Interferon-Gene Family Alterations Following the SARS-Cov Infection in Association with Iron Metabolism and Lymphoid Biology

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ABSTRACT

Interferon (IFN) family has a significant impact on both SARS-CoV and SARS-CoV-2. The aim of this current bioinformatics study is to assess IFN-gene family alterations following the SARS-CoV infection in association with the iron metabolism and lymphoid biology.Gene expression data of human bronchial epithelial cells treated with SARS-CoV for 12, 24, 48 hours were obtained from Array Express (GSE17400). In order to use the obtained data in other targeted analyses,the raw data were normalized by robust multisequence analysis in accordance with the procedure in the Affy package in R. These data consist of 23344 genes (54675 probe sets). In addition, each gene has three repeated expression data values for 12, 24, 48 hours, respectively. For the 48 hours group,positive regulations of the natural killer (NK) cell activation and NK cell-mediated cytotoxicity, as well as hematopoietic stem cells proliferation,were found to be more significant regard to their nominal p-value, family-wise error rate, and false discovery rate (q-value) calculated by gen set enrichment analysis. The gene sets with nominal (NOM) p-value < 0.01, false discovery rate (FDR) q-value ≤ 1, and familywise error rate (FWER) < 1 considered as significantly correlate between compared groups. Our study exhibited that important IFN genes (IFNAR2, IFNA10, IFNA1, IFNLR1, IFNA21, IFNA4, IFNL2, IFNL1, IFNA16, IFNA17) behave like immune genes that show low expression in 12 hours virus exposure, unlike demonstrate high gene expression at 48 hours virus exposure. Likewise, three IFN genes (IFNAR1, IFNGR1, IFNG) have high expression levels at the 12 hours exposure and low expressions at the 48 hours virus expression. All of these interferon genes expression were highly correlated and statistically significant (p< 0.05, pearson r-value > 0.8) with exposure time to the virus. These results suggest that hematopoietic stem cell proliferation pathway is affected by the viral SARS-CoV infection.

Keywords: SARS-CoV, Interferon, Iron metabolism, Lymphoid biology

INTRODUCTION

Coronaviruses represent a family of challenging pathogens affecting the animal and human life on the entire planet. The severe acute respiratory syndrome (SARS) epidemic in 2003 and the Middle East respiratory syndrome (MERS) in 2012 showed that coronavirus family can be globally dangerous if they cross the species barrier and infect humans. At the end of the year 2019, a novel coronavirus (SARS-CoV-2) was identified as the cause of a cluster of pneumonia cases and the World Health Organization (WHO) nominated the disease as COVID-19, which stands for the 'coronavirus disease 2019'.[1] Pathobiological and clinical backgrounds of the previous SARS-CoV infection offer important insights for a better understanding of the currently ongoing SARS-CoV-2 infection. The elucidation of the clinical and biological course of SARS-CoV-2 is important since COVID-19 already had caused a disaster throughout the World.

Interferon (IFN) is actually a defensive mechanism of human body against viral infections. Interferon is considered as the 'scream' of the affected cells during the active viral attack.[2] IFNB1 deficiency was ascribed to the suppression of the sterol pathway in macrophages during viral infections, in relation to the regulation of the lipid metabolism pathway with IFN antiviral defense responses.[3] The mouse model of the SARS-CoV-2 revealed the inflammatory role of the type I IFN signaling. SARS-CoV-2 could damage into the human body via two paradoxical mechanisms that are the depression/ suppression and/or dysregulation/ exaggeration of the immune system.[4] IFN has a great impact on both of the immunogenic mechanisms. IFN, as a drug, may also have a role in the treatment of SARS-CoV-2. In a recently published phase 2 study IFN-beta-1b was administered within the treatment schema for SARS-CoV-2 patients.[5] On the other hand, the lymphoid system has also a critical role in COVID-19 patients which is in close relation with the IFN responses. Different pattern of pre-existing SARS-COV-2 specific T cell immunity in SARS-recovered and uninfected individuals have been demonstrated.[6]

In a study by Turk et al. the whole-genome expression data of the lung epithelial cells infected with SARS-CoV for 12, 24, and 48 hours and a total of 15 renin-angiotensin system (RAS) family and 29 immune genes were analyzed, which were found to be highly correlated with the exposure time to the virus in the studied groups.[7] At the complicating genomic phase of the SARS-CoV, there was an increment in the expressions of some key immune system genes like the IFN gene. Turk et al. had also disclosed that IFN genes were down-regulated in the first 12 hours of SARS-COV and up-regulated in 48 hours.[7] In the literature it was also stated that the increased ferritin and lymphopenia reflect the severity of the COVID-19 clinical course due to SARS-CoV-2.[8]

The aim of this current bioinformatics study is to assess IFN-gene family alterations following the SARS-CoV infection particularly in association with the iron metabolism and lymphoid biology. Herein, we have intended to clarify genomic IFN alterations, since IFNs potentially affect cellular iron regulation, virus-related phospholipid mechanism, lymphocytes and hematopoietic stem cells (HSC) upon viral entry.

MATERIALS AND METHODS

Normalization of Data

Gene expression data of human bronchial epithelial cells treated with SARS-CoV for 12, 24, and 48 hours were obtained from Array Express (GSE17400).[7,9] The data was generated by Yoshikawa et al. to characterize the dynamic, spatial, and temporal changes of the gene expression induced by SARS-CoV using microarray technology.[9] In order to use the obtained data in other targeted analyses, the raw data were normalized by robust multisequence analysis in accordance with the procedure in the Affy package in R. These data consist of 23.344 genes (54.675 probe sets). In addition, each gene has three repeated expression data values for 12, 24, and 48 hours, respectively.

All of the ethical considerations were handled strictly in accordance with the Helsinki Declaration.

Hierarchical Clustering

As a result of the analyzes expressed in the study of Turk et al., some immune genes were showed low expression at 12 hours after infected with SARS-CoV, on the other hand, at 48 hours infection time period, a significant increase in these immune gene expressions has been observed.[7] Additionally, these immune genes, which are highly expressed in 48 hours of infection, also have a statistically significant standard deviation value greater than 0.9. Hierarchical cluster analysis was performed in order to reveal the relationships of these immune genes that were detected significantly as well as the IFN gene family in accordance with the main purpose of this study. Hierarchical clustering of the immune genes which were detected as most variant as well as IFN family genes was applied with the aid of the Euclidean distance Gene Cluster 3.0 program.[10] With the aid of this tool, the expression pattern between the specific immune genes and IFNs was understood.

Gene-Set Enrichment Analysis (GSEA)

The utilized data contains 3 main groups that include SARS-CoV infected gene expression values at 12 hours, 24 hours and 48 hours infection. These 12h, 24h and 48h groups studied triplicated in this data. The GSEA analysis was conducted to determine the most correlated genes between these immune genes and IFN family genes. In order to obtain this aim, two groups which are 12 hours and 48 hours were selected. The underlying reason for these two groups is that these immune genes demonstrate significant as well as clear low expression at 12 hours and significant high expression at 48 hours. For GSEA analysis, the average of triplicated values calculated and utilized for these two groups. With the aid of the GSEA 4.0.3 program, the significant genes which were classified could be realized as most correlated with exposure time.[11] In addition to comprehension of most correlated genes, some associated pathway enriched at the 12 hours as well as 48 hours. These enriched pathways could be noticed in order to understand the specific SARS-CoV-2 virus behavior.

GSEA calculate the enrichment score (ES), normalized enrichment score (NES), nominal p-value (NOM p-value), false discovery rate q-value (FDR q-value), and family wise error rate p-value (FWER). The ES value indicates the gene's maximum deviation in gene sets; in other words, this score helps to find the most upregulated genes. NES value represents the connection or difference between gene sets and gene expression. The higher NES value shows the elevation of permutations. Hence, a higher NES value increases the significance of gene sets. In addition to ES and NES values, the NOM p-value evaluates the importance of ES calculation. Therefore, the NOM p-value directly correlated with ES as well as NES value. Increase in NOM p-value show critical role of ES. On the other hand, the FWER p-value indicates false positives probability of NES and so, lower the FWER p-value directly and significantly correlated with the correctness of NES calculation.

The gene sets with NOM p-value < 0.01, FDR q-value ≤ 1 and FWER < 1 considered as significantly correlate between compared groups.

Table 1. List of interferon genes showing similar and reverse expression patterns with selected immunity genes upon SARS-CoV-2 entry. **(A)** 11 of IFN genes that have similar expression pattern with significant immune genes. **(B)** 3 of IFN genes whose gene expression pattern was different than the immune genes.

(A)-SIMILAR	(B)-REVERSE
IFNA10	IFNAR1
IFNAR2	IFNGR1
IFNA1	IFNG
IFNLR1	
IFNA21	
IFNA4	
IFNL2	
IFNL1	
IFNA16	
IFNL2	
IFNA17	

Network Analysis

Understanding the network between pointing statistically significant immune genes and IFN genes, the network analysis of these genes was conducted via utilizing the Cytoscape application of GeneMANIA[12,13] Cytoscape tool supports analyze these genes both among each other as well as other genes. Therefore, this network analysis assists to demonstrate the connection of both these immune genes and IFN genes with each other as well as with distinct genes. As expected, outcomes of the network analysis of these genes, the visualization of co-expression, co-localization, genetic interaction, pathway relation, physical interaction, shared protein domains could be monitored.

RESULTS

Eleven of the interferon family genes (IFNA10, IFNAR2, IFNA1, IFNLR1, IFNA21, IFNA4, IFNL2, IFNL1, IFNA16, IFNL2, IFNA17) behaved as immune genes that have a low expression at 12 hours and high expression at 48 hours. In other words, the gene expression values are elevated with re-

Table 2. The first 20 list of the related pathways within 12 hours. These are the most correlated pathways with interferon and immune genes

PATHWAYS	ES	NES	NOM p-value	FDR q-value	FWER p-value
GO_VENTRICULAR_TRABECULA_ MYOCARDIUM_ MORPHOGENESIS	-0.55	-1.63	0	1	0.807
GO_VACUOLAR_PROTON_TRANSPORTING_ V_TYPE_ ATPASE_COMPLEX	-0.64	-1.63	0	1	0.861
GO_TRANSFERRIN_TRANSPORT	-0.65	-1.61	0	1	0.861
GO_NADH_METABOLIC_PROCESS	-0.59	-1.61	0	1	0.861
GO_WIDE_PORE_CHANNEL_ACTIVITY	-0.55	-1.6	0	1	0.861
GO_RESPONSE_TO_INCREASED_OXYGEN_ LEVELS	-0.59	-1.58	0	1	0.902
GO_RESPONSE_TO_HYPEROXIA	-0.5	-1.58	0	1	0.902
GO_PROTON_TRANSPORTING_V_TYPE_ ATPASE_COMPLEX	-0.61	-1.58	0	1	0.902
GO_REGULATION_OF_HYDROGEN_PEROXIDE _METABOLIC_PROCESS	-0.58	-1.57	0	1	0.902
GO_PSEUDOPODIUM	-0.44	-1.57	0	1	0.902
GO_IRON_ION_TRANSPORT	-0.55	-1.56	0	1	0.902
GO_ACTIN_FILAMENT_BUNDLE	-0.28	-1.54	0	1	0.952
GO_GLUCOSE_CATABOLIC_PROCESS	-0.55	-1.54	0	1	0.952
GO_TRANSITION_METAL_ION_TRANSPORT	-0.5	-1.53	0	1	0.952
GO_REGULATION_OF_LAMELLIPODIUM_ ORGANIZATION	-0.43	-1.52	0	1	0.952
GO_ATPASE_ACTIVITY_COUPLED_TO_ TRANSMEMBRANE_MOVEMENT_OF_IONS_ ROTATIONAL_MECHANISM	-0.62	-1.51	0	1	0.952
GO_REGULATION_OF_COFACTOR_ METABOLIC_PROCESS	-0.54	-1.51	0	1	0.952
GO_REGULATION_OF_PHOSPHOLIPID_ BIOSYNTHETIC_PROCESS	-0.54	-1.5	0	1	0.952
GO_PROTON_TRANSPORTING_TWO_ SECTOR_ATPASE_COMPLEX	-0.66	-1.5	0	1	0.952
GO_NEGATIVE_REGULATION_OF_ REACTIVE_OXYGEN_SPECIES_METABOLIC_ PROCESS	-0.32	-1.5	0	1	0.952

spect to the viral exposure time. On the other hand, the 3 IFN family genes (IFNAR1, IFNGR1, IFNG) diversely correlated with an exposure time that has low expression in 48 hours.

According to the statistical analysis, the expression pattern of IFN genes was determined for all IFN genes as considering the previously determined immune genes expressions. Table 1 represents the IFN family genes that act similarly as well as differences with regard to previously associated with SARS-CoV virus-infected immune genes.

The outcome of gene-set enrichment analysis which was between 12 hours infection and 48 hours infection groups is demonstrated in Table 2 and Table 3, respectively. This result includes the significant pathways determined according to 12 hours and 48 hours gene expression as well as enriched at 12 hours and at 48 hours.

According to the outcome of gene-set enrichment analysis, three significant pathways were detected for 12 hours groups that are transferrin transport, iron ion transport, regulation of phospholipid biosynthetic process. For 48 hours, positive regula-

Table 3. The first 20 list of the significant pathways which enriched in 48 hours group

PATHWAYS	ES	NES	NOM p-value	FDR q-value	FWER p-value
GO_REGULATION_OF_EXTRACELLULAR_MATRIX_ORGANIZATION	0.46	1.45	0	1	0.946
GO_RESPONSE_TO_UV_C	0.54	1.45	0	1	1
GO_THYROID_HORMONE_GENERATION	0.55	1.45	0	1	1
GO_OXIDOREDUCTION_COENZYME_METABOLIC_PROCESS	0.46	1.44	0	1	1
GO_REGULATION_OF_ACTIVIN_RECEPTOR_SIGNALING_PATHWAY	0.46	1.43	0	1	1
GO_INTERLEUKIN_8_SECRETION	0.55	1.42	0	1	1
GO_ENDOSOME_LUMEN	0.5	1.41	0	1	1
GO_NEGATIVE_REGULATION_OF_TRANSCRIPTION_REGULATORY_REGION_DNA_BINDING	0.68	1.41	0	1	1
GO_NITRIC_OXIDE_SYNTHASE_BIO SYNTHETIC _PROCESS	0.74	1.41	0	1	1
GO_SOMATIC_DIVERSIFICATION_OF_IMMUNE_RECEPTORS	0.41	1.4	0	1	1
GO_AUTONOMIC_NERVOUS_SYSTEM_DEVELOPMENT	0.52	1.4	0	1	1
GO_NITRIC_OXIDE_MEDIATED_SIGNAL_TRANSDUCTION	0.52	1.4	0	1	1
GO_POSITIVE_REGULATION_OF_NATURAL_KILLER_CELL_ACTIVATION	0.65	1.4	0	1	1
GO_CENTRIOLE	0.4	1.4	0	1	1
GO_POSITIVE_REGULATION_OF_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.56	1.39	0.092	1	1
GO_ESTROUS_CYCLE	0.62	1.39	0	1	1
GO_HEMATOPOIETIC_STEM_CELL_PROLIFERATION	0.5	1.39	0	1	1
GO_NEGATIVE_REGULATION_OF_ADENYLATE _CYCLASE_ACTIVITY	0.48	1.39	0.191	1	1
GO_SINGLE_STRANDED_DNA_HELICASE_ACTIVITY	0.53	1.39	0.096	1	1
GO_NEGATIVE_REGULATION_OF_COLD_INDUCED_THERMOGENESIS	0.51	1.39	0.115	1	1

tions of natural killer cell activation, positive regulation of natural killer cell mediated cytotoxicity as well as hematopoietic stem cell proliferation were found more significant.

The network analysis of significant immune genes and IFN genes were performed. This network analysis emphasizes the significant correlation between these immune genes as well as IFN genes. In these outcomes, co-expression as well as pathway analysis demonstrates an influential correlation among IFN genes and selected immune genes.

DISCUSSION

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection is the fundamental source of the global outbreak, coronavirus disease 2019 (COVID-19). Experimental studies of the previous SARS-CoV infection represent the basis for the most potent causative agent of the COVID-19 since SARS-CoV-2 clearly disclosed genetic similarities with the SARS-CoV, However, SARS-CoV-2 has some crucial distinguishing characteristics from the SARS-CoV as well. SARS-CoV-2 could stimulate

a weaker innate anti-viral immune response than SARS-CoV infection.[6,14,15] Fundamentally, innate immunity induces the interferon (IFN) family is the primary immune defense mechanism element, when sensing the infection of the virus.[6,14,16] As depicted in Table 1, several IFN genes play a significant role as a result of the hierarchical clustering of immune genes which were previously determined as significant and IFN family genes found statistically significant in the study conducted by Turk et al.[7] Based on our current results, several genes from IFN family (IFNAR2, IFNA10, IFNA1, IFNLR1, IFNA21, IFNA4, IFNL2, IFNL1, IFNA16, IFNA17) behave like immune genes that show low expression in 12h virus exposure, unlike demonstrate high gene expression at 48h virus exposure. On the other hand, three IFN genes (IFNAR1, IFNGR1, IFNG) have a high expression level at the 12h of exposure and low expressions at the 48h of virus expression.

IFN Elevation Upon Viral Entry

Interferons are the cytokines triggering a signaling pathway upon viral entry for antiviral defense. A host cell produces IFNs when an infection occurs. These infection-induced IFNs target receptors on neighboring cells in order to trigger the rest of the signaling cascade, to produce more IFNs and specific antiviral host proteins. An increase in the expression of specific genes was observed upon SARS-CoV-2 entry into cells. As IFN mechanisms are tightly regulated with viral activities, the relationship between these increasingly expressed genes and interferons was examined. Overall, under normal conditions IFN numbers are expected to increase upon viral entry.

Importantly, many previous studies indicate that IFNAR2 is belonging to the type I IFNs and aid to trigger the propagation of interferon-stimulated genes (ISGs), blocking viral replication. This inhibition also could create the innate immune response of the host against the viral infection. More specifically, as similar to our results, in the study conducted by Utay et al., the increased expression of IFNAR2 after the virus infect to the host was observed, and as a result of this study, in the communication of the virus with ACE2, a vital entry receptor for SARS-CoV-2, the crucial role of IFNs was emphasized.[17] Additionally, IFNAR2 expression is strongly associated with JAK/STAT signaling pathway. This JAK/STAT signaling pathway consist of signal transducer and activator of transcriptions 1 (STAT1) and signal transducer and activator of transcriptions 2 (STAT2), Janus kinase 1 (JAK1) and tyrosine kinase 2 (Tyk2). Especially, the IFNAR2 gene has a role in STAT2 translocation as well as Tyk2 phosphorylation. Similarly with our study's outcomes, up-regulation of IFNAR2 could have affected the efficiency of innate immune response by causing alterations in IFNs.[17-20]

In addition to IFNAR2, IFNLR1 is linked with type III IFNs. IFNLR1 cannot express in whole cells, contrary to IFNAR2. The expression of IFNLR1 is occurred favorably on epithelial cells, neutrophils, in general some immune cells. The moderation of IFNLR1 expression aids in the local examination of the viral entry edge and its replication process. Mordstein et al. proposed that IFNLR1 knockout mice lose their control ability against SARS-CoV replication.[21] Then, the dysfunction of protective mechanism occurs and viral infection develops more severely.[16,21,22] Besides, IFNL1 as well as IFNL2 are other upregulated genes during elevating exposure time. As similar to these, Pizzorno et al. study revealed that after 24h exposure with SARS-CoV-2 infection, up-regulation of IFNL1 and IFNL2 gene was detected and critically, this increase continues with related to the exposure time.[23] Another significant research emphasized that up-regulation of these genes triggers the generation of tumor necrosis factor that could lead to improper functioning of Toll-like receptors (TLRs) that are a fundamental elements of the innate immune response.[23-25] In a recent study it was stated that compared to moderate COVID-19 cases, critical cases exhibited stronger interactions between epithelial and immune cells, as indicated by ligand-receptor expression profiles, and activated immune cells, including inflammatory macrophages expressing IL8, IL1B, and TNF.[26]

IFN Reduction Upon Viral Entry

According to results taken from samples infected with SARS-CoV-2, there were 3 different IFNs ob-

served; IFNAR1, IFNGR1, IFNG, which had reverse expression patterns when compared to other immune cells and most IFNs. Expression of these IFNs decreased in 48 hours when an increase was expected. The reasons and mechanism behind this situation are being questioned with respect to viral infections, especially SARS-CoV-2. Even though the cause underlying this situation is not clearly known, some conclusions can be made. IFN production must be regulated in a way that pro inflammatory damages can be limited.[27] Also, in some viral cases viruses are able to inhibit the host IFN response. It is known that virally produced molecules interact with the host's antiviral protein pathways.[28] Viral system can interfere with various parts of the IFN pathway. It can disturb the upstream components of viral recognitions, production of IFNs and IFN-induced antiviral host protein production.[28] Nevertheless, besides the antagonist actions of viruses, a decrease in some IFN levels could be due to their own regulatory mechanisms. Lastly, the virulence level of viruses also has an effect on the IFN production amount in the cells.

Type I IFN signaling pathway mainly depends on the IFNAR protein, a type I IFN receptor, for initiating the cascade. When pattern recognition receptors recognize microbial organisms, type I IFNs gets triggered. Upon this stimulation, IFNs bind IFNAR.[27] Downstream of this initiation, JAK and STAT pathways get activated, resulting in the activation of interferon stimulated genes (ISGs). Later these ISGs are translated into antiviral host defense proteins. In general, this pathway is supposed to show antiviral protective effects however it also can cause autoimmunity and pro-inflammation, thus needs to be regulated in order to prevent the tissue damage. The severity of the IFNAR signaling is controlled by managing the post-translational modification of IFNAR and proteins belonging to the JAK–STAT signaling pathway. Also, ISG expression is specified with the help of some transcriptional factors that help STAT and alter chromatin at specific loci for specified ISG transcription.[27] Translation of antiviral host proteins is also controlled at the molecular level. As this signaling pathway is controlled in such precise ways, IFNAR is downregulated upon ligand binding in order to prevent the continuous IFN production and consequently pro-inflammatory effects.[29] In severe Covid-19 cases, it is thought that these intense symptoms are caused by the pro-inflammation in the lung epithelia which is causing tissue damage. It was shown that Type I and III IFNs are what is causing the pro-inflammation.[30] However, not all cases show symptoms to that severity. Thus, maybe the regulation of Type I IFNs, especially the IFNAR protein, could be considered as a possible reason.

According to our present results, IFNAR1 is that another type I IFNs, like IFNAR2, down regulated with exposure time, contrary to IFNAR2. In parallel to our findings, Jameel et al. reported that during down regulation time interval, phosphorylation of serine residue would occur.[31] This phosphorylation even stimulates ubiquitination of that as well as lysosomal pathway activates. The activation of the lysosomal pathway links with degradation of receptors due to infection. They also added that further down regulation could lead to the apoptosis pathway.[16,31]

Virulence is a factor affecting how the viral survival will continue in the host cell. High virulence results in a disturbance of protein production in the host cell.[32] Therefore, high virulence results in a decrease in IFN production. However, it would be expected for the virus to interfere with IFN production in general rather than with specific IFNs. In our case, only three genes seem to be downregulated after 48 hours. Nonetheless, coronaviruses can aim specific proteins on hosts, thus interfering with signaling pathways downstream of those proteins.[33] In conclusion, this could result in specific protein down regulations.

As hosts have mechanisms to regulate the IFN response upon viral entry, viruses also have mechanisms similar to those in order to suppress the IFN response for their own benefit. Viral interference could depend on the receptors of host cells. These receptors are crucial in the recognition of these viruses. Inactivation of MDA-5 and RIG-1 signaling by viruses has shown to be affecting the IFN signaling.[28] In a study, it was showed that a respiratory syncytial virus (RSV) inhibits type I IFN signaling temporarily by affecting TLRs.[28] A different idea is that viruses can interfere specifically with the

production of interferons. There are different pathways downstream of receptors that can be inhibited by viruses, such as the JAK-STAT signaling pathway and IRF-3, NF-κB, and AP-1 signaling pathways. Lastly, it is possible for viruses to interfere with the part of the pathway where antiviral host proteins that were IFN-induced are translated. However, our interest is concerned with a decrease in the IFN number, which in this case seems to remain the same. Moreover, our current study outcomes illuminate that in 12h exposure time, transferrin transport, iron ion transport, regulation of phospholipid biosynthetic process pathways, which were enriched.

Effects of IFNs on Iron Regulation in the Cell Upon Viral Entry

Initially, iron takes place in the synthesis of ATP as well as the proper function of mitochondria via oxidative phosphorylation for replication of the virus. In order to the occurrence of viral replication, iron ion supplementation plays a key role. Significantly, in this supplementation, iron ion as well as transferrin association is essential. As indicated, there is a strong correlation between iron ions transport and transferrin transport. Zanella et al. suggested that SARS-CoV-2 infection can propagate the cellular uptake of iron ion, as similar to a great deal of other viral infections.[34] This increased uptake or in other words storage of iron can prevent the starvation of this ion on the cell cycle of SARS-CoV-2. Fundamentally, entry of virus aid to influence the iron-associated proteins functioning so as to increase the efficiency of replication of the virus. Any deficiency in these pathways could trigger anemia, improper replication of virus.[34,35]

In addition to this, iron is also a growth-limiting ion in the microbial world. Viruses need the cells to be full of iron for proper replication. This is important in viral infections as host cells express transferrin (TF) and lactoferrin (LF) proteins.[32] TFs are capable of binding iron and important in regulating the free iron levels in the human body. LFs have antimicrobial activity. They function by binding and transferring iron ions. This results in the depletion of iron in tissues. However, pathogens produce iron-binding molecules, siderophores, which have a high affinity for the iron ion and are able to chelate it, thus increasing the levels of iron in tissues. Therefore, iron regulation is important in order for viral entry and replication to take place. In particular, coronaviruses require iron-containing enzymes for their replicative process.[35] Overall observation was that cytokine effects on iron trafficking resulted in hypoferremia.[36]

In a study conducted with cytokine-stimulated human monocyte cells, effects of IFN-gamma and lipopolysaccharide (LPS), an endotoxin, were examined in relation to iron trafficking. It was observed that IFN-gamma and LPS combined had some pro-inflammatory effects on some components of iron regulatory pathways.[36] It was seen that transferrin receptor (TfR) mRNA levels reduced, expression of divalent metal transporter-1 (DMT1) increased, uptake of non-transferrin bound iron (NTBI) into cells were stimulated, ferroportin mRNA expression down-regulated and iron release from monocytes decreased upon treatment with IFN-gamma and LPS.[36] TfR is a carrier protein, functions in importing iron into cells.[37] Iron is usually bound by transferrin molecules in the cell, rather than floating freely. The decrease in the expression of its mRNA results in an increase in free intracellular iron concentration. Even though the mechanism is still not very clear, DMT1 functions in the transportation of ferrous iron.[38] It is known to uptake iron in most cells. An increase in the expression of DMT1 thus might again result in increased iron levels. NTBI is a critical component in iron overload. Uptake of non-transferrin-bound iron means an increase in free-floating iron in the cell. Ferroportin mRNA expression consequently results in decreased iron release from monocytes. Overall, pro-inflammatory effects were observed as an iron concentration in monocytes increased.

In some viral cases like HIV and AIDS, limiting the free iron in the cells was seen as hopeful therapy. Even though there is no specific study for the infection caused by SARS-CoV-2, it is expected that iron chelation might be beneficial in Covid-19 cases as well, depending on studies about HIV and AIDS.[35]

Effects of IFNs on Phospholipid Mechanism Upon Viral Entry

In general, the lipid composition plays an essential role in exocytosis as well as endocytosis of virus, replication of SARS-CoV-2, and so on, due to its lipid envelop properties. Besides, this property can serve as a receptor or co-factor for cell entry. More specifically, crucial studies illuminate that the phospholipid biosynthetic process pathway has a critical role in constructing the proper replicative organelle for viral replication. Even, any dysfunction in the phospholipid composition could lead to diminishing expression level of ACE2 that trigger the SARS-CoV-2 replication after entry of the virus to the host. Therefore, the phospholipid biosynthetic process pathway is so important for controlling the SARS-CoV-2 replication.[39,40]

The cell membrane is the key for viruses to interact with host cells and membranes mostly rely on lipids. Viruses exploit lipid mechanisms, synthesis, metabolisms, and compartmentalization for their own benefit, viral entry, replication and egress.[41] Phospholipids constitute more than half of the lipids in animal cell membranes. Negatively charged phospholipids can help viruses enter the cell, serving as receptors. IFN-alpha, beta, and gamma are known to have regulatory effects on phospholipid scramblase which can accelerate the bidirectional movement of plasma membrane phospholipids.[42] Phospholipid scramblase activity is important as it promotes the externalization of phosphatidylserine, which is a major phospholipid. It is usually located on the inner leaflet of the membrane however in cases such as cell activation and phagocytosis; it is translocated to the outer side.[43] It was thought that phospholipid scramblase 1 (PLSCR1) might have effects to increase the antiviral effects of IFNs by affecting viral entry, IFN-stimulated cell signaling pathways at the plasma membrane, the transcription of antiviral genes in the nucleus, and by directly blocking specific stages in the viral replication cycle.[43] Expression of some antiviral genes is affected by PLSCR1, which consequently affects viral growth. However, PLSCR1 is also thought to possibly have direct effects on viral replication.[43] Still, as we are to examine the effects of IFNs on phospholipids upon viral entry, it is showed that IFNs have antiviral effects in this case.

In 48 hours, positive regulation of NK cell activation, positive regulation of NK cell-mediated cytotoxicity, as well as hematopoietic stem cell proliferation, are the three fundamental pathways that enriched with 48 hours exposure time. Both the regulation of the NK activation, as well as the cytotoxicity of cell, serves principal stimulator of the innate immune system against SARS-CoV-2 infection. Importantly, the NK cells are significant to adaptive immune response when encountering SARS-CoV-2. The activation of NK cells happens via proper signaling of cytokines as well as some type of IFNs. After stimulation, tumor necrosis factor, chemokines as well as cytokines begin to form. These factors help to communicate the immunity mechanism of cells, especially dendritic cells, and even have the capability to monitor hematopoiesis. According to the research, the amount of NK cells is low during SARS-CoV-2 viral infection. On the other hand, when comparing the level of secretion of NK cytokine as well as NK cytotoxic among a healthy person and SARS-CoV-2 infected people, prevention of these NK cells function and even production of T cells that have a key function in immune response occurs in SARS-CoV-2 infected people. Proportional to these suppressions, innate immune vital element, IFNs amount gets lower. Some further outcomes suggested that insufficient defense mechanisms that targeting the killing or in other words manipulating infected cells, leading to activation of the apoptosis pathway. This pathway demonstrates the death of cells and weaker immunity.[44,45] Essentially, the understanding of the adaptive immunity to SARS-CoV-2 via focusing on interferon genomics is important for vaccine development and disease management.

Effects of IFNs on Hematopoietic Stem Cells Upon Viral Entry

Furthermore, our results suggested that the hematopoietic stem cell proliferation pathway is affected by a viral infection, SARS-CoV-2. The underlying mechanism of this remains unclear but, substantial studies proposed that hematopoietic stem cell plays a highly crucial role in the ACE2 receptor that is critical for entry of viral and initial line of the innate immune response. In addition to these,

targeting of hematopoietic stem cells could affect the elevation of angiotensin II as well as the hyperactivation of some inflammasome factors. The increased function of that factor could trigger pyroptosis that stimulates the death of cells in inflammatory pattern.[46,47] Besides, hematopoietic stem cells are responsible for blood cell development, meaning HSCs differentiate into erythrocytes, granulocytes, monocytes, megakaryocytes, and lymphocytes.[48] A viral infection can result in either the disturbance of hematopoiesis or the virus can use HSCs as a tool for its own genome replication. In a previous paper, it was stated that SARS-CoV-2 can affect HSC and hematopoiesis via local bone marrow RAS system.[49]

IFN-gamma is known to affect the differentiation of most hematopoietic progenitor cells.[50] It was shown that increased expression of IFN-gamma caused impairments in HSC regulations, supporting differentiation rather than self-renewal of HSCs.[51] Overall, IFN-gamma alters gene expression patterns in HSCs. Interestingly, short-term and long-term components of IFN-gamma signaling showed diverse effects. Short-term IFN-gamma signaling increases HSC proliferation, whereas long-term signaling promotes HSC differentiation and impaired self-renewal, which results in opposite effects.[51] Also, IFN-gamma helps cells go under apoptosis.

Type I-IFNs also affect HSCs. According to studies, it was observed that IFN-alpha and beta caused impairment in the bone marrow and resulted in a decrease in HSC number. When samples taken from mice that didn't have the IFNAR1 gene, an IFN-alpha receptor that showed a reverse expression pattern with our genes, were compared to the ones stimulated with IFN-alpha and beta, mice lacking IFNAR1 had more bone marrow.[52] HSCs lacking Type I IFN signaling showed increased numbers of HSCs.

CONCLUSION

In conclusions of our present study, three significant pathways were detected for 12 hours groups that are transferrin transport, iron ion transport, regulation of phospholipid biosynthetic process according to the outcome of gene-set enrichment analysis. For the 48 hours group, positive regulations of the NK cell activation, positive regulation of the NK cell-mediated cytotoxicity as well as HSC proliferation were found to be more significant. Our study exhibited that important IFN genes (IFNAR2, IFNA10, IFNA1, IFNLR1, IFNA21, IFNA4, IFNL2, IFNL1, IFNA16, IFNA17) behave like immune genes that show low expression in 12 hours virus exposure, unlike demonstrate high gene expression at 48 hours virus exposure. Likewise, three IFN genes (IFNAR1, IFNGR1, IFNG) have high expression levels at the 12 hours of exposure and low expressions at the 48 hours virus expression. These results suggest that the hematopoietic stem cell proliferation pathway is affected by the viral SARS-CoV infection. Hematopoietic stem cell plays a highly crucial role in ACE2 receptor that is critical for both the viral entry and the initial line of the innate immune response. Moreover, targeting of HSC could affect the elevation of angiotensin II as well as the hyperactivation of inflammasome factors. Therefore, coronavirus-induced IFN-gene family alterations in association with the iron metabolism and lymphoid biology could be the key elements for a better understanding of the IFN effects on the cellular iron regulation, pathological effects on the phospholipid mechanism inside the infected cell, and diminished HSC upon the viral entry in COVID-19.

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