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BCG vaccination and the risk of COVID 19: A possible correlation

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ABSTRACT

Bacillus Calmette–Guérin (BCG) vaccine is currently used to prevent tuberculosis infection. The vaccine was found to enhance resistance to certain types of infection including positive sense RNA viruses. The current COVID-19 pandemic is caused by positive sense RNA, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A higher mortality rate of COVID-19 patients was reported in countries where BCG vaccination is not routinely administered, when compared to the vaccinated ones. We hypothesized that BCG vaccine may control SARS-CoV2 infection via modulating the monocyte immune response. We analyzed GSE104149 dataset to investigate whether human monocytes of BCG-vaccinated individuals acquire resistance to SARS-CoV-2 infection. Differentially expressed genes obtained from the dataset were used to determine enriched pathways, biological processes, and molecular functions for monocytes post BCG vaccination. Our data show that BCG vaccine promotes a more effective immune response of monocytes against SARS-CoV2, but probably not sufficient to prevent the infection.

1. Introduction

Bacillus Calmette-Guerin (BCG) vaccine is used primarily to treat tuberculosis infection. There are several reports on the role of BCG vaccination in non-specific modulation of the immune responses against various types of infection (Moorlag et al., 2019; Arnoldussen et al., 2011). During tuberculosis outbreaks, a decrease in respiratory tract infections, such as influenza A and yellow fever viruses was observed in BCG vaccinated patients (Moorlag et al., 2019; Arnoldussen et al., 2011; Arts et al., 2018a). A randomized clinical trial showed a decrease in lower respiratory tract infection in patients older than 65 years post-BCG vaccination (Giamarellos-Bourboulis et al., 2020). Other studies reported that BCG vaccine ameliorated the severity of positive sense RNA viruses, such as mengovirus and yellow fever virus (Arts et al., 2018a). The mechanism underlying this effect was attributed to genetic or epigenetic modulation of the innate immune system in a process known as trained immunity (Arts et al., 2018a; Patella, 2020). Trained immunity is initiated through the response of innate immune cells to microbial antigen that causes epigenetic and metabolic changes, that upon re-infection, a more significant response to the antigen occurs (Netea et al., 2011; Sánchez-Ramón et al., 2018).

The current COVID-19 pandemic is caused by the positive sense RNA virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Epidemiological studies reported decreased mortality rate of BCG vaccinated individuals who were infected with SARS-CoV-2 (Curtis et al., 2020 ; Ozdemir et al., 2020). It was thus proposed that BCG vaccine may be effective against SARS-CoV-2. The main mode of SARS-CoV-2 transmission is via droplets and direct contact (Shafaghi et al., 2020; Tung et al., 2021). Patients with COVID-19 present mainly with fever and dry cough that may be complicated with acute respiratory distress syndrome (ARDS), cardiac, gastrointestinal, renal or even central nervous system complications (Wang et al., 2020). Ongoing randomized controlled trials in many countries such as Netherlands and Australia (NCT04327206 and NCT04328441) are designed to determine the effect of BCG vaccine on SARS-COV-2 infection rate. Other study compared the rate of death after COVID-19 in all countries according to their BCG vaccination (Gursel and Gursel, 2020).

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2. BCG immune response

Epidemiological studies have shown that BCG vaccination significantly decreased child mortality in Europe in the 1920's. Randomized controlled trials showed that there is a reduction in BCG-induced mortality in young infants by up to 50% (Aaby et al., 1984, 2011). These studies did not however distinguish between bacterial and viral infections, although it is well documented that viral pathogens are the major cause of respiratory tract infections in children (Stensballe et al., 2005). BCG vaccine boosted the innate immune response upon re-stimulation with different infectious pathogens, in a process known as "trained immunity" (Kleinnijenhuis et al., 2015). Despite the fact that the vaccine has been designed and used against *Mycobacterium tuberculosis*, studies have shown cross-prevention against other non-relevant pathogens, including viruses such as yellow fever (Arts et al., 2018b), hepatitis B, and influenza (H1N1) (Covian et al., 2019) (Zimmermann and Curtis, 2018). Recombinant BCG vaccine was also reported to help against respiratory viruses causing pneumonia and other airway complications (Soto et al., 2018). Vaccination with BCG vaccine has been reported to reduce susceptibility to acute upper and lower respiratory tract infections (Aaby et al., 1984, 2011; Stensballe et al., 2005; Zhu, 2020). The effectiveness of BCG was attributed to epigenetic modifications in stimulated monocytes leading to regulation and induction of cytokines, such as CXCL10, CXCL8, CCL3, CCL3L1 and IL1B.

3. Inflammatory response of monocytes against SARS-COV-2

Serious infection with SARS-CoV-2 causes a gush in pro-inflammatory cytokines, known as cytokine storm (Shin et al., 2009; Spencer et al., 1977; Zhang, 2020). These cytokines include IL-6, TNFα, and IL-10 (Rocha et al., 2013; Thacker and Zdzienicka, 2004). Viremia was also shown to be critical in the pathogenesis of severe COVID-19, where viral particles were present in the brain, kidney and heart of autopsy samples of 27 severely infected patients (Thacker and Zdzienicka, 2003). Entry of viral particles occurs through the angiotensin-converting enzyme-2 (ACE2) receptors on the cell surface of these tissues may explain the severity of the disease (Freund et al., 2010; Campisi and Di Fagagna, 2007). Monocytes/macrophages were reported to play a central role in the fatality of COVID-19 as shown in several recent studies (Kuilman et al., 2008, 2010; Orjalo et al., 2009; Acosta et al., 2013). The immune cell profile of COVID-19 patients with mild, severe, and critical disease in cross sectional study showed a decrease in the total circulating monocytes. Specifically, there was an increase in the percentage of intermediate ($CD14^{+}CD16^{+}$) and non-classical ($CD14^{lo}CD16+$) monocytes in severe and critical cases (Zhou et al., 2020). Pulmonary tissue of transgenic mice with human ACE2 receptors showed greater influx with monocytes/macrophages following SARS-CoV-2 infection (Reaven, 1988). Broncheoalveolar lavage of 9 patients with COVID-19 showed an increase in the proportion of lung macrophages in severe cases relative to mild ones (Jun et al., 1999). These macrophages were not tissue-resident, but were monocyte-derived Fcn1+ cells that displayed an activation of inflammatory pathways (Jun et al., 1999). In another study of 14 hospitalized patients, an increase in hyperactivated pulmonary macrophage was reported in severe cases (Volpe et al., 2018). Another study reported a decrease in monocytes expression of HLA-DR in comparison to patients with milder COVID-19. The decrease in HLA-DR expression was usually observed immediately prior the progress of the case into severe ARDS (Choo et al., 2014).

Interestingly, older individuals were found to suffer a severe form of COVID-19 and higher mortality compared to younger adults (El-Badawy and El-Badri, 2016; El-Badawy et al., 2016; Panina et al., 2018; Elkhenany et al., 2016; Smith and Reid, 2018). A study of 33 COVID-19 patients reported an increase in the peripheral blood $CD14^{+}CD16^{+}$ monocytes, which increased even further in patients with ARDS (Bhansali et al., 2009). $CD14^{+}$ CD16+monocytes are considered the intermediate monocytes that exist in a lower proportion, of around 5% in healthy individuals. Monocytes showed an increased secretion of pro-inflammatory cytokine; IL-6 in mild cases, and increased secretion in severe cases, indicating an important role in COVID-19 cytokine storm. IL-6 induces acute phase response through the stimulation of prostaglandin from brain endothelial cells (Brandl et al., 2011) $CD14^{+}CD16^{+}$ monocytes isolated from blood of 28 patients with COVID-19 acquired macrophage markers suggesting monocytes differentiation (Lee et al., 2019). These cells contributed to the cytokine storm by secreting inflammatory cytokines such as IL-6, TNFα, and IL-10. Patients with severe COVID-19 showed increased proportion of these inflammatory cells (Seo et al., 2017). Because of this role of IL-6 in the cytokine storm, its blockage is now a therapeutic target for severe COVID-19 (Zhang et al., 2012). IL-6 also controls differentiation of monocytes to macrophage by shutting down the switch of monocytes to dendritic cells and transforming them into macrophages (Madonna et al., 2014). Monocytes also express ACE2, which is the main entry receptor for SARS-COV-2 in most of human tissues (Zhang, 2020). ACE2 receptors are involved in the vascular homeostasis via controlling renin-angiotensin-aldosterone system (RAAS) and thus regulating hypertension and vascular inflammation (Yao and Brownlee, 2010; Hao et al., 2013; Chang et al., 2015). Monocytes infection with SARS-COV-2 may impair vascular hemostasis. In this paper, we analyze published data to investigate whether human monocytes of BCG vaccinated individuals are modulated to be more effective against SARS-COV-2.

4. Methods

4.1. Data search

We used the dataset GSE104149 from the Gene Expression Omnibus (GEO) library (Arts et al., 2018b; Mourits et al., 2020). This dataset shows the changes on monocytes epigenome and transcriptome levels after one month of BCG vaccine administration. The data were obtained from six experimental groups, and six controls.

4.2. Data analysis

GEO2R tool only analyzes microarray data and thus could not be used to assess differentially expressed (DE) genes in high throughput sequencing data. We thus used the GEO RNA-seq Experiments Interactive Navigator (GREIN) to perform data analysis for differentially expressed (DE) genes (Mahi et al., 2019).

5. Results

Our analysis generated gene expression profiles of about 26175 genes (including some gene duplicates), which were then filtered by elimination of statistically non-significant data (P-value > 0.05). The use of P-value instead of the adjusted P-value was based on the fact that the sample size was too small for using the latter. This was supported by obtaining the same adjusted value for all genes expression (value $= 1$), which was not sufficient for data filtering. Only 89 genes out of the 26,175 were found to be statistically significant and were used for downstream analysis, as shown in (Supplementary Table 1).

We then used Enrichr online enrichment analysis tool in search for the biological processes, and molecular functions enriched by these genes (Chen et al., 2013; Kuleshov et al., 2016). Kyoto Encyclopedia of genes and genomes database (KEGG 2019) was used to obtain pathway enrichment, while gene ontology for biological processes and molecular functions were obtained from GO Biological Process 2018 and GO Molecular Function 2018 databases, respectively. The top statistically significant outputs, ranked by their adjusted p values (<0.05) were selected (Table 1).

Using OLSVis (an interactive ontology visualizer) and QuickGO, negative regulation of viral genome replication (GO:0045071) was

Table 1
Enriched pathways, biological processes, and molecular functions results using Enrichr analysis.

KEGG 2019 results			
Rank	**Pathway**	**Adj. P-value**	**Genes involved**
1	**Toll-like receptor signaling pathway.**	0.025	**CXCL10, CXCL8, CCL3L1, IL1B, CCL3**
GO Biological Process 2018			
Rank	**Biological process**	**Adj. P-value**	**Genes involved**
1	**Cellular response to type I interferon**	3.93E-08	**IFITM1, RSAD2, IFI27, ISG15, HLA-A, IFIT1, IFIT3, IFIT2, OASL**
2	**Type I interferon signaling pathway**	1.97E-08	**IFITM1, RSAD2, IFI27, ISG15, HLA-A, IFIT1, IFIT3, IFIT2, OASL**
3	**Regulation of viral genome replication**	4.14E-07	**IFITM1, CXCL8, RSAD2, EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL**
4	**Negative regulation of viral genome replication**	2.14E-06	**IFITM1, RSAD2, EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL**
5	**Negative regulation of viral life cycle**	7.20E-06	**IFITM1, RSAD2, EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL**
6	**Cytokine-mediated signaling pathway**	5.34E-05	**IFITM1, CXCL8, RSAD2, CCL3L1, ISG15, HLA-A, PPBP, IFIT1, IFIT3, OASL, IFIT2, CXCL10, IFI27, IL1B, CCL3**
7	**Chemokine-mediated signaling pathway**	0.002	**CXCL10, CXCL8, CCL3L1, CCL3, PPBP**
8	**Inflammatory response**	0.008	**CXCL10, CXCL8, CCL3L1, NLRP7, IL1B, CCL3, PPBP, SIGLEC1**
9	**Cellular response to lipopolysaccharide**	0.024	**CXCL10, CXCL8, NLRP7, IL1B, CCL3**
GO Molecular Function 2018 results			
Rank	**Molecular function**	**Adj. P-value**	**Genes involved**
1	**Chemokine activity**	0.002	**CXCL10, CXCL8, CCL3L1, CCL3, PPBP**
2	**Chemokine receptor binding**	0.001	**CXCL10, CXCL8, CCL3L1, CCL3, PPBP**
3	**CXCR chemokine receptor binding**	0.019	**CXCL10, CXCL8, PPBP**
4	**Cytokine activity**	0.015	**CXCL10, CXCL8, CCL3L1, IL1B, CCL3, PPBP**

found to be a "child term" for regulation of viral genome replication (GO: 0045069), and negative regulation of viral life cycle (GO: 1903901) (Vercruysse et al., 2012; Binns et al., 2009). All of these 3 GO biological processes showed the same set of enriched genes similar the ones obtained from our selected GEO dataset, except for GO:0045071, which obtained an additional gene, CXCL8. We used *Gene ontology* and AmiGO 2 tools to obtain annotations of the previously mentioned common genes in the context of their GO biological process (Ashburner et al., 2000; The Gene Ontology Resourc, 2019; Carbon et al., 2008). Moreover, using PANTHER (a GO enrichment analysis tool), we were able to identify the PANTHER family of these genes, in addition to the biological processes in which each gene is involved (Mi et al., 2018) (Table 2).

Using PANTHER *Gene Ontology* database, we performed another gene ontology enrichment analysis for biological processes in which the 90 genes are involved. This time we obtained 47 statistically significant enriched pathways (adjusted P-value < 0.05). Among the 47 enriched biological processes, 8 were virus-specific. We focused on these pathways that are relevant to viruses (Table 3).

All of the 8 GO biological processes shared a set of 6 commonly enriched genes that were obtained from our selected GEO dataset, while 7 processes shared a set of 7 common enriched genes. We further identified the PANTHER family of these genes in addition to the biological processes in which each gene is involved (Table 4).

We compared the whole set of genes that were enriched for viral biological processes, obtained from GO Biological Processes 2018 and PANTHER overrepresentation test (using VennPainter tool) and found 7 genes to be commonly enriched, while 3 common biological processes to be enriched by these genes which makes these genes and biological

Table 2
PANTHER classification of viral biological processes enriched genes obtained from Enrichr analysis.

GO ID	Gene	PANTHER family	Biological Process
GO:0045069, GO:0045071, GO:1903901	**APOBEC3A**	**mRNA editing enzyme**	• Negative regulation of cellular process • RNA modification • RNA-dependent DNA biosynthetic process • RNA phosphodiester bond hydrolysis, exonucleolytic • Regulation of multi-organism process • Transposition • Defense response to virus • Demethylation
	EIF2AK2	**Eukaryotic translation initiation factor 2-alpha kinase EIF2-alpha kinase-related**	• Protein phosphorylation
	IFIT1	**Interferon-induced protein with tetratricopeptide repeats**	• Defense response to virus
	IFITM1	**Interferon inducible transmembrane protein**	• Regulation of multi-organism process • Negative regulation of biological process • Type I interferon signaling pathway • defense response to virus
	OASL	**2–5 Oligoadenylate synthase**	• regulation of RNA metabolic process • regulation of multi-organism process • negative regulation of biological process • regulation of hydrolase activity • defense response to virus • RNA phosphodiester bond hydrolysis
	RSAD2	**Radical s-adenosyl methionine domain-containing protein 2**	• N/A
	ISG15	**Ubiquitin-like protein ISG15**	• Modification-dependent protein catabolic process • Protein ubiquitination
GO:0045069	**CXCL8**	**Cxc chemokine**	• Cellular response to lipopolysaccharide • Granulocyte chemotaxis • Chemokine-mediated signaling pathway • Antimicrobial humoral immune response mediated by antimicrobial peptide • Inflammatory response • Regulation of molecular function

Table 3
Gene ontology enrichment analysis using PANTHER overrepresentation test.

PANTHER Overrepresentation Test			
Rank	**Biological process**	**Adj. P-value**	**Genes involved**
1	**Defense response to virus (GO:0051607)**	6.70E-11	**IFITM1, RSAD2, IFI27, ISG15, HERC5, IFIT1, IFIT2, OASL, CXCL10, APOBEC3A, EIF2AK2, IFIT3, IFI44L, NT5C3A**
2	**Response to virus (GO:0009615)**	3.19E-09	**IFITM1, RSAD2, IFI27, ISG15, HERC5, IFIT1, IFIT2, OASL, CXCL10, APOBEC3A, EIF2AK2, IFIT3, IFI44L, NT5C3A**
3	**Regulation of viral genome replication (GO:0045069)**	7.45E-08	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, RSAD2, CXCL8, IFITM1, IFI27**
4	**Regulation of viral life cycle (GO:1903900)**	1.56E-06	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, RSAD2, CXCL8, IFITM1, IFI27**
5	**Regulation of viral process (GO:0050792)**	1.59E-06	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, RSAD2, CXCL8, CCL3, IFI27, IFITM1**
6	**Negative regulation of viral genome replication (GO:0045071)**	1.98E-06	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, RSAD2, IFITM1**
7	**Negative regulation of viral process (GO:0048525)**	2.05E-06	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, RSAD2, IFITM1, CCL3**
8	**Negative regulation of viral life cycle (GO:1903901)**	0.008	**EIF2AK2, ISG15, IFIT1, APOBEC3A, OASL, IFITM1, RSAD2**

processes good candidates for further investigation (Lin et al., 2016) (Figs. 1–3). We therefore report the fold change expression of these genes in addition to the rank of each common biological process in (Table 5).

Using STRING tool v11.0, we were able to obtain protein-protein interaction network through functional enrichment analysis for these mutually enriched genes, as depicted in Fig. 4 (Szklarczyk et al., 2019). Edges represent protein interactions while nodes represent proteins. Colored nodes represent first-degree interactions, while white nodes (not included in the figure) represent second-degree interactions. Empty nodes indicate that these proteins have unknown 3D structures. Each edge color indicates the type of interaction that can be known interactions, predicted interactions, and others.

6. Discussion and conclusion

BCG vaccine was shown to modulate the immune responses against tuberculosis and other microbial infection, and was shown to be effective against positive sense-RNA viruses. SARS-CoV2 is a positive sense RNA virus, against which monocytes represent the main cells of the immune response. This paper shows that BCG vaccination modulates the monocytes immune response to be more effective against SARS-CoV2. Our analysis showed differential expression of genes related to toll-like receptor (TLR) pathway in the monocytes of BCG vaccinated individuals, including upregulation of CXCL10 and downregulation of CXCL8, CCL3L1, IL1B, CCL3.

TLR pathway is important for viral infection. TLRs are composed of 10 family members, TLR1 to 10 (Karina et al., 2019), mainly present in macrophages, epithelial cells and fibroblasts (Karina et al., 2019). TLRs stimulation initiates the innate immune response, leading to the production of inflammatory mediators such as CXCL10, CXCL8, IL1B, CCL3 and interferon 1 (Karina et al., 2019). Single stranded RNA viruses interact with TLR7, making it the most likely to recognize SARS-COV-2 (Chollet-Martin et al., 1993a). Stimulation of TLR7 induces the expression of CXCL8 and the secretion of IL-6 and TNFα (Davey et al., 2014).

Table 4
PANTHER classification of viral biological processes enriched genes obtained from PANTHR overrepresentation test.

GO ID	Gene	PANTHER family	Biological Process
GO:0051607, GO:0009615, GO:0045069, GO:1903900, GO:0045071, GO:0048525, GO:1903901, GO:0050792* *Gene IFITM1 was not enriched in GO:0050792	**APOBC3A**	**mRNA editing enzyme**	• Negative regulation of cellular process • RNA modification • RNA-dependent DNA biosynthetic process • RNA phosphodiester bond hydrolysis, exonucleolytic • Regulation of multi-organism process • Transposition • Defense response to virus • Demethylation
	EIF2AK2	**Eukaryotic translation initiation factor 2-alpha kinase EIF2-alpha kinase-related**	• Protein phosphorylation
	IFIT1	**Interferon-induced protein with tetratricopeptide repeats**	• Defense response to virus
	IFITM1	**Interferon inducible transmembrane protein**	• Regulation of multi-organism process • Negative regulation of biological process • Type I interferon signaling pathway • Defense response to virus
	OASL	**2–5 Oligoadenylate synthase**	• Regulation of RNA metabolic process • Regulation of multi-organism process • Negative regulation of biological process • Regulation of hydrolase activity • Defense response to virus • RNA phosphodiester bond hydrolysis
	RSAD2	**Radical s-adenosyl methionine domain-containing protein 2**	**N/A**
	ISG15	**Ubiquitin-like protein ISG15**	• Modification-dependent protein catabolic process • Protein ubiquitination
GO:0051607, GO:0009615, GO:0045069, GO:1903900, GO:0050792	**IFI27**	**Interferon alpha-inducible protein 27**	• Apoptotic signaling pathway
GO:0051607, GO:0009615	**IFIT2**	**Interferon-induced protein with tetratricopeptide repeats**	• Defense response to virus
	IFIT3	**Interferon-induced protein with tetratricopeptide repeats**	• Defense response to virus
	CXCL10	**Cxc chemokine**	• Granulocyte chemotaxis • Cellular response to lipopolysaccharide • Antimicrobial humoral immune response mediated

(*continued on next page*)

Table 4 (*continued*)

GO ID	Gene	PANTHER family	Biological Process
			by antimicrobial peptide • Inflammatory response • Chemokine-mediated signaling pathway
	NT5C3A	**5′-nucleotidase**	**N/A**
	IFI44L	**Interferon-induced protein 44**	• Immune response
	HERC5	**Ubiquitin-protein ligase e3a-related**	**N/A**
GO:0050792	**CCL3**	**Small inducible cytokine A**	• ERK1 and ERK2 cascade • Granulocyte chemotaxis • G protein-coupled receptor signaling pathway • Cellular response to tumor necrosis factor • Innate immune response • Lymphocyte migration • Positive regulation of GTPase activity • Positive regulation of ERK1 and ERK2 cascade • Response to interleukin-1 • Inflammatory response • Chemokine-mediated signaling pathway
GO:0045069, GO:1903900, GO:0050792	**CXCL8**	**Cxc chemokine**	• Cellular response to lipopolysaccharide • Granulocyte chemotaxis • Chemokine-mediated signaling pathway • Antimicrobial humoral immune response mediated by antimicrobial peptide • Inflammatory response • Regulation of molecular function

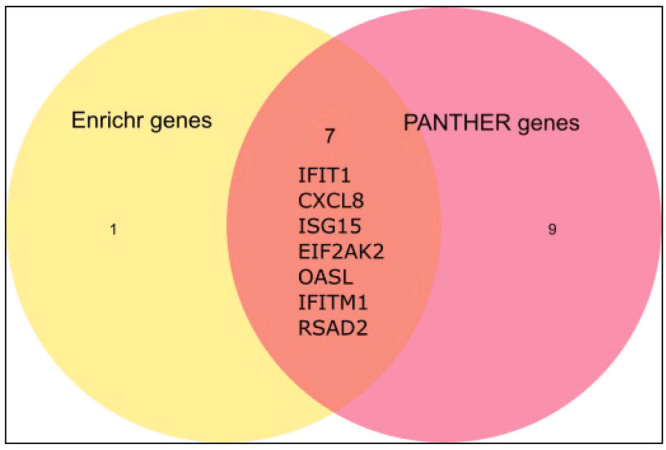

Fig. 1. Commonly enriched genes in both PANTHER Overrepresentation Test and Enrichr Analysis.

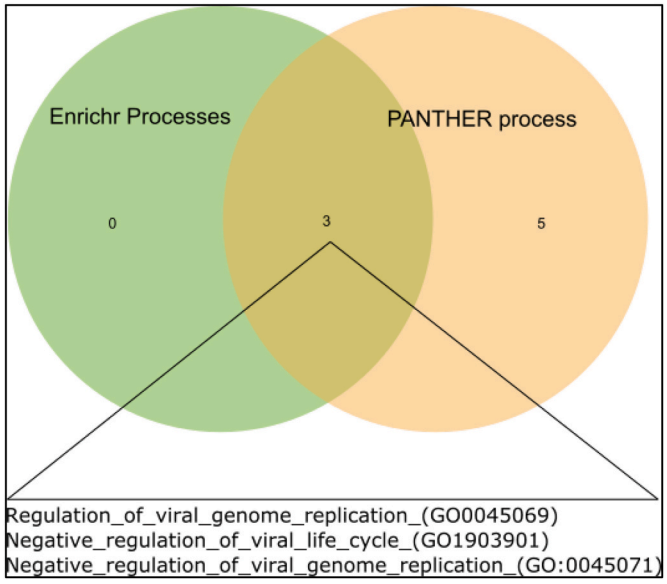

Fig. 2. Commonly enriched biological processes in both PANTHER Overrepresentation Test and Enrichr Analysis.

Inhibition of TLR7, on the other hand, by BCG vaccination, may block the effector of TLR7 through inhibition of CXCL8 (Davey et al., 2014).

IL1B is a pro-inflammatory cytokine that mediates the initiation of the monocyte immune response (Pulugulla et al., 2018) against RNA viruses such as influenza A virus and Sendai virus (Sareneva et al., 1998; Matikainen et al., 2000). Our data showed that monocytes from BCG vaccinated individuals downregulated IL1B expression. Our analysis showed also a decline in the levels of expression of CCL3L1 and CCL3 in monocytes isolated from BCG vaccinated individuals. In severe COVID-19 however, increased levels of CCL3L1 and CCL3 were reported (Arts et al., 2018a; Hernigou et al., 2015). Taken together, these data suggest that BCG vaccination may help in ameliorating the infectivity of SARS-CoV2.

The observed increase in CXCL10 expression is known to have a central role in immune-viral defenses and is correlated with the severity of virus-associated acute respiratory infections (Hayney et al., 2017). CXCL8 is found to be secreted by alveolar macrophages in acute inflammation and respiratory diseases in the lungs, this helps to initiate pro-inflammatory response and recruit other immune cells to the site of inflammation (Gauglitz et al., 2008; Harada et al., 1994). Despite the beneficial immunological response provided by the CXCL8 during inflammatory conditions, it can also contribute to the inflammatory complications and neutrophil-mediated tissue damage (Kaur and Singh, 2013; Moore and Kunkel, 2019). In addition, it was found that upregulated CXCL8 in patients with respiratory problems might correlate with their high risk to develop ARDS and may be associated with increased mortality rate (Chollet-Martin et al., 1993b; Miller et al., 1992; Pugin et al., 1999). Unlike CXCL10, according to our results, CXCL8 was downregulated in monocytes, which may reflect better outcomes to patients previously injected with BCG vaccine (Table 2).

The interferon-induced protein with tetratricopeptide repeats (IFIT) family is composed of 5 members, IFIT 1 through 5 (Brownlee, 1994). These IFITs are regulated by Interferon pathway since they have IFN-stimulated response elements promoters upon viral infection (de Lima et al., 2016). IFIT1 has been shown to be an antiviral protein. It can both detect and react against viral infection by recognizing RNA with their 5′ triphosphate and lacking 2′- O methylation, and sequesters these viral nucleic acids (Pichlmair et al., 2011). Elevated expression levels of IFIT1 upon BCG administration could therefore indicate enhanced

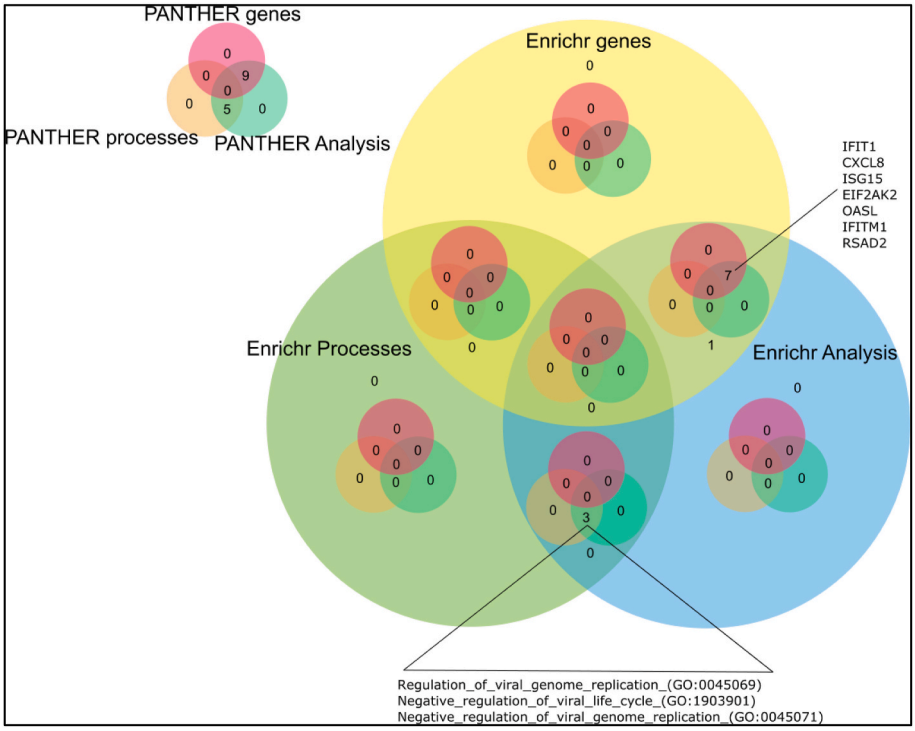

Fig. 3. Nest Venn diagram for the commonly enriched genes and biological processes from PANTHER overrepresentation test and Enrichr analysis.

Table 5
Ranking and Log (FC) of the commonly enriched biological processes and genes.

Mutually enriched biological processes		
Biological Process	**Rank in PANTHER**	**Rank in Enrichr**
Regulation of viral genome replication (GO:0045069)	**3**	**3**
Negative regulation of viral life cycle (GO:1903901)	**4**	**5**
Negative regulation of viral genome replication (GO:0045,071)	**6**	**4**
Mutually enriched genes		
Gene	**Log (FC)**	
IFIT1	**2.119**	
CXCL8	**−2.268**	
ISG15	**1.367**	
EIF2AK2	**0.84**	
OASL	**1.419**	
IFITM1	**2.278**	
RSAD2	**2.001**	

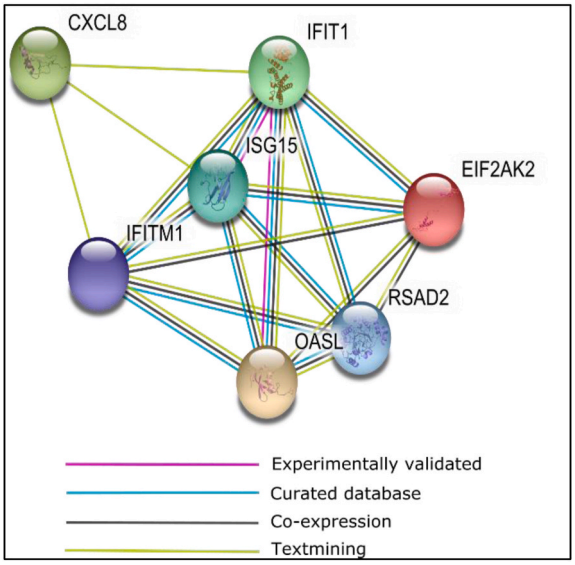

Fig. 4. Protein-protein interaction network of commonly enriched genes, generated by STRING v11.0.

cellular innate immunity against SARS-CoV-2 infection. Different kinds of viruses can evade the action of IFIT1 by having a 2′-O-MTase activity including coronaviruses, as reviewed by Diamond et al. (Diamond, 2014). Thus, targeting viral 2′-O-MTases has been of great interest. For example, therapeutics that target SARS-CoV-2 nsp16 2′-O-MTase have been investigated as potential novel SARS-CoV-2 inhibitors (Yuanyuan et al., 2020). Administration of BCG vaccine along with viral-specific drugs and therapeutics may result in boosted results.

Type 1 interferon pathway is one of the main pathways to be stimulated by viral infection, that is triggered by pathogen-associated molecular patterns (PAMPs) (Fan and Sun, 2016). PAMPs induce the upregulation of Interferon-stimulated gene (ISGs) (Elkhenany et al., 2019; Hayflick, 1975). ISGs stimulation causes inhibition of viral replication in addition to recruitment of immune cells and enhanced tissue repair (Van Tienen et al., 2011; Cianfarani et al., 2013). In SARS-COV-2 infection, there is a delayed and ineffective type 1interferon response (George and Abrahamse, 2019; Yoon et al., 2011; Kozlowska et al., 2019; Han et al., 2014). In serum of patients with SARS-COV-2, there is a decrease in type 1 interferon (Randeria et al., 2019; Cawthorn and Sethi, 2008). This exacerbates the cytokine storm via recruitment of monocytes and macrophages (George and Abrahamse, 2019; Yoon et al., 2011). ISG15 has also been shown to exhibit broad spectrum of antiviral activities through different mechanisms, as reviewed in (Morales and Lenschow, 2013; Harty et al., 2009). It was shown to be upregulated upon administration of BCG vaccine, which further confirms that BCG administration enhances antiviral properties. SARS-CoV2, however, has been shown to alleviate some of ISG15 antiviral activities through the action of papain-like protease which deISGylate viral proteins (Niemeyer et al., 2018; Lindner et al., 2007). This

can be justified by other antiviral activities that ISG15 exerts other than ISGylation of viral proteins. This also can be further supported by the fact that ISG15 can delay coronaviruses replication (Ma et al., 2014). In SARS-CoV2, papain-like protease inhibition enhanced antiviral immunity to SARS-CoV2 (Shin et al., 2020). This indicates that BCG administration along with other therapeutics can substantially foster immunity against SARS-CoV2 infection.

Among the interferon stimulated genes are the IFITM family, which is considered the first line of antiviral defense, and includes IFITM1, as reviewed by Smith et al. (Bailey et al., 2014). IFITM1 protein has been shown to exert antiviral activities among different types of virus families including SARS coronaviruses (Smith et al., 2019; Huang et al., 2011). IFITM1 has been shown to be upregulated upon SARS-CoV2 infection where log2FC = 0.6736, 1.0275 (Loganathan et al., 2020; Blanco-Melo, 2020). Comparing the previous results to our analyzed dataset shows that BCG vaccination can induce more expression of IFITM1 (Log2FC = 2.278) and therefore enhances antiviral immunity and response to SARS-CoV2.

RSAD2, also known as viper in, has been shown to have antiviral activity against several types of viruses via different interferon-dependent and independent pathways, as reviewed by Seo et al. (2011). One of the mechanisms by which RSAD2 acts as an antiviral protein is via the generation of 3'-deoxy-3′,4'-didehydro-CTP (ddhCTP) which acts as a chain terminator for RNA-dependent RNA polymerases (Gizzi et al., 2018). Viperin has been shown to interact with Golgi brefeldin A-resistant guanine nucleotide exchange factor 1 (GBF1) and inhibits its expression (Vonderstein et al., 2018). GBF1 depletion was previously shown to result in reduced SARS-CoV2 infection (de Wilde et al., 2015). Accordingly, higher expression of viperin can be a potential candidate for ameliorating SARS-CoV2 infection. The higher expression of viperin upon BCG administration could also boost host cells immune defense against SARS-CoV2 infection.

OASL is an interferon-induced protein with antiviral activities that include activation of RIG-1 and RNase L (Zhu et al., 2014; Choi et al., 2015). OASL was found to be upregulated in SARS-CoV2 infected cells (Danesh et al., 2011). It was also found to be upregulated after administration of BCG vaccine. This could support the innate cellular antiviral activities in SARS-CoV2 infections. EIF2AK2 is another interferon-induced dsRNA-activated protein that has antiviral activities. EIF2AK2 expression was upregulated upon BCG administration. For its spectrum of antiviral activity, EIF2AK2 gene is suspected to be one of the genes stimulated by BCG vaccine to boost the antiviral cellular innate immunity.

As can be inferred, most of the common enriched genes are interferon-inducible genes that have antiviral mechanisms except for the CXCL8 chemokine. All these genes, except CXCL8, were upregulated as per the performed data analysis upon BCG vaccine administration. This indicates that BCG can foster innate cellular antiviral responses against the novel SARS-CoV2 viral infection. Although SARS-CoV2 has evasion mechanisms against some of these upregulated antiviral factors, BCG administration along with other drugs can overcome such evasion mechanism.

Our data have also shown no significance in the expression of ACE2 in monocytes isolated from BCG vaccinated individuals. ACE2 receptor, which is responsible for infectivity of cells with SARS-CoV2, this suggests that monocyte infection with SARS-CoV2 may still possible in these individuals. Taken together, our data support the premise that trained immunity developed in patients previously vaccinated with BCG can induce a cross-protection mechanism against SARS-CoV2 (Kleinnijenhuis et al., 2015; Covian et al., 2019) Vaccinated BCG patients may thus experience enhanced immune responses against SARS-CoV2 especially in old aged patient segment, albeit not ensuring decreased infectivity of SARS-CoV2. Clinical trials using BCG vaccine to enhance immunity against SARS-CoV2 are essential to confirm these findings.

CRediT authorship contribution statement

Sara M. Ahmed: Methodology, Investigation, Writing – original draft, Writing – review & editing. **Mohamed A. Nasr:** Software, Formal analysis, Writing – original draft. **Shimaa E. Elshenawy:** Writing – original draft. **Alaa E. Hussein:** Writing – original draft. **Ahmed H. El-Betar:** Conceptualization, Writing – original draft. **Rania Hassan Mohamed:** Writing – review & editing. **Nagwa El-Badri:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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