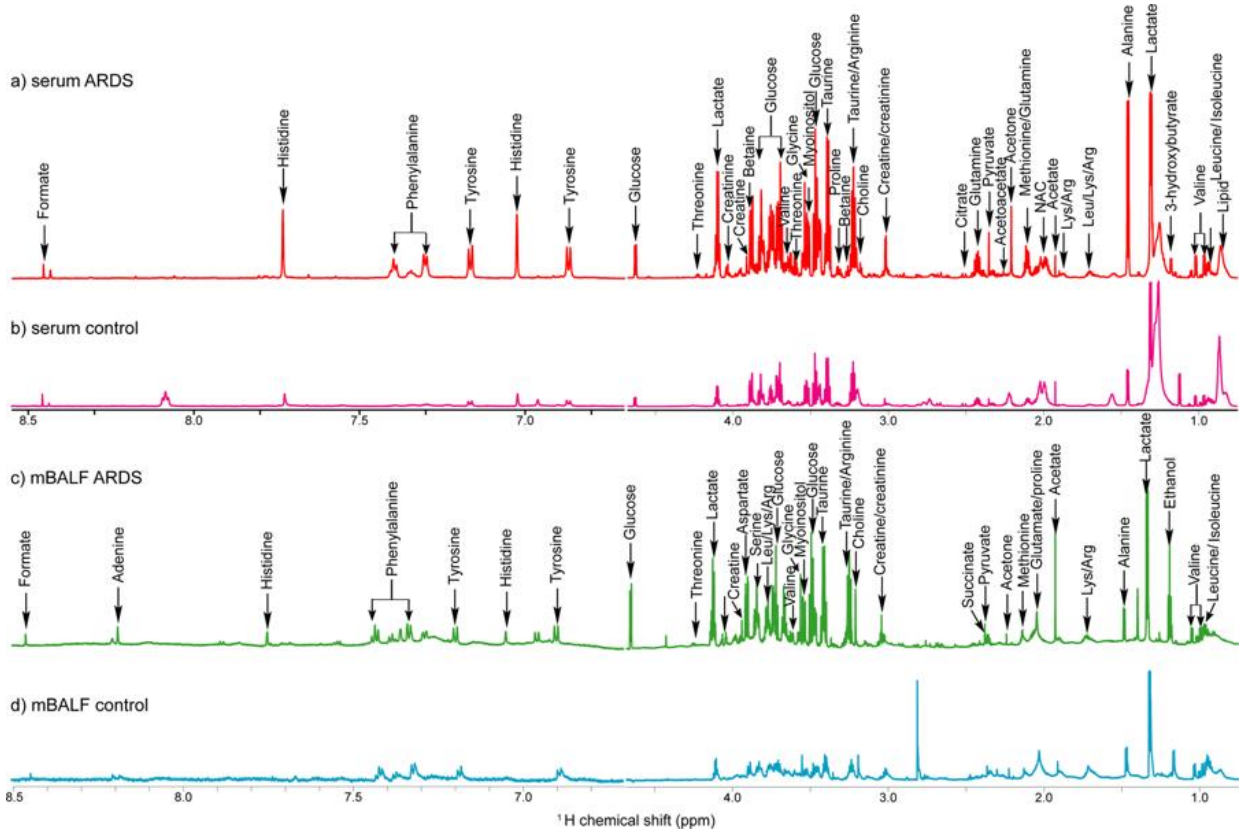
Value of each metabolite is drawn on the map based on KEGG database

* Free for 3 or more samples
* Optional for 2 samples

核磁共振（NMR，Nuclear Magnetic Resonance）
在量子物理中，粒子會以角動量運動，具有「自旋」的內在性質，而變得有磁性。利用這原理，為待測樣品製造出一個恆定的外加磁場，然後以一射頻能量於其上，透過線圈偵測原子核自旋進動的頻率 (測量磁場變化產生的電氣信號，並透過傅立葉轉換得到頻譜)，便能進一步分析出分子中的原子核構成，進一步判讀出分子結構。

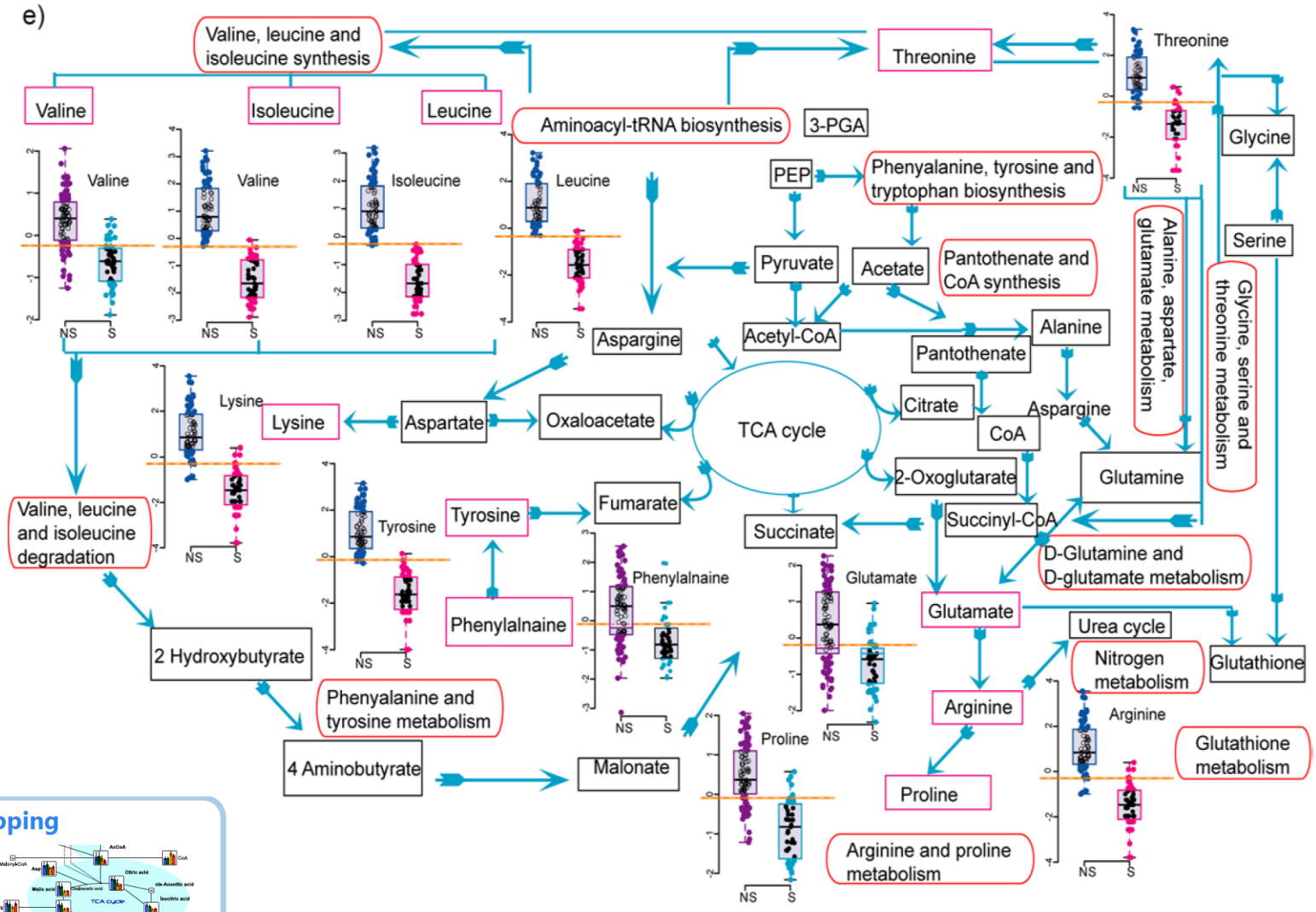
Figure 2. To stratify ARDS in two subphenotypes based on clinical characteristics and its predictability in **determining metabolic endotype** using NMR based metabolomics regardless of clinical heterogeneity.





The difference in metabolite intensity indicates difference in ARDS associated changes with respect to controls

Metabolic pathway of ARDS metabolic endotype

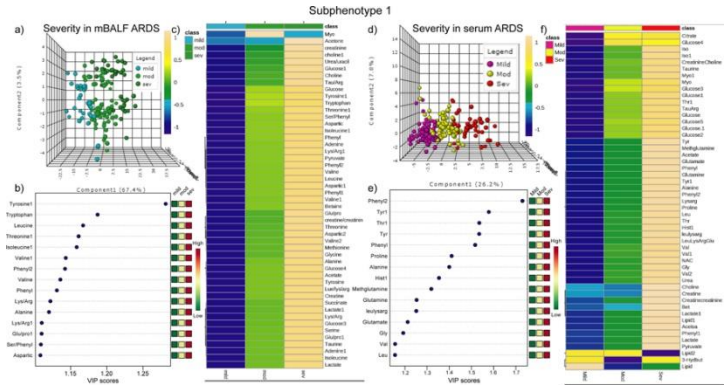


Pathway Mapping

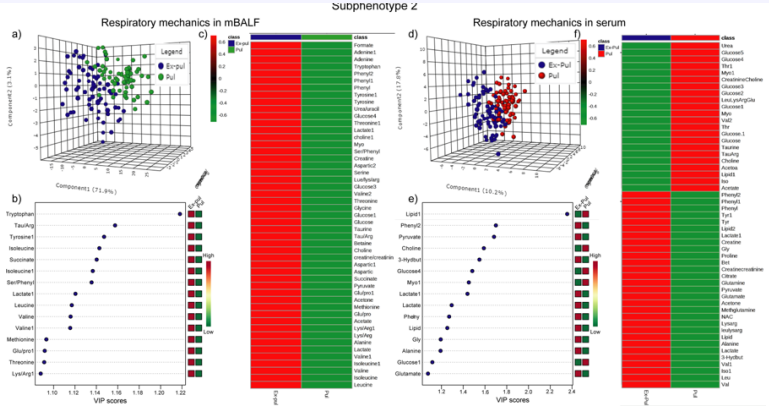
Value of each metabolite is drawn on the map based on KEGG database

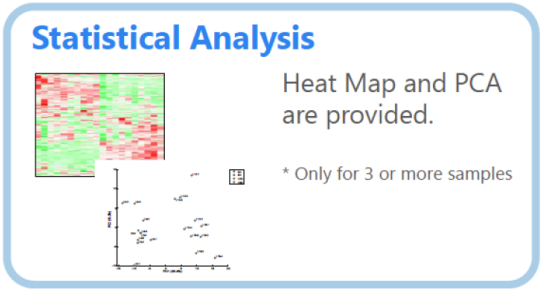
* Free for 3 or more samples
* Optional for 2 samples

Subphenotype1 were classified as **mild, moderate, and severe** subjects based on severity with P/F ratio.



Subphenotype2 were classified as **pulmonary etiologies** and **extra-pulmonary Pulmonary etiologies**

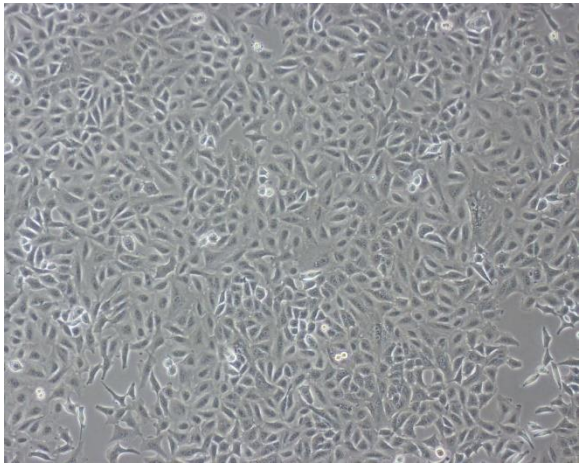


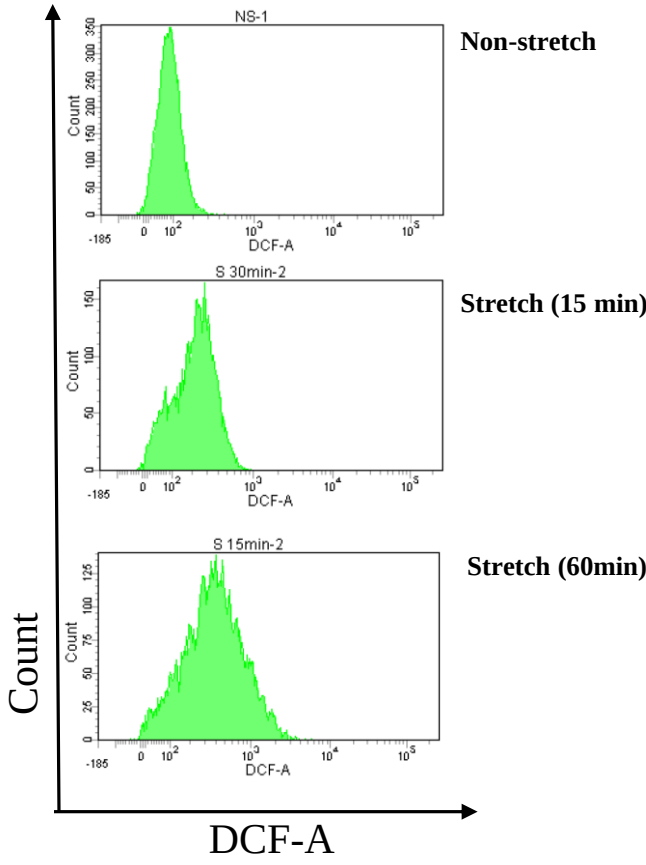


mBALF Metabolites		Serum Metabolites	
Subphenotype1	*Subphenotype2*	*Subphenotype1*	*Subphenotype2*
Lysine/arginine	Isoleucine	Tyrosine	3-hydroxybutyrate
Alanine	Leucine	Phenylalanine	Lactate
Isoleucine	Valine	Leucine/Lysine/Arginine	Alanine
Leucine	Lactate	Methglutamine	Lipid
Phenylalanine	Lysine/Arginine	Alanine	Glutamate
Valine	Methionine	Glutamine	Pyruvate
Tyrosine	Pyruvate	Valine	Choline
Glutamate/proline	Succinate	Proline	Glucose
Aspartic	Betaine	Histidine	Glycine
Tryptophan	Taurine/Arginine	Leucine	Myoinositol
Threonine	Threonine	Glycine	Phenylalanine
Serine/Phenyalnanine	Serine/Phenyalanine	Glutamate	Valine
Lactate	Myoinositol	Threonine	Proline
Methionine	Tyrosine	Isoleucine	Leucine
	Tryptophan		Isoleucine
	Glutamate/proline		Threonine

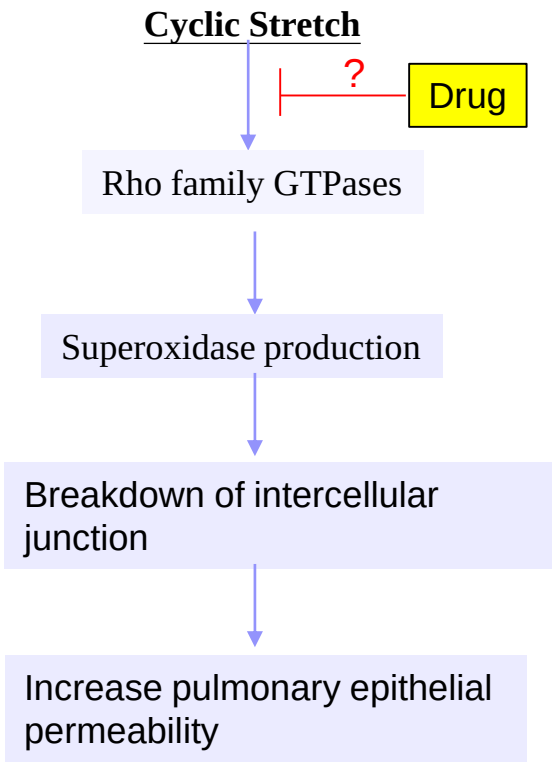
Table 2. List of significant metabolites from subphenotype 1 and subphenotype2 in mB

Current and future work

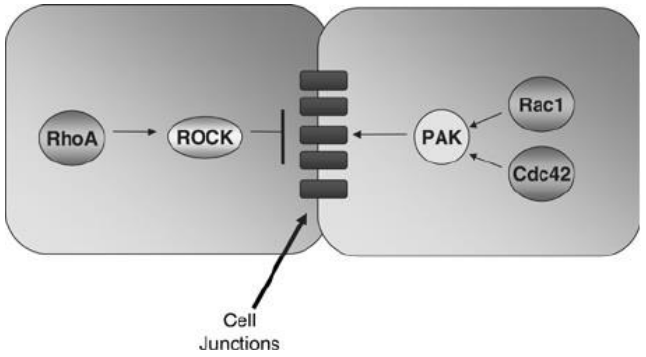


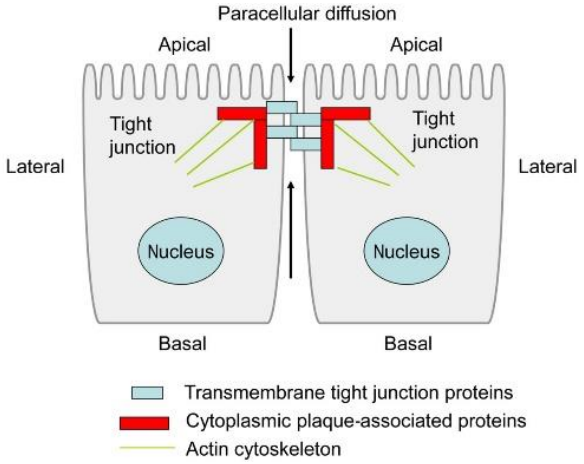


Current and future work



Pulmonary epithelial/endothelial cell





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American Journal of Physiology-Lung Cellular and Molecular Physiology 2013, 305:L141-L153.