Review

MAP Kinases in Immune Responses

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MAP kinases are evolutionarily conserved signaling regulators from budding yeast to mammals and play essential roles in both innate and adaptive immune responses. There are three main families of MAPKs in mammals. Each of them has its own activators, inactivators, substrates and scaffolds, which altogether form a fine signaling network in response to different extracellular or intracellular stimulation. In this review, we summarize recent advances in understanding of the regulation of MAP kinases and the roles of MAP kinases in innate and adaptive immune responses. *Cellular & Molecular Immunology*. 2005;2(1):20-27.

Key Words: MAP kinase, MAP kinase phosphatase, scaffold protein, innate immune response, adaptive immune response

Introduction

There are two wings of immune system, innate and adaptive, in vertebrate animals. The innate immune system detects the presence and the nature of infection, provides the first line of host defence, and controls the initiation and determination of the effector class of the adaptive immune response. A specific immune response, such as the production of antibodies against a particular pathogen, is known as adaptive immune response, because it occurs during the lifetime of an individual as an adaptation to infection with that pathogen. Many signal transduction pathways participate in both innate and adaptive immune responses. The mitogen-activated protein (MAP) kinase signaling cascade is one of the most ancient and evolutionarily conserved signaling pathways and plays essential regulatory roles in both innate and adaptive immune responses (1).

Three main families of MAPKs exist in mammalian species: the extracellular signal-regulated protein kinases (ERK), the p38 MAP kinases, and the c-Jun NH_2-terminal kinases (JNK) (2-5). All the MAPKs consist a Thr-X-Tyr (TXY) motif within their activation loop. The phosphorylation of both threonine and tyrosine within the activation loop is essential and sufficient for their activation. The ERK family contains TEY (Thr-Glu-Tyr) activation motif (6). Members of this family could be further divided into two groups: 1) the classic MAP kinases that consist primarily of a kinase domain such as ERK1 and ERK2; and 2) the big MAP kinases such as ERK3, ERK5, ERK7, and ERK8 that consist of both a kinase domain and a C-terminal domain and range in size from 60 to over 100 kD (7). The p38 family has TGY (Thr-Gly-Tyr) activation motif and includes α, β, γ, δ and ERK6 which is a newly identified member (7, 8). There are three members in the JNK family which all contain Thr-Pro-Tyr (TPY) in their activation motif (9). The expression of JNK1 and JNK2 was found to be ubiquitous, while the expression of JNK3 is brain-specific.

MAPKs respond to a broad range of extracellular and intracellular changes. Cooperating with other signal transduction pathways, MAPKs transduce these changes into alterations in gene expression and regulation of cell function. In this review, first we will summarize recent advance in regulation of MAPKs. Secondly, their roles in innate and adaptive immune responses will be reviewed.

Regulation of MAP kinases

Activation of MAP kinases

Activation of MAP kinase pathway has been well reviewed (1, 4, 10). We will briefly summarize here. The activation of all MAPK pathways is achieved through a core triple kinase cascade: MKKK-MKK-MAPK (Figure 1). They are activated upon phosphorylation on Thr and Tyr by dual-specificity MAP kinase kinases (MKKs). MKKs are activated after phosphorylation on their Ser/Thr residues by MAP kniase kinase kinases (MKKKs). To date, 7 MKK and 14 MKKK have been identified (11). The seven MAPKKs are highly specific to their substrates, allowing minimal variation of the MKK-MAPK part of the cascade. The fourteen MKKKs are more diverse in their structure and can be differentially regulated by a variety of upstream stimulation for their selective regulation of MKKs. Among various upstream

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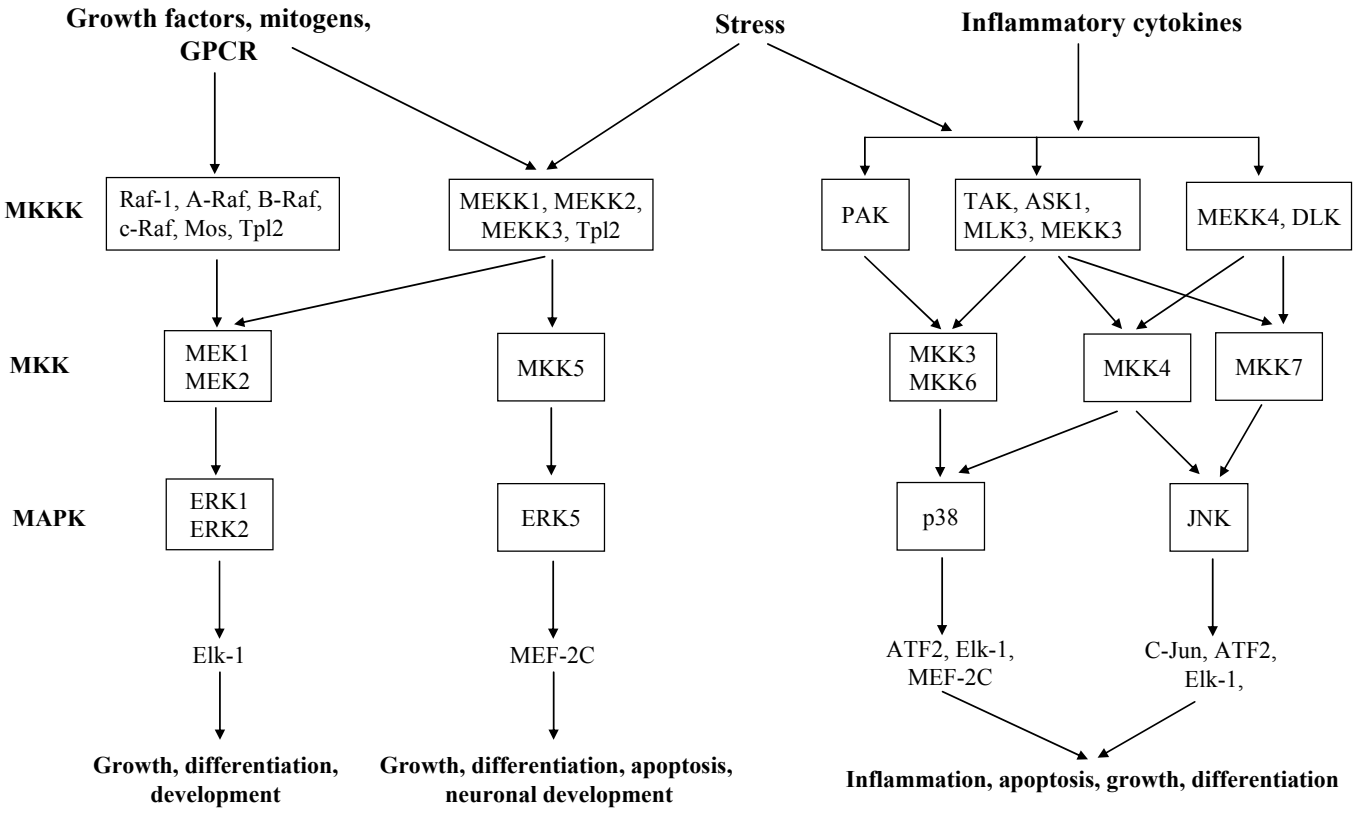

Figure 1. **Mammalian MAP kinase pathways.** The activation of MAPK signaling pathways is achieved through a core triple kinase cascade. The MAPKs are activated by dual phosphorylation on Thr and Tyr caused by specific MKKs. The MKKs are activated, in turn, by MKKKs.

signals, small G proteins have been identified to have prominent roles in MAPK activation. For instance, ERK pathway can be activated by Ras *via* the Raf group of MKKK. In contrast, the p38 and JNK MAP kinases are activated by Rho family GTPases, including Rac and Cdc42.

Inactivation of MAP kinases by MAP kinase phosphatases

The activation of MAP kinases, the magnitude and the duration of their activation, and their inactivation are critical to mediate appropriate immune responses. Over the past several years, important advances have been made in our understanding of the negative regulation on MAP kinases. One new protein family named MAP kinase phosphatases (MKP) or dual specificity phosphatases (DUSP) has been defined as negative regulators of MAP kinases (12-14). They are identified by their common features: a C-terminal catalytic domain containing a highly conserved signature motif HCXXXXXR and two Cdc25-like domains. They inactivate MAP kinases through dephosphorylation of threonine and/or tyrosine residues within the signature sequences –Thr-X-Tyr- located in the activation loop of MAP kinases.

The inactivation of MAPKs by MKPs is a two-step catalytic reaction (15). It begins with nucleophilic attack by thiolate anion of cysteine in the signature motif of MKPs on the phosphorus atom of phosphotyrosine within MAPKs. Meanwhile, an aspartate in the general acid loop of MKPs donates a proton to the phenolic oxygen atom of phosphortyrosine, which results in the formation of a transient MKP-phosphate intermediate and the release of dephosphorylated MAPK (16). In the next step, the same aspartate in the general acid loop accepts a proton from a water molecule and the resulting hydroxyl anion attacks the phosphorus atom of the cysteinyl-phosphate intermediate leading to release of inorganic phosphate and regeneration of a thiolate anion at the active site cysteine in the free enzyme. The dephosphorylation of threonine is believed to be a similar mechanism as the dephosphorylation of tyrosine (15).

Thirteen MKPs have been identified so far with differential substrate specificities, distinct subcellular localizations and modes of regulation (Table 1). For instance, *in vitro* studies indicated that VHR and MKP3 are highly specific for ERK; MKP1, DUSP2, MKP5, MKP7 and VH5 have preference for p38/JNK; MKP2 and MKP4 showed similar preference to the three MAPKs. However, their *in vivo* substrate specificities could be different from their *in vitro* substrate preference. For example, *in vitro* studies indicated that MKP5 substrate preference is as p38~JNK>>ERK (17, 18). Our recent study using MKP5 knockout mice showed that MKP5 is a JNK phosphatase in T cells (19). MKP5-knockout T cells had increased JNK activities but not p38 and ERK. Therefore, the substrates of each MKP need to be further confirmed by *in vivo* study.

Table 1. Features of identified mammalian MKPs

Name	Substrate specificity	Other names	Subcellular localization	Human gene localization	Phenotype of knockout mice
VHR	ERK>>JNK~p38	DUSP3		17q21	
MKP3	ERK>>JNK~p38	DUSP6, PYST1	Cytosolic	12q22-q23	
PacI	ERK>> p38~JNK	DUSP2	Nuclear	2q11	
MKP6	ERK~JNK>>p38	DUSP14, MKP-L		17q12	
MKP2	ERK~JNK~p38	DUSP4, VH2, PYST1	Nuclear	8p11-12	
MKP4	ERK~JNK~p38	DUSP9, PYST3	Nuclear/cytosolic	Xq28	
MKP5	JNK>>p38>> ERK	DUSP10	Nuclear/cytosolic	1q32	Enhanced innate and adaptive immune responses; Resistant to MOG induced EAE; Fatal immune response to 2nd LCMV infection
MKP7	JNK~p38>>ERK	DUSP16	Cytosolic	12p12	
VH5	JNK~p38>>ERK	DUSP8, M3/6	Nuclear/cytosolic	11p15.5	
MKP1	p38~JNK>>ERK	DUSP1, HVH1, CL100	Nuclear	5q35	No obvious phenotype
DSP2	p38~JNK>>ERK	LMW-DSP2			
VH3	N.D.	DUSP5, B23	Nuclear	10q25	
PYST2	N.D.	DUSP7, B59, MKP-X	Cytosolic	3p21	

Modulation of MAP kinase activities by scaffold proteins

Scaffold proteins are organizers and facilitators of the MAPK signaling pathway. They can integrate proteins for their interaction and regulation or conversely may sequester proteins so that they do not interact with other proteins, therefore their regulatory roles could be positive or negative. For example, the JNK-interaction proteins (JIP) have been recently identified as scaffold proteins for the JNK pathway (20-22). Three members of this family, JIP1, JIP2 and JIP3, had been identified. JIP1 and JIP2 share high sequence homology. JIP proteins could interact with JNK, MKKs and members of the mixed-lineage kinases (MLK) group of MKKKs, or with members of MKPs to modulate JNK signaling (21-23). For instance, JIP1 could bind to MLK3, DLK (MLK members, MKKKs) and MKK7. Coexpression of these items enhanced JNK activation. The study of JIP1 knockout mice showed that JIP1 is a JNK activator *in vivo* (21). On the other hand, *in vitro* study showed that JIP1 also could bind the dual-specificity phosphatases MKP7 and M3/6 *via* a region independent of its JNK binding domain (23). The binding of MKP7 to JIP1 results in reduced activity of JNK. Therefore, JIP1 scaffold protein could modulate JNK signaling *via* association with both its protein kinases or protein phosphatases that target JNK. JIP3, also known as JSAP1, has no significant sequence homology with JIP1 and JIP2. In PC12 cells, JIP3 was found to bind to apoptosis signal regulating kinase (ASK) 1 which is a MKKK (25). After phosphorylation of JIP3 by ASK1, it recruits SEK1, MKK7 and JNK3 into its complex, which results in the enhancement of JNK activity. Matsuguchi et al. showed that JIP3 associated with Toll-like receptor 4 (TLR4) significantly increased LPS-induced JNK activation (26). It was also shown that JIP3 associated with other TLRs including TLR2, 4 and 9, indicating the involvement of JIP3 in TLR-mediated JNK activation. Study from Feig group showed that JIP2 is a p38 scaffold protein rather than a JNK-interaction protein because Rac GTPases Tiam1 and Ras-GRF1 bind to JIP2 and activate scaffold- associated p38 instead of JNK (27).

Kinase suppressor of Ras (KSR) is an ERK scaffold that interacts with the components of the Raf/MEK/ERK kinase cascade and positively regulates ERK signaling (28). MEK partner-1 (MP-1) was also identified as a scaffold protein for mammalian ERK (29). Sharma et al. showed that MP1 functions as a regulator of MAP kinase signaling by binding to MEK1 and regulating its association with a larger signaling complex that may sequentially service multiple molecules of ERK (30).

MAP kinases in innate immune responses

The innate immune system detects the presence and the nature of infection, provides the first line of host defense, and controls the initiation and determination of the effector classes of the adaptive immune response. Multicellular organisms have the ability to recognize invading micro-organisms by sense the conserved molecular patterns on microorganisms using so-called pattern recognition receptors such as the Toll-like protein family (31, 32). In *Drosophila*, the 9 Toll-like proteins are the central of innate immunity (32,

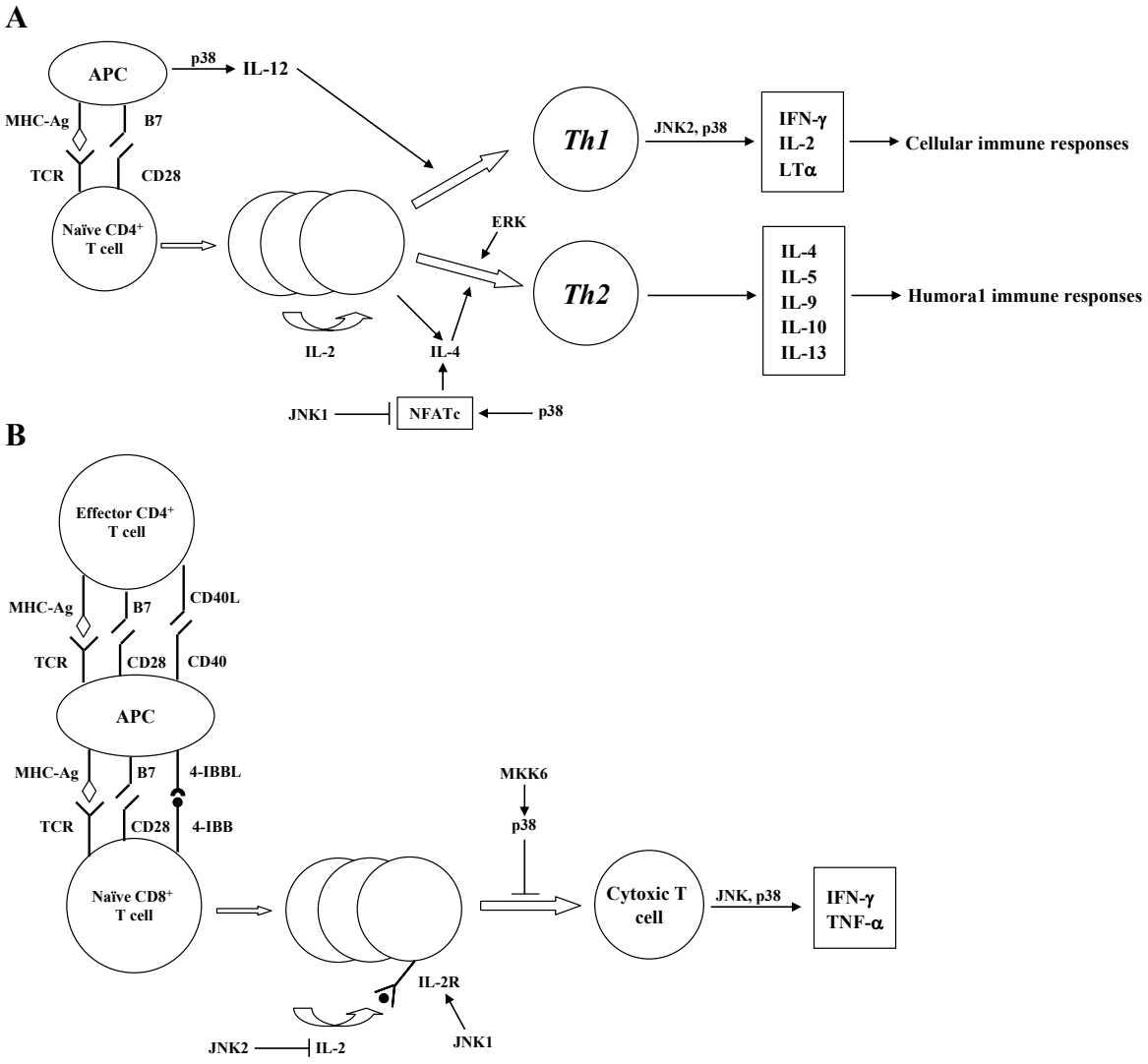

Figure 2. Role of MAP kinases in T cell activation, differentiation and function. (A) MAPKs in $CD4^+$ Th1 and Th2 cell differentiation and function. ERK is required for Th2 differentiation. p38 is required for both Th1 and Th2 differentiation and Th1 IFN-γ production. The role of JNK in $CD4^+$ T cell activation is to reduce the proliferative response of activated Th cells and to potentiate their polarized T cell differentiation into the Th1 lineage. (B) MAPKs in $CD8^+$ activation and function. p38 is involved in IFN-γ production and apoptosis of $CD8^+$ T cells. JNK1 and JNK1 play different roles in $CD8^+$ T cell proliferation.

33). In mammalian species, similar pattern recognition receptors also exist and are named as Toll-like receptors (TLRs). So far, 11 TLRs have been described. Each TLR recognizes specific microbial patterns. For instance, TLR2 recognizes lipoteichoic acids (LTAs) and peptidoglycan (PGN) (34, 35), TLR4 recognizes LPS (36-38), TRL5 recognizes bacterial flagellin (39) and TLR9 recognizes CpG-containing DNA (40). In mammalian species, there is an intracellular pattern recognition protein family called Nod involved in bacterial detection (41). Upon ligation by their specific ligands, TLRs or Nods trigger serial signal transduction events which lead to the activation of NFκB and MAP kinases such as JNK and p38. The activation of NFκB and MAP kinases leads to the production of various inflammatory cytokines including TNF-α, IL-1, IL-6 and IL-12, which are essential for the eradication of the infectious microorganisms.

The essential roles of MAP kinases in innate immune responses have been showed by many studies. Inhibition of p38 by using p38-specific inhibitors (SB 203580, SB 202190, and SB 202474) or by disruption of one of its activator, MKK3 (MAPKK), led to an approximately 40-70% reduction of total p38 activity in macrophages and a selective defect in LPS-induced IL-12 production at both protein and RNA levels (42). The MKK3 deficiency also resulted in a severe reduction of IL-12 secretion by bone marrow-derived DCs activated by CD40L which is a component of upstream of p38 and JNK. Therefore, MKK3-p38 pathway plays a specific role in the production of IL-12 in macrophages and

DCs. MAP-kinase-activated protein kinase (MAPKAP) MK2 is a substrate of p38 (43). The MK2 knockout mice exhibited increased stress resistance and survived LPS shock due to the reduced serum LPS induced TNF-α production (44).

The analysis of Tpl2/Cot, a proto-oncogene and a MAP kinase kinase kinase (MKKK), deficient mice indicated a role of ERK in TNF-α regulation (45). The macrophages from the deficient mice exhibited selective ERK deficits and were deficient in TNF-α production upon LPS stimulation. Further analysis demonstrated that TNF-α mRNA transport from the nucleus to the cytoplasm was inhibited by ERK inhibitor or Tpl2 inactivation.

JNK pathway is evolutionarily conserved in innate immune responses. In *Drosophila* cells, JNK pathway was activated in response to LPS (46). In mammalian species, JNK can be strongly induced in multiple cell types by LPS or inflammatory cytokines such as TNF and IL-1 (4, 47). In fibroblasts, the deficiency of JNK2 resulted in severe reduction of multiple cytokines production including type I interferon and IL-6 (48). MKP5 is a negative regulator of JNK (19). Study from our lab showed that MKP5 deficiency resulted in significant reduction of JNK activities in macrophages upon LPS stimulation (unpublished data). MKP5 knockout macrophages produced significantly increased level of inflammatory cytokines upon various TLR activations. The serum TNF-α level was increased in MKP5 knockout mice in response to LPS injection. Meanwhile, APCs from the knockout mice exhibited increased priming capabilities to OT-I and OT-II cells. Therefore, JNK negative regulator MKP5 plays an essential role in innate immune responses.

MAP kinases in adaptive immune responses

An adaptive immune response is a specific immune response, for instance the production of antibodies against a particular pathogen. Adaptive immune responses depend on lymphocytes including T lymphocytes and B lymphocytes (T and B cells). To participate in an adaptive immune response, T cells need to proliferate and differentiate into $CD4^+$ helper (Th) cells and $CD8^+$ cytotoxic cells from their naïve states after encountering antigen. MAP kinases play important role in the activation, differentiation and function of both $CD4^+$ and $CD8^+$ T cells (Figure 2).

$CD4^+$ Th cells develop in the thymus. Upon recognition of specific MHC-peptide complexes on APCs *via* the T cell receptor (TcR) complex plus receipt of a co-stimulatory signal provided by accessory molecules (B7 molecules) on APCs that bind CD28 or CTLA-4 expressed on T cells, Th cells are triggered to produce IL-2 and enter the cell cycle. Following or during several days of rapid cell division, these T cells differentiate into effector cells which mediate the specific function of the immune system (Figure 2A). Two classes of effector Th cells, Th1 and Th2 cells, have been defined on the basis of their cytokine secretion pattern and their immunomodulatory effects. Th1 cells produce IFN-γ and lymphotoxin-α (LTα), which mediate cellular immune responses. On the other hand, Th2 cells secrete a set of different cytokines including IL-4, IL-5, IL-9, IL-10, IL-13 and so on to mediate B cell activation and differentiation. MAP kinases play important role in these events.

The classic ERK kinases, including ERK1 and ERK2 which are sometimes referred as p44/p42 MAP kinase, were first identified downstream of oncogenic Ras and were often involved in the regulation of cell growth and differentiation. They can be activated by MEK1 and MEK2 upstream kinases and their activation is an important event of T cell activation. The role of Ras/ERK pathway in Th2 differentiation had been indicated by several studies. It was shown that Th2 differentiation was impaired in dominant-negative Ras transgenic T cells (49). The activation of the Ras/ERK pathway is required for IL-4 receptor function and the differentiation of $CD4^+$ Th2 cells. Jorritsma et al. showed that ERK played critical role in IL-4 expression during TCR-induced Th differentiation of naïve $CD4^+$ T cells (50). Reducing ERK activity either by a weak TCR signal or by using its pharmacological inhibitor PD98059 during strong TCR signaling induced early IL-4 expression in naïve $CD4^+$ T cells and subsequent Th2 differentiation. In purified human $CD4^+$ T cells, ERK activator MEK inhibitor effectively reduced alpha-CD3/alpha-CD28 stimulated IL-13 production (51). Intraperitoneal administration of U0126, a specific inhibitor of MEK, reduced lung eosinophila in ovalbum challenged Brown Norway rates by 44%, indicating that inhibition of the MEK-ERK cascade is a therapeutic option for asthma (51).

Several studies using p38 MAP kinase inhibitor SB 203580 showed that it is required for IL-2 expression (52-56). More recently, Wu et al. demonstrated that SB 203580 blocked activation-induced IL-2 production in both immature (thymocytes) and mature (splenic) T cells (57). T cells from B6 mice transgenic expressing a T-specific dominant-negative form of p38-α showed inhibited TCR-mediated p38 activity by 50%. IL-2 production by both thymocytes and splenic T cells from this mouse was largely suppressed upon TCR activation. They further illustrated that p38 MAPK activated NFATc by activating its promoter, stabilizing its mRNA, increasing its translation and promoting its binding to CBP. All together, these studies show that p38 is essential for T cell activation (Figure 2A).

The p38 MAP kinase is also known as an important positive regulator of IFN-γ and Th1 differentiation (Figure 2A) (58-60). It is selectively activated in Th1 effector cells but not in Th2 cells (58). Persistent activation of p38 kinase resulted in increased IFN-γ production by Th1 effector cells (58). Retroviral overexpression of GADD45β, an activator of p38 kinase, leads to sustained p38 activation and enhanced IFN-γ production in IL-12/IL-18-stimulated Th1 cells (61). Imidazole inhibitiors of the p38 kniases block IFN-γ production by Th1 cells in a dose-dependent manner but have no effect on IL-4 production by Th2 cells. Furthermore, cells from dominant negative p38α transgenic mice showed reduced IFN-γ cytokine secretion and mRNA production (58). Inhibition of p38 by its specific inhibitor SC-409 block

IFN-γ production of IL-12/IL-18 activated Th1 cell (62). T cells from mice deficient in the p38 upstream kinase MKK3 have a defect in IFN-γ in production (59). The effect of p38 pathway on IFN-γ is probably transcriptional since IFN-γ promoter reporters are also inhibited by dominant negative p38α in Jurkat cells, indicating that p38 regulation is necessary for IFN-γ expression (59).

In $CD8^+$ T cells, p38 regulates IFN-γ in the similar manner as in $CD4^+$ T cells: inhibition of p38 MAP kinase inhibits IFN-γ production and persistent activation of p38 promotes IFN-γ production (Figure 2B) (63). However, activation of p38 MAP kinase alone has different effects in $CD8^+$ and $CD4^+$ T cells. Expression of the constitutively active MKK6, which actives p38 MAP kinase only, in transgenic mice caused a dramatic reduction in the number of $CD8^+$ T cells *in vivo* and rapid apoptosis of $CD8^+$ T cells *in vitro*, while the $CD4^+$ T cells were unaffected (63). The presence of the p38 MAP kinase inhibitor or a general inhibitor of caspases (zVAD) prevented apoptosis of the transgenic $CD8^+$ T cell *in vitro*, indicating that p38 MAP kinase induces death of $CD8^+$ T cells through a caspase-dependent mechanism (63).

The study of JNK1 and JNK2 knockout mice revealed the critical roles of JNK in $CD4^+$ T cell proliferation and effector T cell function (1, 64). Th cells from the *Jnk1*$^{-/-}$ mice exhibited greatly reduced JNK activity after activation (64). JNK1-deficient mice exhibited an exaggerated Th2 response which led to greatly exacerbated disease with failure to heal skin lesions upon *Leishmania* infection (65). The study of *Jnk2*$^{-/-}$ mice showed that it was required for IFN-γ production (64, 65). The JNK1 and JNK2 double knockout has shown to be embryonically lethal. Dong et al. created animals deficient in both JNK1 and 2 only in T cells and found that these T cells produced 2-3 fold more IL-2 and exhibited greater proliferation than wild-type cells (67). They were also found to produce exaggerated amounts of Th2 cytokines, and preferentially develop along the Th2 lineage. All these studies demonstrate that the role of JNK in T cell activation is to reduce the proliferative response of activated Th cells, and to potentiate their polarized T cell differentiation into the Th1 lineage (67). Furthermore, our analysis of MKP5 knockout mice revealed different roles of JNK in $CD4^+$ T cell activation and effector function (19). After *Mkp5* was knocked out, JNK activity was selectively increased in $CD4^+$ T cells. These cells exhibited hypoproliferation upon TCR-mediated and antigen-specific-mediated activation. However, both MKP5 deficient Th1 and Th2 cells produce greatly increased level of effector cytokines.

In $CD8^+$ T cells, both JNK1 and JNK2 expression could be induced upon activation (Figure 2B) (68). However, they play different roles in $CD8^+$ T cells. Studies using JNK1 deficient mice showed that JNK1$^{-/-}$$CD8^+$ T cells are hypo-proliferative and produce lower levels of IL-2 (68). The JNK1 deficient mice generate lower numbers of virus-specific $CD8^+$ T cells in response to lymphocytic choriomeningitis virus (LCMV) infection (69). The hypoproliferation of JNK1$^{-/-}$$CD8^+$ T cells seems due to the impaired expression of IL-2Rα resulted from a deficient activation of AP1 since the total levels of c-Jun in activated JNK1$^{-/-}$$CD8^+$ T cells were reduced and c-Jun plays an important role in the regulation of IL-2Rα gene expression (68, 70). Conversely, JNK2$^{-/-}$$CD8^+$ cells are hyperproliferative and produce elevated levels of IL-2 than wild-type $CD8^+$ T cells (68). JNK2 knockout mice generate more virus-specific $CD8^+$ T cells in response to LCMV infection than wild-type mice (69). The hyper-proliferation of JNK2 deficient mice is due to its increased production of IL-2 because the addition of exogenous IL-2 results no difference in proliferation between wild-type and JNK2$^{-/-}$$CD8^+$ T cells (68). The MKP5 knockout $CD8^+$ T cells exhibited reduced proliferation *in vitro* (our lab unpublished data). However, upon 2nd LCMV infection, the MKP5 knockout viral specific $CD8^+$ T cells produced significantly increased inflammatory cytokines including TNF-α and IFN-γ, indicating the positive role of JNK in regulation of $CD8^+$ T cell effector unction (19).

Concluding remarks

Great advances have been achieved during the last few years in our understanding of regulation of MAP kinase signaling pathways and their roles in immune responses. More and more new proteins have been and will be defined as components of MAP kinase pathways. However, many questions regarding various aspects of these pathways are still awaited to be resolved. For instance, the negative regulation of MAP kinases by various protein phosphatases has not been fully understood. The *in vivo* roles of most MKPs in MAP kinases regulation are still unknown. Our knowledge about the roles of MAP kinases in innate immunity still awaits for further improvement. Little is presently known about mechanism of MAP kinase modulation in response to different stimuli in different context. The specific targets of the MAP kinases in $CD4^+$ and $CD8^+$ T cells and how they regulate specific gene expression are need to be further elucidated. Future analysis of new components of MAP kinase pathway using traditional and new technologies such as gene knockout and siRNA based gene knockdown will no doubt advance our knowledge of the mechanisms of MAP kinase signaling in immune responses and may help development of therapeutic agents to selectively modulate MAP kinase to treat immune disorders.

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