1. ABSTRACT

Receptors of the various cytokines although structurally diverse, can yet be grouped into four major families of receptor proteins. Most cytokines that function in the immune system bind to either the Class I or Class II receptor families. Two other important receptor families are the Immunoglobulin superfamily receptor and the TNF receptor family. Members of these receptor families also have critical roles in the immune system. A common feature of all these receptor families is that they do not exhibit any intrinsic tyrosine kinase activity. Receptor signaling is initiated through recruitment of kinases and through recruitment of cytosolic proteins to the receptor. In this review we will examine receptor signaling pathways initiated from five receptors that are all involved in either initiating T helper-l (Th1) responses, or in downregulating Th1 responses. The following receptors: Interleukin (IL)-12, Interferon (IFN), IL-4, IL-10, and Tumor necrosis factor (TNF)-alpha will be examined. Signaling initiated from IL-12, IFN-gamma and TNF-alpha are important for inducing Th1 responses, and on the other hand signaling from IL-4 and IL-10 receptors inhibit Th1 responses. We will also discuss human immunodeficiencies resulting from mutations in the genes that encode the Type I cytokine receptors.

2. INTRODUCTION

Cytokines are low molecular weight proteins that serve as a means of communication among varying cell populations. Numerous cells release cytokines in a brief and self-limited manner to mediate a response in a targeted cell. Once released into the environment, cytokines transverse small distances to ligate their high affinity receptors via either autocrine or paracrine fashion. Upon ligation of the cytokine receptor, a signaling cascade is triggered resulting in an alteration in gene transcription by the target cell.

While a variety of cells can synthesize and release cytokines, the two primary producers are CD4+ T helper lymphocytes and macrophages. These two cell populations utilize cytokines to direct, enhance or reduce an inflammatory response. While there is a milieu of cytokines released post antigenic stimulation, the immune response depends on the ligation of the cytokine to the target cell receptor. Without receptor cytokine binding, no signal transduction can take place and therefore no communication among the cells can occur. Thus, the receptor and signaling pathway are as vital as the release of the cytokine.

While all of the cytokine receptors are transmembrane proteins, they vary in the structures found in their extracellular domains. This variance in structure provides a means of characterizing the receptors into one of four families. The families include the Class I receptors, the Class II receptors, the Immunoglobulin superfamily, and the TNF receptor superfamily. The Class I receptors have four conserved cysteine residues and a conserved sequence motif of tryptophan-serine-X-tryptophan-serine...
This review will examine the signaling pathway emanating from IL-12, IFN, TNF-alpha, IL-4 and IL-10 receptors, and also discuss the consequences of defective signaling from some of these receptors in immunity to the intracellular pathogen *Mycobacterium tuberculosis*.

**3. SIGNALING AND CYTOKINE RECEPTORS**

**3.1. Interleukin-4 Receptor**

The T helper type 2 cytokine IL-4 has a type I cytokine receptor consisting of two subunits known as alpha and gamma. The alpha chain has a large cytoplasmic domain while the gamma chain has a shorter one. When IL-4 binds to the alpha chain, higher order oligomers form among the alpha and gamma chains. The oligomers recruit Jak1 and Jak3 to the alpha chain (12). In contrast, Jak3 is constitutively associated with the gamma chain. Once bound to the receptor, the Jak kinases phosphorylate each other and the IL-4R. Phosphorylation of the receptor results in the recruitment and activation of Stat 6. The phosphorylated Stat 6 molecules form dimers and translocate to the nucleus to activate transcription of IL-4 inducible genes. Stat 6 drives transcription by binding to specific GAS-like sequences in IL-4 inducible genes (13).

Beyond the recruitment of Stat 6 to the IL-4R, Jak 1 and Jak 3 tyrosine kinases activate other IL-4 signaling pathways. Both Jak 1 and Jak 3 are required for mediating IL-4 activation of insulin receptor substrate (IRS1/2). Several studies have revealed that IRS1/2 molecules bind to phosphorylated IRS motifs located in the IL-4 R (14). IRS molecules play an integral role in inducing IL-4 cellular proliferation.

In addition to the IRS1/2 pathway, ligation of the IL-4 R increases phosphatidylinositol 3'-kinase (PI3'-K) activity (15). Jak3 has been implicated in recruiting the p85 domain of PI3'-K to the IL-4 R since the kinase has been immunoprecipitated with Jak3 and the IL-4R. Activation of PI3'-K allows for IL-4 mediated cell growth and proliferation via two different pathways. IL-4 induces association of PI3'-K with IRS-2 and the protein tyrosine kinase FES in various cell populations (16). Secondly, IL-4 activation of PI3'-K ensures cell proliferation via upregulation of the anti-apoptotic protein bcl-2 (17).

Down regulation of the IL-4 signaling pathway occurs via four possible mechanisms. The first mechanism implicates Shp-1 as a negative regulator of IL-4 signaling since mice lacking the tyrosine phosphatase had an increase in Stat 6 activity (18). Secondly, SHIP, an Sh2 containing inositol 5'-phosphatase, has been implicated as a negative regulator due to its ability to dephosphorylate the products of PI3'-K. The third regulator implicated is the interleukin Four Receptor Interacting Protein (FRIP). Mice homozygous for the hairless mutation express reduced levels of FRIP and are IL-4 hyper-responsive, thus, implicating FRIP as a negative regulator (19).

The fourth possible regulator is the SOCS regulator implicated is the interleukin Four Receptor Interacting Protein (FRIP). Mice homozygous for the hairless mutation express reduced levels of FRIP and are IL-4 hyper-responsive, thus, implicating FRIP as a negative regulator (19).

The fourth possible regulator is the SOCS family of proteins. IL-4 increases expression of CIS, SOCS-1, SOCS-2, SOCS-3, and SSI. Recent reports have suggested that SOCS-1 is specific for regulating IL-4 signaling (20).
3.2. Interleukin-12 Receptor

IL-12 is synthesized and released from antigen presenting cells such as macrophages and dendritic cells to direct a cell mediated immune response. IL-12 has a type I cytokine receptor consisting of two chains beta 1 and beta 2. The beta 1 chain directly binds to the IL-12 cytokine while the beta 2 chain triggers the signaling pathway (3). The beta 2 chain is the focal point for the early maintenance of IL-12 responsiveness. Beta 2 is not expressed on naive T cells, but is induced on CD4+ and CD8+ T cells for 2 to 4 days following T cell receptor stimulation (4).

IL-12 binds to the beta 1 and beta 2 receptors causing heterodimerization and activation of the receptor associated Jak kinases. Jak2 and Tyk2 phosphorylate the IL-12 receptor creating docking sites on beta 2 for the Stat 4 signaling molecule (5). Stat 4 is phosphorylated by the Jak kinases and then released into the cytoplasm to form homodimers capable of entering the nucleus. Activation of Stat 4 results in an upregulation of IL-12 R beta 2 and the production of IFN gamma (6). The biological effects of Stat 4 activation are an increase in T cell proliferation, NK cell cytolytic activity, and Th1 differentiation.

3.3. Interferon Receptor

The interferon receptors (IFNR) are members of the Class II cytokine receptor family. The receptors are subdivided into two groups based on the ligands they bind. The type I interferons include alpha, beta, tau, and omega; while the type II include gamma (7). Type I and type II interferon receptors contain and alpha and beta chain that are both necessary for IFN binding and signaling. The two IFN receptors differ in the Jak and Stat molecules recruited to the membrane.

The type I interferon receptor transduces the anti-proliferative signals generated by binding of IFN alpha, beta, tau, or omega cytokines. Following cytokine binding, the receptor forms heterodimers capable of recruiting the Jak kinases Tyk2 and Jak1. Tyk2 phosphorylates the alpha subunit at Y466, Y481, Y527, and Y538 while Jak1 phosphorylates the beta subunit (8). The beta subunit can exist in one of three forms – long, short, and soluble (9). The 515 amino acid (aa) long form is necessary for IFN binding and signaling. The two IFN receptors differ in the Jak and Stat molecules recruited to the membrane.

From the receptor enabling them to form heterodimers via their SH2 domains. Unlike other Stat dimers, the Stat2/Stat1 heterodimer cannot enter the nucleus alone. The heterodimer must interact with a small protein known as p48 to form the transcription complex ISGF3 (11). Once formed, ISGF3 enters the nucleus and binds to GAS sites to drive the gene transcription resulting in IFN anti-proliferative effect.

While similar to the Type I receptor, the Type II IFNR differs in its specificity and signaling molecules. The type II receptor is composed of two subunits, IFN-gamma R1 (alpha chain) which is the IFN-gamma binding chain and IFN-gamma R2 (beta chain or accessory molecule) which is the signaling chain. Binding of IFN-gamma to its cognate receptor results in the auto- and transphosphorylation of Jak1 and Jak2 tyrosine kinases previously bound to the receptor. Jak kinases phosphorylate the receptor on specific tyrosine residues including Y440, the docking site for Stat 1 (11). Stat1 binds to the IFN-gamma R and is phosphorylated at Y701. Stat1 is released from the receptor and forms homodimers via the interactions of the SH2 domains. Unlike the Stat 1/Stat2 heterodimer, Stat1 homodimers can access the nucleus without the aid of an accessory protein. Once inside the nucleus, Stat 1 homodimers bind to GAS sites driving transcription of IFN-gamma response genes.

3.4. Interleukin-10 Receptor

The anti-inflammatory cytokine IL-10 binds to its class II receptor to downregulate an immune response. Functional IL-10 receptors (IL-10R) are tetramers consisting of two IL-10R1 polypeptide chains and two IL-10R2 chains (21). IL-10R1 is the signal transducing chain, while IL-10R2 is considered the accessory chain. Although IL-10R2 is termed the accessory chain, ligation of both IL-10R1 and IL-10R2 is necessary for optimal IL-10 signal transduction (22). IL-10 R1 is associated with Jak 1, while IL-10R2 is associated with Tyk2. Ligation of the receptor results in the formation of IL-10R1 and IL-10R2 heterodimers. The close proximity of the receptor chains allows for the phosphorylation of Jak 1, Tyk2, and the receptor. Phosphorylated Y446 and Y496 on IL-10 R1 serve as docking sites for Stat3, while IL-10R2 does not bind the recruited Stat 3. In addition to Stat3 recruitment and phosphorylation, there is a small amount of Stat 1 activated in the IL-10 signaling pathway (23). Activation of Stat 3 and to a lesser degree Stat 1 results in the formation of three distinct dimers: Stat 1 homodimers, Stat 3 homodimers, and Stat1/Stat3 heterodimers. The Stat3 dimers enter the nucleus where they bind GAS sites.

The ability of IL-10 to drive transcription of GAS regulated genes suggests a relationship between IFN-gamma and IL-10 signaling pathways. Studies have shown IL-10 is capable of down-regulating the expression of IFN-gamma, LPS and IL-4 inducible genes. While the mechanism of IL-10 suppression of all of these pro-inflammatory genes has not been clearly defined, IL-10 generated SOCS 3 (Suppressor of Cytokine Signaling) has been implicated. In addition to the GAS sites, Stat 3 homodimers bind to SBE regions of DNA to drive
Signaling from Cytokine Receptors

transcription of SOCS-3 (24). The SOCS-3 molecule has a central SH2 domain enabling it to bind to phosphorylated tyrosine residues of Jak kinases, thus inhibiting the activation of the Jak-Stat pathway (25). The ability of IL-10 to induce expression of SOCS-3 is a possible explanation for how this cytokine inhibits the induction of pro-inflammatory genes.

3.5. Tumor necrosis factor-alpha (TNF-alpha)

Tumor necrosis factor-alpha (TNF-alpha) is a pleiotropic cytokine that signals through two distinct receptors, TNF-receptor (TNFR1) and TNFR2. Activation of TNFR signaling pathway causes apoptosis, and induction of major transcription factors, AP-1 and NF-κB, which induce a variety of genes involved in TNF-alpha induced cellular responses (26,27). Recent data suggest that TNFRs are pre-aggregated before ligand binding through pre-ligand-binding assembly domains (PLAD) (28,29). Upon ligation, the conformational changes of pre-aggregated receptors facilitate signal transduction. Triggering of TNFRs induces receptor aggregation followed by the recruitment of various adaptor molecules. The cytoplasmic domain of TNFRs does not have intrinsic enzymatic activity, but serves as a docking site for signaling molecules. Activation of Death domain (DD) containing TNFR1 leads to the recruitment of TNFR1-associated death domain protein (TRADD), which serves as a platform for three other mediators, receptor-interacting protein 1 (RIP1), Fas-associated death domain protein (FADD), and TNF-receptor-associated protein-2 (TRAF-2) (30,31).

Triggering of TNFR2, which lacks DD domain, can directly induce the recruitment of TRAF-2 and TRAF-1 (32). While FADD mediated signaling is essential for apoptosis, RIP1 and TRAF-2 are involved in the activation of NF-kappaB and AP-1 (30,33-35). AP-1 and NF-kappaB induce genes involved in inflammatory responses. Some of these genes have anti-apoptotic functions, explaining why in most cases, TNF-alpha induced apoptosis depends on the inhibition of protein synthesis (36-38). Both RIP and TRAF-2 deficiencies sensitise TNF-alpha response towards apoptosis, indicating that these molecules may mediate a survival signal (27,34). Furthermore, TRAF-2 recruits anti-apoptotic molecules, cellular inhibitors of apoptosis (cIAP) 1 and 2, which protect cells from apoptosis by inhibiting caspases (39,40). Thus distinct adaptor molecules provide the receptors with ability to induce proinflammatory responses as well as apoptosis.

Whether TNF-alpha induces death or activation of cells seems to be dependent on cell type and a balance of TRAF-2/RIP/FADD recruitment to the receptor complex. The mechanism regulating the balance of these molecules remains to be elucidated. Structural and biochemical studies revealed that TRAF-2 has higher affinity to TRADD than the receptor, which may explain why TNF-alpha is a better activator of TNFR1 than TNFR2 (41). FADD was originally identified as an adaptor molecule of CD95. Like CD95 induced apoptotic pathway, it activates caspase-8, which cleaves downstream caspase molecules to initiate apoptosis. RIP recruits RAIDD through DD-DD interaction, and then N-terminal caspase recruitment domain (CARD) of RAIDD mediates interaction with a similar CARD motif in capase-2 (42). The physiological importance of this apoptotic pathway is unclear because of lack of TNFR1 mediated cell death in caspase-8 deficient mice and also a full apoptotic response in caspase-2 deficient cells (43,44).

TRAF-2 null cells or TRAF-2 dominant negative (DN) transgenic mice, and transfection experiments demonstrate that TRAF-2 is essential for TNF-alpha induced activation of MAPK, JNK and p38, which activate AP-1. Transient overexpression of MAPK kinase kinases (MAPKKKs) suggests that TRAF-2 can be associated with MAPKKKs capable of activating JNK and p38. These reports also showed that MAPKKKs such as NIK, MEKK1, ASK1, GCK and GCKR are involved in JNK and p38 activation (45-47). However, studies with kinase deficient cells failed to show that ASK1, MEKK1, and NIK are responsible for JNK and p38 activation in response to TNF-alpha (48-51).

Thus, none of these MAPKKKs so far have proved to play essential roles in TRAF mediated JNK and p38 activation under physiological condition. Different MAPKKKs may affect MAPKs signaling in a partial and additive way, in a cell type specific way or there may exist other novel MAPKKKs responsible for TNF-alpha induced JNK and p38 activation. Therefore, how TRAF-2 activates MAPKs remains to be elucidated. The RIP proteins also contain DD and serine/threonine protein kinase domain (32). Overexpression of RIPs induces apoptosis and NF-kappa B activation (33,38). However, their primary roles under physiological conditions may be to activate NF-kappa B, because experiments with knockout mice and RIP null cells failed to show TNF-alpha induced NF-kappa B response, but are sensitive to TNF-alpha induced cell death (33,34). These null cells exhibit normal JNK activation response to TNF-alpha, suggesting RIP involves NF-kappa B and not SAPK/JNK activation (33,34). Biochemical analysis of TRAF-2 and RIP deficient fibroblasts showed that both molecules are required for TNFR-1 mediated I kappa B kinase (IKK) activation since neither one of them alone can induce TNF-alpha mediated IKK activation (53,54). These papers also demonstrated that TRAF-2 is required for the recruitment of IKK to the TNFR1, while RIP mediates IKK activation.

The mechanism of how TRAF-2 and RIP1 activate IKK is still not clear since RIP1 kinase activity seems not to be involved in phosphorylation of IKK. RIP may signal to IKK complex, which leads to autoactivation of IKK. The other possible mechanism may be the phosphorylation of recruited IKK complex by TRAF associated kinases. NIK can bind to TRAF-2 and overexpression experiments have showed that NIK is required for TNF-alpha induced NF-xB activation pathway by phosphorylation of IKKα activation loop. But examination of NIK-knockout mice and mice carrying the lymphoplasia (aly) mutation, which maps to the gene encoding NIK, indicated that this kinase is not essential for the activation of NF-kappa B (49,50).
Knock-out studies. Susceptibility appears to be common to all the murine gene pathways that emanate from the receptors of several cytokines that are important in regulating Th1 cellular responses. Significant progress has been made towards defining the signaling pathways activated by several cytokines and in identifying components specific to individual cytokines. However, recent progress in the identification of germ-line mutations in several components of this signaling pathway reveals a crucial role for this pathway in the induction of protective immune responses to intracellular pathogens. The coming years are likely to identify mutations in several other components of the cytokine signal transduction pathway, paving the way to a better understanding of the genetic basis of susceptibility to tuberculosis and to diseases caused by other intracellular pathogens.

4. SUMMARY

In summary, we have discussed signaling pathways that emanate from the receptors of several different cytokines that are important in regulating Th1 cellular responses. Significant progress has been made towards defining the signaling pathways activated by several cytokines and in identifying components specific to individual cytokines. However, recent progress in the identification of germ-line mutations in several components of this signaling pathway reveals a crucial role for this pathway in the induction of protective immune responses to intracellular pathogens. The coming years are likely to identify mutations in several other components of the cytokine signal transduction pathway, paving the way to a better understanding of the genetic basis of susceptibility to tuberculosis and to diseases caused by other intracellular pathogens.

5. ACKNOWLEDGEMENTS

Support by National Institutes of Health Grant HL55972 from the National Heart, Lung, and Blood Institute is acknowledged.

6. REFERENCES

two beta-type cytokine receptor subunits. *Proc Natl Acad Sci USA* 93, 14002-14007 (1996)

1252
Signaling from Cytokine Receptors


Note: Allison M. Hanlon and Sihyung Jang contributed equally to this manuscript

Key Words: Cytokine receptor, Cytokine, Interleukin-1, Interleukin-2, Interleukin-4, Interleukin-6, Interleukin-10, Interleukin-12, Interferon, Tumor necrosis factor-alpha, CD95, Review

Send correspondence to: Padmini Salgame, Ph.D., Associate Professor, Temple University School of Medicine, Department of Microbiology and Immunology, 3400 N. Broad Street, Philadelphia PA 19140, Tel: 215-707-4260, Fax: 215-70-7788, E-mail: salgame@temple.edu