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SIGNALING FROM CYTOKINE RECEPTORS THAT AFFECT TH1 RESPONSES

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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-1 (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

(WSXWS) where X is a nonconserved amino acid. Class I receptors usually consist of two polypeptide chains; one chain for the cytokine binding and the other for the triggering of the signal transduction pathway. The class I receptors are further classified by the number of amino acids contained in their four alpha helical bundle structure. Short chain receptors have helices of 15 amino acids and include the IL-2 family (IL-2, IL-4, IL-7, IL-9, and IL-15). Long chain receptors have helices of 25 amino acids and include IL-6, IL-11, and IL-12. The third group of receptors is known as the Class II or interferon receptors. Like the Class I receptors, the Class II receptors contain conserved cysteine residues. The two families differ in that the Class II contain a fibronectin type III domain rather than the Class I sequence motif of WSXWS. Cytokine receptors belonging to the Class II group include interferon-alpha, -beta, -gamma, and IL-10.

Type I and type II cytokines are similar in their receptor structure and signaling pathways. An important feature of these receptors is that they lack intrinsic enzymatic activity. Nonetheless, binding of cytokine to its specific receptor initiates a series of phosphorylation events within the cell. With the discovery of the Janus family of protein tyrosine kinases, it became apparent that these kinases were involved in the signal transduction. Following receptor ligation, the receptor subunits dimerize allowing for the juxtaposition of the cytosolic domains. Janus tyrosine kinases (Jak) associate with the dimerized receptor to phosphorylate the receptor and each other. There are four known Jak kinases including Jak1, Jak2, Jak3, and Tyk2. Jak3 is only present in lymphohematopoietic cells, but the remaining three are expressed ubiquitously. Following tyrosine phosphorylation, signal transducers and activators of transcription (Stat) are recruited to the receptor. Stats bind to the cytokine receptor where they can be phosphorylated by proximal Jak kinases. Following their tyrosine phosphorylation, Stats dissociate from the receptor to form a dimer via interactions of their SH2 domains. Stat dimers translocate to the nucleus where they drive gene transcription. In addition to their dimer formation, Stat proteins are capable of forming tetramers via interactions of their N terminal domains. Tetramers bind to DNA with a greater affinity than dimers. There are seven known stat proteins: stat1, stat2, stat 3, stat 4, stat 5a, stat 5b, and stat6. Due to involvement of both the Jak tyrosine kinases and the Stat proteins, the cytokine signaling cascade is known as the Jak-Stat pathway. While cytokines generally utilize the Jak-Stat pathway for signal transduction, each cytokine receptor specifically recruits certain Jak and Stat proteins (1). While there may be redundancy amongst the signaling proteins recruited to the receptor, there are differences in the outcome of the signaling pathways.

Cytokine receptors that contain several extracellular Ig domains belong to the immunoglobulin superfamily. Like Class I and Class II receptors, TNF receptor is also devoid of any intrinsic enzymatic activity, and post receptor signaling pathway is characterized by recruitment of cytosolic proteins through specific protein-protein interaction domains.

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 Jak 1 and Jak3 to the alpha chain (12). In contrast, Jak 3 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 I4R 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 (suppressor of cytokine signaling) 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 (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 naïve 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 signaling since Jak1 binds at the Box1 site located at aa 300-346. The short form has identical extracellular and transmembrane domains as the long form, but the cytoplasmic domains differ. There has been no real role elucidated for the short form of the beta chain. The soluble form of the beta chain is a truncated version of the long and short forms since it has a stop codon at the 236 aa. Following Jak1 phosphorylation of the IFNR, the tyrosine receptors of the alpha and beta chains bind Stat 2. There are three known binding sites for Stat 2 to the alpha and beta chains (10). Stat 2 has two binding sites in the beta chain; binding to one site is constitutive while the other is dependent on tyrosine phosphorylation. The third site of Stat 2 binding is on Y466 and Y481 of the alpha chain. Stat1 is also phosphorylated as a result of IFNR ligation, but the sites of Stat1 docking to the receptor are unknown. Both Stat 2 and Stat 1 are phosphorylated and released 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-10R1polypeptide 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/Stat 3 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

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 (TNFR)1 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 sensitize 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 (52). 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-κB activation pathway by phosphorylation of IKKα activation loop. But examination of NIK-knockout mice and mice carrying the alymphoplasia (*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).

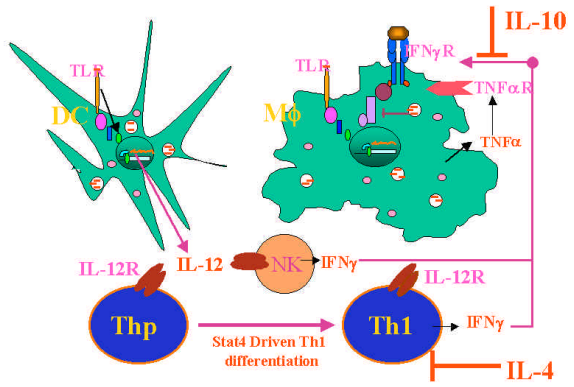

Figure 1. Interactions of IL-12, IFN-gamma, TNF-alpha, IL-4 and IL-10 in generating Th1 responses.

A new model for TRAF mediated IKK activation has been recently proposed (55). TRAF-6 (TRAF-2) complexes with an ubiquitin conjugating enzyme complex composed of Ubc13 and Uev1A and mediates the synthesis of polyubiquitin chains required for the activation of IKK. However, in this report the ubiquitination of IKK complex was not observed. Thus the substrates of this ubiquitination remain to be identified. In addition to the basic pathways discussed above, there are other ways to modulate AP-1 and NF-kappa B activities. Phosphatidylinositol 3'-kinase (PI3'-K) and glycogen synthase kinase 3-beta (GSK3-beta) can contribute to TNF-alpha induced NF- kappa B transcription activity but not to IKK activation (56-58). Dominant negative mutants and antisense experiments show that in response to TNF-alpha, atypical protein kinase C (aPKC) may be involved in IKK activation by binding to RIP1 through p62, which is aPKC-associated protein (59).

3.6. Mutations in IFN-gammaR and IL-12R lead to defects in clearing mycobacterial infections

Protective immunity to intracellular mycobacteria relies on the ability of the host to generate an effective cell-mediated immune response with subsequent containment of the infection in well-organized granulomas. A critical component of the granulomatous response is the activation of mycobacteria-infected macrophages by IFN-gamma, whose production is regulated by IL-12 (60). Dendritic cells and activated macrophages secrete IL-12 following *M.tuberculosis* infection in a TLR-dependent manner (61). The secreted IL-12 binds to its cognate receptors on naïve T cells and through a Stat 4 dependent activation pathway modulates their differentiation towards Th1-type (62). IFN-gamma secreted by Th1 cells, together with TNF-alpha has potent anti-mycobacterial activity (Figure 1). The Th2 cytokines, IL-4 and IL-10 severely diminish Th1 activity, either through direct effects on Th1 cells or through effects on macrophages (63,64). A series of murine studies have shown that the circuitous relationship between IL-12, IFN-gamma, and TNF-alpha is important for combating intracellular mycobacterial infections (65-70). Defective macrophage activation leading to disease susceptibility appears to be common to all the murine gene knock-out studies.

Recently several laboratories have studied individuals who have clinically presented themselves with severe infections caused by either poorly pathogenic or pathogenic mycobacterial species (71). Genetic analysis of these patients revealed that they all had mutations in either the IFN-gamma R or the IL-12R signaling pathway. These "experiments of nature" highlight the essential and non-redundant role of signaling from the IFN-gamma and IL-12 receptors in immunity to mycobacterial infections. Further genetic analysis has identified mutations in five different genes including mutations in the genes that encodes the IL-12p40 subunit (72), the IL-12R beta 1 chain (73-75), in both receptor subunits composing the IFN-gamma R (76-78), and Stat1 molecule (79). These mutations include both point mutations and frame-shift deletions that result in either recessive-null or dominant-negative alleles (71). The comparable clinical, immunological and histopathological phenotypes of patients with mutations in any of these four genes indicates that IL-12-regulated IFN-gamma-dependent signaling by Stat 1 is critical for anti-mycobacterial defense in humans.

Identification of these mutations in the IL-12/IFN-gamma axis forces us to reevaluate the possibility that the development of mycobacterial infections in man may be partly determined by inherited differences in host immune response.

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

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6. REFERENCES

1. Chen E, M. Gadina, M. Chen & O'Shea, J. J. Advances in cytokine signaling: the role of Jaks and STATs. *Transplant Proc* 31, 1482-1487 (1999)
2. Presky D, H. Yang, L. J. Minetti, A. O. Chua, N. Nabavi, C. Y. Wu, M. K. Gately & U. A. Gubler: Functional interleukin 12 receptor complex is composed of

two beta- type cytokine receptor subunits. *Proc Natl Acad Sci U S A* 93, 14002-14007 (1996)
3. Wu C. Y, R. R. Warrier, D. M. Carvaja, A. O. Chua, L. J. Minetti, R. Chizzonite, P. Mongini, P, A. S. Stern, U. Gubler, D. H. Presky & M. K. Gately: Biological function and distribution of human interleukin-12 receptor beta chain. *Eur J Immunol* 26, 345-350 (1996)
4. Szabo S. J, A. S. Dighe, A. Gubler & K. M. Murphy: Regulation of the Interleukin (IL)-12R beta 2 subunit expression in developing T Helper 1 (Th1) and Th2 cells. *J Exp Med* 185, 817-824 (1997)
5. Coccia E. M, N. Passini, A. Battistini, C. Pini, F. Sinigaglia & L. Rogge: Interleukin-12 induces expression of interferon regulatory factor-1 via signal transducer and activator of transcription-4 in human T helper type 1 cells. *J Biol Chem* 274, 6698-6703 (1999)
6. Kaplan M. H, A. L. Wurster & M. J. A. Grusby: Signal transducer and activator of transcription (Stat)4-independent pathway for the development of T helper type 1 cells. *J Exp Med* 188, 1191-1196 (1998)
7. Gadina M, D. Hilton, J. A. Johnston, A. Morinobu, A. Lighvani, Y. J. Zhou, R. Visconti & J. J. O'Shea: Signaling by type I and II cytokine receptors: ten years after. *Curr Opin Immunol* 13, 363-373 (2001)
8. Nadeau O. W, P. Domanski, A. Usacheva, S. Uddin, L. C. Platanias, P. Pitha, R. Raz, D. Levy, B. Majchrzak, E. Fish & O. R. Colamonici: The proximal tyrosines of the cytoplasmic domain of the beta chain of the type I interferon receptor are essential for signal transducer and activator of transcription (Stat) 2 activation. Evidence that two Stat2 sites are required to reach a threshold of interferon alpha-induced Stat2 tyrosine phosphorylation that allows normal formation of interferon-stimulated gene factor 3. *J Biol Chem* 274, 4045-4052 (1999)
9. Lutfalla G, S. J. Holland, E. Cinato, D. Monneron, J. Reboul, N. C. Rogers, J. M. Smith, G. R. Stark, K. Gardiner, K. E. Mogensen, I. M. Kerr & G. Uze: Mutant U5A cells are complemented by an interferon-alpha beta receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluster. *EMBO J* 14, 5100-5108 (1995)
10. Muller M, J. J. Briscoe, C. Laxton, D. Guschin, A. Ziemiecki, O. Silvennoinen, A. G. Harpur, G. Barbieri, B. A. Witthuhn, C. Schindler, S. pelligrini, A. F. Wilks, J. N. Ihle, G. R. Stark & I. M. Kerr: The protein tyrosine kinase JAK1 complements defects in interferon- alpha/beta and -gamma signal transduction. *Nature* 366, 129-135 (1993)
11. Kotenko S. V & S. Pestka: Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene* 19, 2557-2565 (2000)
12. Malabarba M. G, R. A. Kirken, H. Rui, K. Koettnitz, M. Kawamura, J. J. O'Shea, F. S. Kalthoff & W. L. Farrar: Activation of JAK3, but not JAK1, is critical to interleukin-4 (IL4) stimulated proliferation and requires a membrane-proximal region of IL4 receptor alpha. *J Biol Chem* 270, 9630-9637 (1995)
13. Lee C. E & H. J. Park: Interleukin-4 induces two distinct GAS-binding complexes containing STAT6: evidence for DNA binding of STAT6 monomer. *Mol Cells* 11, 28-34 (2001)
14. Chen X. H, B. K. Patel, L. M. Wang, M. Frankel, N. Ellmore, R. A. Flavell, W. J. LaRochelle & J. H. Pierce: Jak1 expression is required for mediating interleukin-4-induced tyrosine phosphorylation of insulin receptor substrate and Stat6 signaling molecules. *J Biol Chem* 272, 6556-6560 (1997)
15. Izuhara K, R. A. Feldman, P. Greer & N. Harada: Interleukin-4 induces association of the c-fes proto-oncogene product with phosphatidylinositol-3 kinase. *Blood* 88, 3910-3918 (1996)
16. Izuhara K, R. A. Feldman, P. Greer & N. Harada: Interaction of the c-fes proto-oncogene product with the interleukin-4 receptor. *J Biol Chem* 269, 18623-18629 (1994)
17. Minshall C, S. Arkins, R. Dantzer, G. G. Freund, & K. W. Kelley: Phosphatidylinositol 3'-kinase, but not S6-kinase, is required for insulin-like growth factor-I and IL-4 to maintain expression of Bcl-2 and promote survival of myeloid progenitors. *J Immunol* 162, 4542-4549 (1999)
18. Haque S. J, P. Harbor, M. Tabrizi, T. Yi & B. R. Williams: Protein-tyrosine phosphatase Shp-1 is a negative regulator of IL-4- and IL-13-dependent signal transduction. *J Biol Chem* 273, 33893-33896 (1998)
19. Nelms K, A. L. Snow, J. Hu-Li & W. E. Paul: FRIP, a hematopoietic cell-specific rasGAP-interacting protein phosphorylated in response to cytokine stimulation. *Immunity* 9, 13-24 (1998)
20. Losman J. A, X. P. Chen, D.Hilton & P. Rothman: Cutting edge: SOCS-1 is a potent inhibitor of IL-4 signal transduction. *J Immunol* 162, 3770-3774 (1999)
21. Donnelly R. P, H. Dickensheets & D. S. Finbloom: The interleukin-10 signal transduction pathway and regulation of gene expression in mononuclear phagocytes. *J Interferon Cytokine Res* 19, 563-573 (1999)
22. Kotenko S. V, C. D. Krause, L. S. Izotova, B. P. Pollack, W. Wu & S. Pestka: Identification and functional characterization of a second chain of the interleukin-10 receptor complex. *EMBO J* 16, 5894-5903 (1997)
23. Finbloom D. S. & K. D. Winestock: IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. *J Immunol* 155, 1079-1090 (1995)
24. Ito S, P. Ansari, M. Sakatsume, H. Dickensheets, N. Vazquez, R. P. Donnelly, A. C. Larner & D. S. Finbloom: Interleukin-10 inhibits expression of both interferon alpha- and interferon gamma-induced genes by suppressing tyrosine phosphorylation of STAT1. *Blood* 93, 1456-1463 (1999)
25. Starr R, T. A. Willson, E. M. Viney, L. J. Murray, J. R. Rayner, B. J. Jenkins, T. J. Gonda, W. S. Alexander, D. Metcalf, N. A. Nicola & D. J. Hilton: A family of cytokine-inducible inhibitors of signalling. *Nature* 387, 917-921 (1997)
26. Lenardo M, K. M. Chan, F. Hornung, H. McFarland, R. Siegel, J. Wang & L. Zheng: Mature T lymphocyte apoptosis-immune regulation in a dynamic and unpredictable antigenic environment. *Annu Rev Immunol* 17, 221-253 (1999)
27. Wallach D, E. Varfolomeev, N. L. Malinin, Y. V. Goltsev, V. Kovalenko & M. P. Boldin: Tumor Necrosis Factor Receptor and Fas signaling mechanisms. *Annu Rev Immuno* 17, 331-367 (1999)
28. Siegel R. M, J. K. Frederiksen, D. A. Zacharias, F. K. Chan, M. Johnson, D. Lynch, R. Y. Tsien & M. J. Lenardo: Fas preassociation required for apoptosis signaling and dominant inhibition by pathogenic mutations. *Science* 288, 2354-2357 (2000)

29. Chan F. K, H. J. Chun, L. Zheng, R. M. Siegel, K. L. Bui & M. J. Lenardo: A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science* 288, 2351-2354 (2000)
30. Hsu H, J. Xiong & D. V. Goeddel: The TNF receptor 1-associated protein TRADD signals cell death and NF-kappa B activation. *Cell* 81, 495-504 (1995)
31. Wallach D, E. E. Varfolomeev, N. L. Malinin, Y. V. Goltsev, A. V. Kovalenko & M. P. Boldin: Tumor necrosis factor receptor and Fas signaling mechanisms. *Annu Rev Immunol* 17, 331-367 (1999)
32. Rothe M, V. Sarma, V. M. Dixit, & D. V. Goeddel: TRAF2-mediated activation of NF-kappa B by TNF receptor 2 and CD40. *Science* 269, 1424-1427 (1995)
33. Ting A. T, F. X. Pimentel-Muinos, B. Seed: RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. *Embo J* 15, 6189-6196 (1996)
34. Kelliher M. A, S. Grimm, Y. Ishida, F. Kuo, B. Z. Stanger & P. Leder: The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunity* 8 297-303 (1998)
35. Lee S. Y, A. Reichlin, A. Santana, K. A. Sokol, M. C. Nussenzweig & Y. Choi: TRAF2 is essential for JNK but not NF-kappaB activation and regulates lymphocyte proliferation and survival. *Immunity* 7, 703-713 (1997)
36. Beg A. A. & D. Baltimore: An essential role for NF-kappaB in preventing TNF-alpha-induced cell death. *Science* 274, 782-784 (1996)
37. Minden, A. & M. Karin: Regulation and function of the JNK subgroup of MAP kinases. *Biochim Biophys Acta* 1333, 85-104 (1997)
38. Liu Z. G, H. Hsu, D. V. Goeddel & M. Karin: Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* 87, 565-576 (1996)
39. Deveraux Q. L, R. Takahashi, G. S. Salvesen & J. C. Reed: X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 388: 300-304 (1997)
40. Roy N, Q. L. Deveraux, R. Takahashi, G. S. Salvesen & J. C. Reed: The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *EMBO J* 16, 6914-6925 (1997)
41. Park Y. C, H. Ye, C. Hsia, D. Segal, R. L. Rich, H. C. Liou, D. G. Myszka, & H. Wu: A novel mechanism of TRAF signaling revealed by structural and functional analyses of the TRADD-TRAF2 interaction. *Cell* 101, 777-787 (2000)
42. Duan H. & V. Dixit: RAIDD is a new 'death' adaptor molecule. *Nature* 385, 86-89 (1997)
43. Bergeron L, G. I. Perez, G. Macdonald, L. Shi, Y. Sun, A. Jurisicova, S. Varmuza, K. E. Latham, J. A. Flaws, J. C. Salter, H. Hara, M. A. Moskowitz, E. Li, A. Greenberg, J. L. Tilly & J. Yuan: Defects in regulation of apoptosis in caspase-2-deficient mice. *Genes Dev* 12, 1304-1314 (1998)
44. Varfolomeev E. E, M. Schuchmann, V. Luria, N. Chiannilkulchai, J. S. Beckman, I. L. Mett, D. Rebrikov, V. M. Brodianski, O. C. Kemper, O. Kollet, T. Lapidot, D. Soffer, T. Sobe, K. B. Avraham, T. Goncharov, H. Holtmann, P. Lonai, & D. Wallach: Targeted disruption of the mouse Caspase 8 gene ablates cell death induction by the TNF receptors, Fas/APO1, and DR3 and is lethal prenatally. *Immunity* 9: 267-276 (1998)
45. Nishitoh H, M. Saitoh, Y. Mochida, K. Takeda, H. Nakano, M. Rothe, K. Miyazono & H. Ichijo: ASK1 is essential for JNK/SAPK activation by TRAF2. *Mol Cell* 2, 389-395 (1998)
46. Yuasa T, S. Ohno, J. H. Kehrl, & J. M. Kyriakis: Tumor necrosis factor signaling to stress-activated protein kinase (SAPK)/Jun NH2-terminal kinase (JNK) and p38. Germinal center kinase couples TRAF2 to mitogen-activated protein kinase/ERK kinase kinase 1 and SAPK while receptor interacting protein associates with a mitogen- activated protein kinase kinase kinase upstream of MKK6 and p38. *J Biol Chem* 273, 22681-22692 (1998)
47. Wajant H, F. Henkler & P. Scheurich: The TNF-receptor-associated factor family: scaffold molecules for cytokine receptors, kinases and their regulators. *Cell Signal* 13, 389-400 (2001)
48. Yujiri T, M. Ware, C. Widmann, R. Oyer, D. Russell, E. Chan, Y. Zaitsu, P. Clarke, K. Tyler, Y. Oka, G. R. Fanger, P. Henson & G. L. Johnson: MEK kinase 1 gene disruption alters cell migration and c-Jun NH2- terminal kinase regulation but does not cause a measurable defect in NF- kappa B activation. *Proc Natl Acad Sci U S A* 97, 7272-7277 (2000)
49. Shinkura R, K. Kitada, F. Matsuda, K. Tashiro, K. Ikuta, M. Suzuki, K. Kogishi, T. Serikawa, T & T. Honjo: Alymphoplasia is caused by a point mutation in the mouse gene encoding NF-kappa b-inducing kinase. *Nat Genet* 22, 74-77 (1999)
50. Yin L, L. Wu, H. Wesche, C. D. Arthur, J. M. White, D. V. Goeddel, & R. D. Schreiber: Defective lymphotoxin-beta receptor-induced NF-kappaB transcriptional activity in NIK-deficient mice. *Science* 291, 2162-2165 (2001)
51. Tobiume K, A. Matsuzawa, T. Takahashi, H. Nishitoh, K. Morita, K. Takeda, O. Minowa, K. Miyazono, T. Noda & H. Ichijo: ASK1 is required for sustained activations of JNK/p38 MAP kinases and apoptosis. *EMBO J* 2, 222-228 (2001)
52. Stanger B. Z, P. Leder, T-H. Lee, E. Kim, E. & B. Seed: RIP: A novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. *Cell* 81, 513-523 (1995)
53. Devin A, A. Cook, Y. Lin, Y. Rodriguez, M. Kelliher & Z. Liu: The distinct roles of TRAF2 and RIP in IKK activation by TNF-R1: TRAF2 recruits IKK to TNF-R1 while RIP mediates IKK activation. *Immunity* 12, 419-429 (2000)
54. Devin A, Y. Lin, S. Yamaoka, Z. Li, M. Karin & Z. Liu: The alpha and beta subunits of IkappaB kinase (IKK) mediate TRAF2-dependent IKK recruitment to tumor necrosis factor (TNF) receptor 1 in response to TNF. *Mol Cell Biol* 21, 3986-3994 (2001)
55. Deng L, C. Wang, E. Spencer, L. Yang, A. Braun, J. You, C. Slaughter, C. Pickart & Z. J. Chen: Activation of the IkappaB kinase complex by TRAF6 requires a dimeric ubiquitin-conjugating enzyme complex and a unique polyubiquitin chain. *Cell* 103, 351-361 (2000)
56. Madrid L. V, M. W. Mayo, J. Y. Reuther, & A. S. J. Baldwin: Akt stimulates the transactivation potential of the RelA/p65 Subunit of NF-kappa B through utilization of the

Ikappa B kinase and activation of the mitogen-activated protein kinase p38. *J Biol Chem* 276, 18934-18940 (2001)
57. Delhase M, N. Li & M. Karin: Kinase regulation in inflammatory response. *Nature* 406, 367-368 (2000)
58. Hoeflich K. P, J. Luo, E. A. Rubie, M. S. Tsao, O. Jin & J. R. Woodgett: Requirement for glycogen synthase kinase-3beta in cell survival and NF- kappaB activation. *Nature* 406: 86-90 (2000)
59. Sanz L, P. Sanchez, M. J. Lallena, M. T. Diaz-Meco & J. Moscat: The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. *EMBO J* 18, 3044-3053 (1999)
60. Flynn J. L. & J. Chan: Immunology of Tuberculosis. *Annu Rev Immunol* 19, 93-129 (2001)
61. Thomas-Uszynski, S, S. M. Kiertscher, M. T. Ochoa, D. A. Bouis, M. V. Norgard, K. Miyake, P. J. Godowski, M. D. Roth & R. L. Modlin: Activation of Toll-like receptor 2 on human dendritic cells triggers induction of IL-12 but not IL-10. *J Immunol* 165, 3804 (2000)
62. A. O'Garra: Cytokines Induce the Development of Functionally Heterogeneous T Helper Cell Subsets. *Immunity* 8, 275 (1998)
63. Fiorentino D. F, A. Zlotnik, P. Vieira, T. R. Mosmann, M. Howard, K. W. Moore & A. O'Garra: IL-10 acts on the antigen-presenting cell to inhibit cytokine production by Th1 cells. *J Immunol* 146, 3444-3451 (1991)
64. D'Andrea A, M. Aste-Amezaga, N. M. Valiante, X. Ma, M. Kubin & G. Trinchieri: Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 178, 1041-1048 (1993)
65. Flynn J. L, J. Chan, K. J. Triebold, D. K. Dalton, Y. A. Stewart & B. R. Bloom: An essential role for interferon g in resistance to *Mycobacterium tuberculosis* infection. *J Exp Med* 178, 2249-2254 (1993)
66. Cooper A. M, D. K. Dalton, T. A. Stewart, J. P. Griffin, D. G. Russell & I. M. Orme: Disseminated tuberculosis in gamma interferon gene-disrupted mice. *J Exp Med* 178, 2243 (1993)
67. Flynn J. L, M. M. Goldstein, J. Chan, K. J. Triebold, K. Pfeffer, C. L. Loenstein, R. Schreiber, T. W. Mak & B. R. Bloom: Tumor necrosis factor is required in the protective immune response against Mycobacterium tuberculosis in mice. *Immunity* 2, 561-572 (1995)
68. Flynn J. L, M. M. Goldstein, K. J. Triebold, J. Sypek, W. Stanley & B. R. Bloom: IL-12 increases resistance of BALB/c mice to Mycobacterium tuberculosis infection. *J Immunol* 155, 2515-2524 (1994)
69. Cooper A. M, A. D. Roberts, A. R. Rhoades, J. E. Callahan, D. M. Getzy & I. M. Orme: The role of interleukin-12 in acquired immunity to *Mycobacterium tuberculosis* infection. *Immunology* 84, 423-432 (1995)
70. Flynn J. L, C. A. Scanga, K. E. Tanaka & J. Chan: Effects of aminoguanidine on latent murine tuberculois. *J Immunol* 160, 1796-1803 (1998)
71. Ottenhoff T. H, T. de Boer, C. E. Verhagen, F. A. Verreck & J. T. van Dissel: Human deficiencies in type 1 cytokine receptors reveal the essential role of type 1 cytokines in immunity to intracellular bacteria. *Microbes Infect* 2, 1559-1566. (2000)
72. Altare F, D. Lammas, P. Revy, E. Jouanguy, R. Doffinger, S. Lamhamedi, P. Drysdale, D. Scheel-Toellner, J. Girdlestone, P. Darbyshire, M. Wadhwa, H. Dockrell, M. Salmon, A. Fischer, A. Durandy, J. L. Casanova, J. L & D. S. Kumararatne: Inherited interleukin 12 deficiency in a child with bacille Calmette-Guerin and Salmonella enteritidis disseminated infection. *J Clin Invest* 102, 2035-2040 (1998)
73. Altare F, A. Durandy, D. Lammas, J. F. Emile, S. Lamhamedi, F. Le Deist, P. Drysdale, E. Jouanguy, R. Doffinger, F. Bernaudin, O. Jeppsson, J. A. Gollob, E. Meinl, A. W. Segal, A. Fischer, D. Kumararatne & J. L. Casanova: Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 280, 1432-1435 (1998)
74. Picard C, C. Fieschi, F. Altare, S. Al-Jumaah, S. Al-Hajjar, J. Feinberg, S. Dupuis, C. Soudais, I. Z. Al-Mohsen, E. Genin, D. Lammas, D. S. Kumararatne, T. Leclerc, A. Rafii, H. Frayha, B. Murugasu, L. B. Wah, R. Sinniah, M. Loubser, E. Okamoto, A. Al-Ghonaium, H. Tufenkeji, L. Abel & J. L. Casanova: Inherited Interleukin-12 Deficiency: IL12B Genotype and Clinical Phenotype of 13 Patients from Six Kindreds. *Am J Hum Genet* 70, 336-348. (2002)
75. Altare F, A. Ensser, A. Breiman, J. Reichenbach, J. E. Baghdadi, A. Fischer, J. F. Emile, J. L. Gaillard, E. Meinl & J. L. Casanova: Interleukin-12 receptor beta1 deficiency in a patient with abdominal tuberculosis. *J Infect Dis* 184, 231-236 (2001)
76. Jouanguy E, F. Altare, S. Lamhamedi, P. Revy, J. F. Emile, M. Newport, M. Levin, S. Blanche, E. Seboun, A. Fischer & J. L. Casanova: Interferon-gamma-receptor deficiency in an infant with fatal bacille Calmette-Guerin infection. *N Engl J Med* 335, 1956-1961 (1996)
77. Jouanguy E, S. Lamhamedi-Cherradi, D. Lammas, S. E. Dorman, M. C. Fondaneche, S. Dupuis, R. Doffinger, F. Altare, J. Girdlestone, J. F. Emile, H. Ducoulombier, D. Edgar, J. Clarke, V. A. Oxelius, M. Brai, V. Novelli, K. Heyne, A. Fischer, S. M. Holland, D. S. Kumararatne, R. D. Schreiber & J. L. Casanova: A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. *Nat Genet* 21, 370-378. (1999)
78. Doffinger R, E. Jouanguy, S. Dupuis, M. C. Fondaneche, J. L. Stephan, J. F. Emile, S. Lamhamedi-Cherradi, F. Altare, A. Pallier, G. Barcenas-Morales, E. Meinl, C. Krause, S. Pestka, R. D. Schreiber, F. Novelli & J. L. Casanova: Partial interferon-gamma receptor signaling chain deficiency in a patient with bacille Calmette-Guerin and Mycobacterium abscessus infection. *J Infect Dis* 181, 379-384 (2000)
79. Dupuis S, C. Dargemont, C. Fieschi, N. Thomassin, S. Rosenzweig, J. Harris, S. M. Holland, R. D. Schreiber & J. L. Casanova: Impairment of mycobacterial but not viral immunity by a germline human STAT1 mutation. *Science* 293, 300-303 (2001)

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