EFFECT OF PARAINFLUENZA VIRUS INFECTION ON HUMAN IMMUNO-INFLAMMATORY GENES EXPRESSION AND GENE ONTOLOGY ANALYSIS

ABDULRHEM THONNON AL-GHAZAL[1], SAID IBRAHEM ISMAIL[2], YOUSUF IBRAHEM UMARY[3], AND RULA FAIEK AL-KHUZIE[4].
[1]Dept. of Biology, Faculty of Science, University of Mosul, Iraq.
[2]Dept. of Biochemistry, Faculty of Medicine, University of Jordan.
[3]Dept. of Biology, Faculty of Science, University of Jordan.
[4]Dept. of Nutrition and food Science, Faculty of Allied Medical Sciences, Applied Sciences University.
(Accepted for publication: June 9, 2013)

ABSTRACT

Parainfluenza virus (PIV) causes respiratory infections with high rates of morbidity and mortality in Children and immune-deficiency patients worldwide. The aim of this study is to detect the changes of mRNA expression of 84 human inflammatory genes and gene ontology analysis. From 90 patients only 2 gave positive IgM –PIV test. PIV infection caused over expression of 25 prominent genes (from 168 genes) affect functional pathways, ten of these genes regard as proinflammatory genes. Three genes were down-regulated; this suggests that the host-viral interaction is a cellular response against viral infection. In conclusion: Gene ontology analysis revealed that PIV stimulated 4 different key role immunological pathways represented by cytokine-cytokine receptor interaction, NOD-like receptor signaling, Toll-like receptor signaling, and asthma pathway. The patients are mostly infected with related subtypes of PIV. These results may help in further analysis of viral-host interaction.

Keywords: *RT-PCR-arrays; Parainfluenza virus- white blood cell interaction; inflammatory gene expression; cytokines; cytokines receptors*

INTRODUCTION

Parainfluenza viruses (PIV) are single stranded RNA viruses cause viral respiratory tract infections and stimulate cells to secrete cytokines that mediate signals between cells. These signals lead to alterations in the gene expression by the target cell and end up to influence functional pathways (Green, 2000). Up-regulated and down-regulated genes provide insights into functional responses of both host and pathogen (Penelope, *et al*., 2004). Detection of these pathogens and determination of their effects on immune-gene expression in white blood cells is very important for ; gene ontology analysis, initiating antiviral therapy, avoiding unnecessary antimicrobial therapy, preventing nosocomial spread, decreasing the duration of hospital stays, and reducing management costs (Adams, *et al*., 2010; Henrickson, 2005). Parainfluenza virus with adenovirus cause 5% of respiratory infections (Heikkinen and Jarvinen, 2003). Expression microarray analysis is a powerful method to determine global profiles of gene in cells and tissues under a variety of complex biological conditions (Clewley, 2004). Most of recent medical and scientific debates have concentrated on: which genes will be up-regulated or down-regulated that may lead to stimulate or affect inflammatory pathways (Andrejeva *et al*., 2004).

Human infection with PIV stimulates the host cell to initiate early antiviral response represented by increase the expression of interferon with high level at the initial period of infection. Interferon type 1 is released by infected cells to prevent the spreading of virus from one infected site to another (Andrejeva *et al*., 2002). PIV infection to human respiratory tract stimulates high expression of anti-inflammatory interleukins that play an important immune-regulatory role such as; inhibit the antigen presentation, interleukins secretion, immune blastogenesis and proliferation pathways (Sieg *et al.,* 1996). The expression of many human chemokines is increased after infection with PIV especially those chemokines that play important role in initial recruitment of immune cells and adhesion molecules to infected foci (Jacob *et al*., 2008; Liao *et al*., 1999). PIV induces expression of tumor necrosis factors pathways in a high rate after infection. This cytokine stimulates a major pro-inflammatory cytokine that inhibit the viral infection through activating the NF-kB translocation to the nucleus which stimulates the transcription of many inflammatory genes that activate proinflammatory and apoptosis activity in the infected cells (Mehta *et al*., 2003; Bienhof *et al*.,

2002; Lin, *et al*., 2007). The aim of the present study is to analyze proinflammatory gene-expression profiles in the peripheral WBCs after infection with PIV. In addition, analyze the gene ontology after PIV infection to determine genes that influence the main functional immune-inflammatory pathways.

MATERIALS AND METHODS

Patients, blood samples and ELISA test

Through the period from May 17 to July 8/ 2009, 90 patients were hospitalized at Ibn AL-Haytham Hospital in Amman/Jordan for respiratory tract infection symptoms. EDTA-blood from the 90 patients was included in this study in the acute phase of the disease (within 48 hrs of infection) with high-grade fever. Serum was separated for indirect ELISA-IgM kit (IBL-Hamburg Corporation) test to confirm the viral type infection. Another EDTA blood sample after I month (after recovery as control samples) was taken from those who shown a positive result to analyze the gene expression changes in leukocytes in response to PIV infection.

Extraction of mRNA from patient's leukocyte

The RNA was extracted within 24h of blood collection as follows: RNA-extraction phase, treat the leukocytes concentrate (isolated from the blood Buffy coat) with Trizol reagent (Phenol-guanidin-isothiocyanate); Separation phase, using chloroform; RNA precipitation phase, using Isopropanol Alcohol; washing phase, using 70% ethanol, and dissolving phase; using Rnase-Dnase free water (Invitrogen) (Rinyet *et al*., 2005).

RNA quantitation

Spectrophotometer (Bio-Rad) analysis was carried out to determine the concentration (μg/ml) of RNA by measuring the optical density (O.D) at λ_{260} and according to the following equation: RNA concentration = 40 (O.D factor) x 25 (dilution factor) x O.D at λ_{260}. RNA quality was tested using two methods, spectrophotometer (Bio-Rad) and gel-electrophoresis. In spectrophotometer the $O.D_{260/280}$ ratio was checked. RNA was considered pure if the O.D ratio was between 1.8 and 2.0.

RNA efficiency test

Before performing the RT PCR-Array, the RNA samples were tested for the efficiency of the reverse transcription to produce the cDNA on the RNA samples using 1.0μg of random primer, PCR components and master mixture (Promega USA) (Sebastian and Johanson, 2009).

Real time- PCR array

Real time-PCR array were performed to calculate the expression level of human cytokine and chemokine in presence or absence of PIA infection to monitor the mRNA expression levels of 84 different cytokines and chemokines (Table1) in patient`s leukocytes concentrate. Isolated cDNA were synthesized using SABioscince kit. Each cDNA sample was added to RT-q PCR master Mix containing SYBR Green and reference dye (SABioscince). 25μL of cDNA-Master Mix mixture was added to each well across the PCR-arrays (two RT-PCR-array plates for each patient; one during PIA infection and the second for control, after recovery). Each PCR-array profiles the expression of 84 pathway-specific gene plus 12 internal controls (SA Bioscience). Thermocycling conditions were 95°C for 10 min and 40 cycles of (95 °C for 15 sec, 60 °C for 1 min) using Bio-Rad-cycler real-time PCR detection systems.

Real-Time PCR detection

The threshold cycle values (Ct values) were calculated for each well using iQ cycler Bio-Rad software. The data were exported to a blank Excel Spread-sheet for use with SABioscience Data Analysis Template Excel File. Melting curve program (cycle 1, 1 repeat, 95C for 1 min. cycle 2, 1 repeat, 55C for 1 min. cycle 3, 80 repeat, 55C for 10 sec.) is run immediately after the cycling program, and generates a first derivative dissociation curve for each well in the entire plate. The fold-change is calculated for each gene in control and experimental groups as 2 ^ (-ΔΔCt). If the fold-change is greater than 1, then the result may be reported as a fold up-regulation. If the fold-change is less than 1, then the negative inverse of the result may be reported as a fold down-regulation.

Statistical analysis

Since each gene (of 84 immune genes) has 2 Ct-values (Ct-values of experimental sample and Ct-values of control sample) the difference between the two Ct-values for each gene is analyzed using the Non-parametric Wilcoxon signed ranks-test to calculate the Z-value and P-value for each gene (Joshua *et al*., 2006).

RESULTS

From 90 patients only 2 patients gave positive ELISA-IgM test for PIV (2.22 %). The RNA

concentrations of the 2 patients (acute samples and control samples) were 397.9 and 1195 µg/ml for acute samples, and between 130.5 and 499.3 µg/ml for control samples. The RNA quality (purity) of the 2 patients (acute samples and control samples) ranged from 1.7660 to 1.8332 for acute samples and from 1.7298 to 1.9273 for control samples. The volumes (µL) of RNA samples (acute and control) are calculated from the RNA concentrations that were calculated by the spectrophotometer. Each volume of each sample must contains µg/µL and then used in the RT-PCR-Array as illustrated in Table 1.

Table (1): The concentrations of acute and control RNA samples that are calculated by spectrophotometer (µg/ml) and then converted into RNA volume (contains 1 µg/ml) for each sample and then used in the RT-PCR Array.

	During infection (Acute samples)		**After recovery (Control samples)**	
Sample Number	RNA concentration µg/ml	RNA volume (µL) used in the RT-PCR Array	RNA concentration µg/ml	RNA volume (µL) used in the RT-PCR Array
1	1195.0665	0.8	130.5861	7.6
2	367.9903	2.7	499.3196	2.0

The result of gel electrophoresis test showed good quality RNA samples represented by two sharp and clear bands (28S and 18S) rRNA (Figure1).

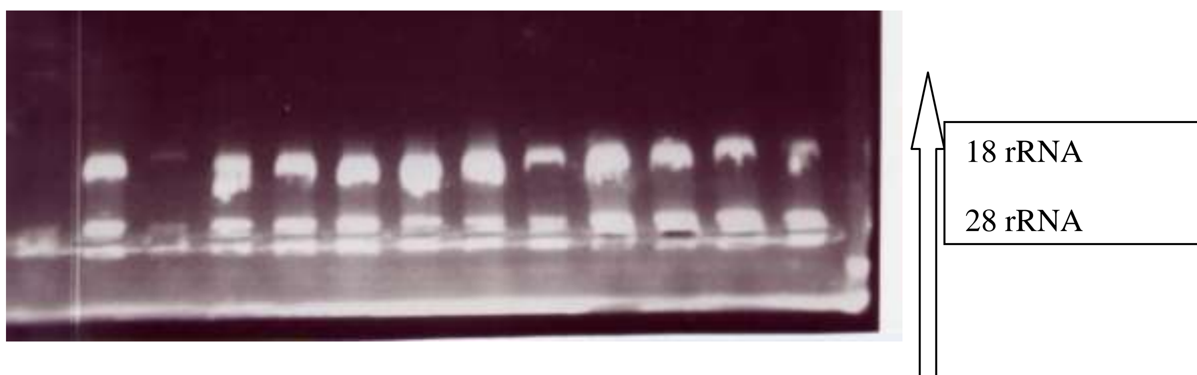


Figure (1): Gel electrophoresis for RNA (acute and control samples)
Showing the two rRNA bands (28S and 18S). These bands give indication that the quality of RNA samples is adequate and can be used to produce cDNA and perform RT-PCR-Array.

These bands give indication that the quality of RNA samples is adequate and can be used to produce cDNA and perform RT-PCR-Array. The RNA samples were tested for the efficiency of the reverse transcription to produce the cDNA. The results showed a clear 500 bp DNA band for each samples (Figure 2). The RNA samples are of high quality and can be used to produce the cDNA strand to be used for the RT-PCR Array technique.

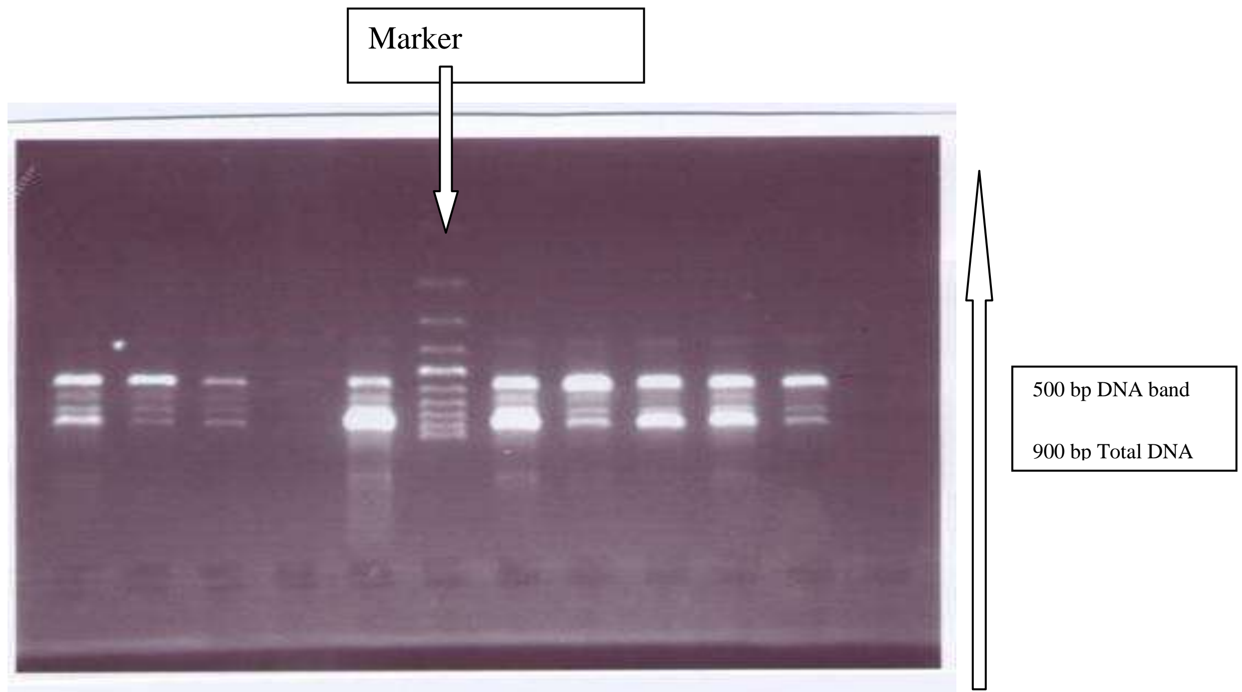


Figure (2): Gel electrophoresis test for the quality of RNA acute samples to produce the cDNA with 500 bp DNA band. The production of 500 bp DNA band indicates that the RNA samples (for acute and control) are efficient to produce cDNA from RNA samples with presence of reverse transcriptase enzyme. The 900 bp DNA bands represent the total DNA that used and these bands appear because DNase was not used.

Real time PCR –Array

Ct values of the fold change for each gene are calculated using SABioscince Excel-Analysis of RT-PCR-Array.

Leukocyte gene expression analysis after PIV infection:

Parainfluenza virus infected two samples and caused up regulation of 48 (57.14%) genes in patient 1 and down-regulation of 20 (23.8%) genes. The fold changes of up regulated genes ranged from 1.11 fold as in secreted phosphoprotein 1 and 101.13 fold in Interleukin 1, alpha (IL1A) (Figure 3). The fold change of the 20 down regulated genes ranged between -1.91 in Chemokine (C-X-C motif) ligand 14 and -1.11 in Chemokine (C-C motif) receptor 6 (Figure 4). In patient 2 the PIV infection caused up regulation to 75 (89.28%) genes and down regulated 7 (8.33%) genes (Figure 5). The up regulated 75 genes ranged between 1.13 fold as in Lymphotoxin beta (TNF superfamily, member 3) and 1428.22 fold as in Interferon, alpha 2 (IFNA2) which represents the most highly up regulated gene in this study. The down regulated 7 genes in patient 2 after 48 h of PIV infection are ranged between – 37.27 fold as in each of Interleukin 13 receptor, alpha 1 (IL13RA1). Macrophage migration inhibitory factor (glycosylation-inhibiting factor) (MIF) and -2.67 fold in Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating) (Figure 6).

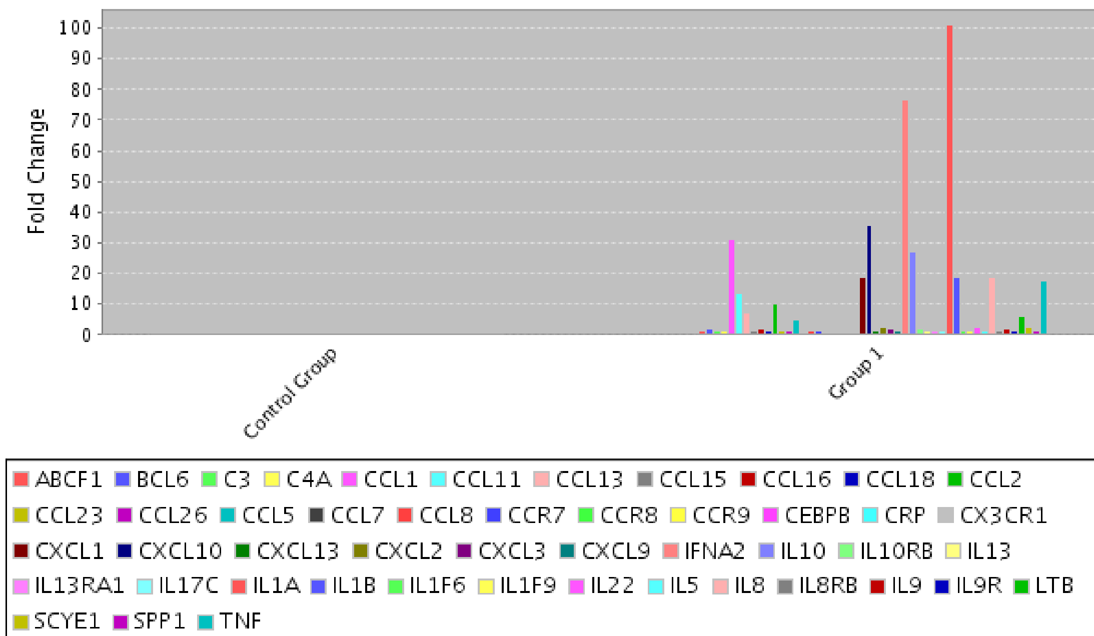


Figure (3): The up-regulated 44 genes in patient 1 after 48 h of PIV infection. The most up-regulated gene is IL1A gene (101.13 fold change) and the lower up-regulated gene is SPP1 gene (1.11 fold change).

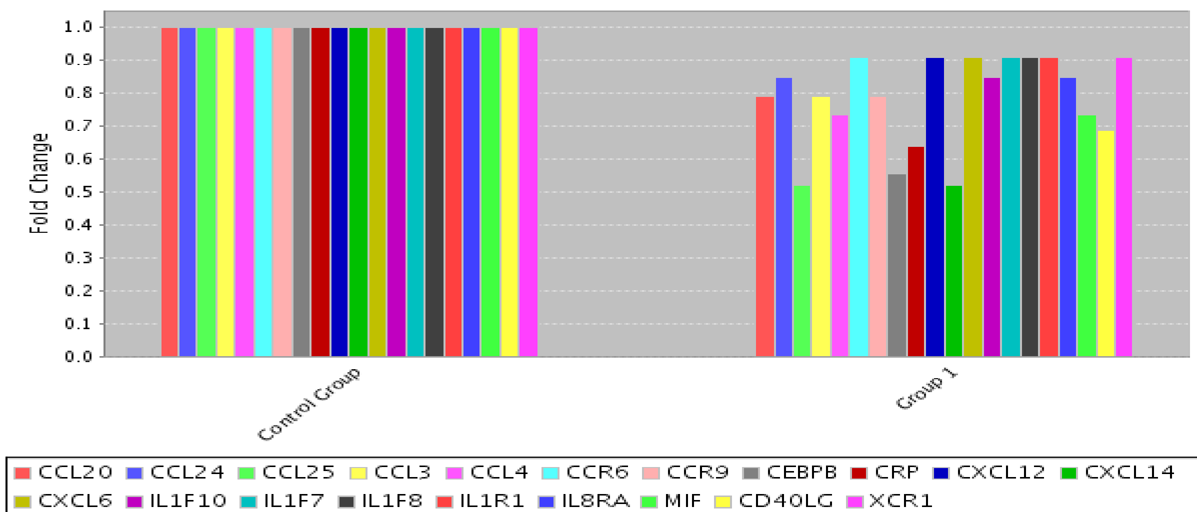
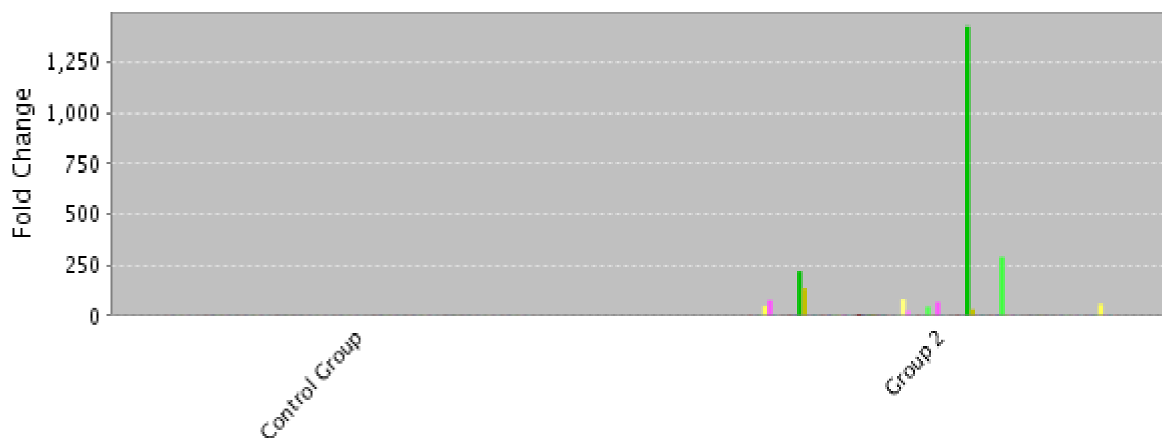


Figure (4): The down-regulated 20 genes by PIV infection in patient 1. The upper down-regulated gene is CXCL14 gene (-1.91 fold change) and the lower down-regulated gene is CCR6 gene (-1.11fold change).



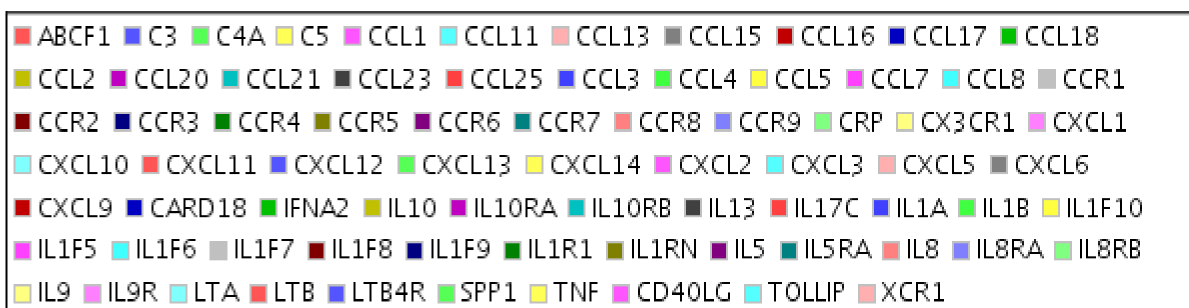


Figure (5): The up-regulated 75 genes in patient 2 after 48h of PIV infection. The most up-regulated gene is IFNA2 gene (1428.22 fold change) and the lower up-regulated gene is LTB gene (1.13 fold change).

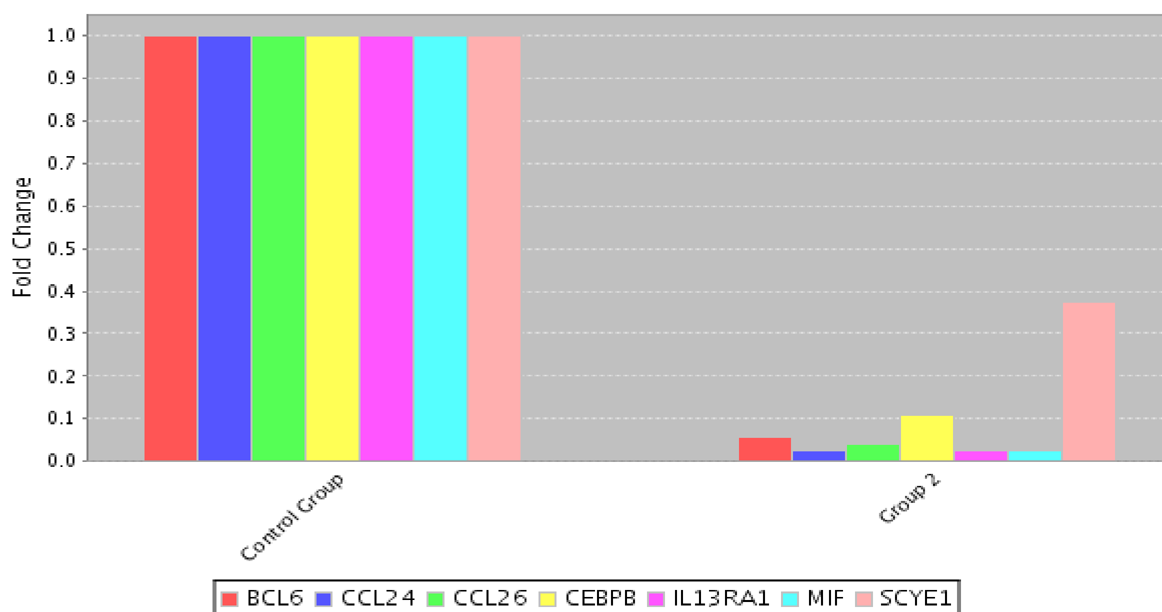


Figure (6): The down-regulated 7 genes in patient 2 after 48 h of PIV infection. The upper down-regulated gene is IL13RA1 and MIF genes (-37.27 fold change) and the lower down-regulated gene is SCYE1gene (- 2.67 fold change).

Common up regulated genes in PIV infected patients.

The most prominent and common up-regulated genes in the two patients infected with PIV is 25 genes with different fold changes ranged between 752.42 in IFNA2 and 2.22 in CCR7 (Table 2). The remaining 23 common up-regulated genes with fold change between 1.84 and 1.13 fold (Table 2).

Table (2): The most 25 prominent and common up-regulated genes in PIV infected samples after 48h of infection with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.211575	Interferon, alpha 2	752.42	-2.944, ($P < 0.003$)	Receptor binding, cytokine activity, interferon-alpha/beta receptor binding, protein, protein binding
2	Hs.126256	Interleukin 1, beta	154.57	-3.061 ($P \leq 0.002$)	Immune system process, gene expression, response to stimulus.
3	Hs.143961	Chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)	110.44	-2.511, ($P < 0.012$)	G-protein- coupled receptor binding, cytokine activity, chemokine activity
4	Hs.303649	Chemokine (C-C motif) ligand 2	72.76	-3.061, ($P < 0.002$)	G-protein- coupled receptor binding, signal transducer activity, chemokine receptor activity, cytokine binding.
5	Hs.72918	Chemokine (C-C motif) ligand 1	54.41	-3.061, ($P < 0.002$)	G-protein- coupled receptor binding, receptor binding, cytokine activity, chemokine chemokine receptor binding.
6	Hs.1722	Interleukin 1, alpha	51.30	-2.943, ($P < 0.003$)	Signal transducer activity Receptor binding, cytokine activity, Interleukin 1 receptor binding molecular transducer activity.
7	Hs.494997	Complement component 5	51.26 (1Frequency)	-2.197, ($P < 0.028$)	Immune system process, metabolic process, cellular process, developmental process, response to stimulus, and biological regulation
8	Hs.789	Chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	46.62	-2.983, ($P < 0.003$)	Immune system process, cellular process, multicellular organism process, response to stimulus, biological regulation
9	Hs.78913	Chemokine (C-X3-C motif) receptor 1	42.5	-2.119, ($P < 0.034$)	Cellular process, biological adhesion, and response to stimulus
10	Hs.241570	Tumor necrosis factor (TNF superfamily, member 2)	38.38	-3.061, ($P < 0.002$)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation
11	Hs.590921	Chemokine (C-X-C motif) ligand 2	35.11	-3.059, ($P < 0.002$)	Immune system process, viral reproduction, biological adhesion, response to stimulus.
12	Hs.193717	Interleukin 10	30.45	-2.433, ($P < 0.015$)	Immune system, cellular process, gene expression, developmental process, and response to stimulus.
13	Hs.654593	Interleukin 10 receptor, beta	30.45	-2.136, ($P < 0.033$)	Signal transducer activity, interleukin receptor activity, and growth factor binding, cytokine binding, and interleukin-10 binding, molecular transducer activity.
14	Hs.100431	Chemokine (C-X-C motif) ligand 13	24.88	-2.046, ($P < 0.041$)	G-protein- coupled receptor binding, receptor binding, cytokine activity, chemokine activity, chemokine receptor binding
15	Hs.632586	Chemokine (C-X-C motif) ligand 10	18.5	-3.061, ($P < 0.002$)	Pleiotropic effects; stimulation of monocytes, natural killer T-cell

					migration, modulation of adhesion molecule and expression within cytokine-cytokine interaction pathway
16	Hs.624	Interleukin 8	10.2	-3.061, (P< 0.002)	Immune system process, viral reproduction, response to stimulus.
17	Hs.511794	Chemokine (C-C motif) receptor 2	8.45 (1Frequency)	-2.669, (P< 0.008)	G-protein- coupled receptor binding, chemokine receptor binding, molecular transducer activity.
18	Hs.54460	Chemokine (C-C motif) ligand 11	7.62	-2.864, (P< 0.004)	G-protein- coupled receptor binding, receptor binding, cytokine activity, protein binding, chemokine activity, chemokine receptor binding.
19	Hs.414629	Chemokine (C-C motif) ligand 13	4	-2.510, (P< 0.012)	G-protein- coupled receptor binding, chemokine activity, cytokine activity, molecular transducer activity. Chemokine receptor binding, molecular transducer activity.
20	Hs.376208	Lymphotoxin beta (TNF superfamily, member 3)	3.9	-2.312, (P< 0.021)	Immune system process, cellular process, viral reproduction, biological adhesion, and response to stimulus, and biological regulation, tumor necrosis factor receptor binding.
21	Hs.514821	Chemokine (C-C motif) ligand 5	3.31	-3.059, (P< 0.002)	Immune system process, response to stimulus. Biological regulation
22	Hs.184926	Chemokine (C-C motif) receptor 4	2.57	-2.756, (P< 0.006)	G-protein- coupled receptor binding, chemokine receptor binding, cytokine activity, peptide binding, molecular transducer activity.
23	Hs.368527	Toll interacting protein	2.42 1Frequency	-2.845, (P< 0.004)	Signal transducer activity. Toll receptor binding, protein binding, molecular transducer activity.
24	Hs.287369	Interleukin 22	2.39 1Frequency	-2.981, (P< 0.003)	Receptor binding, cytokine activity, interleukin-22 receptor binding.
25	Hs.370036	Chemokine (C-C motif) receptor 7	2.22	-2.981, (P< 0.003)	G-protein- coupled receptor binding, chemokine receptor binding, cytokine activity, peptide binding, molecular transducer activity,

The common down-regulated genes in PIV infected patients

The common down regulated genes in the 2 patients infected by PIV are represented by only 3 genes with fold changes ranged between 19.30 for MIF and 5.56 for CEPBP gene (Table 3). The complete gene expression profile of 84 inflammatory genes in the 2 PIV infected patients gathered in a cluster (Figure 7).

Table (3): The common three down-regulated genes in PIV infected samples with statistical analysis, fold change and functions for each gene.

No.	Unigene	Gene name	Mean of fold Change	Wilcoxon t-value with P-value	Gene function
1	Hs.407995	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)	19.30	-2.120, (P< 0.034)	Immune system process, response to stimulus, biological regulation

2	Hs.247838	Chemokine (C-C motif) ligand 24	19.25	-1.609, (P< 0.108	Immune system process, cellular process, response to stimulus.
3	Hs.517106	CCAAT/enhancer binding protein (C/EBP). beta	5.56	-1.569, (P< 0.117)	Immune system response, cellular process, gene expression, response to stimulus.

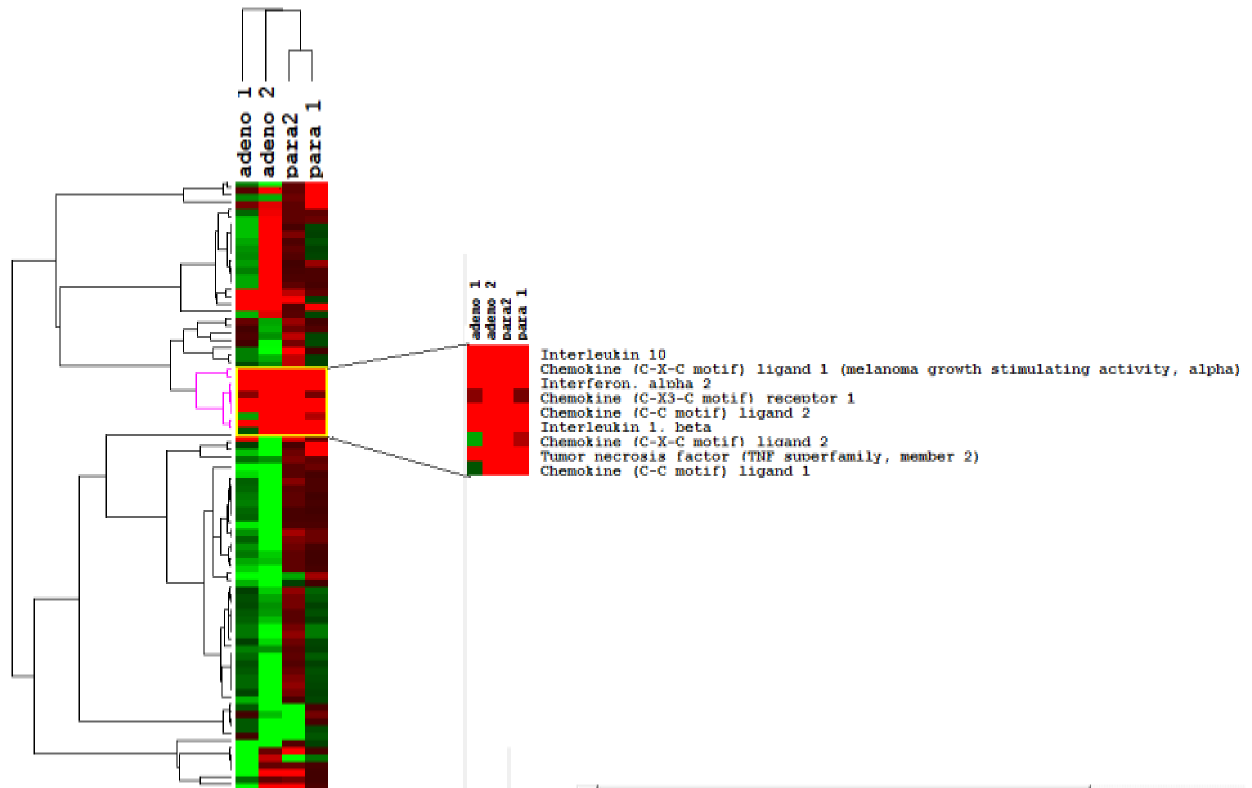


Figure (7): Cluster of 84 inflammatory gene expressions in 2 patients of the human leukocytes after PIV infection (represented by para 1 and para 2). Each panel represents one sample with up regulated genes (Red color) and down regulated genes (Green color).

DISCUSSION

The response of human leukocytes to PIV infections includes the induction of different cytokines, chemokines and other inflammatory genes (Dennis *et al*., 2003). The up-regulation of most viral inducible genes in this study were10 proinflammatory genes (IFNA2, IL1B, IL8, CCL2, IL1 A, CXCL1, CX3CR1, TNF, IL10, and CCL 5) (Table 2) which stimulated at initial infection. These results are in agreement with previous study showed that the infection of respiratory cells lead to increase the production of proinflammatory genes and apoptosis genes (Geiler *et al*., 2010). The early cellular response in this study (24- 48 h), suggests that the expression of these genes may initiate the cascades of an antiviral responses that result in upregulation of different inflammatory genes. Some of these upregulated genes cause asthma complication with PIV infection and play a key role in the cytokine-cytokine receptor interaction pathway (Huang *et al*., 2009; Dennis *et al*., 2003).

The infection with PIV viruses caused up-regulation of inflammatory genes with fold change ranged between 2.22 fold for CCR7 and 752.42 fold for IFNA2 (Table 2). Interferon alpha 2 acts as; a natural cell-signaling protein produced by the cells of the immune system in response to the presence of double-stranded RNA, a key indicator of viral infection (Kotenko *et al*., 2003). The infection of PIV stimulates the upregulation of IFNA2 gene which degrades the mRNA in host cell and blocks the spreading of PIV infection in the body (Andrejeva *et al*., 2002). Interleukin 1-B cytokine is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis (Stuart and Nancy, 2001). The high significant mRNA expression (154.57 fold) (Table 2) of IL1B gene in this study suggests its immune role against the PIV by activation of

many immune response activities which may lead to asthma symptoms. PIV infection stimulates the IL1B cytokine production by activated macrophages as a proinflammatory protein. This cytokine is released during inflammation of the central nervous system (Asadullah *et al.,* 2003).

Interleukin 8 cytokine is up-regulated in 2 infected patients (10.2 fold) (Table 2). The activation of IL8 was found to cause bronchiolitis and ends with asthma (Huang *et al*., 2009). PIV infection up-regulates the IL8 gene which in turn activates immune system processes to overcome the PIV infection and participates in the NFkB activation pathway that activates many transcriptional factors in nucleus and thereby stimulates various inflammatory gene expressions (Weiss *et al*., 2004). Another study also showed expression of IL8 after PIV infection and may activate immune system processes to overcome the viral infection (Young *et al.,* 2006). CCL 2 is up-regulated with high significance (72.76 fold) (Table 2). The CCL2 has direct and indirect effect on immune responses toward viral infections through its functions as a chemotactic factor for monocytes and basophiles. CCL2 transcription is also upregulated in human monocyte culture after IAV infection (Bubfeld *et al*., 1998). Another studies showed that the expression of CCL2 chemokine was increased after infection with PIV and other respiratory viruses (Jacob *et al*., 2008). It stimulates the migration primarily of monocytes and T cells to infected sites (Zlotnik *et al*., 2006). In this study the interleukin-1A is up-regulated with high significance (51.30 fold) (Table 2). Interleukin-1A is a member of the IL-1 family which seems to participate in the pulmonary immune response against pathogens (Rosseau *et al*., 2007). The cytokine IL1 A is up-regulated and increased in A549 cells after PIV infection. High level of IL1A increases the activity of class I MHC which enhances the immune response against PIV (Garofalo *et al*., 1996).

CXCL1 chemokine up-regulated in this study with high significance (46.62 fold) (Table 2). The function of CXCL1 chemokine gene (the melanoma growth stimulatory activity/growth-regulated protein) is to regulate the cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane G protein-coupled receptors. It also plays fundamental roles in the development, homeostasis, and function of the immune system (Huang *et al*., 2009). Previous studies reported that CXCL1 acts through G-protein to activate IL-6 gene expression since the viral infection induce up-regulation of IL-6 and IL-8 gene expression which are positively correlated to the onset of acute inflammatory pain and lead to asthma complication (Wang *et al*., 2009; Papdopoulos *et al*., 2001). Another study showed that the infection with PIV and other respiratory viruses increase the expression of these CXCL1 after 24 h of infection with PIV (Jacob *et al*., 2008). This increase may enhance its ability to specifically recruit polymorphonuclear leucocytes (PMNLs) into inflamed tissues (Constantin *et al*., 2000).

CX3CR1 is up-regulated with high significance (42.5 fold) (Table 2). CX3CR1 (chemokine fractalkine) gene has two important functions, one acts as a mediator for both adhesive and migratory leukocytes to activate endothelial cells, where it is primarily expressed and second acts as a co-receptor for viral envelope protein (Huang *et al*., 2009).PIA infection up-regulates CX_3CR1 significantly, this chemokine acts as a receptor for CX3CL1 (fractalkine). The binding of this receptor with its ligant induce leukocyte chemotaxis and displaying a range of CX_3C biological activities (Tripp *et al*., 2001). TNF is upregulated in this study with high significance (38.38 fold) (Table 2). This gene encodes a multifunctional proinflammatory cytokines through binding to its receptors TNFRA and TNFRB leading to the translocation of transcriptional factor NF-kB to the nucleus which turns on the transcription of more than 60 known genes that participate in activation of proinflammatory activities and apoptosis of infected cells (Mehta *et al.,* 2003). It was recorded previously that TNF mRNA was significantly increased by infection with PIV and plays important roles in clearing virus infection through inflammatory responses (Lin, *et al*., 2007). Another study showed that PIV induces expression of TNF in high rate after PIV infection (Bienhof *et al*., 2002). TNF also creates signal transduction pathways after viral infection and lead to activation of transcription factors, such as NF-kB that induce secretion of inflammatory cytokines like IL-6, TNFα, MIP1α, MIP1β and IL-1β (Sallusto *et al*., 2000).

Interleukin-10 cytokine up-regulated with 30.45 fold (Table 2) . The up-regulation of this potent anti-inflammatory cytokine may be induced by the invading virus to make use of its anti-inflammatory function and to provide a safe

environment for viral replication. IL10 represses the expression of TNF-α, IL6 and IL1 by activating macrophages. Another study showed a significant increase in IL10 mRNA expression in virus infection accompanied with acute asthma, but not observed in virus infection without asthma (Grissell *et al*., 2005). Previous studies showed that PIV infection also stimulates high expression of Interleukine-10 and activates signal transducer activity to stimulate growth factor binding and cytokine binding (Sieg *et al.,* 1996; De Waal *et al.,* 1991). Another published work revealed a wide involvement of interleukin 10 to prevent viral infections and produce regulatory cells that are involved in protection against allergic diseases (Mege *et al*., 2006). CCL 5 is up-regulated in patients infected with PIV with high significant (3.31 fold) (Table 2). Another study also showed that the expression of CCL5 chemokine was increased after infection with PIV (Jacob *et al*., 2008).

Gene ontology and up-regulated genes after PIA infection

The function of gene ontology (GO) is to classify functional categories based on co-occurrence with sets of genes in a gene list and rapidly unraveling new biological processes associated with cellular functions and pathways. GO also provides investigators with much more power to analyze their genes using many different biological aspects in a single space (Huang *et al.,* 2009). The infection with PIV caused considerable changes in 25 gene expression of human WBCs. Four groups of these 25 genes stimulate four inflammatory pathways including; 22 (91.7%) genes, activate the cytokine-cytokine receptor interaction pathway, 8 (42.1%) genes involved in the NOD-like receptor signaling pathway (Figure 9), 6 (25%) genes involved in Toll-like receptor signaling pathway , and 3(21.5%) genes involved in asthma pathway (Umetsu *et al*., 2002) (table 4). These pathways can be determined using gene ontology (GO) with KEGG tool (Grosu *et al*., 2002; Huang *et al.,* 2009) (Table 4).

Table (4): Gene ontology for up-regulated genes after PIV infections. The virus up-regulated specific number of genes which stimulate specific inflammatory pathways.

Stimulated Gene pathway	Upregulated genes by PIV infection	
	Gene No. and (%)	Gene name
Cytokine-cytokine receptor interaction pathway	22/25 (91.7)	CCL1,CCL11, CCL13, CCL18,CCL2, CCL5, CCR2, CCR4, CCR7, CXCL1, CXCL10, CXCL13, CX3CR1, IFNA2, IL1A, IL1B, IL8, IL10, IL10RB, IL22, LTB, TNF
NOD-like receptor signaling pathway	8/25 (42.1)	CCL2, CCL5, CCL11,CCLl3, CXCL1, IL1b, IL8, TNF
Toll-like receptor signaling pathway.	6/25 (25)	CCL5,CXCL10, IFNA2, IL1B, IL8, TNF
Asthma pathway	3/25 (21.5)	CCL11, IL10, TNF

CONCLUSIONS

The generation of RT-PCR-Array allows the comparison of viruses with respect to their impact on cellular pro-inflammatory gene expression. Gene ontology showed that PIV viruses stimulate four different inflammatory pathways with specific genes for each virus. Human WBC gene expression profiles, induced by PIV demonstrate different alterations of inflammatory genes which are involved in the complex interaction between these viruses and human WBCs. The infection with PIV stimulates asthma pathway which leads to asthma complication. A complete analysis of changes in cellular functions including the production of gene products and protein- protein interactions within the stimulated cells will be required to explore the underlying mechanism of the effects ofPIV infection during the course of infection.

REFFERENCES

Adams S., Xing Z., Li J., Cardona C. (2010). Immune-related gene expression in response to H11N9 low pathogenic avian influenza virus infection in chicken and Pekin duck peripheral blood

mononuclear cells, **Molecular Immunology**, 46,8-9, 1744-1749.

Andrejeva, J., Childs, K., Young, D., Carlos, T., Stock, N., Goodbourn, S. and Randall, R. (2004). The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-β promoter, **Proceedings of National Academy of Sciences of the United State of America,** 101 (49). 17264-17269**.**

Andrejeva, J., Young, D. , Goodbourn, S., and Randall, R. (2002). Degradation of STAT1 and STAT2 by the V proteins of simian virus 5 and human parainfluenza virus type 2, respectively: consequences for virus replication in the presence of alpha/beta and gamma interferons, **Journal of Virology**, 76**,** 2159-2167.

Asadullah, K., Sterry, W., and Volkh, D. (2003). Interleukin-10 therapy-review of a new approach, **Pharmacological Review**, 55, 241–269.

Bienhoff, S., Allen, G., and Berg, J., (1992). Release of tumor necrosis factor- alpha from bovine alveolar macrophages stimulated with bovine respiratory viruses and bacterial endotoxins, **Veterinary Immunology and Immunopathology**, 30(4). 341-357.

Bubfeld, D., Kaufmann, A., Meyer, R., Gemsa, D., Sprenger, H., (1998). Differential mononuclear leukocyte attracting chemokine production after stimulation with active and inactivated influenza A virus, **Cell Immunology**, 186, 1–7.

Campeau, P., Rafei, M., Boivin, M., Sun, Y., Grabowski, G., Galipeau, J. (2009). Characterization of Gaucher disease bone marrow mesenchymal stromal cells reveals an altered inflammatory secretome, **Blood**, 114(15). 3181-3190.

Clewley J. (2004). A role for arrays in clinical virology: fact or fiction, **Journal of Clinical Virology,** 29, 2-12.

Constantin, G., Majeed, M., Giagulli, C., Piccio, L., Kim, J., and Butcher, E. (2000). Chemokines trigger immediate β2 integrin affinity and mobility changes: differential regulation and roles in lymphocyte arrest under flow, **Immunity**, 13, 759–769.

Dennis, G Jr., Sherman, BT., Hosack, DA., Yang, J., Gao, W., Lane, HC., Lempicki, RA. (2003). DAVID: Database for Annotation, Visualization, and Integrated Discovery. **Genome Biology,** 4 (5). 3

De Waal M., Haanen, J., Spits, H., Roncarolo, M., Te Velde, A., Figdor, C., Johnson, K., Kastelein, R., Yssel, H., and De Vries, J. (1991). Interleukin 10 (IL-10) and viral IL-10 strongly reduce Ag-specific human T cell proliferation by diminishing the antigen presenting capacity of monocytes via down-regulation of class II major histocompatibility complex expression, **Journal of Experimental Medicine**, 174, 915.

Garofalo, R., Mei, F., Espejo, R., Haeberle, G., Ye, H., Baron, S., Ogra, P., and Reyes V. (1996). Respiratory syncytial virus infection of human respiratory epithelial cells up-regulates class I MHC expression through the induction of IFN- beta and IL-1 alpha, **The Journal of Immunology**, 157 (6). 2506-2513.

Geiler J., Michaelis M., Naczk P., Leutz A., Langer K., Doerr H., and Jr J. (2010) N-acetyl-L-cysteine (NAC) inhibits virus replication and expression of pro-inflammatory molecules in A549 cells infected with highly pathogenic H5N1 influenza A virus, **Biochemical Pharmacology**, 79, 413-420.

Green, DR. (2000). Apoptotic Pathways: paper wraps stone blunts scissors, **Cell,** 102:1-4.

Grissell T., Heather P., Darren R., Michael J. Boyle, M., Peter D., Bruce F. and Peter G. (2005). Interleukin-10 gene expression in acute virus-induced asthma, ***American Journal of Respiratory and Critical Care Medicine,*** 172, 433-439.

Grosu P, Townsend J, Hartl D, and Cavalieri D: Pathway processor: a tool for integrating whole-genome expression results into metabolic networks**. Genome Research**, 2002, **12:**1121-1126.

Haller O, Kochs G., and Weber F., (2006). The interferon response circuit: Induction and suppression by pathogenic viruses, **Virology, 344,** 119–130.

Henrickson, K**.** (2005). Cost-effective use of rapid diagnostic techniques in the treatment and prevention of viral respiratory infections, **Pediatrics Annual**, 34**,** 24-31.

Huang H., Li H., Zhou P., and Ju D. (2010). Protective effects of recombinant human granulocyte macrophage colony stimulating factor on H1N1 influenza virus-induced pneumonia in mice, **Cytokine**, 51, 2, 151-157.

Huang D, Sherman B, Lempicki R. (2009). Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources, **Nature Protocol,** 4(1). 44-57.

Jacob E., Shannon C. Miller, J., Boa L, Craig G., Tres C., Alan D. and David L. (2008). The chemokine receptor CCR5 plays a key role in the early memory CD8+T-cell response to respiratory virus infections, **Cell**, 29(1). 101-113.

Joshua S., Ann R., Feng C., and Neal S. (2006). Statistical analysis of real-time PCR data ***BMC Bioinformatics***,**7:**85doi:10.1186/1471-2105-7-85.

Kotenko S., Gallagher G., Baurin V., Lewis-Antes A., Shen M, Shah N., Langer J., Sheikh F., Dickensheets H., and Donnelly R., (2003). IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex, **Nature Immunology,** 4, 69-75.

Liao F, Rabin R, Smith C, Sharma G, Nutman T., Farber J. (1999). CC-chemokine receptor 6 is expressed on diverse memory subsets of T cells and determines

responsiveness to macrophage inflammatory protein 3 alpha, ***Journal of Immunology,***; **162**, 186–94.

Lin Y., Minghao S. , Sandra M., Celia D., Terri R., and Biao H. (2007). Inhibition of interleukin-6 expression by the V protein of parainfluenza virus 5. **Virology**, 368, (2): 262-272.

Mege J., Soraya M., Amelie H., Christian C., and Didier R. (2006). The two faces of interleukin 10 in human infectious diseases, **The Lancet Infectious Diseases**, 6 (9). 557-569.

Mehta, D., Wurster A., Whitters M., Young D., Collins M., and Grusby M.

(2003). IL-21 induces the apoptosis of resting and activated primary B cells**. Journal of Immunology**, 170, 4111.

Myskiw C, Arsenio J, van Bruggen R, Deschambault Y, Cao J. (2009). Vaccinia virus E3 suppresses expression of diverse cytokines through inhibition. Journal of Virology**, 83(13):6757-68.**

Papandopoulos N, Papi A, Meyer J, Stanciu L, Salvi S,Holgate S, Johanston S, (2001). Rhinovirus infection up-regulates eotaxin and eotaxin-2 expression in bronchial epithelial cells, **Clincal and Experimental Allergy**, 31(7). 1060-6.

Penelope A., Bryant D., Roy R., and Nigel C. (2004). Chips with everything: DNA microarrays infectious diseases, **Lancet Infectious Diseases**, 4, 100–111.

Rinyet J., Jeroen P., Hennie H., Annemarie B. Marijke O., Lia d, Kemal O., Jan D, Tjeerd K., and Hoebee, B. (2005). Host transcription profiles upon primary respiratory syncytial virus infection, **Journal of Virology**, 81, 5958-5967.

Rosseau Simone, Andreas Hocke, Hans Mollenkopf, Bernd Schmeck, Norbert Suttorp, Stefan H E Kaufmann, and Jens Zerrahn (2007). Comparative transcriptional profiling of the lung reveals shared and distinct features of *Streptococcus pneumoniae* and influenza A virus infection, **Immunology**, 120 (3), 380–391.

Sallusto F, Lanzavecchia A. (2000). Understanding dendritic cell and T-lymphocyte traffic through the analysis of chemokine receptor expression, Immunology Review, **177, 134–140.**

Schaller M., Cory M., Nicholas L and Steven L. (2006). Respiratory viral infections drive chemokine expression and exacerbate the asthmatic response, **Journal of Allergy and Clinical Immunology**, 118, 295-302.

Sebastian T., and Johanson P. (2009). RasV12-mediated down-regulation of CCAAT/enhancer binding protein beta in immortalized fibroblasts requires loss of p19Arf and facilitates bypass of oncogene-induced senescence, **Cancer Research**, 15; 69(6): 2588-2598.

Sieg S., King C., Huang Y., and Kaplan D., (1996). The role of interleukin-10 in the inhibition of T-cell proliferation and apoptosis mediated by parainfluenza virus type 3, **Journal of Virology**, 70, 4845.

Simpson J., Scott R., Boyle M., and Gibson G. (2006). Inflammatory subtypes in asthma: assessment and identification using induced sputum, **Respirology**, 11, 54−61.

Stuart A., and Nancy R. (2001). Cytokines and Acute Neurodegeneration. **Nature reviews**, Neuroscience, 2, 734.

Tripp R., Les P., Lia M., HaoQiang Z., Philip M. and Larry J. (2004). CX_3C chemokine mimicry by respiratory syncytialvirus G glycoprotein. **Natural Immunology, 2**: 732–738.

Umetsu D., McIntire J., Akbari O., Macaubas C., and DeKruyff R. (2002). Asthma: An epidemic of dysregulated immunity, **Nature Immunology**, 3, 715−720.

Wang X, Hamza M, Wu T, Dionne R, (2009). Upregulation of IL-6, IL-8 and CCL2 gene expression after acute inflammation: Correlation to clinical pain, **Pain**, 142 (3), 275-83.

Weiss Elliot, Adam Joseph Mamelak, Stephania La Morgia, Binghe Wang, Claudio Feliciani, Antonio Tulli, and Daniel Nathan Sauder, (2004). The role of interleukin 10 in the pathogenesis andpotential treatment of skin diseases. **Journal of American Academy Dermatology**, 50(5), 657-675.

Young D., Didcock L., Goodbourn S., and Randall R., (2000). Paramyxoviridae Use Distinct Virus-Specific Mechanisms to Circumvent the Interferon Response, **Virology**, 269 (2), 383-390.

Zlotnik A., Yoshie O., and Nomiyama H., (2006). The chemokine and chemokine receptor superfamilies and their molecular evolution, **Genome Biology, 7**, 243.

تأثير الاصابة بفايروس نظير الانفلونزا على التعبير الجيني للجينات المناعية الالتهابية للانسان وتحليل الجينات معلوماتيا

الخلاصة

يسبب فايروس نظير الانفلونزا اصابات تنفسية بمعدلات عالية من الامراضية والموت في الاطفال وذوي العوز المناعي في مختلف انحاء العالم. الهدف الرئيسي من هذه الدراسة هو تحديد التغيرات الحاصلة في تعبير الحامض النووي الرايبوزي الرسول والناتج من **84** جين مناعي التهابي اضافة الى تحليل هذه الجينات معلوماتيا. من مجموع **90** مريض اظهر مريضان فقط فحصا موجبا للاجسام المضادة نوع IgM تجاه هذا الفيروس. ادت الاصابة الى زيادة في التعبير الجيني ل 25 جين من مجموع 168 جين والتي اثرت على مسارات ايضية مهمة حيث ان عشرة من هذه الجينات تعتبر جينات محفزة للمناعة الاولية. اظهرت الدراسة انخفاض في التعبير الجيني لثلاثة جينات فقط ، هذه النتائج تدل على ان العلاقة بين الفيروس والخلية المصابة هي علاقة استجابة مناعية تجاه الفيروس. من التحليل الجيني المعلوماتي نستنتج ان الاصابة بهذا الفيروس تسبب تحفيزا لاربعة مسارات مناعية التهابية مهمة جدا هي: مسار تفاعل السايتوكاين مع مستقبل السايتوكاين ومسار مستقبل الاشارة للمركب شبيه ال NOD ومسار مستقبل الاشارة للمركب شبيه ال Toll ومسار الازمة (الربو). كما اظهرت الدراسة ان الفايروسات التي سببت الاصابات تعود لانواع مصلية متقاربة لفايروس نظير الانفلونزا. هذه النتائج قد تساهم في زيادة فهم العلاقة بين الفيروس والمضيف.