Acute Respiratory Distress Syndrome is a TH17 and Treg immune disease

By Wan-Jiung Hu
Postdoctorate
Genomics Research Center
Academia Sinica
No 128 Academia Road section2
Nangang 115, Taipei, Taiwan

Previous Institutes:

Department of Pediatrics
Taipei Municipal Chung-Hsin Hospital

Department of Internal Medicine
Taipei Tzu-Chi Buddhist Hospital (Medical Center)

Department of Neurology
Taipei Mackay Memorial Hospital (Medical Center)

Graduate Institute of Immunology
National Taiwan University College of Medicine

Department of International Health (Vaccine science track)
Johns Hopkins University Bloomberg School of Public Health

Abstract

Acute Respiratory Distress Syndrome (ARDS) is a very severe syndrome leading to respiratory failure and subsequent mortality. Sepsis is the leading cause of acute respiratory distress syndrome. Thus, extracellular bacteria play an important role in the pathophysiology of ARDS. Overactivated neutrophils are the major effector cells in ARDS. Thus, extracellular bacteria triggered TH17 host immunity with neutrophil activation counts for the etiology of ARDS. Here, I use microarray analysis to describe TH17 innate immunity related cytokine including TGF-β and IL-6 up-regulation in whole blood of ARDS patients. Innate TH17 related TLR1,2,4,5,8, HSP70, G-CSF, GM-CSF, complements, defensin, PMN chemokines, cathepsins, Fc receptors, NCFs, STAT5B, FOS, JunB, CEBPs, NFkB, and leukotriene B4 are all up-regulated. TGF-β

secreting Treg cells play important roles in lung fibrosis. Up-regulation of STAT5B and TGF-β with down-regulation of MHC genes, TCR genes, and costimulation molecule CD86 are noted. Many fibrosis promoting genes are also up-regulated including MMP8, MMP9, FGF13, TIMP1, TIMP2, PLOD1, P4HB, P4HA1, PDGFC, HMMR, HS2ST1, CHSY1, and CSGALNACT. Failure to induce successful adaptive immunity could also attribute to ARDS pathogenesis. Thus, ARDS is actually a TH17 and Treg immune disorder.

About the author

Wan-Jiung Hu is a MD PhD. His former name is Wan-Chung Hu. His MD degree was awarded from National Taiwan University. His PhD degree was awarded from Vaccine science track of Department of International Health of Johns Hopkins University. His PhD thesis was using microarray to identify the host immunological pathway after malaria infection. His first first-author paper: “Common and divergent immune response signaling pathways discovered in peripheral blood mononuclear cell gene expression patterns in presymptomatic and clinically apparent malaria” is published in Infection and Immunity in 2006 October. Thus, he first proposed the THαβ immunity which is host immunity against viruses. A subsequent paper in 2008 called it TH9 immunity. However, TH9 immunity is not a good name since IL-9 is a TH2 cytokine. He was trained as a neurology resident in Department of Neurology of Taipei Mackay Memorial Hospital of Taiwan. Currently, he is doing postdoc research in Genomic Research Center of Academia Sinica, Taiwan. His current research topic is cancer immunotherapy. Besides, he is doing functional genomics studies. The author would like to publish this manuscript. If journal editors are interested in this paper, please feel free to contact me.

Introduction

Acute respiratory distress syndrome (ARDS) is a severe cause of respiratory failure. Despite of current treatment, the mortality rate is very high. We still don’t have successful management strategies to deal with ARDS. Most important of all, we still don’t know the exact pathophysiology of ARDS. Sepsis or bacteremia is the leading cause of ARDS. Besides, neutrophil activation is reported in many studies of lung of ARDS patients. Thus, extracellular bacteria induced TH17 immunity overactivation should be the etiology of ARDS. Here, I use a microarray analysis to study immune-related gene profiles in peripheral leukocytes of ARDS patients. I found several TH17 related effector molecules are activated in ARDS. That supports that

ARDS is a TH17 dominant inflammatory disease.

Material and Methods

Microarray dataset

According to Dr. J. A. Howrylak's research in Physiol Genomics 2009, he collected total RNA from whole blood in sepsis and sepsis induced ARDS patients.(Howrylak, Dolinay et al. 2009) He tried to find out molecular signature of ARDS compared to sepsis patients. His dataset is available in Gene Expression Omnibus (GEO) www.ncbi.nlm.nih.gov/geo (assession number GSE 10474). The total number of his sepsis induced ARDS is 13. The overall mortality of these patients is 38%. The second dataset is from GSE20189 of Gene Expression Omnibus. This dataset was collected by Dr. Melissa Rotunno in Cancer Prevention Research 2011.(Rotunno, Hu et al. 2011) Molecular signature of early stage of lung adenocarcinoma was studied by microarray. I use the healthy control (sample size 21)whole blood RNA from this dataset to compare the ARDS patients. In this study, I perform further analysis to study peripheral leukocyte gene expression profiles of ARDS compared to those of healthy controls.

Statistical analysis

Affymetrix HG-U133A 2.0 genechip was used in both samples. RMA express software(UC Berkeley, Board Institute) is used to do normalization and to rule out the outliners of the above dataset. I rule out the potential outliners of samples due to the following criteria:

1. Remove samples which have strong deviation in NUSE plot
2. Remove samples which have broad spectrum in RLE value plot
3. Remove samples which have strong deviation in RLE-NUSE mutiplot
4. Remove samples which exceed 99% line in RLE-NUSE T2 plot

Then, Genespring XI software was done to analysis the significant expressed genes between ARDS and healthy control leukocytes. P value cut-off point is less than 0.05. Fold changecut-off point is >2.0 fold change. Benjamini-hochberg corrected false discovery rate was used during the analysis. Totally, a genelist of 3348 genes was generated from the HGU133A2.0 chip with 18400 transcripts including 14500 well-characterized human genes.

RT-PCR confirmation

Dr. J. A. Howrylak performed real time PCR for selected transcripts (cip1, kip2) by using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA). In the second dataset, Dr. Melissa Rotunno also performed qRT-PCR test to validate the microarray results. RNA quantity and quality was determined by using RNA 600 LabChip-Aligent 2100 Bioanalyzer. RNA purification was done by the reagents from Qiagen Inc. All real-time PCRs were conducted by using an ABI Prism 7000 Sequence Detection System with the designed primers and probes for target genes and an internal control gene-GAPDH. This confirms that their microarray results are convincing compared to RT-PCR results.

Results

RMA analysis of whole blood from healthy normal control

The RMA analysis was performed for RNA samples from whole blood of healthy control of the lung adenocarcinoma dataset. Raw boxplot, NUSE plot, RLE value plot, RLE-NUSE multiplot, and RLE-NUSE T2 plot were generated. Then, sample was included and excluded by using these graphs(Figure 1A, 1B, 1C, 1D, 1E). Because of the strong deviation in the T2 plot, the sample GSM506435 was removed for the further analysis.

RMA analysis of whole blood from acute lung injury patients

The RMA analysis was performed for RNA samples from whole blood of healthy control of the ARDS dataset. Raw boxplot, NUSE plot, RLE value plot, RLE-NUSE multiplot, and RLE-NUSE T2 plot were generated. Then, sample was included and excluded by using these graphs(Figure 2A, 2B, 2C, 2D, 2E)

TH17 and Treg related genes are up-regulated in ARDS

Based on the microarray analysis, I find out that many TH17 related genes are up-regulated in ARDS including Toll-like receptors 1,2,4,5,8, complement, heat shock protein 70, cathpesin, S100A proteins, leukotrienes, defensins, TH17 chemokines, and MMPs. Many fibrosis related genes are also up-regulated including key collagen synthesis enzymes and fibroblast growth factor. Key TH17 initiating cytokines including TGF beta and IL-6 are also up-regulated. This explains that TH17 immunity

is initiated in ARDS. NK cell and T cell related genes are down-regulated. This explains that TH1 or THαβ immunological pathway is not triggered in ARDS. (Table 1-14)

In the table1, we can see the up-regulation of TLR1, 2,4,5,8 with the expression of IRAK4. Thus, toll-like receptor signaling is generated during ARDS. It is worth noting that TLR1,2,4,5,8 are all anti-bacteria TH17 signaling (TLR8 is against CpG rich oligonucleotides). Thus, strong TH17 related Toll signaling can be activated.

In table2, we can see differentiated expression of heat shock proteins. Most important of all, HSPA1A, HSPA1B, and HSPA4 are up-regulated. HSPA1A and HSPA1B is greater than 6.0 fold up-regulation. These heat shock proteins are HSP70 family which can activated TH17 related toll-like receptors (TLR2 and TLR4) to generate anti-bacterial immunity. Because of the co-upregulation of TLR2/4 and HSP70, we can see the proinflammatory signaling is generated in acute lung injury.

In table3, chemokine and chemokine receptor genes are differentially regulated. TH1 T cell chemotaxis factor CCL5 with its receptor CCR1 and CCL4 with its receptor CCR5 are both down-regulated. TH2 eosinophil chemotaxic factor receptor CCR3 and THαβ NK cell chemotaxic factors XCL1 and XCL2 are all down-regulated. However, TH17 related chemokines such as PAF related molecules and S100A binding proteins are up-regulated. It suggests that TH17 related response to recruit neutrophils is initiated.

In table 4, strikingly and surprisingly, all the MHC related genes are down-regulated during acute lung injury. These genes include HLA-DRB, HLA-DRA, HLA-DQB, HLA-DPA, HLA-DQA, and HLA-DMB. HLA-DQA1 has the lowest expression level with greater than 5.8 fold down-regulation. Thus, MHC antigen presentation genes are down-regulated in acute lung injury.

In table 5, many immune-related transcription factors are up-regulated or down-regulated. Treg related key transcription factor, STAT5B, is up-regulated. And, THαβ and TH1 related key transcription factor, STAT1, is down-regulated. In addition, TH2 related transcription factor, GATA3, is also down-regulated. Surprisingly, TH17 related transcription factor, STAT3, is down-regulated. It suggests that full TH17 immunity may not be activated during ARDS. Other innate immunity related genes for myeloid or granulocyte lineages are up-regulated including AP1(Fos and Jun), CEBP family genes, and NFIL3. It is worth noting that the inhibitor of NFkB, key innate immunity mediator, is down-regulated in acute lung injury. Besides, T cell related

transcription factors including NFATC3, NFAT5, and NFATC2IP are down-regulated in ARDS. JAK2, a signal transduction for all cytokines and STATs proteins, is also up-regulated.

In the table 6, we can see many chemotaxic factors, leukotrienes and prostaglandins, are up-regulated or down-regulated. The key enzyme: leukotriene A4 hydrolase for leukotriene B4, a potent PMN chemoattractant, is up-regulated. Besides, leukotriene B4 receptor is also up-regulated. Besides, the receptor of PGD2, a TH2 related effector molecule, is 9 fold down-regulated. In addition, the gene 15-hydroxyprostaglandin dehydrogenase (HPGD), which is responsible for shutting down prostaglandin, is 27 fold up-regulated. Key molecules including phospholipase A 2 and arachidonate 5-lipoxygenase to initiate leukotriene synthesis are also up-regulated in ARDS. And, formyl peptide receptor 2, another chemotaxic receptor of PMN, is also up-regulated. Innate immune initiators, SERPINB1 and SERPINB2, are also up-regulated in acute lung injury.

In table7, many fibrosis related genes are up-regulated during ARDS. Most strikingly, MMP8 has greater than 28 fold up-regulation. MMP9 has greater than 11 fold up-regulation. Matrix metalloproteinase 8 & 9 play key roles in the pathogenesis of ARDS(Kong, Li et al. 2011). In addition, MMP25, TIMP1, and TIMP2 are also up-regulated in ARDS. Thus, severe extracellular matrix destruction happens in ARDS. Fibroblast growth factors including FGF13 (5 fold up-regulation) and PDGFc (12 fold up-regulation) are also significantly expressed. Chondroitin sulfate deposition is reported in pulmonary fibrosis(Venkatesan, Ouzzine et al. 2011). In this study, chondroitin sulfate synthetase and chondroitin sulfate N-acetylgalactosaminyltransferase 1& 2 are also found up-regulated. There is 7.5 fold up-regulation in chondroitin sulfate N-acetylgalactosaminyltransferase 1 and 13 fold up-regulation in chondroitin sulfate N-acetylgalactosaminyltransferase 2. Carboxypeptidase D, which can up-regulate nitric oxide, is also up-regulated in acute lung injury.(Hadkar, Sangsree et al. 2004) Previous studies also found up-regulation of iNOS as well as nitric oxide during the inflammation in ARDS.(Kobayashi, Hashimoto et al. 1998) Heparanse and heparan sulfate 2-O-sulfotransferase 1 are also up-regulated in ARDS. Heparan sulfate sulfation is a potent stimulation of FGF signaling to cause fibrosis(Li, Shworak et al. 2002; Escobar Galvis, Jia et al. 2007). Hyaluronan-mediated motility receptor (RHAMM) is also up-regulated. Hyaluronan plays an important role in pulmonary fibrosis. In addition, key collagen synthesis enzymes, prolyl-4-hydroxylases and procollagen-lysine 1, 2-oxoglutarate 5-dioxygenase are also up-regulated in ARDS.(Turpeenniemi-Hujanen 1981;

Myllyharju 2008)

In table 8, many complement related genes are up-regulated including CD59, C1QB, ITGAM, CR1, C3AR1, ITGAX, C1QA, C1RL, and C5AR1. Complements are important in the TH17 arm to kill extracellular bacteria. Thus, the whole complement machinery is activated in ARDS. In addition, two defensin genes, DEFA1B3 and DEFA4, are also up-regulated to defend the possible bacterial infection. Neutrophil overactivity with up-regulated complements and defensins plays a vital role in acute lung injury.

In table9, many cathepsin genes are up-regulated in acute respiratory distress syndrome including CTSK, CTSG, CTSZ, CTSA, CTSD, and CTSC except CTSO and CTSW. Cathepsins are important proteases in antigen processing. Thus, antigen processing is likely to be activated in ARDS. However, MHC related genes are down-regulated in acute lung injury. In addition, myeloperoxidase, which is the enzyme responsible for ingested bacteria killing, is upregulated in ARDS. Neutrophil cytosolic factor 1&4, the subunit of NADPH oxidase for ingested bacteria killing, are also up-regulated in ARDS.

In table 10, two CSF receptors are up-regulated in acute lung injury. CSF2 receptor (GM-CSF receptor) is more than two fold up-regulated. And, CSF3 receptor (G-CSF receptor) is also more than two fold up-regulated. GM-CSF and G-CSF can stimulate granulocyte and monocyte proliferation. It means that myeloid and granulocyte lineages are proliferative in acute respiratory distress syndrome.

In table 11, Fc receptor related genes including IgG Fc receptor 2A, IgA Fc receptor, IgG Fc receptor 2C, IgG Fc receptor 1B, and IgG Fc receptor 1A/1C are up-regulated in ARDS. These Fc receptors are related to TH17 immunological pathway. Besides, TH2 related IgE Fc receptor 1A is down-regulated. These support that TH17 armed immune response is activated in acute lung injury.

In table 12, many TH17 related cytokine genes are up-regulated in acute respiratory distress syndrome. Most important of key, the central TH17 cytokine initiators, TGFB1 and IL-6, are up-regulated in ARDS. Thrombospondin(THBS1), the activator of TGFB, is also strongly up-regulated. In addition, TH22 related cytokines such as IL32 and IL1A are down-regulated. And, IL1RN, an IL1 antagonist, is up-regulated. The receptor of TH17 immunity is also down-regulated including IL17RA, IL6R, and TGFBR3. The receptors for Treg pathway are also down-regulated including IL2RB and TGFBR3. This suggests that TH17 immunity is up-regulated. Other immunological

pathway cytokine receptors are up-regulated including IL1RA, IL1R2, IL1R1, IL4R, IL18R1, IFNGR1, IFNGR2, IFNAR1, and TNFRSF1A. Besides, TH1 or THαβ associated interferon related genes are down-regulated including ISG20L2, IFI16, GVINP1, GBP1, IFI44L, and IFIT3. In addition, FAS is up-regulated and Fas apoptic inhibitory molecule 3 (FAIM3) is down-regulated. It suggests that apoptosis machinery is activated in ARDS.

In table 13, many important CD molecules are differentially regulated. CD molecules are important in mediating host immune reaction. Thus, the up-regulation or down-regulation of these CD molecules suggests the status of host immunity. From this table, we can see many T cell activation molecules are down-regulated including CD8A, CD3G, CD3D, and CD86. CD8A is the molecule of cytotoxic T cell activation. CD3G is the molecule for helper or killer T cell activation. CD86 is the key co-stimulation signal to activate B cells and T cells. Thus, we can see adoptive immunity including B and T lymphocytes are not likely to be activated in ARDS.

In the table14, we see many NK cells and T cell related molecules are down-regulated. Molecules related to NK cell activation include grazymes(GZMK, GZMM, GZMB, and GZMH), perforin(PRF1), and killer cell receptors(KLRK1, KLRD1, KLRG1, KLRB1, NKTR, and KLRF1). NKTR is 13 fold down-regulated and granulysin (GNLY) is 5 fold down-regulated. NK cells are the key effector cells in THαβ immunity. Thus, this data suggests that THαβ immunity is not activated or even down-regulated in ARDS. In addition, many TCR related genes are also down-regulated including TRBC1, TRAC/J17/V20, TRBC2, TRD@, TRAP/TRGC2, and TRDV3. Several TCR related genes have greater than 5 fold down-regulation. We can see the down-regulations of MHC genes, CD costimulation molecules, STAT3, and TCR genes as well as the up-regulation of TGFB1 and STAT5B. These suggest that T cells are not activated in the acute lung injury. Thus, adaptive immunity cannot be successfully triggered in ARDS. It also suggests that Treg plays an important role in ARDS pathophysiology.

Discussion

Acute respiratory distress syndrome (ARDS) is a very severe respiratory complication. Sepsis is the major risk factor of ARDS. Sepsis is the uncontrolled bacteremia by extracellular bacteria infection. In addition, PMNs overactivation is very important in the pathogenesis of ARDS. Thus, extracellular bacteria induced TH17 immunity with neutrophil activation should be the key in the pathophysiology of ARDS.

According to Harrison's internal medicine, the time course of ARDS can be divided into three stages.(Kong, Chui et al. 2009) First, the exudative phase. In this phase, injured alveolar capillary endothelium and type I pneumocytes cause the loss of tight alveolar barrier. Thus, edema fluid rich in protein accumulate in the interstitial alveolar spaces. It has been reported that cytokines (IL-1, IL-6, and TNF-α) and chemokines (IL-8, and leukotriene B4) are present in lung in this phase(Papadakos 2002; Lee, Lim et al. 2012). A great numbers of neutrophils traffic into the pulmonary interstitium and alveoli.(Downey, Dong et al. 1999; Lee and Downey 2001) Alveolar edema predominantly leads to diminished aeration and atelectasis. Hyaline membranes start to develop. Then, intrapulmonary shunting and hypoxemia develop. The situation is even worse with microvascular occlusion which leads to increasing dead space and pulmonary hypertension. The exudative phase encompasses the first seven days of disease after exposure to a precipitating ARDS risk factor such as sepsis, aspiration pneumonia, bacteria pneumonia, pulmonary contusion, near drowning, toxic inhalation injury, severe trauma, burns, multiple transfusions, drug overdose, pancreatitis, and post-cardiopulmonary bypass.

Second, proliferative phase. This phase usually lasts from day 7 to day 21. Although many patients could recover during this stage, some patients develop progressive lung injury and early change of pulmonary fibrosis. Histologically, this phase is the initiation of lung repair, organization of alveolar exudates, and a shift from a neutrophil to a lymphocyte dominant pulmonary infiltrate. There is a proliferation of type II pneumocytes which can synthesize new pulmonary surfactants. They can also differentiate into type I pneumocytes. In addition, there is beginning of type III procollagen peptide presence which is the marker of pulmonary fibrosis.

Third, fibrotic phase. Although many patients with ARDS recover lung function three weeks after the initial lung injury, some enter a fibrotic phase that may require long term support on mechanical ventilators. Histologically, the alveolar edema and inflammatory exudates in early phases are converted to extensive alveolar duct and interstitial fibrosis. Intimal fibroproliferation in the pulmonary microvascular system leads to progressive vascular occlusion and pulmonary hypertension.

Here, I propose a detail pathogenesis to explain the three stages of ARDS. In the first exudative stage, neutrophils are attracted to lung due to chemotaxic agents such as IL-8 or C5 or leukotriene B4. During sepsis, bacterial infection in pulmonary tissue can trigger pulmonary epithelial cells, pulmonary endothelial cells, pulmonary fibroblast, and alveolar macrophage to be activated. Toll-like receptors 1,2,4,5 as well as heat

shock proteins (HSP60, HSP70) are key molecules to trigger TH17 host immunity(Jiang, Liang et al. 2005; Fan, Li et al. 2006; Togbe, Schnyder-Candrian et al. 2006; Imai, Kuba et al. 2008; Lv, Shen et al. 2009; Sharif, Dawra et al. 2009; Charles, Tissieres et al. 2011; Reino, Pisarenko et al. 2011; Wu, Chen et al. 2011). Heat shock proteins are important stress proteins in situation such as burn, trauma, hemorrhagic shock, near drowning or acute pancreatitis.(Ganter, Ware et al. 2006; Chase, Wheeler et al. 2007; Krzyzaniak, Cheadle et al. 2011) HSP60 and HSP70 can activate TH-17 related Toll-like receptors. Thus, TH17 related cytokines such as IL-17, IL-1, TNF-α, and IL-6 as well as TH-17 related chemokines such as IL-8 and other CXCL group chemokines will be triggered. Bacterial infection can let pulmonary epithelial cells to release chemokines and cytokines.(Smart and Casale 1994; Khair, Davies et al. 1996; Thorley, Ford et al. 2007) TH17 cytokines will start to activate TH17 immunity including activating PMN effector function for immunity against extracellular bacteria. The cytokine storm during ARDS now explained. However, in this study, I find out that CD86 costimulation signal, TCR genes, and majority of MHC genes are down-regulated in ARDS. Thus, adaptive immunity related effective and specific antibody and TCR response against bacteria may not be triggered. However, a paper in JI found that there is IL-8 autoantibody in ARDS patients.(Kurdowska, Miller et al. 1996) IL-8 as well as leukotriene B4 is the main chemoattractant in pulmonary tissue.(Standiford, Kunkel et al. 1990; Strieter, Chensue et al. 1990; Kunkel, Standiford et al. 1991; Nakamura, Yoshimura et al. 1991; Donnelly, Strieter et al. 1993; Lin, Pearson et al. 1994; De Luca, Minucci et al. 2011) It was first identified in lung giant cell lines.(Suzuki, Miyasaka et al. 1989) Besides, IL-8 has high affinity to bind to the heparin sulfate and chondroitin sulfate enriched lung tissue.(Frevert, Goodman et al. 2002; Frevert, Kinsella et al. 2003) And, IL-8 retention in pulmonary tissue can further recruit neutrophils to lung. It can explain why IL-8 secreted from distant site such as pancreas during acute pancreatitis can cause ARDS. However, IL-8 itself is not changed in this study. And, there are several defects in the IL8 autoantibody paper in JI. First, antiIL8-IL8 complex can be detected in 55% of healthy control serum. There is no significant difference of IL8-antiIL8 complex between ARDS patients' serum and healthy controls' serum. In addition, IL8 autoantibody can suppress IL8 binding activity for neutrophils and it can reduce IL8's chemotaxic activity. Thus, IL8 autoantibody's importance in ARDS pathogenesis is doubtful. Besides, several studies can support that TH17 immunity and the pathogenesis of ARDS. G-CSF, the growth factor of neutrophils, can cause the common symptoms of ARDS.(Takatsuka, Takemoto et al. 2002) And, suppression of NFkB can attenuate ARDS progression.(von Bismarck, Klemm et al. 2009; Tanaka, Nishiumi et al. 2010) Key THαβ cytokine, IL-10, can reduce the severity of ARDS.(Wu, Lin et al. 2009)

Bacterial infection is the most common risk factor of ARDS. However, certain pathogens other than bacteria also are risks for developing ARDS. Plasmodium falciparum malarial infection can also cause the complication of ARDS. The reason for this is that Plasmodium falciparum can activate heat shock proteins to trigger TH17 immunity to cause ARDS.(author's paper in press)(Van den Steen, Geurts et al. 2010) SARS-CoV and H1N1 Avian flu virus can also down-regulate normal anti-viral interferon-α/β and up-regulate TH17 immunity to trigger ARDS. (author's paper in press: Viral Immunology)(Rockx, Baas et al. 2009; Zhang, Sun et al. 2012) Thus, the above phenomonons suggest that TH17 inflammation is the key to the pathogenesis of ARDS. If different pathogens lead to a common pathway of TH17 immunity, they will cause the same consequence of ARDS. It is also seen in burn, trauma, or pancreatitis when TH17 autoimmunity is also activated.

In the second proliferative stage, lymphocytes replace neutrophils and become the dominant population in ARDS. These lymphocytes are TH17 lymphocytes and subsequent Treg lymphocytes. TH17 helper cells can secrete TH17 cytokines such as IL-17, IL-1, IL-6, and TNF-α to continue the inflammatory process. However, once the bacterial antigen during sepsis is cleared. Toll-like receptor signaling is stopped, and no further proinflammatory cytokines such as IL-6 is synthesized. In addition, there is no TCR signal found in this study. Thus, TH17 adaptive immunity may not be successfully generated. This could be very important in ARDS pathogenesis. In TH17 immunity, both TGF-β and IL-6 are two important triggering cytokines. If there is no longer IL-6 signaling, only TGF-β is generated. IL-6 is the key factor to regulate the balance between Treg cells and TH17 cells. If there is enough IL-6, Treg cells will become TH17 cells. If there is not enough IL-6, TGF-β secreting Treg cells will be maintained. Thus, in the third fibrosis stage, TGF-β secreting Treg cells are the dominant effector cells in ARDS.(Fahy, Lichtenberger et al. 2003) TGF-β is a very strong fibrosis promoting agent and is the most important and potent stimulant in tissue fibrosis.(Border and Noble 1994; Daniels, Wilkes et al. 2004) TGF-β will promote the synthesis of multiple collagen genes.(Kitamura, Cambier et al. 2011) Thus, overproduction of TGF-β in lung tissue will cause pulmonary fibrosis. TGF-β caused fibrosis is usually a process for repairing cavity after bacterial infection locus such as abscess. This mechanism can solve many controversial studies before. Several studies found that TLR4 and heat shock proteins can aggravate ARDS.(Imai, Kuba et al. 2008) However, another studies found that TLR4 or heat shock protein can protect from pulmonary fibrosis after acute lung injury.(Hagiwara, Iwasaka et al. 2007; Hilberath, Carlo et al. 2011; Yang, Wang et al. 2012) It is because TLR and heat shock

signaling can maintain the activation of proinflammatory cytokines such as IL-6. Thus, no solely TGF-β overproduction happens for lung fibrosis. In an animal study, neutrophil inhibitor can attenuate the progression of acute lung injury(Sakashita, Nishimura et al. 2007). Thus, TH17 and Treg inflammatory process can fully explain the pathogenesis of ARDS.

Recently, two papers in JCI and JI suggested that Treg cells play protective roles in acute lung injury.(D'Alessio, Tsushima et al. 2009; Venet, Chung et al. 2009) I disagree with their suggestions. First of all, they used Rag-/- mice and found out that ARDS is reduced in Rag-/- mice. However, both B cells and T cells are absent in Rag-/- mice. This finding can be explained that adaptive immune T & B lymphocytes relieve the TH17 innate ARDS pathological change. They also found out that CD8 T cells have protective roles in ARDS. In my study, I find out that antigen specific T cells are not activated in ARDS. Thus, specific TCR or antibody response against bacteria antigen could not be successfully triggered in acute lung injury. If the adaptive immunity can be triggered, it can limit ARDS pathology. Second, they used a lung injury scoring to access the severity of ARDS. However, the scoring system only included lung congestion and inflammatory infiltration. The most important sequel of ARDS: pulmonary fibrosis is not included. Thus, they made a wrong conclusion that TGF beta secreting Treg cells can protect ARDS. Actually, antiTGFB antibody can prevent mice from lung fibrosis.(Shenkar, Coulson et al. 1994) After knowing the complete pathophysiology of acute respiratory distress syndrome, we can develop better treatment strategies to managing this highly detrimental disease.

Figure legends

Figure 1. RMA express plot for selecting samples in normal healthy controls.
1-A NUSE boxplot for normal control
1-B RLE boxplot for normal control
1-C RLE-NUSE multiplot for normal control
1-D RLE-NUSE T2 plot for normal control
1-E Raw data Boxpolt for normal control

Figure 2. RMA express plot for selecting samples in ARDS patients.
2-A NUSE boxplot for ARDS patients
2-B RLE boxplot for ARDS patients
2-C RLE-NUSE multiplot for ARDS patients
2-D RLE-NUSE T2 plot for ARDS patients
2-E Raw data Boxplot for ARDS patients

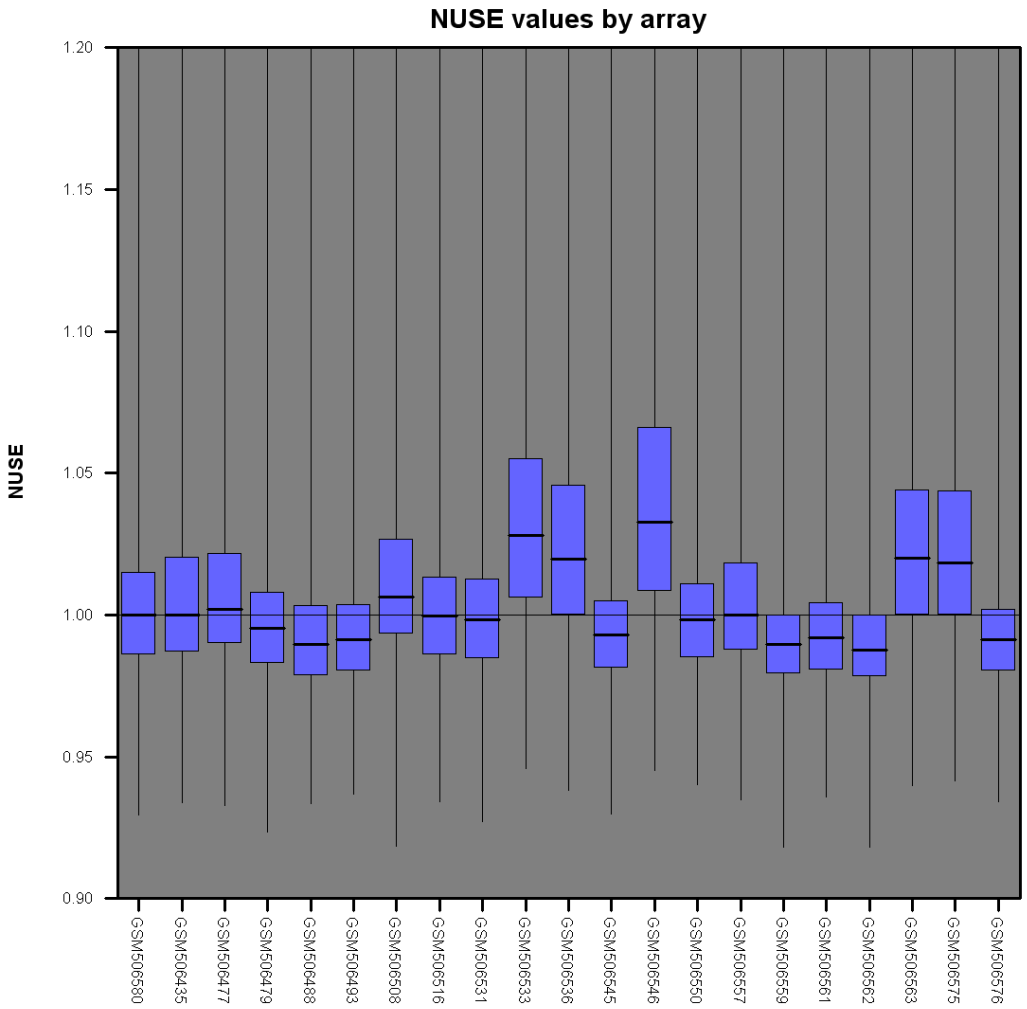
NUSE values by array
NUSE
1.20
1.15
1.10
1.05
1.00
0.95
0.90
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-A

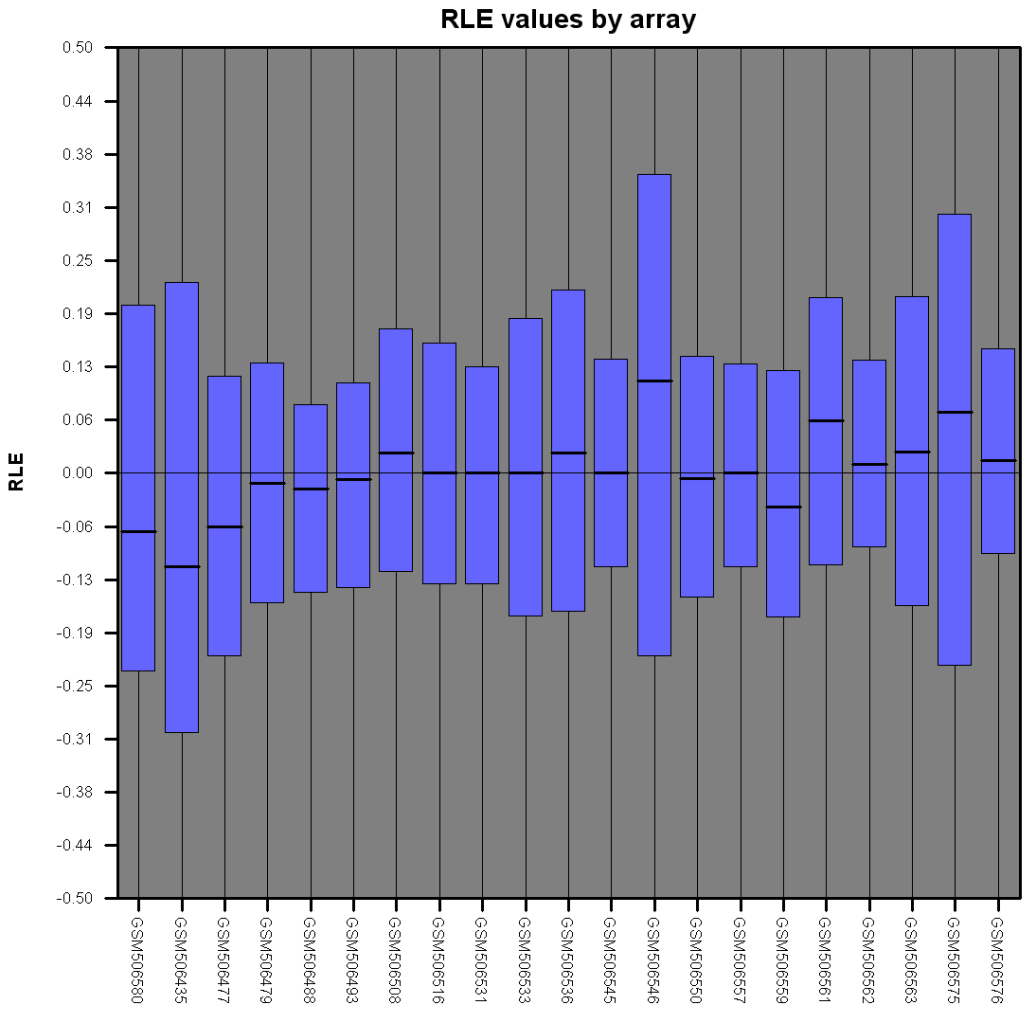
RLE values by array
RLE
0.50
0.44
0.38
0.31
0.25
0.19
0.13
0.06
0.00
-0.06
-0.13
-0.19
-0.25
-0.31
-0.38
-0.44
-0.50
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-B

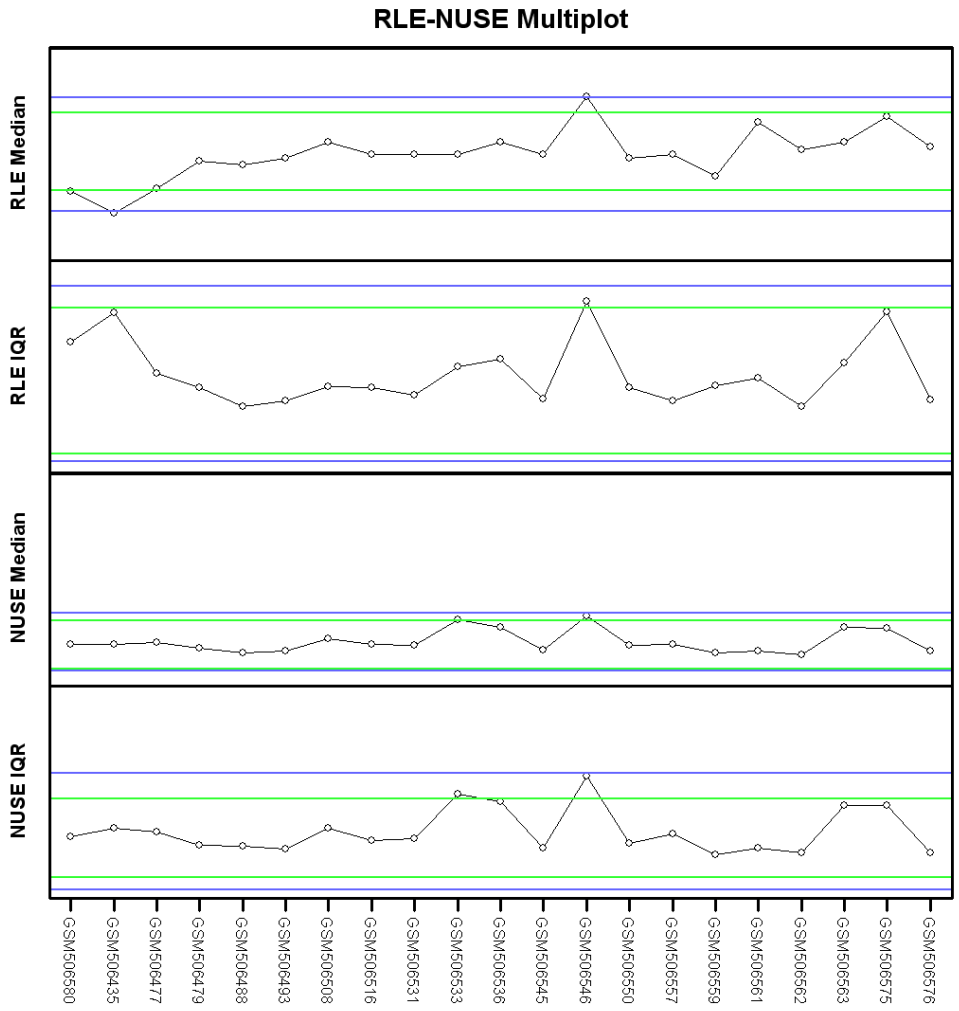
RLE-NUSE Multiplot
RLE Median
RLE IQR
NUSE Median
NUSE IQR
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-C

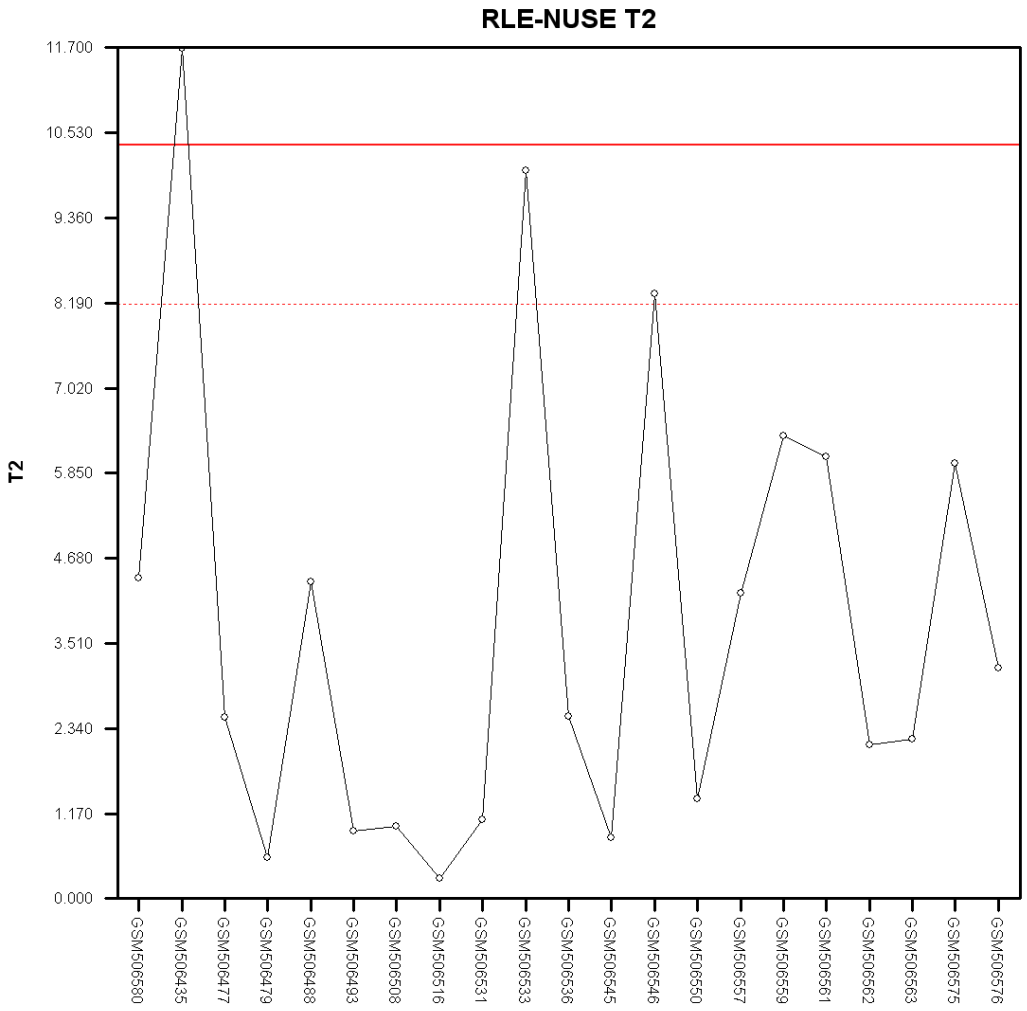
RLE-NUSE T2
T2
11.700
10.530
9.360
8.190
7.020
5.850
4.680
3.510
2.340
1.170
0.000
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-D

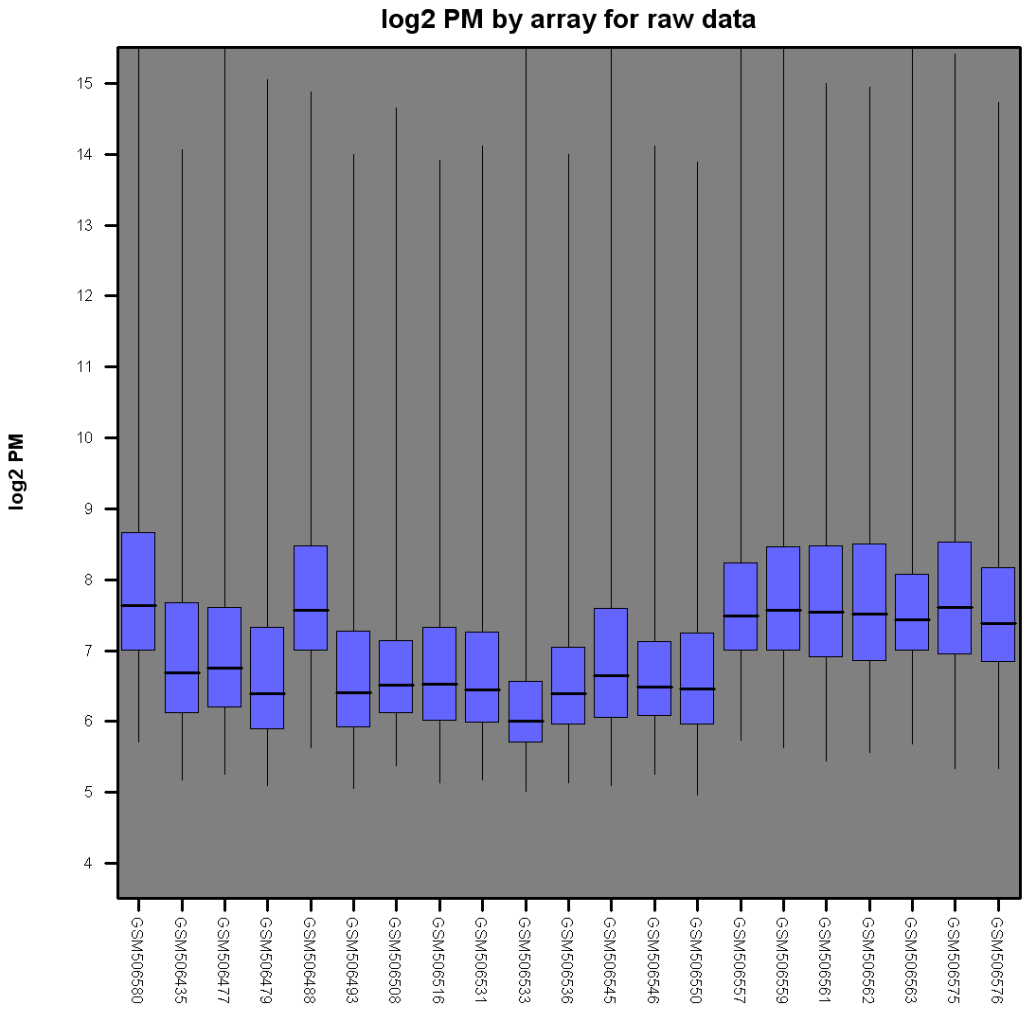
log2 PM by array for raw data
log2 PM
15
14
13
12
11
10
9
8
7
6
5
4
GSM506580
GSM506435
GSM506477
GSM506479
GSM506488
GSM506493
GSM506508
GSM506516
GSM506531
GSM506533
GSM506536
GSM506545
GSM506546
GSM506550
GSM506557
GSM506559
GSM506561
GSM506562
GSM506563
GSM506575
GSM506576

Figure 1-E

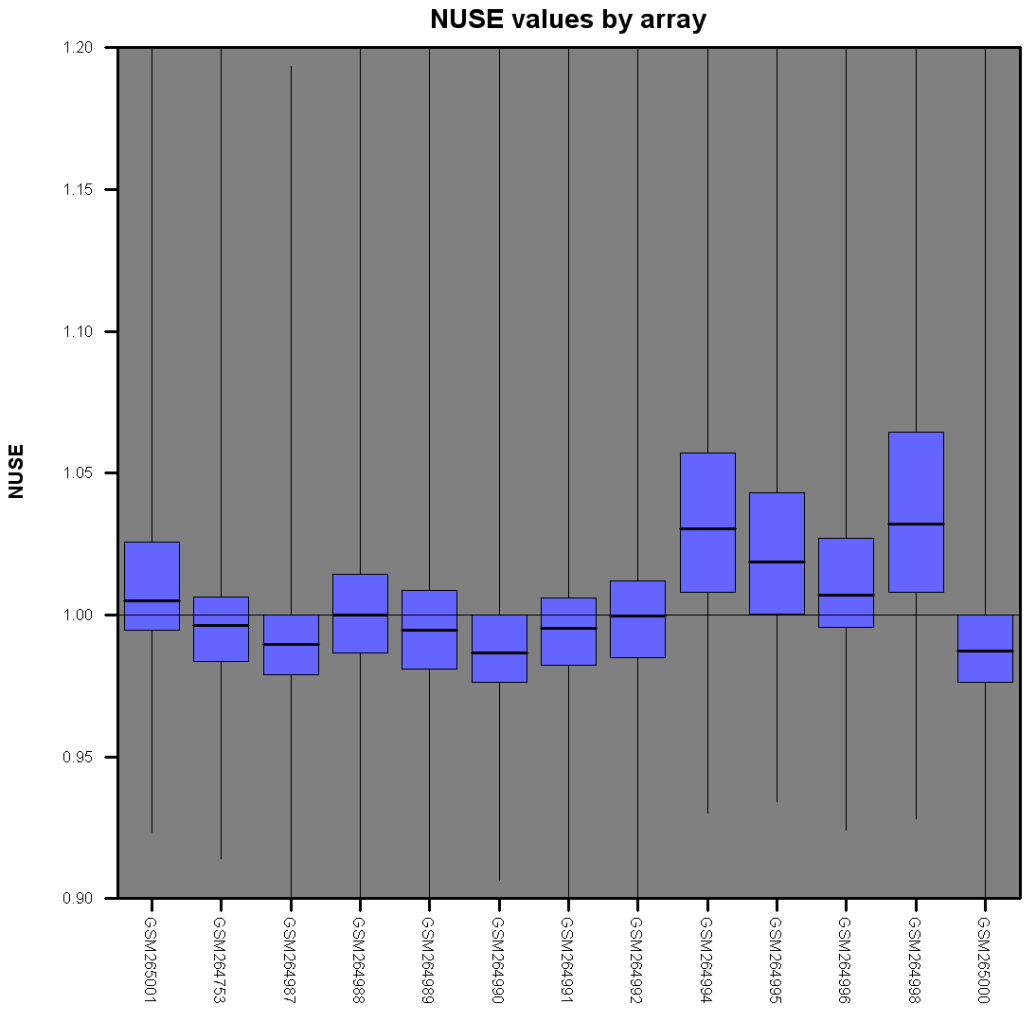
NUSE values by array
NUSE
1.20
1.15
1.10
1.05
1.00
0.95
0.90
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-A

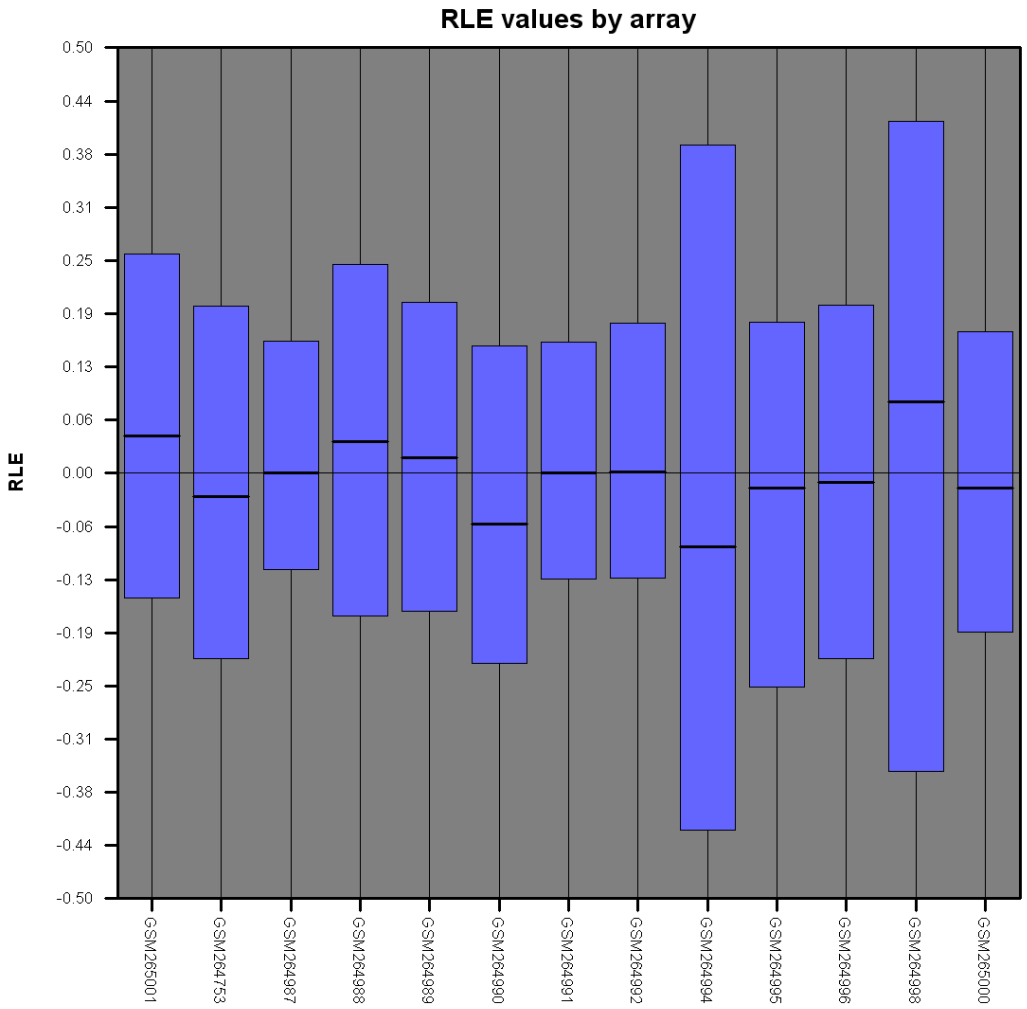
RLE values by array
RLE
0.50
0.44
0.38
0.31
0.25
0.19
0.13
0.06
0.00
-0.06
-0.13
-0.19
-0.25
-0.31
-0.38
-0.44
-0.50
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-B

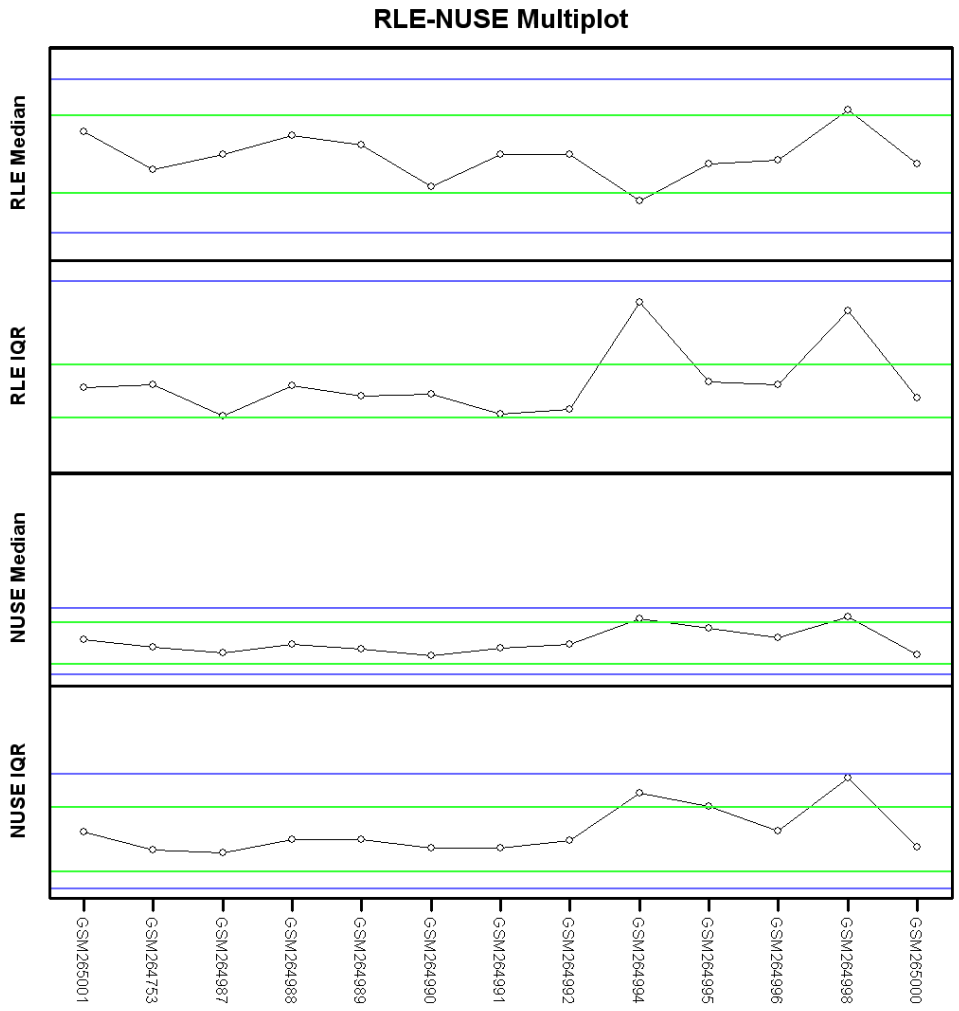
RLE-NUSE Multiplot
RLE Median
RLE IQR
NUSE Median
NUSE IQR
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-C

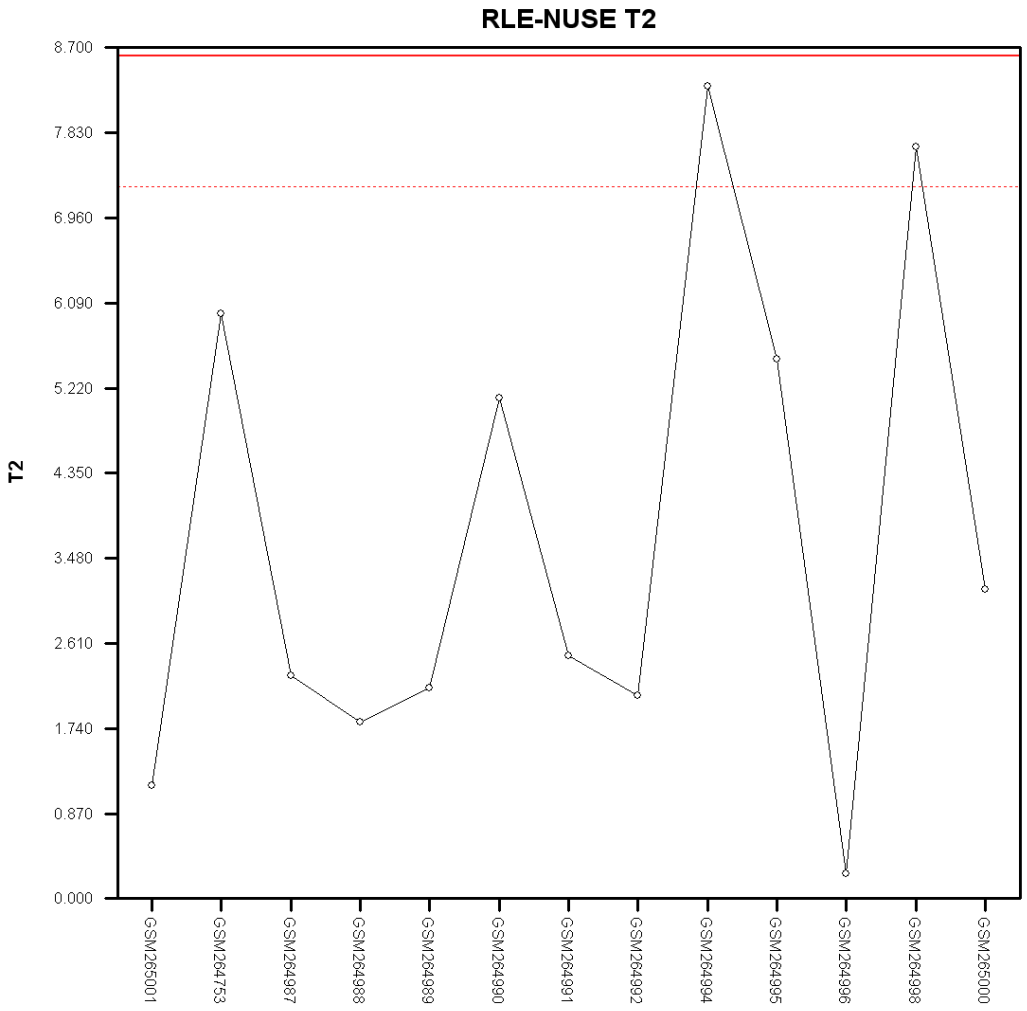
RLE-NUSE T2
T2
8.700
7.830
6.960
6.090
5.220
4.350
3.480
2.610
1.740
0.870
0.000
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-D

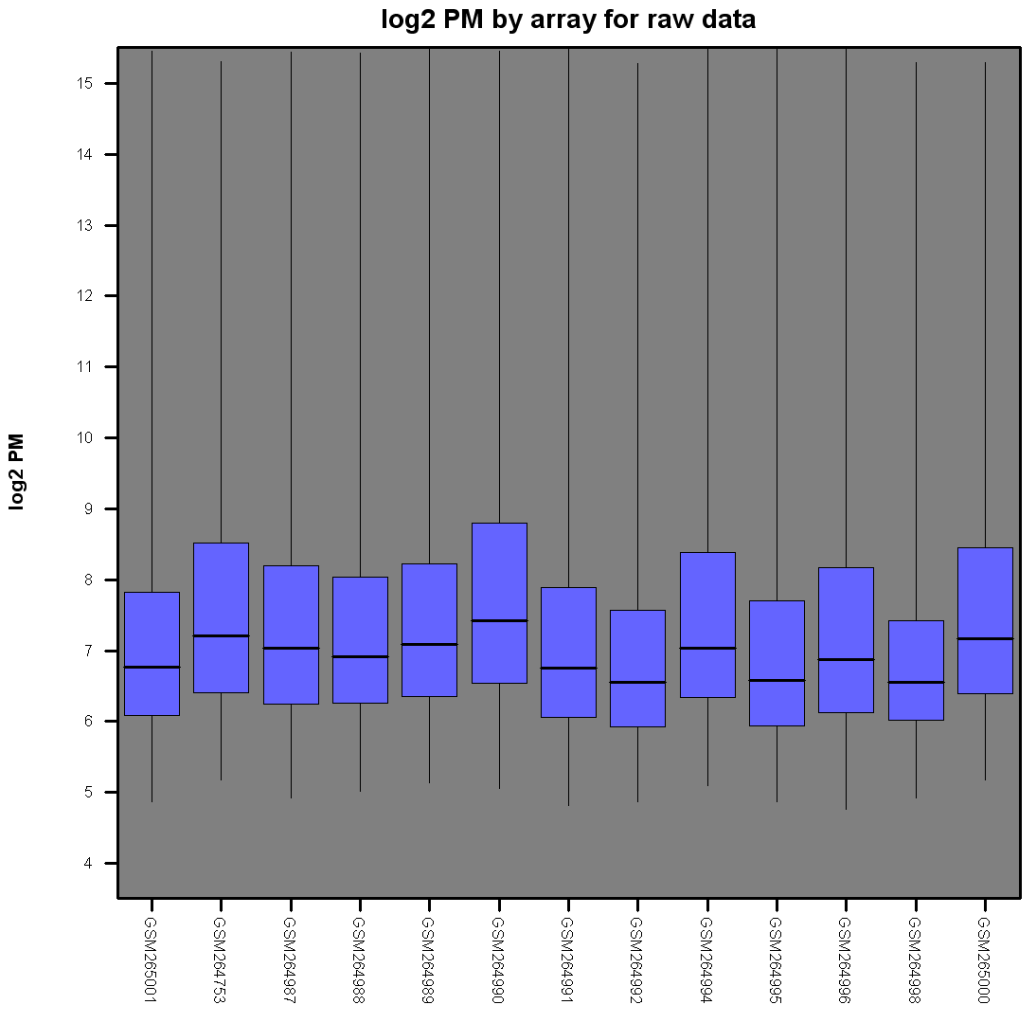
log2 PM by array for raw data
log2 PM
15
14
13
12
11
10
9
8
7
6
5
4
GSM265001
GSM264753
GSM264987
GSM264988
GSM264989
GSM264990
GSM264991
GSM264992
GSM264994
GSM264995
GSM264996
GSM264998
GSM265000

Figure 2-E

Table 1.Toll-likereceptor

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
204924_at	6.89E-09	up	3.412607	TLR2
210166_at	1.85E-07	up	2.732714	TLR5
210176_at	3.49E-04	up	2.256822	TLR1
220832_at	1.23E-07	up	5.041227	TLR8
221060_s_at	8.48E-06	up	2.713024	TLR4
219618_at	1.79E-09	up	3.059633	IRAK4

Table 2.HeatShock Protein

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
200598_s_at	5.09E-07	down	2.56764	HSP90B1
200800_s_at	8.10E-08	up	3.152098	HSPA1A /// HSPA1B
202557_at	2.10E-04	up	2.438485	HSPA13
202581_at	1.79E-11	up	6.14778	HSPA1A /// HSPA1B
208744_x_at	1.45E-08	down	2.109862	HSPH1
208815_x_at	6.07E-07	up	2.042983	HSPA4
210338_s_at	4.69E-08	down	2.607109	HSPA8
211969_at	9.22E-14	down	15.02711	HSP90AA1
219284_at	4.74E-04	up	2.277097	HSPBAP1
200941_at	7.08E-09	up	2.341059	HSBP1
200942_s_at	1.40E-05	up	2.092604	HSBP1
				DNAJB6 ///
208810_at	9.82E-04	up	2.226605	TMEM135
209015_s_at	2.14E-07	up	2.790782	DNAJB6
209157_at	9.23E-10	up	3.232302	DNAJA2
212467_at	6.04E-10	up	4.209722	DNAJC13
212908_at	1.42E-10	down	3.075077	DNAJC16
212911_at	3.41E-09	up	3.145019	DNAJC16
202842_s_at	6.90E-04	up	2.138169	DNAJB9
206782_s_at	6.78E-09	up	2.321206	DNAJC4

Table 3.Chemokine

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
1405_i_at	5.89E-04	down	2.63913	CCL5
204103_at	8.18E-05	down	2.515743	CCL4
204655_at	7.65E-04	down	2.245439	CCL5
205099_s_at	1.52E-06	down	2.461891	CCR1
205898_at	7.09E-06	down	4.04859	CX3CR1
206337_at	1.20E-07	down	4.466033	CCR7
206366_x_at	5.25E-11	down	5.07455	XCL1
206991_s_at	1.51E-04	down	2.039673	CCR5
208304_at	4.89E-06	down	4.671167	CCR3
				XCL1 ///
214567_s_at	1.62E-08	down	3.339736	XCL2
219161_s_at	4.46E-07	up	2.387246	CKLF
221058_s_at	9.57E-08	up	2.487534	CKLF
200660_at	9.31E-10	up	2.113736	S100A11
200815_s_at	1.43E-11	up	2.871307	PAFAH1B1
202917_s_at	3.16E-09	up	2.744783	S100A8
203535_at	2.25E-13	up	2.882588	S100A9
204351_at	4.60E-04	up	2.44348	S100P
205863_at	7.00E-10	up	4.3815	S100A12

Table4.MHC

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
204670_x_at	9.95E-11	down	3.865348	HLA-DRB1/4
208306_x_at	9.05E-09	down	2.986262	HLA-DRB1
208894_at	3.15E-10	down	4.546559	HLA-DRA
209312_x_at	4.77E-09	down	3.655453	HLA-DRB1/4/5
209823_x_at	1.81E-04	down	2.484667	HLA-DQB1
210982_s_at	8.58E-08	down	3.12086	HLA-DRA
211656_x_at	4.91E-05	down	2.002577	HLA-DQB1
211990_at	1.49E-06	down	3.785754	HLA-DPA1
211991_s_at	1.37E-08	down	3.178668	HLA-DPA1
212671_s_at	3.46E-04	down	2.569759	HLA-DQA1/2
212998_x_at	2.28E-05	down	2.456309	HLA-DQB1
213537_at	2.69E-05	down	2.602025	HLA-DPA1
215193_x_at	1.62E-08	down	3.284869	HLA-DRB1/3/4
217478_s_at	1.25E-07	down	2.783175	HLA-DMA
221491_x_at	4.58E-06	down	2.600531	HLA-DRB1/3/4/5
201137_s_at	1.25E-06	down	2.815927	HLA-DPB1
203290_at	4.64E-08	down	5.873323	HLA-DQA1
203932_at	7.50E-07	down	2.382645	HLA-DMB

Table5.Transcription factor

Probe Set ID	Pvalue	Arrow	Fold	GeneSymbol
205026_at	2.14E-10	up	2.427908	STAT5B
208991_at	5.85E-09	down	3.745264	STAT3
209969_s_at	1.14E-05	down	3.752185	STAT1
212549_at	4.02E-11	up	2.520162	STAT5B
212550_at	3.90E-09	up	2.643262	STAT5B
209189_at	1.67E-04	up	2.499937	FOS
218880_at	4.95E-08	up	3.472536	FOSL2
201473_at	7.18E-08	up	2.59816	JUNB
212501_at	7.61E-09	up	2.240303	CEBPB
213006_at	1.41E-09	up	3.735119	CEBPD
214523_at	2.02E-06	up	2.091157	CEBPE
204039_at	1.40E-08	up	2.398913	CEBPA
204203_at	1.31E-08	up	2.358698	CEBPG
203574_at	1.13E-08	up	4.640286	NFIL3
201502_s_at	5.83E-06	down	2.115988	NFKBIA
205841_at	2.40E-13	up	5.992293	JAK2
205842_s_at	2.73E-06	up	3.288805	JAK2
209604_s_at	4.81E-16	down	6.909352	GATA3
210555_s_at	1.03E-05	down	2.547481	NFATC3
210556_at	5.20E-05	down	2.429433	NFATC3
215092_s_at	1.12E-05	down	2.197351	NFAT5
217526_at	3.36E-08	down	3.459827	NFATC2IP
217527_s_at	1.86E-10	down	4.586211	NFATC2IP

Table6.Leukotriene & prostaglandin

Probe Set ID	Pvalue	Arrow	Fold	GeneSymbol
208771_s_at	1.53E-08	up	2.726662	LTA4H
210128_s_at	8.83E-10	up	3.067644	LTB4R
216388_s_at	4.30E-09	up	2.518544	LTB4R
215894_at	1.13E-11	down	9.422997	PTGDR
203913_s_at	3.16E-10	up	27.23115	HPGD
203914_x_at	3.10E-09	up	19.87965	HPGD
204445_s_at	1.10E-06	up	2.227303	ALOX5
204446_s_at	3.10E-08	up	2.322821	ALOX5
204614_at	3.94E-06	up	2.905599	SERPINB2
209533_s_at	1.09E-08	up	2.612623	PLAA
210145_at	1.31E-09	up	3.562516	PLA2G4A
210772_at	3.05E-08	up	4.401296	FPR2
210773_s_at	3.42E-06	up	3.867929	FPR2
213572_s_at	2.12E-12	up	6.133628	SERPINB1
214366_s_at	4.55E-09	up	3.975351	ALOX5

Table7.MMP and FGF

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
207329_at	1.17E-08	up	28.02386	MMP8
207890_s_at	1.46E-09	up	3.310085	MMP25
203936_s_at	2.84E-12	up	11.50853	MMP9
205110_s_at	2.26E-06	up	5.236618	FGF13
201666_at	1.46E-08	up	2.519345	TIMP1
203167_at	2.47E-10	up	3.173297	TIMP2
219295_s_at	7.61E-07	up	6.184153	PCOLCE2
200827_at	2.27E-07	up	2.158958	PLOD1
200654_at	1.47E-09	up	2.244832	P4HB
201940_at	2.24E-08	up	5.123088	CPD
201941_at	8.36E-08	up	4.763787	CPD
201942_s_at	2.76E-06	up	3.149759	CPD
201943_s_at	6.79E-10	up	6.419939	CPD
202304_at	1.23E-09	up	3.617562	FNDC3A
203044_at	1.34E-05	up	3.342165	CHSY1
203284_s_at	2.64E-08	up	3.515453	HS2ST1
203285_s_at	9.15E-10	up	2.626396	HS2ST1
207165_at	7.94E-05	up	2.063695	HMMR
207543_s_at	9.43E-07	up	2.718742	P4HA1
211945_s_at	0.001738	up	2.065411	ITGB1
218718_at	2.24E-10	up	12.04417	PDGFC
219049_at	1.52E-08	up	7.475137	CSGALNACT1
219403_s_at	1.33E-07	up	5.223431	HPSE
222235_s_at	3.83E-10	up	13.31196	CSGALNACT2

Table8.Complement

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
200983_x_at	1.18E-09	up	4.196889	CD59
200984_s_at	1.67E-10	up	4.910066	CD59
200985_s_at	3.02E-11	up	8.311746	CD59
201925_s_at	3.14E-07	up	6.090309	CD55
201926_s_at	4.95E-09	up	4.097339	CD55
202953_at	5.10E-04	up	2.016919	C1QB
205786_s_at	9.34E-11	up	3.896006	ITGAM
206244_at	5.96E-11	up	7.560091	CR1
209906_at	5.21E-09	up	5.687038	C3AR1
210184_at	2.03E-05	up	2.185833	ITGAX
212463_at	3.67E-08	up	3.248518	CD59
217552_x_at	1.83E-09	up	3.938783	CR1
218232_at	2.16E-05	up	3.030927	C1QA
218983_at	2.04E-07	up	3.029844	C1RL
220088_at	2.45E-06	up	2.500003	C5AR1
205033_s_at	7.03E-06	up	6.365769	DEFA1/1B/3
207269_at	1.49E-04	up	6.195451	DEFA4

Table9.Cathepsin

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
202450_s_at	4.73E-06	up	2.020977	CTSK
203758_at	3.84E-07	down	2.476479	CTSO
205653_at	4.28E-04	up	3.634934	CTSG
210042_s_at	5.41E-05	up	2.824491	CTSZ
214450_at	9.49E-04	down	2.150796	CTSW
200661_at	1.10E-07	up	2.603046	CTSA
200766_at	3.02E-11	up	3.793397	CTSD
201487_at	2.22E-07	up	3.024736	CTSC
203948_s_at	6.35E-04	up	2.112642	MPO
203949_at	5.14E-06	up	4.61238	MPO
204961_s_at	5.23E-06	up	2.092114	NCF1B1C
207677_s_at	5.21E-09	up	3.07743	NCF4

Table10.CSF

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
205159_at	7.47E-05	up	2.272558	CSF2RB
210340_s_at	6.06E-10	up	2.727757	CSF2RA
203591_s_at	4.27E-06	up	2.365631	CSF3R

Table11.Fc receptor

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
203561_at	5.57E-08	up	2.06512	FCGR2A
204232_at	6.41E-10	up	2.89912	FCER1G
207674_at	4.21E-07	up	6.511793	FCAR
210992_x_at	2.25E-05	up	2.003132	FCGR2C
211307_s_at	3.73E-07	up	4.462972	FCAR
211734_s_at	7.11E-05	down	4.276542	FCER1A
211816_x_at	2.46E-05	up	2.47651	FCAR
214511_x_at	1.06E-05	up	3.171251	FCGR1B
216950_s_at	4.67E-08	up	5.148257	FCGR1A/1C

Table12.Cytokine & receptor

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
203828_s_at	6.11E-04	down	2.216758	IL32
205227_at	4.73E-04	up	2.365252	IL1RAP
205291_at	2.41E-06	down	3.160494	IL2RB
205403_at	5.96E-11	up	9.990063	IL1R2
205707_at	5.70E-06	down	2.016105	IL17RA
205798_at	2.19E-21	down	28.62358	IL7R
205926_at	2.78E-10	down	2.211585	IL27RA
205945_at	9.53E-14	down	13.61186	IL6R
205992_s_at	3.86E-06	up	3.345045	IL15
206618_at	1.05E-11	up	17.52686	IL18R1
207072_at	3.95E-11	up	6.322352	IL18RAP
208200_at	3.63E-09	down	4.584162	IL1A
208930_s_at	7.16E-10	down	4.59278	ILF3
211372_s_at	2.35E-10	up	17.05508	IL1R2
212195_at	9.18E-05	up	2.753398	IL6ST
212196_at	1.44E-05	up	2.060642	IL6ST
212657_s_at	3.37E-06	up	2.343125	IL1RN
217489_s_at	9.59E-13	down	3.415206	IL6R
202948_at	1.06E-10	up	9.925212	IL1R1
203233_at	1.18E-09	up	3.541053	IL4R
205016_at	6.86E-09	up	4.867131	TGFA
201506_at	1.41E-04	down	2.289291	TGFBI
203085_s_at	1.35E-05	up	2.13325	TGFB1
204731_at	3.89E-15	down	10.80744	TGFBR3
206026_s_at	7.23E-06	up	4.685213	TNFAIP6
206222_at	3.55E-07	down	2.189329	TNFRSF10C
207536_s_at	4.78E-06	down	2.923358	TNFRSF9
207643_s_at	1.78E-08	up	2.618468	TNFRSF1A
207907_at	3.57E-08	down	3.31319	TNFSF14
208296_x_at	8.56E-05	up	2.646926	TNFAIP8
210260_s_at	5.80E-05	up	2.88896	TNFAIP8
214329_x_at	2.56E-04	up	2.679365	TNFSF10
202509_s_at	8.86E-10	down	2.258532	TNFAIP2
208114_s_at	6.33E-16	down	6.286403	ISG20L2
208965_s_at	1.52E-08	down	6.302218	IFI16
211676_s_at	1.42E-07	up	4.556334	IFNGR1

220577_at	8.19E-07	down	2.331832	GVINP1
201642_at	8.60E-08	up	2.225393	IFNGR2
202269_x_at	1.73E-05	down	4.987527	GBP1
202727_s_at	9.68E-08	up	3.452631	IFNGR1
204191_at	2.33E-07	up	2.031339	IFNAR1
204415_at	0.004538	up	2.774495	IFI6
204439_at	0.004491	down	3.991523	IFI44L
204747_at	5.65E-04	down	3.737294	IFIT3
204786_s_at	5.62E-17	down	6.491528	IFNAR2
200704_at	1.66E-07	up	2.056096	LITAF
201108_s_at	3.76E-05	up	2.314963	THBS1
201109_s_at	2.60E-05	up	3.128824	THBS1
201110_s_at	1.47E-08	up	7.142229	THBS1
204780_s_at	3.86E-04	up	2.720255	FAS
204781_s_at	9.11E-05	up	2.032212	FAS
221601_s_at	1.46E-09	down	4.684259	FAIM3
221602_s_at	2.90E-10	down	3.944784	FAIM3

Table13.CD molecules

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
200663_at	1.23E-10	up	2.64625	CD63
201005_at	1.19E-06	up	4.053223	CD9
202878_s_at	1.20E-04	up	2.316249	CD93
202910_s_at	1.39E-04	up	2.078598	CD97
203645_s_at	1.60E-06	up	7.818829	CD163
203799_at	0.002884	up	2.038077	CD302
204489_s_at	3.25E-10	up	2.475646	CD44
204490_s_at	1.31E-08	up	2.582747	CD44
204627_s_at	3.45E-05	up	3.667203	ITGB3
204661_at	7.10E-06	down	2.483425	CD52
205173_x_at	7.28E-08	up	4.151193	CD58
205758_at	1.12E-04	down	3.211919	CD8A
205789_at	4.22E-05	up	2.844588	CD1D
205831_at	2.21E-07	down	4.421892	CD2
205987_at	1.13E-07	down	2.078875	CD1C
205988_at	1.13E-15	down	5.71907	CD84
206150_at	2.30E-08	down	2.506513	CD27
206488_s_at	6.10E-04	up	2.481129	CD36
206493_at	5.05E-06	up	3.000028	ITGA2B
206494_s_at	7.02E-04	up	3.137038	ITGA2B
206761_at	3.30E-04	down	2.113857	CD96
206804_at	6.16E-10	down	4.490583	CD3G
208405_s_at	8.52E-05	up	2.330166	CD164
208650_s_at	7.09E-07	up	6.548884	CD24
208651_x_at	6.39E-08	up	4.732706	CD24
208652_at	1.21E-07	up	2.66052	PPP2CA
208653_s_at	6.26E-10	up	5.120652	CD164
208654_s_at	3.97E-06	up	5.608513	CD164
209555_s_at	2.95E-04	up	2.950439	CD36
209771_x_at	1.32E-07	up	6.559978	CD24
209835_x_at	1.06E-06	up	2.202673	CD44
210031_at	1.34E-06	down	3.413696	CD247
210184_at	2.03E-05	up	2.185833	ITGAX
210895_s_at	2.39E-04	down	2.251445	CD86
211744_s_at	5.92E-08	up	4.172478	CD58
211893_x_at	8.92E-10	down	2.082775	CD6

211900_x_at	5.14E-12	down	2.56049	CD6
212014_x_at	7.54E-07	up	2.308336	CD44
212063_at	4.06E-06	up	2.119029	CD44
213958_at	1.17E-06	down	2.205519	CD6
215049_x_at	2.45E-06	up	7.967604	CD163
215240_at	1.19E-08	up	2.467146	ITGB3
216233_at	4.99E-06	up	6.556412	CD163
216331_at	0.001993	up	2.434621	ITGA7
216379_x_at	5.86E-08	up	7.598393	CD24
216942_s_at	1.49E-05	up	3.193467	CD58
216956_s_at	1.65E-04	up	2.29709	ITGA2B
217523_at	9.19E-10	down	6.259623	CD44
219669_at	4.80E-13	up	52.83338	CD177
222061_at	2.36E-09	up	3.870316	CD58
266_s_at	6.15E-09	up	9.687818	CD24
213539_at	1.57E-06	down	3.240318	CD3D

Table14.NK/CTL molecules

Probe Set ID	Pvalue	Arrow	Fold	Gene Symbol
205821_at	8.68E-07	down	3.717665	KLRK1
206666_at	6.47E-06	down	3.898898	GZMK
207460_at	5.21E-06	down	2.196101	GZMM
207795_s_at	8.11E-05	down	2.646551	KLRD1
210164_at	3.84E-06	down	4.671744	GZMB
210288_at	2.27E-09	down	5.673069	KLRG1
210321_at	2.14E-05	down	6.098404	GZMH
210606_x_at	2.18E-05	down	2.995347	KLRD1
210915_x_at	2.72E-06	down	3.166231	TRBC1
210972_x_at	3.84E-07	down	3.223779	TRAC/J17/V20
211796_s_at	2.86E-06	down	3.233982	TRBC1/C2
211902_x_at	3.75E-06	down	2.651879	TRD@
213193_x_at	8.35E-07	down	3.394675	TRBC1
213830_at	1.16E-08	down	3.694018	TRD@
214470_at	4.30E-04	down	2.832583	KLRB1
214617_at	2.56E-04	down	3.489038	PRF1
215338_s_at	2.44E-19	down	13.36511	NKTR
215806_x_at	2.27E-05	down	3.907998	TARP/TRGC2
216191_s_at	3.85E-06	down	5.215065	TRDV3
216920_s_at	1.31E-06	down	5.017736	TARP/TRGC2
217143_s_at	6.84E-08	down	6.090665	TRD@
220646_s_at	0.004097	down	2.571194	KLRF1
37145_at	4.61E-06	down	5.027259	GNLY

References

Border, W. A. and N. A. Noble (1994). "Transforming growth factor beta in tissue fibrosis." N Engl J Med **331**(19): 1286-1292.

Charles, P. E., P. Tissieres, et al. (2011). "Mild-stretch mechanical ventilation upregulates toll-like receptor 2 and sensitizes the lung to bacterial lipopeptide." Crit Care **15**(4): R181.

Chase, M. A., D. S. Wheeler, et al. (2007). "Hsp72 induces inflammation and regulates cytokine production in airway epithelium through a TLR4- and NF-kappaB-dependent mechanism." J Immunol **179**(9): 6318-6324.

D'Alessio, F. R., K. Tsushima, et al. (2009). "CD4+CD25+Foxp3+ Tregs resolve experimental lung injury in mice and are present in humans with acute lung injury." J Clin Invest **119**(10): 2898-2913.

Daniels, C. E., M. C. Wilkes, et al. (2004). "Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis." J Clin Invest **114**(9): 1308-1316.

De Luca, D., A. Minucci, et al. (2011). "Secretory phospholipase A pathway during pediatric acute respiratory distress syndrome: a preliminary study." Pediatr Crit Care Med **12**(1): e20-24.

Donnelly, S. C., R. M. Strieter, et al. (1993). "Interleukin-8 and development of adult respiratory distress syndrome in at-risk patient groups." Lancet **341**(8846): 643-647.

Downey, G. P., Q. Dong, et al. (1999). "Regulation of neutrophil activation in acute lung injury." Chest **116**(1 Suppl): 46S-54S.

Escobar Galvis, M. L., J. Jia, et al. (2007). "Transgenic or tumor-induced expression of heparanase upregulates sulfation of heparan sulfate." Nat Chem Biol **3**(12): 773-778.

Fahy, R. J., F. Lichtenberger, et al. (2003). "The acute respiratory distress syndrome: a role for transforming growth factor-beta 1." Am J Respir Cell Mol Biol **28**(4): 499-503.

Fan, J., Y. Li, et al. (2006). "Hemorrhagic shock-activated neutrophils augment TLR4 signaling-induced TLR2 upregulation in alveolar macrophages: role in hemorrhage-primed lung inflammation." Am J Physiol Lung Cell Mol Physiol **290**(4): L738-L746.

Frevert, C. W., R. B. Goodman, et al. (2002). "Tissue-specific mechanisms control the retention of IL-8 in lungs and skin." J Immunol **168**(7): 3550-3556.

Frevert, C. W., M. G. Kinsella, et al. (2003). "Binding of interleukin-8 to heparan sulfate and chondroitin sulfate in lung tissue." Am J Respir Cell Mol Biol **28**(4):

464-472.

Ganter, M. T., L. B. Ware, et al. (2006). "Extracellular heat shock protein 72 is a marker of the stress protein response in acute lung injury." Am J Physiol Lung Cell Mol Physiol **291**(3): L354-361.

Hadkar, V., S. Sangsree, et al. (2004). "Carboxypeptidase-mediated enhancement of nitric oxide production in rat lungs and microvascular endothelial cells." Am J Physiol Lung Cell Mol Physiol **287**(1): L35-45.

Hagiwara, S., H. Iwasaka, et al. (2007). "Association between heat stress protein 70 induction and decreased pulmonary fibrosis in an animal model of acute lung injury." Lung **185**(5): 287-293.

Hilberath, J. N., T. Carlo, et al. (2011). "Resolution of Toll-like receptor 4-mediated acute lung injury is linked to eicosanoids and suppressor of cytokine signaling 3." FASEB J **25**(6): 1827-1835.

Howrylak, J. A., T. Dolinay, et al. (2009). "Discovery of the gene signature for acute lung injury in patients with sepsis." Physiol Genomics **37**(2): 133-139.

Imai, Y., K. Kuba, et al. (2008). "Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury." Cell **133**(2): 235-249.

Jiang, D., J. Liang, et al. (2005). "Regulation of lung injury and repair by Toll-like receptors and hyaluronan." Nat Med **11**(11): 1173-1179.

Khair, O. A., R. J. Davies, et al. (1996). "Bacterial-induced release of inflammatory mediators by bronchial epithelial cells." Eur Respir J **9**(9): 1913-1922.

Kitamura, H., S. Cambier, et al. (2011). "Mouse and human lung fibroblasts regulate dendritic cell trafficking, airway inflammation, and fibrosis through integrin alphavbeta8-mediated activation of TGF-beta." J Clin Invest **121**(7): 2863-2875.

Kobayashi, A., S. Hashimoto, et al. (1998). "Expression of inducible nitric oxide synthase and inflammatory cytokines in alveolar macrophages of ARDS following sepsis." Chest **113**(6): 1632-1639.

Kong, M. Y., Y. Li, et al. (2011). "Early elevation of matrix metalloproteinase-8 and -9 in pediatric ARDS is associated with an increased risk of prolonged mechanical ventilation." PLoS One **6**(8): e22596.

Kong, S. L., P. Chui, et al. (2009). "Elucidating the molecular physiopathology of acute respiratory distress syndrome in severe acute respiratory syndrome patients." Virus Res **145**(2): 260-269.

Krzyzaniak, M., G. Cheadle, et al. (2011). "Burn-induced acute lung injury requires a functional Toll-like receptor 4." Shock **36**(1): 24-29.

Kunkel, S. L., T. Standiford, et al. (1991). "Interleukin-8 (IL-8): the major neutrophil chemotactic factor in the lung." Exp Lung Res **17**(1): 17-23.

Kurdowska, A., E. J. Miller, et al. (1996). "Anti-IL-8 autoantibodies in alveolar fluid from patients with the adult respiratory distress syndrome." J Immunol **157**(6): 2699-2706.

Lee, E. J., J. Y. Lim, et al. (2012). "The expression of HSPs, anti-oxidants, and cytokines in plasma and bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome." Clin Biochem **45**(6): 493-498.

Lee, W. L. and G. P. Downey (2001). "Neutrophil activation and acute lung injury." Curr Opin Crit Care **7**(1): 1-7.

Li, J., N. W. Shworak, et al. (2002). "Increased responsiveness of hypoxic endothelial cells to FGF2 is mediated by HIF-1alpha-dependent regulation of enzymes involved in synthesis of heparan sulfate FGF2-binding sites." J Cell Sci **115**(Pt 9): 1951-1959.

Lin, G., A. E. Pearson, et al. (1994). "Regulation of interleukin-8 expression in porcine alveolar macrophages by bacterial lipopolysaccharide." J Biol Chem **269**(1): 77-85.

Lv, T., X. Shen, et al. (2009). "TLR4 is essential in acute lung injury induced by unresuscitated hemorrhagic shock." J Trauma **66**(1): 124-131.

Myllyharju, J. (2008). "Prolyl 4-hydroxylases, key enzymes in the synthesis of collagens and regulation of the response to hypoxia, and their roles as treatment targets." Ann Med **40**(6): 402-417.

Nakamura, H., K. Yoshimura, et al. (1991). "Interleukin-8 gene expression in human bronchial epithelial cells." J Biol Chem **266**(29): 19611-19617.

Papadakos, P. J. (2002). "Cytokines, genes, and ARDS." Chest **121**(5): 1391-1392.

Reino, D. C., V. Pisarenko, et al. (2011). "Trauma hemorrhagic shock-induced lung injury involves a gut-lymph-induced TLR4 pathway in mice." PLoS One **6**(8): e14829.

Rockx, B., T. Baas, et al. (2009). "Early upregulation of acute respiratory distress syndrome-associated cytokines promotes lethal disease in an aged-mouse model of severe acute respiratory syndrome coronavirus infection." J Virol **83**(14): 7062-7074.

Rotunno, M., N. Hu, et al. (2011). "A gene expression signature from peripheral whole blood for stage I lung adenocarcinoma." Cancer Prev Res (Phila) **4**(10): 1599-1608.

Sakashita, A., Y. Nishimura, et al. (2007). "Neutrophil elastase inhibitor (sivelestat) attenuates subsequent ventilator-induced lung injury in mice." Eur J Pharmacol **571**(1): 62-71.

Sharif, R., R. Dawra, et al. (2009). "Impact of toll-like receptor 4 on the severity of acute pancreatitis and pancreatitis-associated lung injury in mice." Gut **58**(6):

813-819.

Shenkar, R., W. F. Coulson, et al. (1994). "Anti-transforming growth factor-beta monoclonal antibodies prevent lung injury in hemorrhaged mice." Am J Respir Cell Mol Biol **11**(3): 351-357.

Smart, S. J. and T. B. Casale (1994). "Pulmonary epithelial cells facilitate TNF-alpha-induced neutrophil chemotaxis. A role for cytokine networking." J Immunol **152**(8): 4087-4094.

Standiford, T. J., S. L. Kunkel, et al. (1990). "Interleukin-8 gene expression by a pulmonary epithelial cell line. A model for cytokine networks in the lung." J Clin Invest **86**(6): 1945-1953.

Strieter, R. M., S. W. Chensue, et al. (1990). "Human alveolar macrophage gene expression of interleukin-8 by tumor necrosis factor-alpha, lipopolysaccharide, and interleukin-1 beta." Am J Respir Cell Mol Biol **2**(4): 321-326.

Suzuki, K., H. Miyasaka, et al. (1989). "Purification and partial primary sequence of a chemotactic protein for polymorphonuclear leukocytes derived from human lung giant cell carcinoma LU65C cells." J Exp Med **169**(6): 1895-1901.

Takatsuka, H., Y. Takemoto, et al. (2002). "Common features in the onset of ARDS after administration of granulocyte colony-stimulating factor." Chest **121**(5): 1716-1720.

Tanaka, S., S. Nishiumi, et al. (2010). "Vitamin K3 attenuates lipopolysaccharide-induced acute lung injury through inhibition of nuclear factor-kappaB activation." Clin Exp Immunol **160**(2): 283-292.

Thorley, A. J., P. A. Ford, et al. (2007). "Differential regulation of cytokine release and leukocyte migration by lipopolysaccharide-stimulated primary human lung alveolar type II epithelial cells and macrophages." J Immunol **178**(1): 463-473.

Togbe, D., S. Schnyder-Candrian, et al. (2006). "TLR4 gene dosage contributes to endotoxin-induced acute respiratory inflammation." J Leukoc Biol **80**(3): 451-457.

Turpeenniemi-Hujanen, T. M. (1981). "Immunological characterization of lysyl hydroxylase, an enzyme of collagen synthesis." Biochem J **195**(3): 669-676.

Van den Steen, P. E., N. Geurts, et al. (2010). "Immunopathology and dexamethasone therapy in a new model for malaria-associated acute respiratory distress syndrome." Am J Respir Crit Care Med **181**(9): 957-968.

Venet, F., C. S. Chung, et al. (2009). "Lymphocytes in the development of lung inflammation: a role for regulatory CD4+ T cells in indirect pulmonary lung injury." J Immunol **183**(5): 3472-3480.

Venkatesan, N., M. Ouzzine, et al. (2011). "Increased deposition of chondroitin/dermatan sulfate glycosaminoglycan and upregulation of

beta1,3-glucuronosyltransferase I in pulmonary fibrosis." Am J Physiol Lung Cell Mol Physiol **300**(2): L191-203.

von Bismarck, P., K. Klemm, et al. (2009). "Selective NF-kappaB inhibition, but not dexamethasone, decreases acute lung injury in a newborn piglet airway inflammation model." Pulm Pharmacol Ther **22**(4): 297-304.

Wu, C. L., L. Y. Lin, et al. (2009). "Attenuation of lipopolysaccharide-induced acute lung injury by treatment with IL-10." Respirology **14**(4): 511-521.

Wu, T. T., T. L. Chen, et al. (2011). "Lipopolysaccharide stimulates syntheses of toll-like receptor 2 and surfactant protein-A in human alveolar epithelial A549 cells through upregulating phosphorylation of MEK1 and ERK1/2 and sequential activation of NF-kappaB." Cytokine **55**(1): 40-47.

Yang, H. Z., J. P. Wang, et al. (2012). "TLR4 activity is required in the resolution of pulmonary inflammation and fibrosis after acute and chronic lung injury." Am J Pathol **180**(1): 275-292.

Zhang, Y., H. Sun, et al. (2012). "Acute respiratory distress syndrome induced by a swine 2009 H1N1 variant in mice." PLoS One **7**(1): e29347.