Changes of gene expression profiles of infants inflammatory genes after Rota virus infection.

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Abstract. Rotavirus is a medical important pediatric pathogen causes severe gastroenteritis in infants, leading to 450,000 deaths globally each year. RT-PCR-Array technique analysis is a powerful method to determine global profiles of gene expression in cells and tissues after viral infections. Using this analysis, the Ct values of 14 immune inflammatory genes were subjected to determine their gene expression rates compared with Ct values of control (non-infected persons). Five housekeeping genes were used as internal control genes. All mRNA transcripts of these genes were upregulated (from 2.2974 fold change for CCL3, CCR1, CCR3, CXCL2, IFN-a, IFNA-g, IL1B, IL1R and 21.1121 fold change for TNF-b) after infection with rotavirus. This results revealed that rotavirus infection increase the gene expression rate of 14 inflammatory genes and lead to high impact on key immune pathways in infected infants.

Key words: Rotavirus, RT-PCR-Array, Gene expression.

Introduction

Rotavirus is a major cause of severe gastroenteritis among young children. In developing countries, it is a major cause of under-5 year old mortality, accounting for up to 20% of all childhood deaths in countries with high diarrheal disease burden (Langa et al., 2016). In developing countries, first rotavirus infections usually occur between 6-9 months of age, and 80% occur among infants <1 year old. Developing countries often have year-round transmission, intense rotavirus exposure, and a diversity of circulating rotavirus strains. Each year, rotavirus causes >500,000 deaths worldwide among infants and very young children, with 90% of these deaths occurring in Africa and Asia alone (Tayeba et al., 2011). Worldwide, around 40% of all pediatric hospitalizations for diarrhea are attributable to rotavirus infections (Salim et al., 2014). 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: how some Rotavirus infections develop into immune sensitivity, if this correlation occurs what are the molecular changes that take place at the
gene level, which genes will be up-regulated or down-regulated that may lead to immune sensitivity, which virus will be the most effective to stimulate such genes, what is the interpretation for these complications and if there is a possibility to treat these cases at the molecular gene level. (Simpson et al., 2006). In this study RT-PCR-Array technique was chosen for a number of reasons. First, little is known about the immune cells genomic response to viral infections Second, only microarrays and RT-PCR-Arrays could definitely determine whether the immune cell-viral infections will be accompanied by a particular pattern of change in gene expression. Third, it would be necessary to have data on immune cell's gene regulation (Myskiw et al., 2009). The aims of this study are; to detect the effect of rotavirus infection on the mRNA expression of inflammatory cytokines genes (Ajuebor and Swain, 2002; Geiser et al., 1993). This lead to determine the relationship between human inflammatory gene expression and Rotavirus.

Materials and methods

From bioinformatics data concerned with studying the effect of rotavirus on human immune gene expression (1- 6) fourteen immune inflammatory genes with different fold change (FC) were subjected to this study and regard as acute sample. The Ct values (threshold value) of acute samples calculated from bioinformatics data as standard tables (Al-Ghazal, 2010) (Table 1).

| No. | Gene symbol | Gene name                      | Fold change | Standard Ct values |
|-----|-------------|--------------------------------|-------------|--------------------|
| 1   | CXCL10      | Chemokine ligand-10            | 2.6         | 28.7               |
| 2   | CCL5        | Chemokine ligand-5             | 2.6         | 30.9               |
| 3   | CCL2        | Chemokine ligand-2             | 2.6         | 25.3               |
| 4   | IL1B        | Interleukine-1 beta            | 2           | 25.7               |
| 5   | IL5         | Interleukine-5                 | 2.5         | 32.7               |
| 6   | TNF-a       | Tumor necrosis factor- alpha   | 2.5         | 32.7               |
| 7   | TNF-b       | Tumor necrosis factor- beta    | 2           | 33.7               |
| 8   | IFNA-g      | Interferon- gamma              | 2           | 24.4               |
| 9   | IL1R        | Interleukine-1 receptor        | 2           | 25.7               |
| 10  | CCL3        | Chemokine ligand-3             | 2           | 25.0               |
| 11  | IFN-a       | Interferon-alpha               | 2           | 25.0               |
| 12  | CXCL2       | Chemokine ligand-2             | 2           | 24.4               |
| 13  | CCR1        | Chemokine receptor -1          | 2           | 24.4               |
| 14  | CCR3        | Chemokine receptor -3          | 2           | 25.7               |

Ct values of control genes represent the complete gene expression analysis of healthy persons. 5ml of blood samples were taken and the white blood cells (buffy coat) were extracted to get purified total RNA using blood RNA kits (Invitrogen, USA) according to the manufacturer's protocols (Diepen et al 2010; Riny, 2005). Spectrophotometer analysis is carried out to determine the amount of RNA by measuring the optical density (O.D) at 260. The O.D 260/280 ratio (Ratio of RNA to protein) is checked and RNA is considered pure if the O.D ratio is between 1.7 – 2.0. Lower ratio (O.D< 1.7) indicates presence of protein contamination. Gel electrophoresis was performed to test the purity of RNA. Good quality RNA appears as a sharp clear two bands (28S and 18S) of ribosomal RNA.
Real time PCR-Array

The real time PCR-array takes advantage of real-time PCR performance and combines it with the ability of microarrays to detect the expression of many biological genes lines in human cells. The same amount of total RNA in this reaction is used for every sample starting with 0.5 or 1.0 g of total RNA for 96-well plate formats. The contents are mixed gently with a pipette followed by brief centrifugation. The mixture is incubated at 42°C for 5 min and chilled on ice immediately for at least one minute. The cDNA is prepared from RNA samples then cDNA is added to RT-qPCR Master Mix (Master mixes contain SYBER Green and reference dye). Then the master mix is used to prepare the experimental mixture (cDNA and RT-qPCR Master Mix as 25 l to each well of the PCR-array) which is aliquot across the PCR-arrays (SAbioscience). The plate is placed in real-time thermal cycler and programed (95°C, 1 min.,1 cycle; 95°C, 15 sec., 40 cycle; 60°C, 1min.). The is a two-step cycling used for Bio-Rad-iCycler.

Using the instrument’s software the threshold cycle (Ct) is calculated for each well. The baseline and threshold values were determined. The resulting threshold cycle (Ct) values of controls and acute samples for all wells were analyzed using a Blank Excel Spread-sheet SABioscience Data Analysis Template Excel File.

Results

Gene expression analysis (SABioscience) revealed upregulation of 14 immune inflammatory genes ranged between 2.2974 fold change (FC) for CCL3, CCR1, CCR3, CXCL2, IFN-a, IFN-g, IL1B, IL1R and 21.1121 FC for TNF-b (Table 2, Figure1, 2: A and B). Eight inflammatory genes (CCL3, CCR1, CCR3, CXCL2, IFN-a, IFN-G, IL1B, IL1R) were upregulated with low rate (2 FC). Three inflammatory genes (CCL2, IL5, TNF-a) were upregulated with moderate rate (4-7 FC). Three inflammatory genes (CCL5, CXCL10, TNF-b) were upregulated with high rate (18–21 FC).

Table 2: Gene position, Gene name, Ct values (Acute and control) and the new fold change of gene expression

| No. | Gene position in Array | Gene name | Ct - values | New fold change |
|-----|------------------------|-----------|-------------|-----------------|
|     |                        |           | Controls    | Samples         |                |
| 1   | A10                    | CCL2      | 25.3        | 22.9            | 4.9246         |
| 2   | B04                    | CCL3      | 25.0        | 23.7            | 2.2974         |
| 3   | B06                    | CCL5      | 30.9        | 26.6            | 18.3792        |
| 4   | B09                    | CCR1      | 24.4        | 23.1            | 2.2974         |
| 5   | B11                    | CCR3      | 25.7        | 24.4            | 2.2974         |
| 6   | C11                    | CXCL10    | 28.7        | 24.4            | 18.3792        |
| 7   | D03                    | CXCL2     | 24.4        | 23.1            | 2.2974         |
| 8   | D11                    | IFN-a     | 25.0        | 23.7            | 2.2974         |
| 9   | D12                    | IFN-g     | 24.4        | 23.1            | 2.2974         |
| 10  | E10                    | IL1B      | 25.7        | 24.4            | 2.2974         |
| 11  | E11                    | IL1R      | 25.7        | 24.4            | 2.2974         |
| 12  | F05                    | IL5       | 32.7        | 29.7            | 7.4643         |
| 13  | G05                    | TNF-a     | 32.7        | 29.7            | 7.4643         |
| 14  | G06                    | TNF-b     | 33.7        | 29.2            | 21.1121        |
Figure 1: Scatter plot revealed upregulation (red color) of 14 human immune inflammatory genes after rotavirus infection (some dot color represent more than gene in same loci).

Figure 2: A- Upregulation of gene expression in 9 inflammatory genes after Rotavirus infection compared with control.

Figure 2: B- Upregulation of gene expression in 5 inflammatory genes after Rotavirus infection compared with control.
Tumor necrosis factors alpha and beta (TNF a and b), genes are upregulated with 7.46 and 21.1121 FC respectively. This upregulation after rotavirus infection reflects the key importance of both types of TNF which produced from macrophages in response to infection and activates NF-kB which leads to transcription of more than 60 known genes that participate in activation of proinflammatory mediators and apoptosis of infected cells (Mehta et al., 2003; Alberts et al., 2002). Another study also revealed that rotavirus can induce the expression of TNF-a which acts as anti-viral properties (Hakima et al., 2018; Dinarello, 1996).

CCL5 was upregulated with 18.37 FC for each gene. CCL5 stimulates two key immune pathways, the NOD-like receptor signaling pathway and Toll-like receptor signaling pathway. In addition this gene stimulates antimicrobial barrier formation, activation of neutrophil recruitment, T-cell differentiation and antigen-specific T and B-cell response (Huang et al., 2009). For these reasons CCL5 expression was increased to supply more immune responses against rotavirus. Another study reported increasing of CCL5 expression with low rate (McDermott, 2001) whereas another study showed high rate of expression (25-fold change) during rotavirus infection (Casola et al., 2002) These variations in CCL5 expression may belong to immune state of patients, viral strains and time of sampling.

CXCL10 codes protein function stimulation of monocytes, natural killer and T-cell migration. Rotavirus infection stimulates upregulation of this gene with high rate (18.37 FC). Cuadras et al., 2002 recorded increasing of CXCL10-mRNA with low rate after infection with Rotavirus. This variation may be related to the state of infant immunity, time of blood sampling, or load of virus. CCL2 gene displays chemotactic activity for monocytes and basophils during microbial infection. Our results showed moderate rate increasing of CCL2 expression (4.92 FC) after rotavirus infection. Another study also recorded low rate of expression during rotavirus (Julien and Di Fiore 2016).

IL1B gene function as mediator of the immune response, and is played many cellular activities, such as cell proliferation, differentiation, and apoptosis and mediate the immunopathogenesis of various infectious or inflammatory diseases. It was upregulated with 2.29 FC. Another study revealed that rotavirus infection stimulate increasing of IL1B expression in haematopoietic cells (Holloway et al. 2013). Dinarello, 1996, recorded that IL1B expression increased during viral infection to function as potent signaling molecules that induce the synthesis of acute-phase proteins and mediate the immunopathogenesis of various infectious or inflammatory diseases.

ILIR gene is an important mediator involved in many cytokine-induced immune and inflammatory responses, thus it is upregulated with 2 FC to perform its inflammatory action. Another study revealed moderate increasing of ILIR mRNA after rotavirus infection (Tang Kevin et al., 2007). In 2007, Yuhuan recorded that Rotavirus infection up-regulates genes encoding IL-1R cytokine that is involved in anti-inflammatory and antiviral activities. IL5 gene upregulated with 7.4643FC after rotavirus infection. This moderate increasing of expression reflects its major role in the regulation of eosinophil formation, maturation, recruitment and survival during inflammatory diseases. Another study also revealed increasing in the IL5-mRNA after rotavirus infection (Yu et al., 2012).

IFN-alpha and IFN-gamma expression increased with low rate (2 FC) and produced by macrophages and has antiviral activity. Previous study illustrated that IFNA gene expression increased with low rat (2 FC) during rotavirus infection (Bass, 1997). Another study recorded increasing of IFNA expression after rotavirus infection (Puccetti et al., 2018). CCL3 gene expression increased with 2 FC after rotavirus infection to stimulate inflammatory responses.
through binding to the receptors CCR1, CCR4 and CCR5 during viral infections therefore in this study CCR1, CCR3 genes were also upregulated along with the upregulation of CCL3 because of their connected functions. Similar results were recorded from other studies (Tange et al., 2007).

CXCL2 gene is antimicrobial gene encodes secreted proteins involved in immunoregulatory and inflammatory processes and up regulated with 2 FC. In 2013 Dolcino et al showed similar increasing in the CXCL2 gene expression after rotavirus infection.

References

[1] 1-Alberts, B., Johnson, A., Lewis, J., Raff, M., Roperts, K., and Walter, P. (2002). Molecular biology of the cell, (4th ed). United State of America, Garland Science, Tylor and Francis Group.

[2] 2-AL-Ghazal, A. T. (2010). Effect of viral respiratory infections by influenza A virus, Parainfluenza viruses and Adenoviruses on Immuno-inflammatory gene expression /inhibitory of human white blood cells.Ph.D thesis. University of Jordan, Amman, Jordan, 179-190.

[3] 3-Ajuebor, M. N., and M. G. Swain. 2002. Role of chemokines and chemokine receptors in the gastrointestinal tract. Immunology 105:137–143.

[4] 4-Bass DM (1997). Interferon gamma and interleukin 1, but not interferon alfa, inhibit rotavirus entry into human intestinal cell lines Gastroenterology, Volume 113, Issue 1, Pages 81-89.

[5] 5-Casola Antonella, Garofalo Roberto P., Crawford Sue E., Estes Mary K., Frank Sheila E. Crowe, and Mercurio Allan R, (2002). Interleukin-8 Gene Regulation in Intestinal Epithelial Cells Infected with Rotavirus: Role of Viral-Induced I_B Kinase Activation. . Brasier Virology 298, 8–19 (2002), doi:10.1006/viro.2002.1475.

[6] 6-Clewley J. (2004). A role for arrays in clinical virology: fact or fiction, Journal of Clinical Virology, 29, 2-12.

[7] 7- Cuadras Mariela A., Feigelstock Dino A., An Sungwhan, and Greenberg Harry B. (2002) Gene Expression Pattern in Caco-2 Cells following Rotavirus Infection. J. Virology, p. 4467–4482 Vol. 76, No. 9. DOI: 10.1128/JVI.76.9.4467–4482.2002.

[8] 8-Dinarello, C. A. 1996. Biologic basis for interleukin-1 in disease. Blood, 87:2095–2147.

[9] 9-Dolcino Marzia, Zanoni Giovanna, Bason Caterina, Tinazzi Elisa, Boccola Elisa, Valletta Enrico, Contreras Giovanna, Lunardi Claudio, Puccetti Antonio ( ). A subset of anti-rotavirus antibodies directed against the viral protein VP7 predicts the onset of celiac disease and induces typical features of the disease in the intestinal epithelial cell line T84. Immunol Res. DOI 10.1007/s12026-013-8420-0.

[10] 10-Geiser, T., B. Dewald, M. U. Ehrengruber, I. Clark-Lewis, and M. Baggioioli. 1993. The interleukin-8-related chemoatctic cytokines GRO alpha, GRO beta, and GRO gamma activate human neutrophil and basophil leukocytes. J. Biol. Chem. 268:15419–15424.

[11] 11-Hakima S., Mohamad, Ding Shihao, Chen Sunrui, Yin Yuebang, Sua Junhong , Woude C. Janneke van der, Fuhler Gwenny M., Peppelenbosch Maikel P., Pan Qiwei, Wang Wenshi (2018) TNF- exerts potent anti-rotavirus effects via the activation of classical NF- B pathway. Virus Research, 223, 28-37.

[12] 12-Holloway Gavan and Coulson Barbara S. (2013) Innate cellular responses to rotavirus infection. Journal of General Virology, 94, 1151–1160. DOI 10.1099/vir.0.051276-0.

[13] 13-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.
[14] 14-Julien Izabel and Di Fiore Martini (2016). Innate immune responses to rotavirus and viral countermeasures in infected macrophages and intestinal cells. Submitted in total fulfillment of the requirements of the degree of Doctor of Philosophy.
[15] 15-Langa JS, Thompson R, Arnaldo P, et al. Epidemiology of Rotavirus A diarrhea in Chókwè, Southern Mozambique, from February to September, 2011. J Med Virol 2016;88:1751–8
[16] 16-McDermott, M. F. 2001. TNF and TNFR biology in health and disease. Cell Mol. Biol. 47:619–635.
[17] 17-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.
[18] 18-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.
[19] 19-Puccetti Antonio, Saverino Daniele, Opri Roberta, Gabrielli Oretta, Zanoni Giovanna, Pelosi Andrea, Fiore Piera Filomena Moretta Francesca, Lunardi Claudio, and Dolcino Marzia (2018) Immune Response to Rotavirus and Gluten Sensitivity Journal of Immunology Research Volume-ID 9419204, 26 pages. https://doi.org/10.1155/2018/9419204
[20] 20-Salim H, Karyana IPG, Sanjaya-Putra IG, et al. Risk factors of rotavirus diarrhea in hospitalized children in Sanglah Hospital, Denpasar: a prospective cohort study. BMC Gastroenterol 2014;14:51.
[21] 21-Simpson J., Scott R., Boyle M., and Gibson G. (2006). Inflammatory subtypes in asthma: assessment and identification using induced sputum. Respirology, 11, 54–61.
[22] 22-Tang Kevin, Gentsch Jon R., Glass Roger I. and Wang Baoming, Yuhuan, Penelope H. Dennehy, Harry L. Keyserling (2007). Diarrhea Homeostasis in Children with Acute Rotavirus Infection Alters Peripheral T-Cell. J. Virol. 2007, 81(8):3904. DOI: 10.1128/JVI.01887-06.
[23] 23-Tayeb HT, Balkhy HH, Aljuhani SM, et al. Increased prevalence of rotavirus among children associated gastroenteritis in Riyadh Saudi Arabia. Virol J 2011;8:548
[24] 24-Yuhuan Wang, Penelope Dennehy H., Harry Keyserling L., Kevin Tang, Jon Gentsch Jon R., Roger Glass I. and Baoming Jiang (2007) Rotavirus Infection Alters Peripheral T-Cell Homeostasis in Children with Acute Diarrhea. J. Virol. 2007, 81(8):3904. DOI: 10.1128/JVI.01887-06.
[25] 25-Yu Tsung-Han, Tsai Chi-Neu, Lai Ming-Wei, Chen Chien-Chang, Chao Hsun-Chin, Lin Che-Wei, Chiu Cheng-Hsun, Chen Shih-Yen. (2012). Antigenemia and cytokine expression in rotavirus gastroenteritis in children. Journal of Microbiology, Immunology and Infection (2012) 45, 265e270.