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PREDICTION OF FUNCTIONAL NETWORKS OF INFLAMMASOME AND APOPTOSOME DURING A PARASITIC INFECTION: FOR LEISHMANIA

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ABSTRACT: One of the many challenges of bioinformatics, now a day; is the integration of biological data. The establishment of large quantities of data concerning the differentially expressed genes during infection with an intracellular pathogen requires the implementation of integration strategies to gather all the data and therefore a better understanding of the functioning of the Intracellular infection. This work aims to exploit the bioinformatics approach, to provide a new interpretation of the data available in the literature as well as databases on the effect of intracellular infection, cases of the Leishmania parasite, on the host. A total of 30 genes expressed differently from a Leishmania parasite infection were used for functional analysis using the bioinformatics tool. The latter provides rapid assessment of signaling and metabolic pathways, molecular networks and biological processes that are more significantly disrupted in a data set of interest. Bioinformatics analysis revealed that apoptosis, cytokine production, and signaling were altered following infection with the Leishmania parasite. The genes of IL-10, IL-4, IL-6, SOD1, EIF2AK2, NF-kB and PI3K/akt were most activated after Infection by the Leishmania parasite. The results suggest that the Leishmania parasite reverses immune and inflammatory responses, meaning the ability to induce programmed cell death and microbicidal functions of macrophages such as inhibition of nitric oxide (NO), And the functions of the inducible cytokines of macrophages. Analyzing and interpreting data using the bioinformatics approach helps to unlock knowledge buried in experimental data by quickly identifying links, mechanisms, functions and main pathways to move beyond Statistical analysis to new biological analyzes.

KEYWORDS: Infection, Inflammatory response, bioinformatics, network, interactions.

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1.INTRODUCTION

The Infection with intracellular pathogens has a global distribution. These intracellular pathogens agents can infect humans as well as practically all warm-blooded animals, including mammals [1], [2]. Intracellular survival capacity, is crucial for several pathogens after the invasion of their eukaryotic target cells. Once the infection has begun, exchanges of information will take place between the host and the intracellular pathogen. It will be a matter for the host to try to eliminate this microorganism as quickly as possible by using its immune system, and for the microorganism to try to escape the immune system while modifying its environment (the host) In order to create favorable conditions. This exchange of information, which takes place through the exchange of molecules between the two actors, forms a genuine molecular dialogue [3]–[8].Studies of these emerging infections reveal the evolutionary properties of pathogenic micro-organisms and the dynamic relationships between micro-organisms, their hosts and the environment. Thereby, an in-depth understanding of infectious mechanisms is an indispensable tool for the implementation of targeted therapeutic and preventive approaches. It requires a biology systems approach, concentrating on molecular interactions between pathogenic organisms and host organisms [9]–[11].Biology Systems aims to understand and model complex biological systems as a whole. This last is a field of interdisciplinary research in life sciences that focuses on the study of nonlinear interactions between biological entities by integrating and combining biomolecular and medical sciences with mathematical, computer science, Engineering [12]. The approach of biology systems can focus on one or more levels, including genes, proteins, cells, tissues, whole organisms or populations. This field of research emerged at the beginning of the 21st century [12]–[14]. It is characterized by a strong connection of laboratory experiments and computer analysis, where the analysis and modeling of experimental data results in new hypotheses that lead to new experiments [12].In biology systems, there are multiple widely recognized approaches to the elucidation of key molecules and their interactions within and between organisms. Among them, network inference for the identification of relationships between molecules, thus discovering potential drug targets [15]. This approach aims to understand the molecular mechanisms underlying all biological processes at the system level through mathematical modeling. These mechanisms are usually represented in a network composed of nodes designating molecules (for example proteins, DNA, RNA or its metabolites) and edges representing the interactions between the connected nodes (for example: protein-protein, Protein-DNA). We can distinguish two main sectors in omic data, supported by prior knowledge, and secondly, Interactions concerning the discovery of disease modules supported by prior knowledge and omic data [15]–[17]. The different levels of omic data collected from infected pathogens and / or cells are essential components that lead to bioinformatics analyzes facilitating the construction and analysis of the regulator, metabolic and protein-protein gene networks, specific to the infection [18]–[20]. In a constant context of established infections, epidemics of new and old infectious diseases emerge

periodically, greatly amplifying the global burden of infections. The exploitation of the approach of biology systems, unlocks the knowledge buried in the experimental data, recruiting algorithms and statistical models, in order to create new ways of understanding the inflammasome and apoptosome involved. This can help predict the type of immune response and subsequently the mode of intervention to remedy this public health problem. The objective of this work is to study, using a computer-statistical approach, the representation of biological data in order to make their integration more effective in the case of a leishmania infection. Therefore, predict the functional networks of inflammasome and apoptosome that are set up during this parasitic infection.

2. MATERIALS AND METHODS

This study consists on studying, using a data process-statistical approach, the representation of biological data in order to make them have a more effective integration in the case of an infection with Leishmania. For that, this work is carried out on a list of differently expressed genes (DEG) selected from various databases and scientific publications. The results were subsequently processed by different software: STRING (https://string-db.org/), Cystoscape software and the BiNGO plugin, and Ingenuity Pathway Analysis (IPA) (Ingenuity® Systems, http://www.ingenuity.com).In a first part, we will start with the set of genes selected from different databases, then in the second part we will present the ontological analysis of these genes thanks to 3 bio-computer software.

I. List of genes

The infection with Leishmania parasite in the host induces a DEG leading to the alteration of several signaling pathways and molecular functions of the cell. A gene list seems to have a relationship with the immune response during the infection with Leishmania is obtained after consulting various scientific databases and analyzing several scientific articles. The genes were listed in the following **tables I, II** and **III**.

Table I: Differently Expressed Genes (DEG) during infection leishmaniasis, selected from various databases and scientific publications.

Abbreviation	Genes	Discription
IL-1B	Interleukin 1, beta	Mediator of the inflammatory response, involved in cell proliferation, differentiation and apoptosis (https://ghr.nlm.nih.gov/gene/IL1B).
IL-12B	Interleukin 12B	Maintaining a sufficient number of cells Th1 memory / effector to meditate a long-term protection against a pathogenic intracellular (https://www.ncbi.nlm.nih.gov/gene/3593).
IL-4	Interleukin 4	Pleiotropique cytokine produced by activated T cells (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL4 ; https://www.ncbi.nlm.nih.gov/gene/3565).
IL-10	Interleukin 10	Reduces the expression of cytokines of the Th1, the Ags of the MHC of class II, and molecules of co-stimulation in macrophages (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL10).

IL-17A	Interleukin 17A	Stimulates the expression of the IL6 and cyclo-oxygenase-2 (PTGS2/COX-2), as well as the improvement of the production of nitric oxide (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL17A ; https://www.ncbi.nlm.nih.gov/gene/3605).
IL-6	Interleukin 6	Involved in a variety of inflammation (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL6 ; https://www.ncbi.nlm.nih.gov/gene/3569).
IFNγ	Interferon, gamma	Soluble cytokine with anti-viral properties, immunoregulatory and anti-tumor and it is a powerful activator of macrophages (https://www.ncbi.nlm.nih.gov/gene/3458).
TNF	Tumornecrosis factor	Cytokine is involved in the regulation of a wide range of biological processes, including cell proliferation, differentiation, apoptosis, metabolism of lipids and clotting (http://www.genecards.org/cgi-bin/carddisp.pl?gene=TNF ; https://www.ncbi.nlm.nih.gov/gene/7124).
CD40	CD40 molecule, TNF receptor superfamily member 5	This receptor is critical in switching the immunoglobulin class dependent on T cells and development of memory B cells (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CD40 ; https://www.ncbi.nlm.nih.gov/gene/958).
CCL 1	Chemokine (C-C motif) ligand 1	This chemokine, a member of the CC subfamily, is secreted by activated T cells and displays chemotactic activity for monocytes but not for neutrophils (https://www.ncbi.nlm.nih.gov/gene/6346).
CCL 2	Chemokine (C-C motif) ligand 2	This chemotactic activity for monocytes and basophils. It has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCL2 ; https://www.ncbi.nlm.nih.gov/gene/6347).

Table II: Differently Expressed Genes (DEG) during infection leishmansiasis selected from various databases and scientific publications (continued)

Abbreviation	Genes	Discription
CCL 3	Chemokine (C-C motif) ligand 3	Plays a role in inflammatory responses through binding to receptors CCR1, CCR4 and CCR5 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCL3 ; https://www.ncbi.nlm.nih.gov/gene/6348).
CCL 4	Chemokine (C-C motif) ligand 4	It has chemokinetic and inflammatory functions (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCL4 ; https://www.ncbi.nlm.nih.gov/gene/20303).
CCL 16	Chemokine (C-C motif) ligand 16	This gene shows a potent myelosuppressive activity and suppresses proliferation of myeloid progenitor cells (https://www.ncbi.nlm.nih.gov/gene/6360).

CXCL2	Chemokine (C-X-C motif) ligand 2	Also called (MIP2-alpha) macrophage inflammatory protein 2-alpha. This chemokine is secreted by monocytes and macrophages and is chemotactic for polymorphonuclear leukocytes and hematopoietic stem cells (https://www.ncbi.nlm.nih.gov/gene/2920 ; http://www.genecards.org/cgi-bin/carddisp.pl?gene=CXCL2).
TNFSF10	Tumor necrosis factor (ligand) superfamily, member 10	This protein preferentially induces apoptosis in transformed and tumor cells (https://www.ncbi.nlm.nih.gov/gene/8743 ; http://www.genecards.org/cgi-bin/carddisp.pl?gene=TNFSF10).
CD200	Molecule CD200	Belongs to the immunoglobulin superfamily, it can regulate the activity of myeloid cells and delivers an inhibitory signal for the macrophage lineage in various tissues (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CD200 ; https://www.ncbi.nlm.nih.gov/gene/4345).
SPP 1	Secreted phosphoprotein 1 Osteopontine	Involved in the binding of osteoclasts to mineralized bone matrix. Also increases the expression of interferon-gamma and interleukin-12 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SPP1 ; https://www.ncbi.nlm.nih.gov/gene/6696).
CXCR 1	Chemokine (C-X-C motif) receptor 1	Member of the family of receptors coupled to the G protein. This protein is a receptor with high affinity for interleukin 8 (http://www.genecards.org/cgi-bin/carddisp.pl?gene=CXCR1 ; https://www.ncbi.nlm.nih.gov/gene/3577).
FASLG	Fas ligand (TNF superfamily, member 6)	The protein encoded by this gene is a transmembrane protein, FAS ligand. The interaction between the two is critical in induction of apoptosis in certain cell types such as lymphocytes (http://www.genecards.org/cgi-bin/carddisp.pl?gene=FASLG ; https://www.ncbi.nlm.nih.gov/gene/356).
IRF1	Interferon regulatory factor 1	Transcriptional activator of genes induced by the interferons alpha, beta and gamma. it plays a role in the regulation of apoptosis and tumor suppression (http://www.genecards.org/cgi-bin/carddisp.pl?gene=IRF1 ; https://www.ncbi.nlm.nih.gov/gene/3659).

Table III: Differently Expressed Genes (DEG) during infection leishmansiasis selected from various databases and scientific publications (continued)

Abbreviation	Genes	Discription
PRF1	Perforin 1 (pore forming protein)	One of the main cytolytiques proteins of cytolytiques granules, it is known to be a molecule key effector cells cytotoxic T and NK cells. Creates a transmembrane tubules and is capable of lysing of nonspecific way a variety of target cells (http://www.genecards.org/cgi-bin/carddisp.pl?gene=PRF1 ; https://www.ncbi.nlm.nih.gov/gene/5551).
GZMB	Granzyme B	Both regulates the inflammatory tissue reaction and control of the replication of the parasite in the cell [21]. What triggers apoptosis by a mechanism of activation of Caspases [22].

SLC11A1	Solute carrier family 11 (proton-coupled divalent metal ion transporter), member 1	Works like a metal carrier divalent, involved in the metabolism of iron and the resistance of the host to certain pathogens. Mutations in this gene have been associated with susceptibility to infectious diseases (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SLC11A1 ; https://www.ncbi.nlm.nih.gov/gene/6556).
SOD 1	Superoxide dismutase 1, soluble	the protein encoded by this gene sets the zinc and copper ions and is one of the two isozymes responsible of the destruction of superoxydes free radicals in the body (http://www.genecards.org/cgi-bin/carddisp.pl?gene=SOD1 ; https://www.ncbi.nlm.nih.gov/gene/6647).
EIF2AK2	Eukaryotic translation initiation factor 2-alpha kinase 2	The protein encoded by this gene is a kinase protein serine / threonine. The activated form of the protein inhibits protein synthesis (http://www.genecards.org/cgi-bin/carddisp.pl?gene=EIF2AK2; https://www.ncbi.nlm.nih.gov/gene/5610).
FOXP3	Forkhead box P3	Deficits of this gene are the cause of the immunodeficiency polyendocrinopathy, enteropathy, syndrome X-linked (IPEX) (https://www.ncbi.nlm.nih.gov/gene/50943).

II. The Analysis with Bioinformatics Software

1. Study of Co-expression and gene ontology

This study was complemented in two STRING and Cytoscape software with it function plugin BiNGO, which allowed us to classify and group the genes according to Their co-expression while determining the different Deontological levels resulting from it.

a. Co-expression analysis by STRING software: The analysis of co-expression was performed By the String software on genes.

Visualization of gene interactions: We used the default parameters, focusing on specific criteria such as co-occurrence, co-expression, experimental evidence, existing databases, and text mining.

Enriched statistical analyzes: The terms are classified by their enrichment P-value, which is calculated using a hypergeometric test. The p-values are corrected for multiple tests using the method of Benjamini and Hochberg, but the software also offers options either to disable this correction or to select a more rigorous statistical test.

b. Ontological analysis by the Cytoscape software

• **Data import:** The results obtained by the String software were imported in txt file. The network is imported as an unformatted, pre-existing table that we saved on the STRING site in a local file.

• We used **the Network Analyzer** plugin to customize the network**.** The size and color of the nodes were customized according to the values of the chosen parameter: here, depending on the number of connections of the node with other proteins, large size and light color for low connectivity.

• **The Use of the BiNGO function for network customization**: The genes were selected manually from Cytoscape, in order to have a functional profile in the form of a network and then in the form

of DAG. We chose the ontological level "biological process" as a query for analysis with the BiNGO plugin function.

• **Statistical analysis:** P-values give a good indication of the importance of a given functional category. However, we can not draw conclusions based solely on p-values, these last, returned by Bingo, can give us additional clues, which must be interpreted in the light of the other elements related to the study.

2. **Ingenuity Pathway Analysis with Analysis (IPA)**

The Ingenuity Pathway Analysis software (IPA), is used to evaluate the functional relationship between the 28 genes selected for the study. The IPA software was able to map and mark the 28 genes emblem. After the annotation: The differential expressed genes of our list input were analyzed by the "IPA-Core analysis". The statistical significance of genes obtained networks was established by calculating p-value. The score of interconnected genes and pathways over represented (number of molecules involved in the network) was used to classify the different networks obtained.At the end, the data set was filtered to perform a specific analysis. At this level, other parameters are fixed as follows: The organism or species, tissue or cell type in question “Tissues and cell lines”, the type of pathology and type of targeted biological process or pathway.

3.RESULT AND DISCUSSION

I. Results obtained by STRING and Cytoscape

The analysis of the interactions of gene products was done in the first place thanks to the software STRING, the results obtained will be treated later by the software Cystoscape and the plugin BiNGO. The purpose of this analysis is to determine the GO terms that are clearly over-presented in the set of genes and to review the biological processes altered during infection.

a. **STRING**

• **Identification of protein-protein interactions:** The differentially expressed genes were imported and analyzed by the STRING software. The results of networks showing the different interactions between proteins **(Figure :1)**. 30 genes are annotated on STRING, of which 28 have formed a single network and 2 genes remain outside this network. The result obtained shows the interaction between the proteins identified without additional proteins. 28 proteins were found to be bound either directly or indirectly through one or more interaction proteins, suggesting the existence of functional linkages between them. The thickness of the blue line between two nodes indicates the level of association confidence of these two nodes. The central hubs of this network are:

- Interleukin 6 (IL6) which is activated by 9 proteins.
- Fasligand (FASLG) is activated by 5 proteins and inhibited by 3.
- Forkhead box P3 (FOXP3), inhibited by 3 proteins.
- Chemokine (C-C motif) ligand 2 (CCL2) is activated by 5 proteins and inhibited by 3.
- IFNG occupies a central role in the network; it is linked with most proteins.

A group of proteins in reaction (black lines) consists of: CCL5 CCL16 CXCL2 CXCL10 CXCR1, which plays the common chemotactic role for monocytes and other lymphocytes to infection site, as an attempt by the host to fight disease.

• **Biological processes:** The analysis of the biological processes revealed the presence of different processes that were determined to participate significantly in this network (p-value <0.05). The cellular response to a biotic stimulus, the regulation of the immune response and the positive regulation of apoptosis **(figure :2)** are the three processes that have been investigated for their close relationship with our study.

b. CYTOSCAPE:

The genes treated by Cytoscape software were imported in TXT from STRING. This file contains the list of genes with the interactions that link them. The **Network Analyzer plugin** was used for network visualization **(figure :3)**. The central genes for this network according to the chosen parameter are: IL6, IFNG and CXCL10, which is in agreement with the results obtained by the STRING software **(figure :1)**.

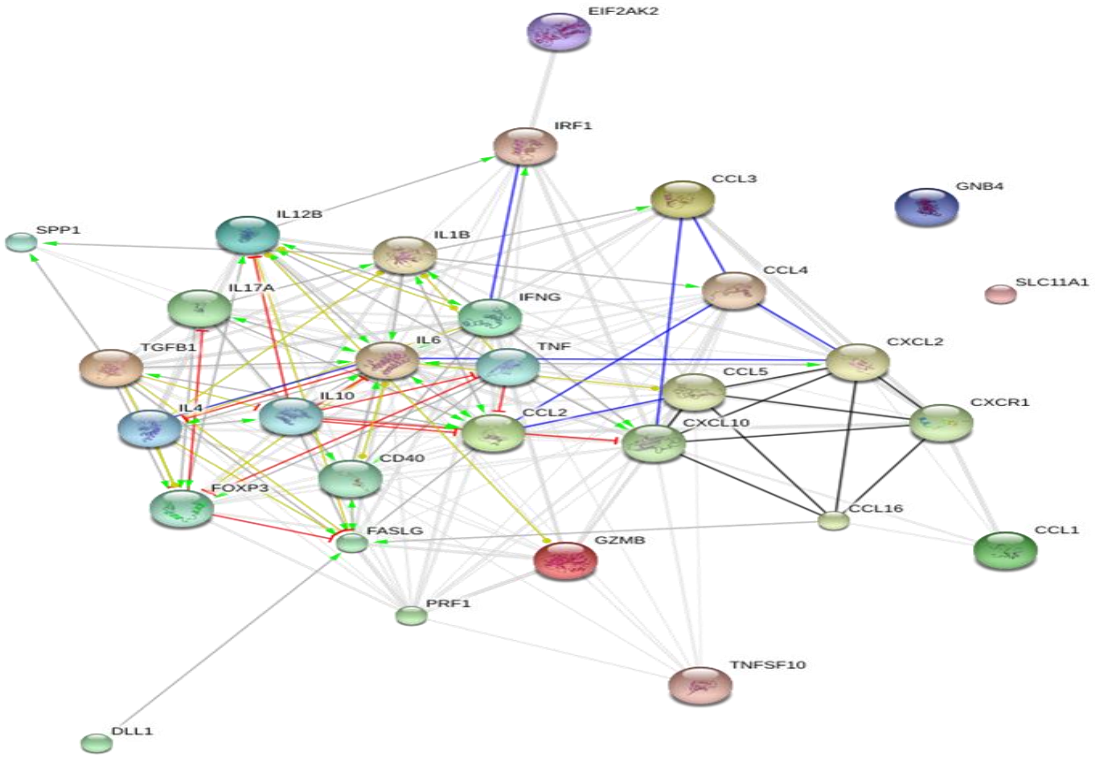

Figure-1: Types of interactions between the proteins of the network on STRING.

The nodes of the network are proteins. A confidence score is assigned for each predicted association. The higher it is, the higher the confidence level. The confidence score for each interaction is the probability that a predicted link exists between two nodes on the same metabolic plane in the KEGG database. The size of the nodes is the same for all the proteins whose three-dimensional structure is known, and it can be seen in transparency inside the node. The nodes whose three-dimensional

structure is not yet available are of small size.

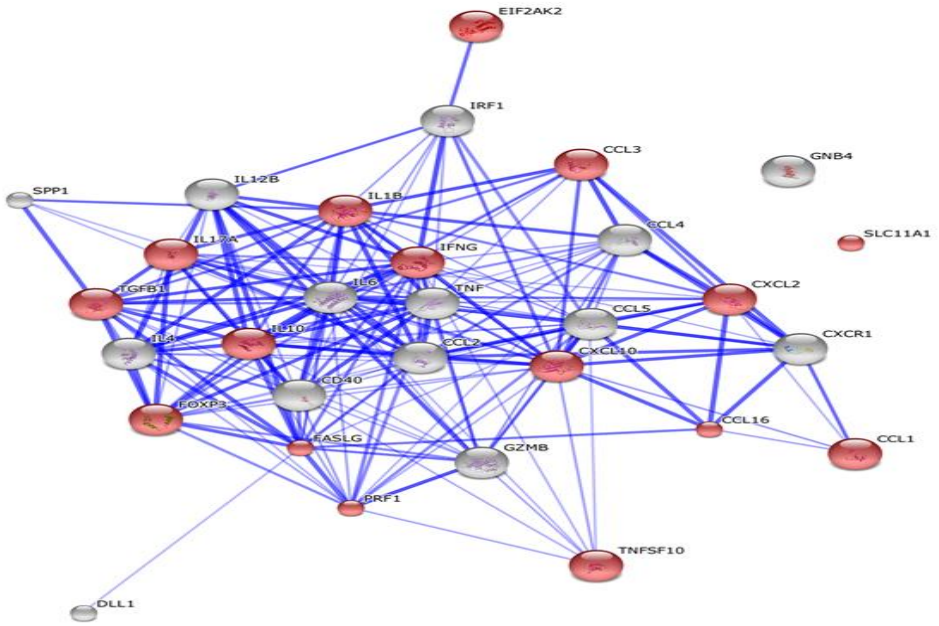

Figure-2: GO biological process on STRING: positive regulation of the apoptosis process.

The genes included in each biological process are colored red by selecting the process on the table.

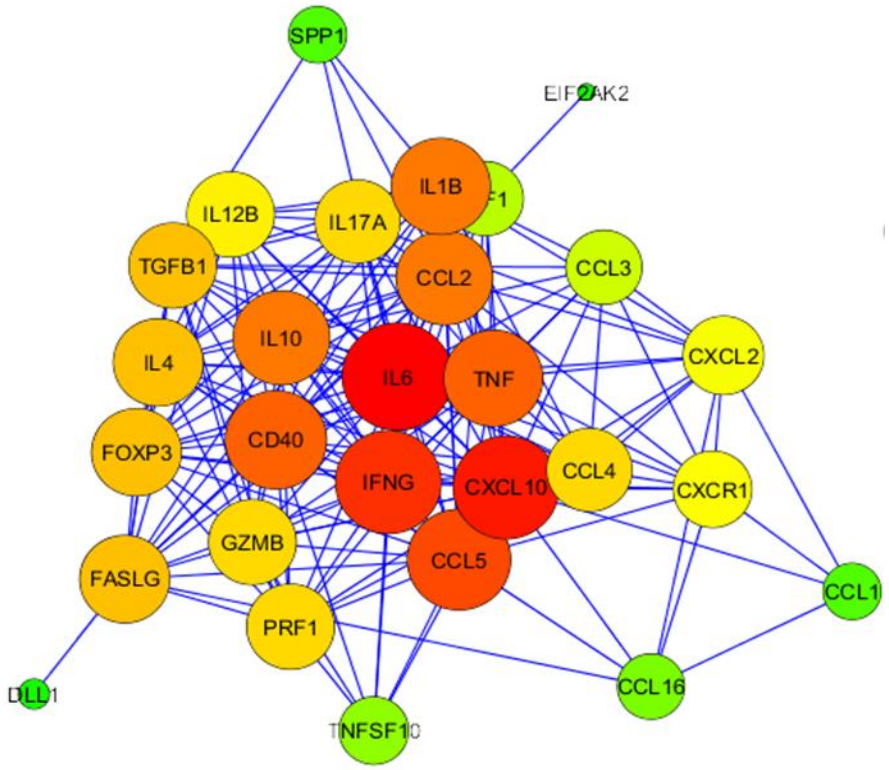

Figure-3: Gene network customized by the Network Analyzer plugin on Cytoscape.

On the network obtained, the size and color of the nodes was customized according to the values of the chosen parameter: here, according to the number of connections of the node with other proteins, large size and light color for low connectivity.

c. BiNGO:

• **Table and network of GO terms:** After the launch of BiNGO, the results of the GO terms found are displayed in two ways: The first is an array of GO terms found **(figure :4)** and the second is an acyclic direct graph; In which the nodes are the terms GO found and the directed lines link the terms parent to the terms child **(Data not shown)**. Significant GO terms in the table **(figure:4)** that are closely related to parasitic infection include response to a stimulus, inflammatory response, and regulation of apoptosis.

• **"TOP" functions:** The functions are grouped into biological processes and the most interesting according to the table are: response to a stimulus; The process of the immunity system and the process of apoptosis.

- Response to a stimulus: Of the 28 genes in interactions in the network, 24 genes are annotated in this biological process according to the table **(figure :4)**. This biological process has a very significant p-value of 3.3080E-11. The response to a stimulus is linked with other child terms in the DAG, the more significant are: response to an external stimulus, stress response and immune response **(figure :5)**. These results agree well with our current understanding of the in vivo functions of genes after infection by the parasite. The three processes that derive from the parent term **"response to a stimulus"** are the major steps that should be followed by macrophage after parasite infection, so changes in the expression of the genes involved in these processes will disrupt the functions of the cell to the benefit of the parasite.

- Immunity system process: Among the 28 genes in interactions in the network, 23 genes are annotated in this biological process according to the table **(figure:4)**. This biological process has a very significant p-value of 4.3177E-23. The process of the immunity system is linked with other child terms in the DAG, the most significant are: leucocytes migration, leukocyte activation and immune response **(figure:6)**. These three processes are associated with both the recruitment and activation of specific leukocyte subsets to the site of infection, as an attempt by the host to fight the disease, according to several studies Experiments. These results are in agreement and follow the chain of previous results.

- The process of apoptosis: Among the 28 genes in interactions in the network, 10 genes are annotated in this biological process according to the table **(figure :4)**. This biological process has a very significant p-value of 6.0906E-8. The process of apoptosis is linked with other terms child and a parent term in the DAG, the most significant are: programmed cell death, inflammatory cell apoptosis and apoptosis of myeloid cells **(figure :7)**. These 3 apoptotic processes are in agreement with several experimental studies, which showed that during the infection there is an immune suppression, and apoptosis of the lymphocytes is involved in this process. The upset expression of the genes associated with this lymphocyte depletion would therefore benefit the parasite.

GO,Homo Sapiens,default,bingo,namespace close

GO-ID	Description	p-val	corr p-val	cluster freq	total freq	genes
2376	immune system process	3.3784E-24	5.0169E-21	24/29 82.7%	948/14304 6.6%	IL4 CCL1 IL6 CCL3 CCL2 TNF CXCL2 FASLG DLL1 CD40 FOXP3 CCL16 CCL5 CCL4 IL10 ...
6954	inflammatory response	1.7915E-21	1.3302E-18	17/29 58.6%	315/14304 2.2%	IL6 CCL3 CCL2 TNF CXCL2 CXCR1 CD40 CCL16 CCL5 CCL4 IL10 TGFB1 CXCL10 SLC11...
6952	defense response	2.8251E-21	1.3984E-18	20/29 68.9%	620/14304 4.3%	IL4 PRF1 IL6 CCL3 CCL2 TNF CXCL2 CXCR1 CD40 CCL16 CCL5 CCL4 IL10 TGFB1 CXCL...
6935	chemotaxis	3.9790E-20	1.1818E-17	14/29 48.2%	169/14304 1.1%	CCL1 IL4 IL6 CCL3 CCL2 CXCL2 CXCR1 CCL16 CCL5 CCL4 IL10 CXCL10 IFNG IL1B
42330	taxis	3.9790E-20	1.1818E-17	14/29 48.2%	169/14304 1.1%	CCL1 IL4 IL6 CCL3 CCL2 CXCL2 CXCR1 CCL16 CCL5 CCL4 IL10 CXCL10 IFNG IL1B
6955	immune response	1.2511E-19	3.0966E-17	19/29 65.5%	619/14304 4.3%	IL4 CCL1 IL6 CCL3 CCL2 TNF CXCL2 FASLG CCL16 CCL5 CCL4 IL10 TGFB1 CXCL10 SLC...
40011	locomotion	5.3883E-19	1.1431E-16	17/29 58.6%	440/14304 3.0%	IL4 CCL1 IL6 CCL3 CCL2 TNF CXCL2 CXCR1 CCL16 CCL5 CCL4 IL10 TGFB1 CXCL10 IFN...
9611	response to wounding	1.7655E-17	3.2772E-15	17/29 58.6%	541/14304 3.7%	IL6 CCL3 CCL2 TNF CXCL2 CXCR1 CD40 CCL16 CCL5 CCL4 IL10 TGFB1 CXCL10 SLC11...
9605	response to external stimulus	2.4018E-17	3.9630E-15	17/29 58.6%	551/14304 3.8%	IL4 CCL1 IL6 CCL3 CCL2 TNF CXCL2 CXCR1 CCL16 CCL5 CCL4 IL10 TGFB1 CXCL10 IFN...
7626	locomotory behavior	3.6637E-17	5.4406E-15	14/29 48.2%	273/14304 1.9%	CCL1 IL4 IL6 CCL3 CCL2 CXCL2 CXCR1 CCL16 CCL5 CCL4 IL10 CXCL10 IFNG IL1B
1817	regulation of cytokine production	1.9056E-15	2.5726E-13	12/29 41.3%	202/14304 1.4%	SLC11A1 IL6 TNF CCL2 IFNG IRF1 IL1B IL12B CD40 FOXP3 TGFB1 IL10

Figure-4: Table of GO terms found by the BiNGO plugin.

The table showed us the most over-represented GO terms, sorted by their p-value (ascending from top to bottom). In the table, there is a list of GO terms (with their names and GO-IDs) the uncorrected p-value and p-value corrected. In addition, the total frequency values and a list of the corresponding proteins are listed for each term and listed under the heading "genes". Since the list is sorted just by p-value, many general terms, (less descriptive conditions) go back to the surface of the table, making it difficult to see more specific terms that are more useful.

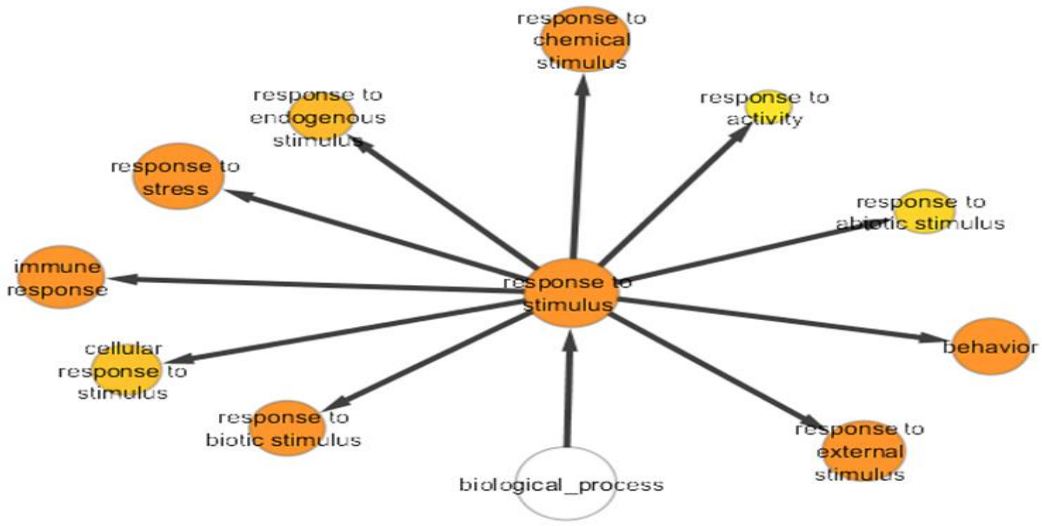

Figure-5: Acyclic oriented graph of GO terms overrepresented for "response to a stimulus".

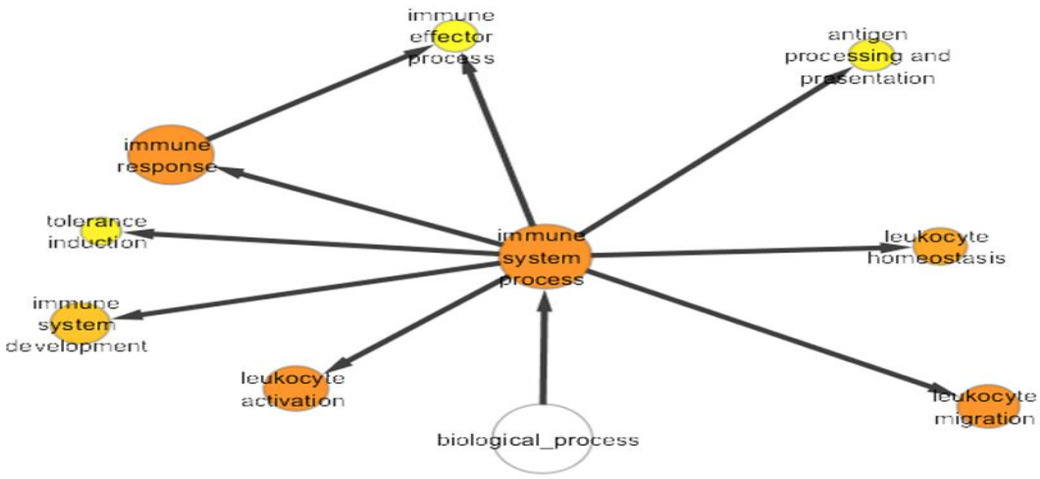

Figure-6: Acyclic oriented graph of GO terms overrepresented for the immunity system process.

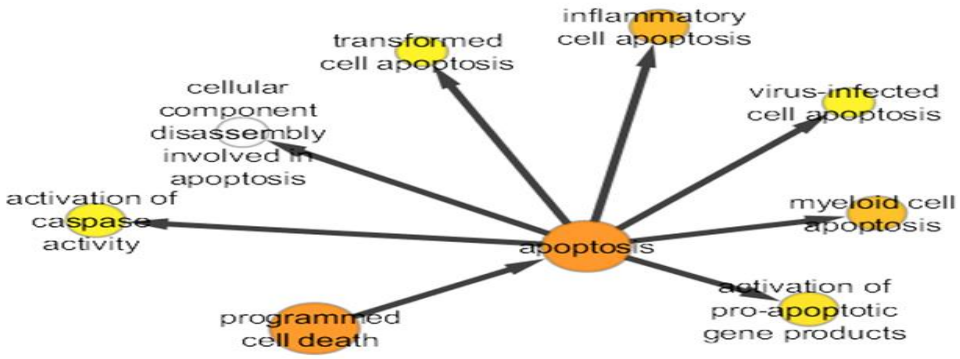

Figure-7: Acyclic oriented graph of GO terms overrepresented for the apoptosis process.

II. Results obtained by Ingenuity Pathway Analysis (IPA)

A list of 28 numbers acquisition human sequence was recovered and used as input to the IPA. The IPA software has mapped and annotated all of the 28 genes. The result is given in the form of networks. The network is a connection of several genes and the number of these genes represents the network score. Genes involved in each network are related by function.The result of analysis of the revelation about the involvement of all genes in seven networks. This last presents an interconnection with a score of 8 molecules (figure: 8). It is the largest network since it has the highest score. These molecules or genes have functions according to their location which is demonstrated in the network. In this network, the IL-10 and nuclear factor kappa B (NF-kB) are the central cores. Thus, they link the genes involved in cellular movement, hematological system development and function and immune cells trafficking. In particular, CCL 1, CCL16, DLL1, GZMB, IL-12b, IL-17A, IL-10 et SLC11A1.

In the network, there is four compartments with four types of clusters.

Compartment 1 "extracellular space": IL-10 is in the form of central node. is regulated by 13 different cellular localization genes. Four genes of the extracellular compartment (HLA-DQ, IL-23, IL-12, IL-17A), Five genes encoding membrane proteins (DLL1, GM-CSF, HDL, IFNγ, Notch), STAT5 a/b, secretase γ and JAK cytoplasmic and nuclear level there NF-kB. IL-10 is a pleiotropic mediator, it inhibits cytokine production by macrophages. At expressed under state acts as a suppressor of the immune response [23]. It also inhibits the ability of macrophages to kill intracellular organisms and maturation of dendritic cells from monocyte precursors [24]. **IL-17A** is regulated within this network by 11 genes. Three genes with an inhibitory effect The cytokine (IL-10, HDL, arginase) [25]–[27]. IL-17A is associated with inflammation of neutrophils. This cytokine may stimulate the expression of IL-6 and several chemokines, improve the production of nitric oxide (NO) and thereafter, potentiate the inflammatory reaction. However, its role remains controversial and poorly defined [28], [29].

Compartment 2 "Plasma Membrane": The **SLC11A** membrane protein, molecular transporter, is associated with infectious diseases and autoimmune and plays an important role in the activation of macrophages. In addition it is involved in the growth inhibition of intracellular pathogens [30], [31]. **Compartment 3 "cytoplasm": Granzyme B** (GZMB) is Activated by JAK and IL-12b, regulates both the inflammatory tissue reaction and control parasite replication in the cell [32]. Which triggers apoptosis by a caspase activation mechanism [33]. The second network **(figure: 9)** presents an interconnection with a score of 5 molecules. The interconnected molecules have functions according to their location. the top Functions, are **hematological system development and function; immune cells trafficking** and **inflammatory response**. The interconnected molecules are a part of our list of genes which includes: **CD 200, EIF2AK2, IRF-1, LTA, TNFSF10**. **EIF2AK2** is regulated within this network by 6 genes of different cellular localization Two genes from the extracellular compartment (TNSF10, IFN α / β). Two genes coding for Membrane proteins (TLR, TNF receptor), at the nuclear level there is IRF-1 and STAT1-STAT2. **The regulatory factor interferon 1** (IRF1), is a member of the interferon 1 regulator of transcription factor family. It is a transcription factor that fits in the regulation of gene expression at the level of the nucleus. Its role is the activation of transcription of genes induced by the interferons alpha, beta and gamma. It also plays a role in the regulation of apoptosis and tumor suppression [34]. **TNFSF10** is regulated within this network by 5 genes of different cellular localization. Two genes of the extracellular compartment (IFN α / β, IFNβ). A gene encoding Membrane protein (IFNAR), at the nuclear level there is IRF-1 and NF-kB. Within this **CD 200** network, inhibition of the ERK pathway[35]–[38]. CD200 is a type 1 membrane glycoprotein that has been identified as a molecule immunosuppressant, expressed by a wide range of cell types, including lymphoid cells, neurons and endothelium [39], [40]. is the ligand of a receptor (CD200R), the expression of which is restricted to hematopoietic cells, especially myeloid cells [38], [40], [41]. Various immunomodulatory roles for CD200 have been reported; these include the responses antigen-specific T cells, suppression of regulatory T cells [42], tumor suppression mediated by cytotoxic T lymphocytes [43] and the immune tolerance associated with apoptosis [44]. The way agents pathogens regulate the expression of CD200 is not clear [45]. **The third network (figure :10)** contains the molecules: ***INFγ, IL-6, SOD1, Foxp3, IL-4***, which regulate the transcription of genes that codes for the development and Functioning of the hematological system, immune cell traffic and response inflammatory. In the **fourth network (figure :11)**, the genes: ***CCL3, CCL4, CD40, PRF1*** are linked to the development and functioning of the hematological system, cellular movement and Immune response to cell mediation. The **Fifth Network** is involved in the development and functioning of the hematological system, cell traffic Immune and cellular movement. The **sixth network** includes genes linked to death and Cell survival, signaling and cell-cell interaction. The **seventh network** is involved in the inflammatory response, cell signaling and molecular transport. We note that this network is linked to the fifth by the molecule IL-8r.

DISCUSSION

The networking analysis of the various genes selected in the case of infection by Leishmania, showed an interconnection of the majority of genes **(figure: 1, 3, 8, 9,10)**. Include chemokines CCL 2 **(figure :1, 3)**, 3 and 4 which are secreted **(figure: 11)** in response to signals. The CCL2 has chemotactic activity for monocytes and basophils. He was involved in the pathogenesis of diseases characterized by monocytic infiltrates [46]. The **CCL3** and **CCL4** are inducible in most immune cells in response to various pro-inflammatory stimuli and are powerful chimoattractants to vital cells for innate and adaptive immunity. They can activate several chemokine receptors (CCR1 and CCR5), which initiate various cellular responses and regulate acute and chronic inflammation [47]. However, Leishmania can promote the secretion of chemokines to recruit their host cells, macrophages in the immune response [48]. The expression of genes coding for **CD40 (figure: 1, 3, 11)** and **MHC II (figure: 11)**, are required for antigen presentation and the induction of lymphoproliferation [49]. Thus, the transcription of IL-12, IL-1b, IL-23, IL-17A, GM-CSF **(figure :8)** and Toll-like receptor (TLR) **(figure: 9)** indicates, initiation of protective immunity Th1 against Leishmania [50], [51]. **L'IL-4, IL-10** mediated activation of macrophage (M2) [52]. Mφ M2 is labeled by the expression of several surface molecules, including *the scavenger A receptor* **(figure :11)**, *CXCR1* **(data not shown)** [52]–[54]. *CXCR1* is a receptor for chemokines that attracts PMN to inflammatory sites [55]. *CCL1* **(figure :8)**, *CCL2* **(figure :1, 3)** and *IL-1Ra* **(figure :9),** *Vegf* **(figure :10)** are also performed by Mφ that are alternately activated [54]. The expression of IL-4 and IL-10, and the BCR complex **(figure: 11)** that induce the production of immunoglobulins, are indicators of activation of the DCT 4+ Th2 cells triggering humoral immune response [49]. These two interleukins **(IL-4 and IL-10)** can also induce the expression of arginase-1 **(figure :8)**, which disrupts the generation of nitric oxide (NO), a mechanism considered as a Decreased resistance of macrophages to pathogens sensitive to this radical [56], [57]. Maximum gene expression of IL-10 only occurs after histone phosphorylation **(figure :10)** associated with specific sites in the IL-10 promoter [58]. It inhibits the production of inflammatory cytokines, particularly TNF, by macrophages [53]. So, in the presence of IL-4 **(figure: 1, 3, 10)**, the engagement of CD40 not only causes the B cell proliferation, but also increased production of cytokine LTA [59]. **The lymphotoxin alpha** (LTa) **(figure :9)** is a family member of the tumor necrosis factor, it is necessary to control the growth of the parasite in the liver. It also plays a key role in the formation of granulomas, which facilitates the trafficking of lymphocytes perivascular area of the liver Kupffer cells infected [60]. The soluble lymphotoxin participates in inflammatory responses, including the activation of macrophages and endothelial cells [61].

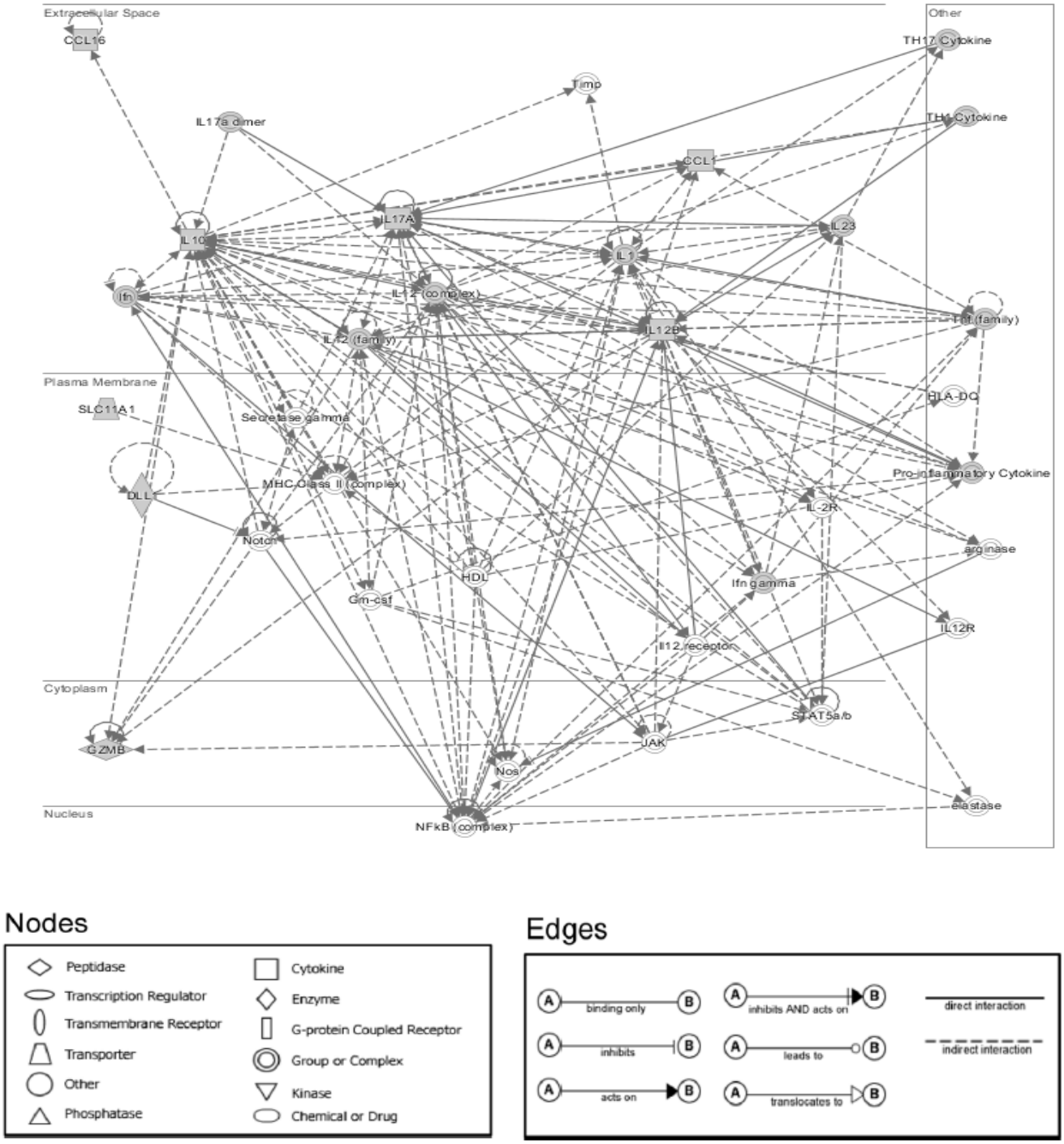

Figure-8: Network with the highest likelihood score (17) shows the interaction between 8 genes. These genes have been found linked either directly or indirectly.

The network shows the genes as nodes, having different shapes, which represent the functional class of the gene. Connections between nodes are represented by continuous or discontinuous lines. These lines show the direct interactions (solid lines) and indirect (dotted lines) between the genes. The nodes are normally represented by a red and green shading indicating the genes expressed and repressed, respectively, and the intensity of the color indicates the degree of modulation. Since this analysis was based on the identifiers of genes (ID) only and devoid of gene expression values, these nodes are shaded.

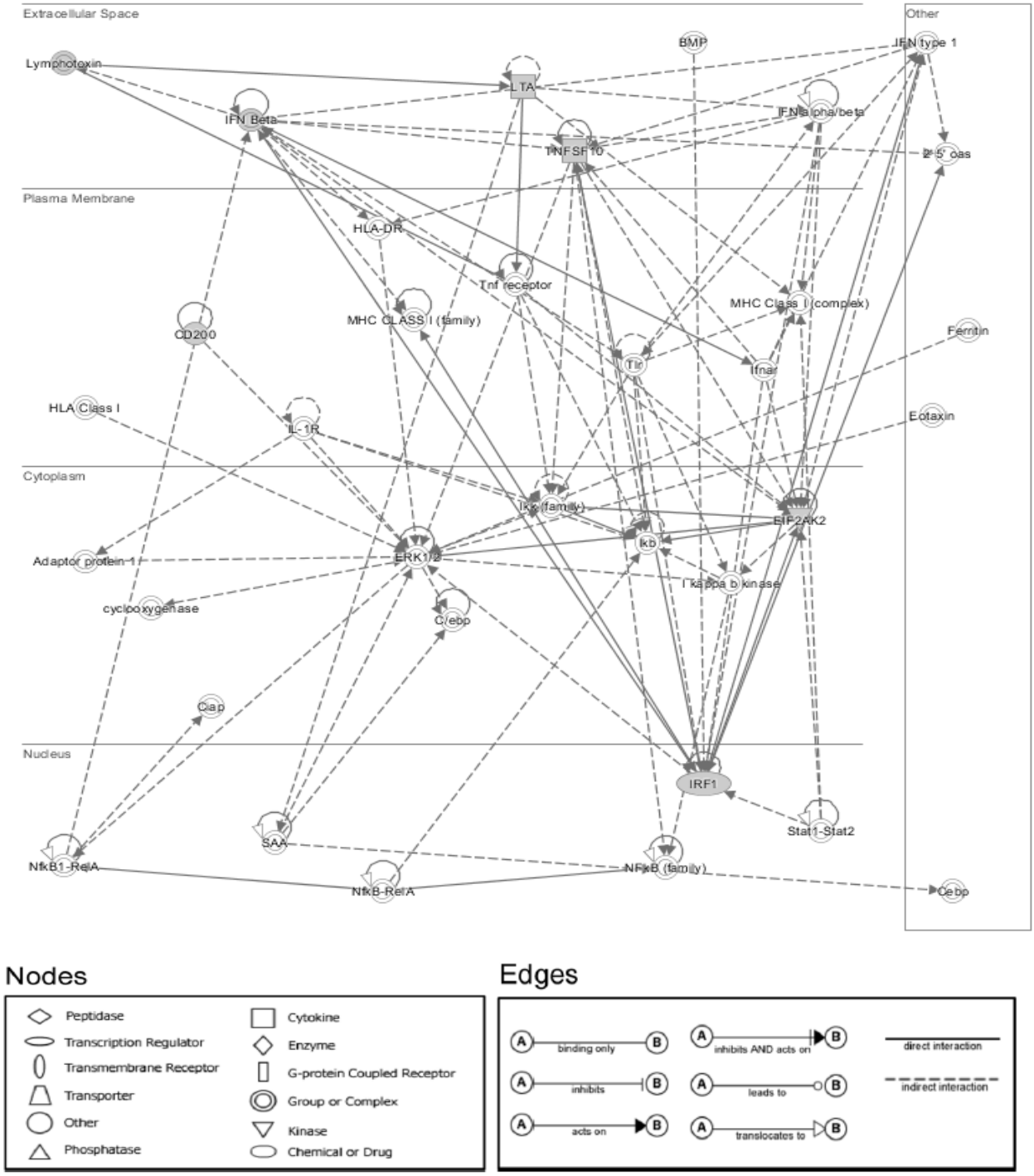

Figure-9: Second network with a score of 10, shows the interaction between 5 genes. These genes have been found linked either directly or indirectly

The network shows the genes as nodes, having different shapes, which represent the functional class of the gene. Connections between nodes are represented by continuous or discontinuous lines. These lines show the direct interactions (solid lines) and indirect (dotted lines) between the genes. The nodes are normally represented by a red and green shading indicating the genes expressed and repressed, respectively, and the intensity of the color indicates the degree of modulation. Since this analysis was based on the identifiers of genes (ID) only and devoid of gene expression values, these nodes are shaded.

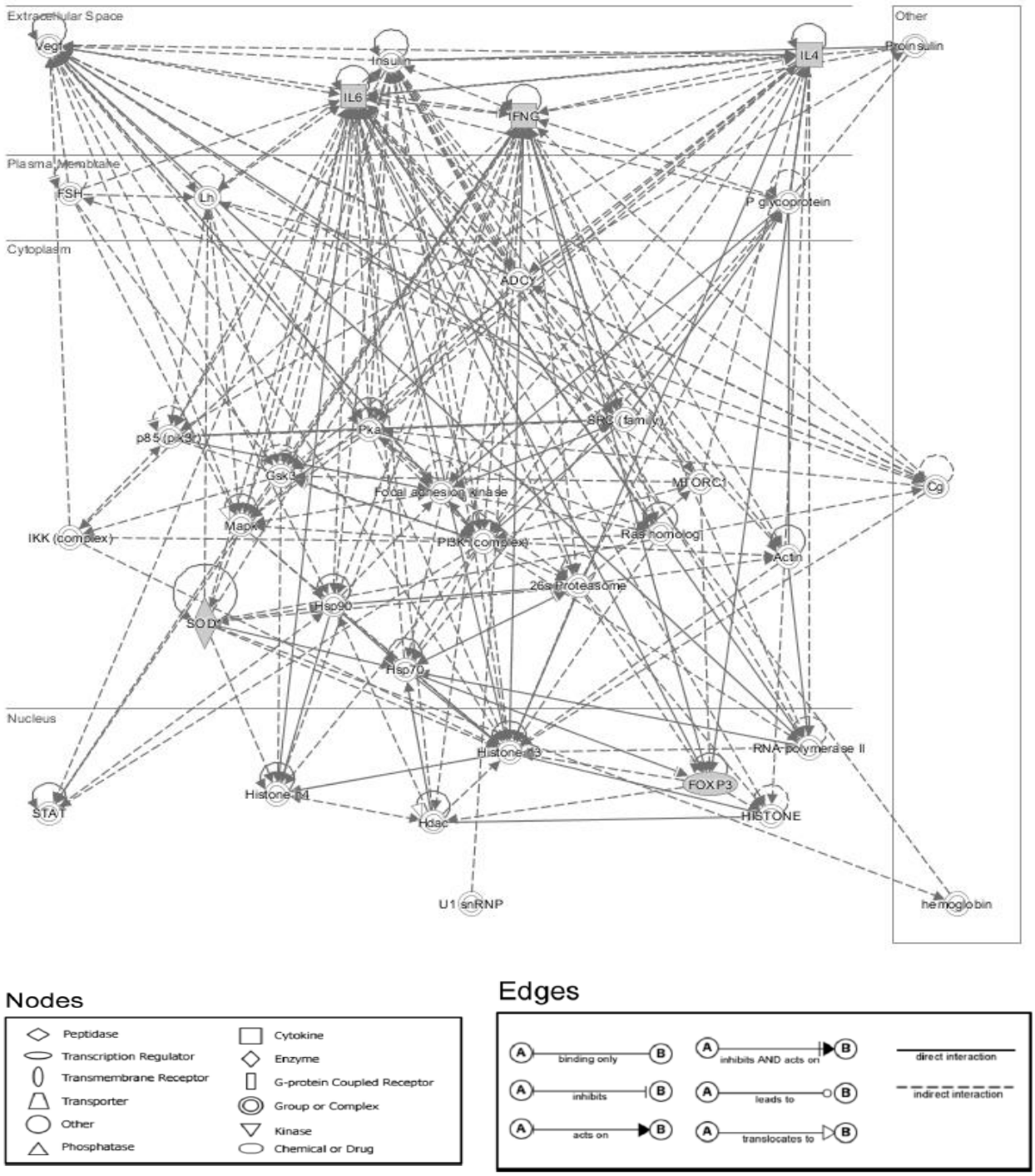

Figure-10: Third network with a score of 10. These genes have been found associated either directly or indirectly.

The network shows the genes as nodes, having different shapes, which represent the functional class of the gene. Connections between nodes are represented by continuous or discontinuous lines. These lines show the direct interactions (solid lines) and indirect (dotted lines) between the genes. The nodes are normally represented by a red and green shading indicating the genes expressed and repressed, respectively, and the intensity of the color indicates the degree of modulation. Since this analysis was based on the identifiers of genes (ID) only and devoid of gene expression values, these nodes are shaded.

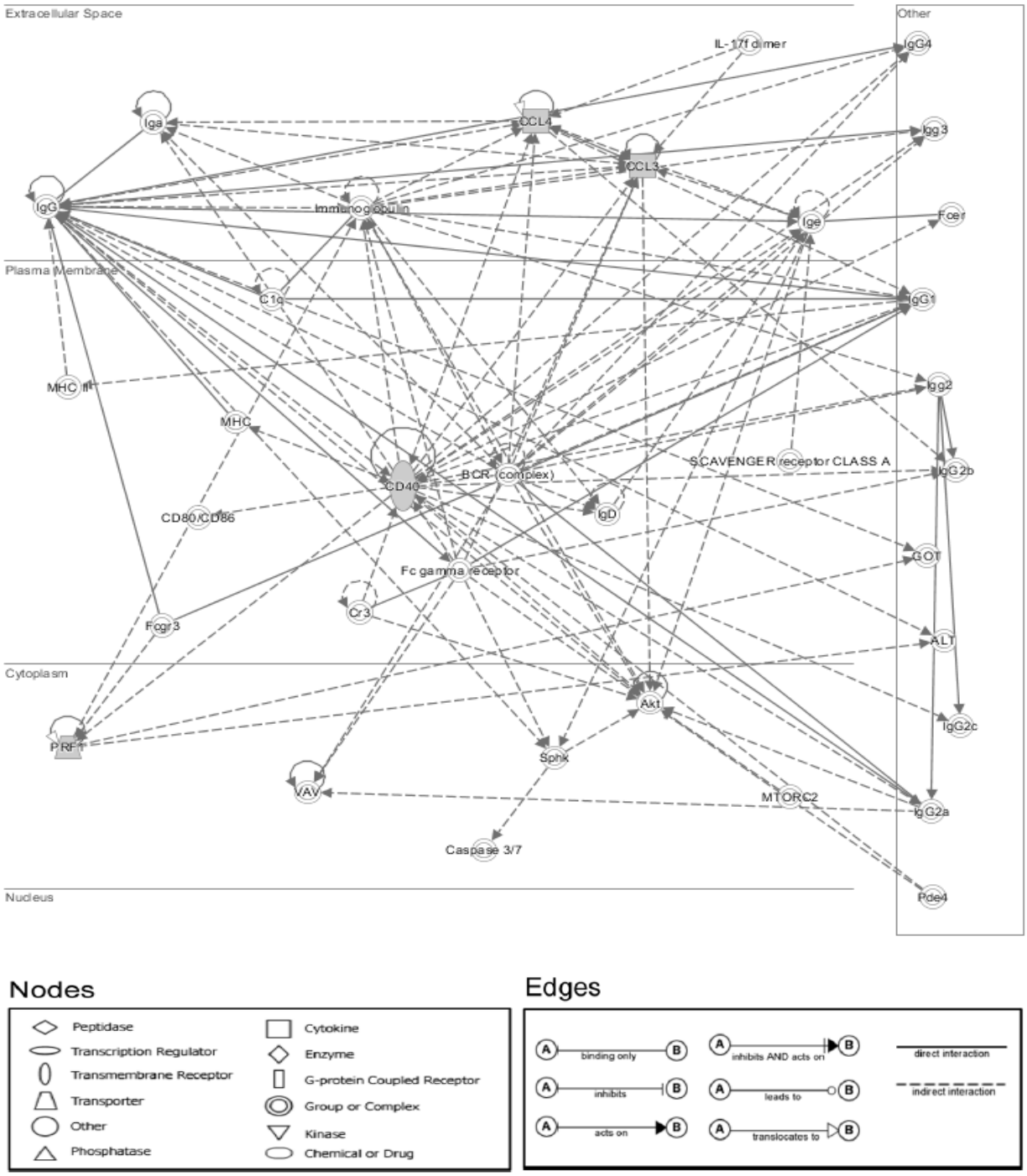

Figure-11: Fourth network, with a score of 7. These genes have been found associated either directly or indirectly.

The network shows the genes as nodes, having different shapes, which represent the functional class of the gene. Connections between nodes are represented by continuous or discontinuous lines. These lines show the direct interactions (solid lines) and indirect (dotted lines) between the genes. The nodes are normally represented by a red and green shading indicating the genes expressed and repressed, respectively, and the intensity of the color indicates the degree of modulation. Since this analysis was based on the identifiers of genes (ID) only and devoid of gene expression values, these nodes are shaded.

The **DLL1 (figure :8)** belongs to the family of ligands Notch, selectively causes type CD4 T helper cell 1 independently of cytokine signaling. They play an important role in the protective immune response against leishmaniasis [62], [63]. When interacting with its ligand, the intracellular domain of Notch active is released from the membrane by proteolytic cleavage and translocates into the nucleus [62]. **Nuclear factor kappa B** (NF-kB) **(figure :8, 9)** is activated during infection with Leishmania [33], [64]. It plays an important role in the initiation of innate immune responses [65], [66]. It also regulates the expression of a number of immunological mediators [67], [68]. It also has an anti-inflammatory role in directly inhibiting the expression of pro-inflammatory genes and by modulating the expression or activity of anti-inflammatory cytokines such as IL-10 [68]. The prediction obtained as a network shows that the Leishmania parasite activates NF-KB via the PI3K/Akt, leading eventually to the transcriptional repression of iNOS [69]. In addition, activation of the transcription of NF-kb seems to be crucial in protecting against apoptosis of cells infected with intracellular pathogens [64], [70]–[72]. On the other hand, NF-kb induced IRF-1 activation **(figure :9)** [73]. This transcription factor is an immunomodulatory factor, it works downstream of the pathogen recognition receptor signaling [74]. Furthermore, activation of **PI3K** is due to the inhibition of the production of IL-12 **(figure :10)** [75], [76]. Thus, PI3K is known to mediate the recruitment and activation of Akt [77], [78]. One of the targets of Akt is glycogen synthase kinase 3b (GSK-3b) **(figure: 10)**, which undergoes inactivation [79]. The inhibition of GSK-3b promotes the production of an excess of IL-10 at the expense of pro-inflammatory cytokines [80], [81]. Therefore, the PI3K/Akt confers resistance to the host cell, macrophage, apoptosis [64]. Many protozoan pathogens use this method to block certain pro-apoptotic molecules or high regulatory proteins which are anti-apoptotic drugs, such as Akt promote their own survival and intracellular persistence [82], [83]. The **IFN-α/β (figure :9)** are responsible for the early induction of iNOS [33]. Thus, interleukin 12 (IL-12) is a critical cytokine required for the development of CD4 + Th1 and IFN-γ [84]. The **IFNγ (figure :10)** occupies a central role in the networks, it is linked with most genes. This result agrees with the results obtained by the software STRING **(figure: 1)** and Cytoscape **(figure :3)**. Its role as an activator of macrophages powerful puts this result in line with the reaction of the body facing the infection by the parasite [85]. **Interleukin-6** (IL-6), **(figure :1, 3, 10)** a pleiotropic cytokine developed in response to a wide range of inflammatory stimuli, including intracellular infection. It is usually seen as a pro-inflammatory factor [86]. IL-6 activates the JAK-STAT pathway. Outside of the signaling pathway JAK-STAT, also activates the ERK-MAPK pathway [87], [88]. In addition, IL-6 can activate the PI3K/Akt pathways that mediate the anti-apoptotic signal [88], [89]. The transmission of extracellular signals to their intracellular targets is conveyed by a protein interaction network that regulates many cellular processes. The complex MAPK **(figure :10)** (ERK1/2, JNK and p38), is part of the signal transducers, they regulate macrophage functions including the production of pro-inflammatory cytokines and NO [90]. The transducers also regulate Activated Protein-1 (AP-1)

through the JNK and ERK [91]–[93]. **The Activated Protein-1** (AP-1) **(Data not shown)** is an important transcription factor involved in the regulation of several genes (TNFa, iNOS and IL-12) in response to external or internal stimuli. These genes are involved in the activation of biological functions of the macrophage cell during Leishmania infection [94]–[96]. **PI3K/Akt (figure :10)** has an inhibitory effect on the MAP kinases, p38 and JNK. By cons may induce activation of the ERK signaling **(figure :9)**. The inhibition of JNK may cause inactivation of AP-1 and therefore FasL **(figure : 1, 3, sixth network IPA: Data not shown)** [92], [97], [98]. However, ERK, keeps the activation of AP-1 [91]. These results suggest that altering the activity of AP-1 can significantly contribute to the suppression of innate immune function observed in the early stages of infection with Leishmania. **The eukaryotic initiation factor 2α kinase2** (EIF2AK2) **(figure :1, 3, 9)**, is phosphorylated in response to stress signals, primarily due to viral infections. The activation of the latter induced inhibition of protein synthesis [99], [100]. The expression of the **superoxide dismutase 1** (SOD1) **(figure :10)**, is induced by the activation of MEK-ERK [101]. SOD1, belonging to the family of superoxide dismutase and catalysts for the dismutation reaction of superoxide anions, reduces the release of superoxide [102], [103]. **The perforin** (PRF) **(figure :11)** induce cell death with granzyme forming a pore in the target cell [33]. In contrast, inhibition of p38 pathway counteract its activity during infection by Leishmania [104]. These results are consistent with the role of the parasite in the alteration of macrophage functions after the infection. **Fc gamma receptors** (FcγR) **(figure :11)** expressed on the membrane surface of macrophages, bind to the Fc portion of antibodies and activates ERK signaling, leading subsequently to the production of IL-10 [105], and activation of the PI3K [106]. The **TCR-CD3 (Data not shown)** complex can induce the dephosphorylation of STAT5 and also activates ERK and MEK via inducing under-expression of Foxp3. PI3K/Akt inhibitor has the same effect on FoxP3 [107], [108]. However, the expression of Foxp3 control immune responses by their suppressive activity [108]. GM-CSF, TLR ligands and IL-4 promote activation of **mTORC1 (figure :10)** and **mTORC2 (figure :11)** in the immune cells, more specifically DCs, neutrophils, monocytes and macrophages [109]–[120]. The activation of mTORC1 and mTORC2 controls a wide range of basic cellular processes such as protein translation and synthesis, cell growth, metabolism and anabolic processes. MTORC2 also improves glycolytic metabolism by activating the AKT [121], [122]. Thus mTOR negatively regulates the GSK-3 activity to limit production of IL-12 [114], [117]. Our data is consistent with mTORC1 acting as negative regulator of IL-12 and facilitator of production of IL-10 after exposure to microbial agents. Overall, the functions related to the majority of IPA software networks are involved in the development and functioning of the haematological system, trafficking in immune cells, cellular movement, cell-mediated immune response, inflammatory response and death and cell survival. These top functions are coherent with the biological processes suggested by cytoscape : the response to a stimulus ; The process of immunity system and the process of apoptosis. Taken together, these results suggest that infection with

Leishmania, mobilize the immune system to respond to infection by the secretion of cytokines and proinflammatory chemokines. These cytokine (IL-12, IL-1β, IL-17A, IL-23, IL-6 and INFγ) and pro-inflammatory chemokines (CCL2, CCL3 and CCL4) ensure communication between immune cells and modulate the inflammatory response by the release of several intracellular signaling pathways (p38 and JNK, JAK, MTORC1 and 2, NOTCH, STAT5) via membrane receptors (CD-40, TCR-CD3, GM-CSF, HLA-DR, BCR, MHC II, CXCR1, Fcγr3, SLC11A1, DLL1-NOTCH1, Scavenger receptor class A, TLR) to induce an inflammatory response. Accordingly, the Th1 cytokines (IL-12, IL-1b and INFγ) appears to have a protective effect against infection by Leishmania and, conversely, the Th2 cytokine profile, IL-10 and IL 4 could therefore contribute to the progression of the disease. The results of this study are consistent with other results that affirm that genes and other molecules do not work independently. Thus, the study of the gene in its environment gives the opportunity to know its expression and to understand the complex relations between the biological entities in order to decipher the function of the genes, the process in which they intervene and to be able to understand the functioning of the cell.

4.CONCLUSION

In this study, we were interested in exploiting the data analysis and retrieval capabilities of the bioinformatics tool to analyze a set of genes differentially expressed in the host during the infection by the parasite Leishmania. This set of genes, obtained from different data bases and targeted literature. This approach has allowed us to establish a global network of interactions between differentially genes expressed during the infection. Thus, the results provided by this study, showed some response mechanisms to infection with Leishmania, and Amastigote strategies to reverse the immune and inflammatory responses.

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