Interleukin-10 signaling modulation - a survival strategy of MTB

5.1 Overview

5.1.1 Interleukin-10: An anti-inflammatory cytokine

Interleukin-10 (IL-10) is an anti-inflammatory, pleiotropic cytokine with important immunoregulatory functions. It is also known as 'cytokine synthesis inhibitory factor' (CSIF) and influences the activity of several cell types of the immune system. IL-10 is secreted by various cells of the immune system which include activated T-cells, macrophages, monocytes, B-cells and natural killer cells [214]. This cytokine is released when the immune cells are activated by endogenous or exogenous mediators such as bacterial toxins or antigens, catecholamines and cAMP-elevating drugs [215, 216]. During an infection, when immune cells are exposed to foreign antigens, cells of immune system produce pro-inflammatory cytokines such as interferon gamma and tumor necrosis factor alpha. These cytokines lead to rapid pathogen clearance and cell damage at the site of infection. Studies have shown that prolonged inflammation and excessive tissue damage can be fatal and in extreme cases cause death [217]. Interleukin-10 is an anti-inflammatory cytokine which dampens the inflammation caused during infection which otherwise can lead to potential tissue damage.

Interleukin-10 plays an important role as an immunoregulatory cytokine for the host immunity during tuberculosis infection. The survival of MTB in host macrophage is regulated by interleukin-10 in several ways. Earlier reports on IL-10 transgenic mice have demonstrated that mice fail to control MTB infection and consequently develop severe lung disease when compared with non transgenic mice [218]. Role of IL-10 in promoting the survival of MTB is further supported by the fact that IL-10 knockout mice are more resistant to MTB infection and can limit the growth in comparison with the wild type. It has been previously reported that IL-10 knockout mice also show stronger Th1 response and excessive inflammation [219].
5.1.2 Significance of generating Interleukin-10 signaling network

Several studies have reported the molecular events associated with IL-10 signaling and their role in tuberculosis infection. However, a well-annotated pathway map which can further be used by the scientific community to study the molecular mechanisms pertaining to IL-10 pathway has been lacking. Considering the significance of this molecule in immunological events related to several diseases, pathway map availability in public domain is much desired. In order to develop a comprehensive pathway map, molecules associated with IL-10 signaling available in published literature were manually curated. The series of molecular events in the IL-10 pathway that take place once the cell is exposed to foreign a antigen have been discussed. Only those interactions for which there is experimental evidence available in literature were considered.

5.1.3 Structure of human Interleukin-10

The human interleukin-10 gene containing 5 exons is located on chromosome 1. IL-10 exists as a homodimer of 36Kd molecular weight. The IL-10 homodimer interacts with tetrameric IL-10 receptor complex containing two of each IL10RA and IL10RB. IL-10 acts against inflammation by suppressing chemokines, pro-inflammatory cytokines, cell adhesion and co-stimulatory molecules [220]. Furthermore, IL-10 is also known to prevent differentiation of monocytes into dendritic cells. These cells are one of the important antigen presenting cells in the immune system [216]. Downregulation of co-stimulatory molecules such as ICAM-1 (intracellular adhesion molecule-1) and CD86 has also been found to be controlled by IL-10. Due to downregulation of these molecules, the cells are unable to present antigens on their surface. Another strategy of IL-10 to control inflammation is inducing the expression of heme oxygenase-1 (HMOX1) which is a potent anti-inflammatory agent [221].
5.1.3 Role of interleukin-10 in MTB infection

Interleukin -10 is known to play an important role in survival and persistence of intracellular pathogens such as MTB, *Leishmania donovani* and *Trypanosoma cruzi* in the host cell [219, 222, 223]. These pathogens have evolved various mechanisms for modulating the production of IL-10 by immune cells during infection. Several studies have indicated the correlation between IL-10 and tuberculosis susceptibility in the host [215, 224, 225]. In response to MTB infection, IL-10 is produced by hematopoietic and non-hematopoietic cells. When an individual gets infected by a pathogen, the immune cells of the body start releasing pro-inflammatory cytokines such as TNF-alpha, interferon gamma to kill the pathogen. IL-10 reduces the production of these cytokines and prevents the pathogen killing [226].

The mycobacterial antigens such as ESAT-6 activate pattern recognition receptors (PRR) present on the surface of alveolar macrophage. The antigen binds to TLR2 and TLR4 receptors on the macrophage surface and activates glycogen synthesis kinase-3 (GSK) by PI3K-Akt system. This promotes the production of IL-10 [227]. In neutrophils, when TLR2-MyD88 and C-type lectin receptors (CLR) are co-activated, IL-10 is upregulated via phosphorylation of p38 MAP an Akt kinases [228]. Studies have also shown that interaction between mycobacterial antigen infected dendritic cells (DC) with neutrophils produce large amounts of IL-10 [229]. Several proteins of MTB directly affect the IL-10 expression in macrophages. Rv1265, for example, is important for activation of ERK which further affects the expression of IL-10. Additionally, binding of MTB heat shock protein 60 (HSP60) to TLR2 is known to activate macrophage p38 MAPK which in turn increases the production of IL-10.

![Figure 26: Key pathways modulated by MTB during infection](image-url)
Table 30: List of MTB antigens involved in IL-10 signaling

<table>
<thead>
<tr>
<th>MTB antigen</th>
<th>Role in IL-10 signaling</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFP32</td>
<td>Increase levels of IL-10 was observed in pulmonary TB patients which positively correlated with levels of CFP32 antigen</td>
<td>[230]</td>
</tr>
<tr>
<td>Rv1265</td>
<td>Rv1265 is critical in activating ERK which further induces the expression of IL-10 in macrophages. This promotes intracellular survival of MTB</td>
<td>[230]</td>
</tr>
<tr>
<td>ESAT6</td>
<td>Peripheral blood mononuclear cells (PBMCs) obtained from multi-drug resistant TB patients stimulated by ESAT6 displayed a lower frequency of IFN-γ-producing T cells and a higher frequency of regulatory T cells (Treg) correlated with increased IL-10 secretion.</td>
<td>[231]</td>
</tr>
<tr>
<td>Hsp60</td>
<td>Binding of the Mtb heat shock protein 60 to the TLR2 can activate macrophage p38 MAPK to increase the IL-10 production</td>
<td>[232]</td>
</tr>
<tr>
<td>PPE family</td>
<td>Physical binding of Rv1808 to TLR2 results in increase in the secretion of anti-inflammatory cytokine interleukin-10 (IL-10)</td>
<td>[233]</td>
</tr>
</tbody>
</table>

5.2 Methodology

5.2.1 Literature survey and pathway curation

Studies that report IL-10 signaling were gathered through literature survey in PubMed and Google Scholar. The search terms included- (IL-10 OR “Interleukin-10”) AND (Signaling OR Pathway OR Signal OR “Signal Transduction”) NOT ‘Review’. Literature describing downstream molecular events taking place after IL-10 binds to its receptor were shortlisted. The information gathered was then manually curated and added to PathBuilder [234] which is an open source software for annotating and developing pathway resources developed in-house. A stringent annotation criterion was followed containing following guidelines:

- Only those reactions which are proved to be induced or enhanced by IL10-IL10RA-IL10RB receptor were catalogued
- Reactions should either be induced or enhanced in vivo
- Proteins considered for pathway should be reported in human system for those interactions. In case of proteins in other mammals, the orthologous proteins identified in human should be reported
The IL-10 pathway resource also contains annotated information for catalytic reaction, protein-protein interactions, gene regulation and activation/inhibition studies related to IL-10 signaling. All the experimental details such as the type of cell lines used, experimental conditions were also documented. Additionally, in case of post-translational modification (PTM), information about the PTM site and amino acid residue was also added to the database. In order to avoid any error or misinterpretation of data, an internal review system was followed in which the curated examples were manually validated by an expert. In addition, the pathway was also reviewed by Pathway authority- Dr. Sheetal Gandotra, CSIR-IGIB, New Delhi, who has a proven expertise in the field of study.

Snapshot from Netpath: http://www.netpath.org/pathways?path_id=NetPath_132

Source: Netpath; http://www.netpath.org/pathways?path_id=NetPath_132
5.2.2 Interleukin-10 pathway map generation and visualization

A comprehensive IL-10 pathway map was developed using the information derived from literature. The IL-10 pathway can be visualized using PathVisio [235]. PathVisio is a visualization tool for biological pathways based on GenMAPP tool that allows integration of multiple open source databases.

5.3 Results and discussion

For annotating the IL-10 signaling pathway, around 3,000 articles were screened from PubMed and Google Scholar. In all, 87 gene regulation events, 30 catalytic reactions, 4 activation/inhibition interactions and 4 protein-protein interactions were depicted in the signaling network. The pathway provided as a public resource is linked to their respective PubMed articles referred to construct the map. The molecules mentioned are linked to HPRD (Human Protein Reference Database). Also, the transcriptionally regulated genes are linked to their corresponding gene pages in NCBI.

After annotating the iterations curated from literature, IL-10 signaling pathway overall depicts that when immune cells of the host are exposed to a foreign antigen, pro-inflammatory cytokines are released to control infection. In order to avoid excessive damage, IL-10 is released which binds to extracellular domain of IL-10R-alpha. This leads to phosphorylation of JAK1 (Janus Kinase1) associated with IL-10R-alpha and TYK2 (tyrosine kinase2) associated with IL-10R beta. The intracellular domain of IL-10RA gets phosphorylated at Y446 and Y496 by these kinases. The phosphorylated residues act as temporary docking sites for STAT3 (Signal Transducer and Activator of Transcription-3). STAT3 is further phosphorylated by JAK1 which helps in dimerization of STAT3 and translocate to the nucleus. In the nucleus, it regulates the cell cycle progression genes such a BCL2 and other anti-apoptotic genes. IL-10 dampens the pro-inflammation in tuberculosis infection by suppressing the expression of pro-inflammatory cytokines such as IFN-gamma, TNF-alpha, IL-6, IL-8. Interleukin-10 also prevents the differentiation of monocytes into dendritic cells which are involved in antigen presentation during infection. From IL-10 signaling analysis, it was also found that IL-10 down-regulates co-stimulatory molecules such as CD86 and intracellular adhesion molecule-1 (ICAM1) resulting in making the cells incapable of presenting antigen on their surface [236].
Figure 27: Schematic representation of IL-10 signaling pathway - The map represents the NetPath reactions of IL-10 signaling pathway. The different types of reactions are distinguished by colors as described in the legend.

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