



Reconstruction of Intercellular Signaling Network by Cytokine-Receptor Interactions

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Background: The immune system function depends on the coordination activity of the components of system and communications between them which leads to the formation of a complex communication network between immune cells. In this network, cytokines have an important role in the communication between immune cells through the interaction to their specific receptors. These molecules cause to cellular communications and normal function of a tissue. Reconstruction of such a complex network can be a way to provide a better understanding of cytokines' function.

Objective: Our main goal from reconstructing such a network was investigation of expressed cytokines and cytokines receptors in various lineage and tissues of immune cells and identifying the lineage and tissue with the highest expression of cytokines and their receptors

Materials and Methods: In this study, gene expression data related to part of the Immunological Genome Project (ImmGen) and receptor-ligand interactions dataset were used to reconstruct the immune network in mouse. In next step, the topological properties of reconstructed network, expression specificity of cytokines and their receptors and interactions specificity were analyzed.

Results: The results of the network analysis were indicated that non-hematopoietic stromal cells have the highest expression of cytokines and cytokine receptors and interactions specificity is very high. Our results show that chemokine receptor of Ccr1 receives the largest number of signals between receptors and only expressed in three hematopoietic lineages.

Conclusions: The most of the network communications belonged to non-hematopoietic stromal and macrophage cells. The relationships between stromal cells and macrophages are necessary to create an appropriate environment for differentiation of immune cells. Studying the cellular expression specificity of receptor and ligand genes reveal the high degree of specificity of these genes that indicate non-random transfer of information between cells in multicellular organisms.

Keywords: Cytokines, Intercellular Signaling, Immune system, Network reconstruction

1. Background

For responding to internal and external stimuli through immune system, various types of cells interact with each other by their specific synthesized molecules. Hence the function of interacting network depends on the coordinated activity and communications between network components (1, 2). Communications between different types of immune cells are carried out through secretory molecules and their receptors. For instance, cytokines and their receptors allow the immune cells to communicate with each other (3). These molecules shaped the cellular organization of mammals CNS in

response to invasive agents (4, 5).

The Cytokines have significant role in regulating cellular communications and normal function of a tissue. Disruptions in regulating of cytokines is associated with the incidence of neuroinflammation diseases and pathogenesis of skin inflammatory diseases (6-8). In recent years, studies related to cancer have demonstrated that group of cytokines known as interleukins have anti-cancer activity which can be used to treat cancer (3, 9,10, 11, 12, 13, 14). Cytokines have dual function which can activate and suppress the immune system (3, 15). Reconstruction of such a complex network can be

a way to provide a better understanding of cytokines multifaceted function (16).

We used transcriptome and receptor-ligand interaction data for reconstruction the immune network in mouse. Cytokine-receptor interactions and gene expression data related to them were gathered from public protein-protein interaction databases by iRefWeb interface and GEO database respectively. The results of the network analysis were indicated that interactions specificity is very high. Our results show that chemokines receptor of Ccr1 is signal commune receiver and only expressed in three hematopoietic lineages.

2. Objective

In this study, cytokine-cytokine receptor network in immune system reconstructed to investigate and identify of expressed cytokines and cytokines receptors in various lineage and tissues of immune cells and identifying cytokines and their receptors with the highest expression level. In order to investigate the specificity of intercellular signaling process, cytokines-cytokines receptor cell-type expression specificity was calculated.

3. Materials and Methods

3.1. Data Collection

For reconstructing the intercellular interaction network through cytokines and their receptors, our datasets include cytokine-receptor interaction and gene expression of cytokines and their receptors were needed. For creating the interaction dataset, first the cytokines and cytokine receptors datasets were created by data extraction from IUPHAR (<https://www.guidetopharmacology.org/>) (17) and KEGG (<https://www.genome.jp/kegg/>) (18) databases. In the next step, to investigate the interactions between cytokines and receptors, we used protein-protein interaction dataset which was extracted from the iRefWeb interface (<http://wodaklab.org/iRefWeb/>) (19), and by cytokine and their receptors datasets which was collected from KEGG and IUPHAR databases, cytokine-receptor interactions obtained of protein-protein interaction dataset. Finally, we completed our datasets by adding cytokines-cytokines receptors interaction dataset from KEGG database that included 484 interactions between 158 cytokines and 154 cytokine receptors.

For creating transcriptome dataset, we selected a dataset with GSE15907 serial number which was related to Immunological Genome Project (<http://www.immgen.org/>) (20) for constructing expression datasets from GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) (21). In this project, the Affymetrix 1.0 ST

MuGene arrays was used for gene expression profiling. This dataset includes 653 cell samples which consist of hematopoietic lineages and non- hematopoietic stromal cells. The hematopoietic lineages were created based on lineages tree and include lymphocyte B and T, monocyte, macrophage, granulocyte, natural killer cells, dendritic cells, hematopoietic progenitor and stem cells (22).

These samples were extracted from lymph node, spleen, liver, kidney, bone marrow, lung, pancreas and skin. For analyzing this dataset, we used limma package in R and RMA normalization method (23, 24). Finally, a gene expression set include 25751 probes was created which each of them related to a specific gene name. Probes which related to receptors and ligands were extracted by using 312 cytokine receptor-ligand gene ID in receptor-ligand interaction datasets. After identifying receptor-ligand genes in each sample we used threshold for identifying receptor and ligand genes.

3.2. Network Reconstruction

For reconstructing our network, we extracted the interactions which receptor and ligand expression were more than threshold so the intercellular communications were investigated by identifying interactions. After network reconstruction, the specificity of ligand and receptor genes expression, interaction specificity and also the network topological features were analyzed. Reconstructed network visualized by cytoscape software (25).

4. Results

The reconstructed network includes 3376 communications between 120 cells that these communications were established by 167 interactions between 85 cytokines and 82 cytokine receptors. In this network there are 25 cells with 77 autocrine communication (77 loops) in which the secreted cytokines by each cell activate the membrane receptors of the same cell. From these 25 cells, 2 granulocyte cells which related to synovial fluid and peritoneal cavity tissues have the highest number of loop.

In this network, stromal cell of skin tissue (FI.SK) communicated with other cells through 285 communication pathways and as sender and receiver of signal respectively in 145 and 140 of pathways had the most communications. The most transmitted signals by FI.SK were sent through Ccl8 and Ccl7 chemokine with 25 and 16 communications respectively and the most signals were received by interleukin and bone morphogenetic protein receptors like Il1rap and Bmpr1a with 29 communications for each one.

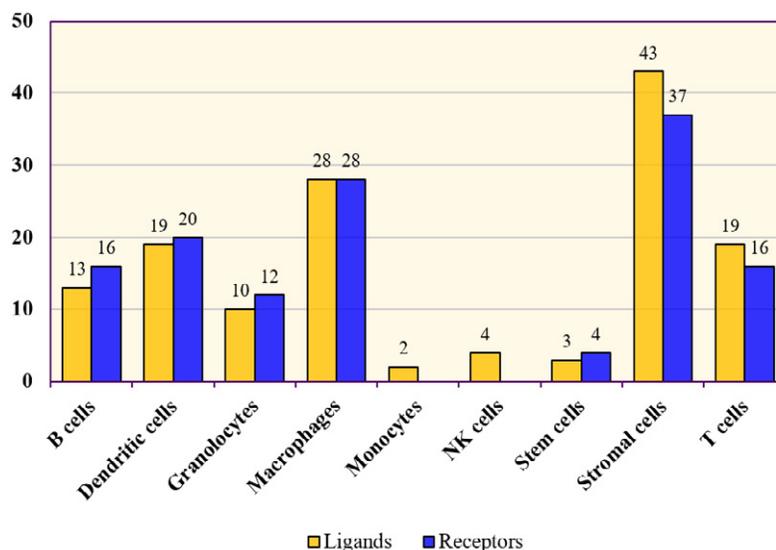


Figure 1. Evaluation of receptors and ligands expression in different lineages

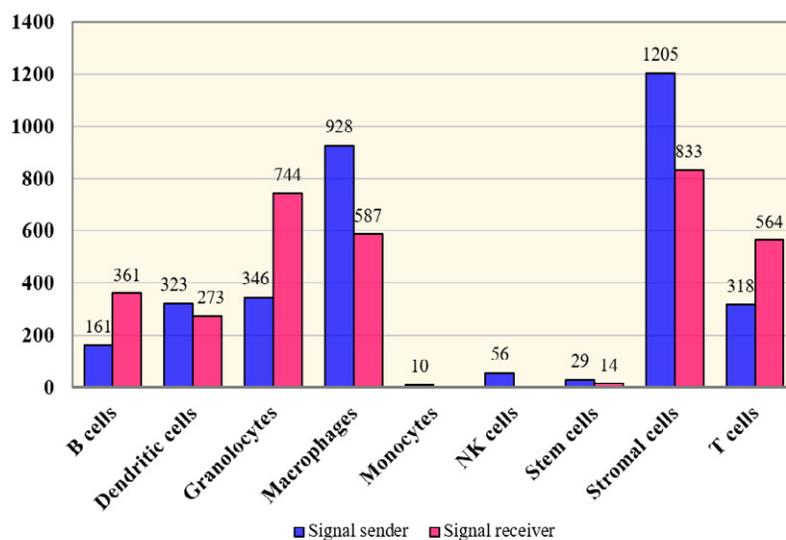


Figure 2. The number of transmitting and receiving signals by 9 cell lineages

4.1. Non- Hematopoietic Stromal Cells Have the Highest Expression of Receptor and Ligand Genes.

The analysis of receptor and ligand gene expression data show that non- hematopoietic stromal cells have the highest expression (**Fig. 1**). Among hematopoietic lineages cells, macrophages have the highest receptor and ligand gene expression. According to **Figure 1**, natural killer cells and monocyte have the lowest expression so that they just express ligand genes and act as signal senders.

Analysis of transmitted and received signals by different cell lineages shows that the stromal cells with sending and receiving 1205 and 833 signals respectively, have the most communications in network (Figure

2). Among hematopoietic cell lineages, macrophages and granulocytes have the most transmitted and received signals respectively. The monocytes lineage with transmitting two signals have the least number of transmitted signals among all cell lineages. This lineage and natural killer cells lineage don't receive any signals of other cells (**Fig. 2**).

Among 3376 communications in network, Ccr1 (chemokine receptor) act as receptor in 504 communications. Although Ccr1 has the highest expression among all receptors in our network, it doesn't express in stromal cells which are the most signal receiver cells in the network. Ccr1 is only expressed in macrophage, granulocyte and lymphocyte

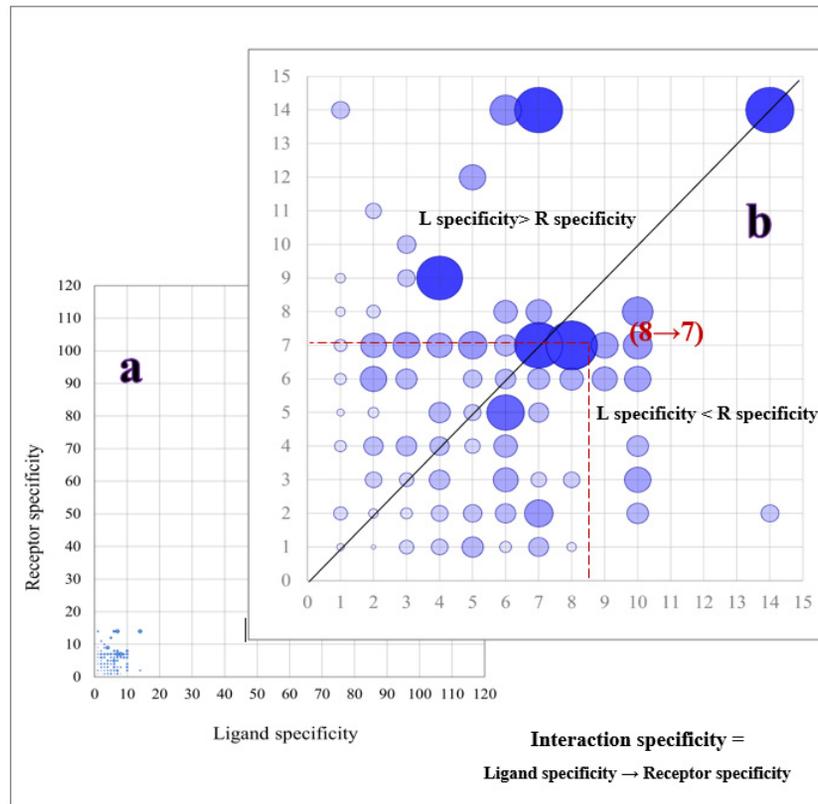


Figure 3. Evaluation of expression specificity of 167 interacting cytokine–cytokine receptor pairs. (a) Based on 120 expression profiles, expression specificity of 167 interacting ligand–receptor pairs in these profiles have been shown. (b) The frequency of interactions in each specificity degree.

T hematopoietic lineage cells. In 224 communications, Ccl2 act as ligand for sending signal from stromal, dendritic cells and macrophages to other cells.

4.2. In Network, Cellular Expression Specificity of Receptor and Ligand Is High.

The analysis of gene expression specificity of receptor and ligand which involved in each interaction is shown in **Figure 3a**. The lowest specificity degree of interactions in intercellular interaction network with 120 cells is 120 for both receptor and ligand genes. Distribution of receptor and ligand interactions specificity among 120 cells indicates the high specificity of these interactions, so that interactions in which the ligand and receptor specificity is equal to fourteen (14→14) are interactions with the lowest specificity degree.

In the next step, the frequency of interactions (the total frequency of interactions is equal to the number of communications in our network) at each levels of specificity were measured (**Fig. 3b**). The highest frequency of interactions belongs to the specificity

degree of 8→7 (8 specificity for ligand and 7 for receptor) with 224 interactions and the lowest frequency is related to the specificity degree of →1 (2 interactions). From 3376 interactions, the frequency of interactions in which the receptor's specificity is higher than the ligand is 1,382. In 1478 interactions, we also observed the specificity of the ligand is greater than the receptor and in 516 interactions, the specificity degree of receptors and ligands are equal (circles on the main axis of the scatter plot). In **Figure 3b**, each circle in the scatter plot represents a degree of specificity which is shown by two numbers, the first and second numbers represent the ligand specificity and receptor specificity, respectively. Blue color Intensity of circles scales with the number of interactions in each specificity degree. Circles located in the above area of the main diameter of the plot show the frequency of interactions in which the receptor specificity is greater than the ligand and vice versa, in the lower region of the main diameter, there are interactions with the higher ligand specificity than receptor.

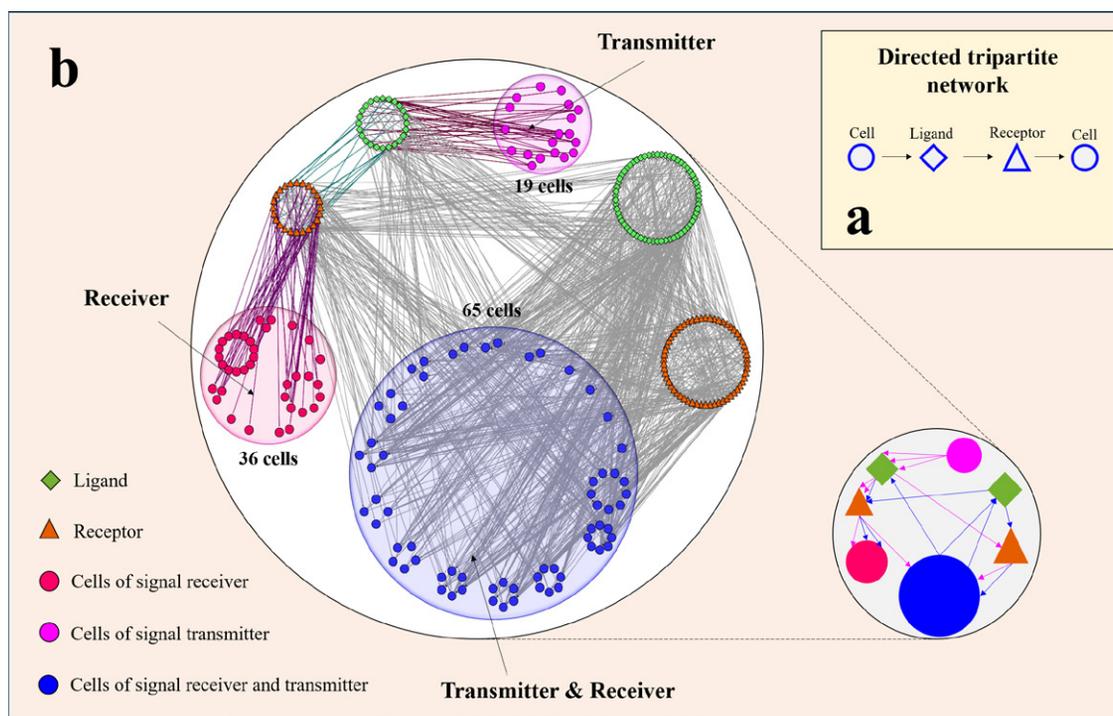


Figure 4. Tripartite network of intercellular communication among 120 cells. (a) The network consists of three components of the cell, receptor and ligand, and therefore there is not edge between the members of each component. (b) Reconstructed network of intercellular communication that three components of it was represented with different colors.

4.3. More than Half of the Cells Are Transmitter and Receiver Signal.

From 120 cells in our network, 19 and 36 cells are just the signal transmitter and signal receiver respectively, and the rest of them (65 cells) act as both receiver and transmitter of signal. This directed network is visualized based of tripartite template that include three components of cells, receptors and ligands (**Fig. 4a**). The number of nodes and interactions are 287 (120 cells, 85 ligands and 82 receptors) and 883 respectively (**Fig. 4b**). Cells that are only sender of the signal are involved in cellular communication through 24 ligands, and cells that are only receiver of the signal are received signals through 24 receptors.

5. Discussion

In reconstructed network, stromal cells have the most network communication compared to hematopoietic cells and it seems that hematopoietic cells use fewer pathways to communicate with one another (26). The most expressed genes in stromal cells are *Ccl8* and *Ccl7* chemokine and *Il1r1*, *Il1rap* and *Bmpr1a* receptors. Chemokine ligands and their receptors have the most number of sent and received messages. Chemokines play an important role in inflammatory conditions

and their connection to specific receptors increase intracellular calcium and cellular responses by target cells (27, 28). Activated chemokine receptors cause the activation of the MAP-kinase pathway which is important in chemotaxis and this pathway changes the cell adhesive proteins (29).

Among expressed receptors by network cells, *Ccr1* receptor has the highest expression but in stromal cells not express. This receptor commonly expressed in bone marrow stromal cells that expression data these cells not available for network reconstruction (30, 31). Stromal cells acts only as the sender of the signal to the cells containing this receptor such as macrophage, granulocyte and lymphocyte. Expression of *Ccr1* in granulocytes for response to inflammatory stimuli is essential (32) and in macrophages acts as chemotactic receptor (33). High cell-type expression specificity of receptors and ligands as PM and secreted proteins respectively approve the obtained results of expression specificity investigation of PM and secreted proteins (26).

6. Conclusion

One way of the understanding the function of cells in response to external stimuli is reconstruction of

intercellular interactions and study those interactions (34,35). In this study, we have constructed the network of communication between immune cells and non-hematopoietic cells. Most of network communications belonged to non-hematopoietic stromal and macrophage cells. Immune cells are affected by their interaction with stromal cells which are essential for the development of the immune system and the formation of immune tissues (36,37). The relationships between stromal cells and macrophages are necessary to create an appropriate environment for differentiation of immune cells (38). Therefore, one of the reasons of the high communication between them can be their role in differentiation of immune cells.

Studying the cellular expression specificity of receptor and ligand genes indicates the high degree of specificity of these genes. This high degree could be an indication of non-random transfer of information between cells in multicellular organisms (26). Therefore, the coherent behavior of cells to maintain the stable state of multicellular organisms might need a selective transmission of messages between cells. This is possible when the expression of the components involved in the transmission of the message has a high specificity of receptors and ligand molecules.

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Conflicts of Interest

There is no conflict of interest.

Author's Contribution

The initial idea of this work was presented by Dr. Zahiri then with consultation of Dr. Arab and Dr. Hassan Sajedi was conducted and analyzed by Dr. Zahiri and Shemaye Azadian.

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