ELSEVIER

Immunopharmacology 47 (2000) 119–125

Immunopharmacology

www.elsevier.com/locate/immpharm

Review

Mechanisms of action of cyclosporine

Satoshi Matsuda, Shigeo Koyasu *

Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Received 30 August 1999; accepted 1 September 1999

Abstract

Cyclosporine (cyclosporin A, CsA) has potent immunosuppressive properties, reflecting its ability to block the transcription of cytokine genes in activated T cells. It is well established that CsA through formation of a complex with cyclophilin inhibits the phosphatase activity of calcineurin, which regulates nuclear translocation and subsequent activation of NFAT transcription factors. In addition to the calcineurin/NFAT pathway, recent studies indicate that CsA also blocks the activation of JNK and p38 signaling pathways triggered by antigen recognition, making CsA a highly specific inhibitor of T cell activation. Here we discuss the action of CsA on JNK and p38 activation pathways. We also argue the potential of CsA and its natural counterparts as pharmacological probes.

Keywords: MAPK; JNK; p38; NFAT

1. Action on the calcineurin/NFAT pathway

Cyclosporine (cyclosporin A, CsA), a neutral lipophilic cyclic undecapeptide isolated from the fungus *Hypocladium inflatum gams*, has been widely used for the treatment of allograft rejection and graft-vs.-host disease since Borel et al. (1976) reported its immunosuppressive activity. Early biological studies revealed that CsA inhibits T cell activation by blocking the transcription of cytokine genes, including those of IL-2 and IL-4 (Kronke et al., 1984; Herold et al., 1986; Granelli-Piperno, 1988). Upon entering T cells, CsA binds with high affinity to cyclophilins, especially to the cytosolic 17 kDa cyclophilin A which is the most abundant cyclophilin in T cells (Handschumacher et al., 1984; Schreiber, 1991). Cyclophilins are ubiquitous cytosolic proteins possessing peptidyl-proline-*cis-trans* isomerase (PPIase) activity, an enzymatic activity possibly mediating protein folding (Schmid, 1995). Although CsA inhibits the PPIase activity of cyclophilins, inhibition of PPIase is not involved in the mechanism of immunosuppression because some of CsA analogues which fail to block T cell activation are still able to inhibit the PPIase activity (Bierer et al., 1990; Sigal et al., 1991).

Liu et al. (1991) found that the cyclophilin–CsA complex, but not cyclophilin alone, can associate with another cytosolic protein, calcineurin. Calcineurin (also termed PP2B) belongs to a superfamily of protein serine/threonine phosphatases, and its activity is tightly regulated by Ca^{2+}/calmodulin (Klee et al., 1988; Cohen, 1989; Shenolikar, 1994).

* Corresponding author. Tel.: +81-3-3353-1211; fax: +81-3-5361-7658.

E-mail address: koyasu@sun.microb.med.keio.ac.jp (S. Koyasu).

0162-3109/00/$ - see front matter
PII: S0162-3109(00)00192-2

Calcineurin consists of two subunits, a catalytic subunit (calcineurin A, CnA) and a regulatory subunit (calcineurin B, CnB). Note that there are two genes encoding closely related (approximately 80% identical) CnA isoforms, CnAα and CnAβ, and that CnAα is the predominant isoform expressed in T cells (Guerini and Klee, 1991; Zhang et al., 1996). Engagement of the T cell receptor (TCR) with its cognate ligand induces the elevation of intracellular calcium concentration, and subsequent activation of calmodulin. Activated calmodulin, then, interacts with CnA and releases the autoinhibitory domain of CnA from its active site, leading to the activation of its phosphatase activity. The cyclophilin–CsA complex directly binds to CnA and inhibits the phosphatase activity. In contrast to TCR-induced T cell activation (Clipstone and Crabtree, 1992; O'Keefe et al., 1992), CsA fails to inhibit certain types of Ca^{2+}-independent T cell activation pathways such as stimulation through CD28 in the presence of PMA (Lin et al., 1991).

Calcineurin dephosphorylates NFAT family members, allowing them to translocate into the nucleus and activate gene expression through the *cis*-element named NF-AT (Flanagan et al., 1991; Northrop et al., 1994; Shaw et al., 1995; Loh et al., 1996; Timmerman et al., 1996). Recent studies have also shown that activated calcineurin also translocates into the nucleus together with NFAT family members, where it may maintain the sustained activation of NFAT proteins (Shibasaki et al., 1996). Among NFAT family members, NFAT1, NFAT2, and NFAT4 are involved in the transcriptional activation of genes encoding cytokines including IL-2 and IL-4, and CD40L (Rao et al., 1997). By preventing their calcineurin-mediated dephosphorylation, CsA inhibits the nuclear translocation of these NFAT family members and subsequent gene expression in activated T cells. Undoubtedly, inhibition of the calcineurin/NFAT pathway is one of the mechanisms of CsA-mediated immunosuppression.

2. Action on JNK and p38 signaling pathways

Transcriptional activation of the IL-2 gene requires cooperative interaction of several transcription factors, including AP-1, NF-κB, and NFAT (Crabtree, 1989). It has been shown that CsA affects the activities of AP-1 and NF-κB in addition to NFAT, implying the presence of another target(s) of CsA as well as the calcineurin/NFAT pathway (Mattila et al., 1990; Rincon and Flavell, 1994). It has also been shown that CsA can inhibit an antigen-specific and Ca^{2+}-independent response (Metcalfe et al., 1994). Accordingly, recent studies provided evidence that CsA blocks both JNK and p38 signaling pathways in addition to the calcineurin/NFAT pathway (Su et al., 1994; Matsuda et al., 1998).

The mitogen-activated protein kinase (MAPK) pathway is a conserved eukaryotic signaling cascade that participates in a diverse array of biological processes. This pathway consists of three protein kinases, MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKK-K); MAPKK-K phosphorylates and activates MAPKK, which in turn phosphorylates and activates MAPK (Nishida and Gotoh, 1993; Avruch et al., 1994; Blumer and Johnson, 1994; Marshall, 1995; Waskiewicz and Cooper, 1995; Su and Karin, 1996). Three distinct subgroups of the MAPK superfamily, ERK (also termed classical MAPK), JNK (also termed SAPK) and p38 (also termed MPK2, RK, HOG1, and CSBP), have been defined in detail in mammalian cells (Davis, 1994; Woodgett et al., 1996; Robinson and Cobb, 1997; Ip and Davis, 1998; Lewis et al., 1998). Although JNK and p38 are thought to function primarily in stress responses, such as inflammation and apoptosis, we and others have shown that both are activated when T cell responses are triggered through both the TCR and CD28 costimulatory receptor, and that these pathways are sensitive to CsA (Su et al., 1994; Matsuda et al., 1998). Cooperative activation of JNK and p38 in conjunction with ERK leads to activation of transcription factors including AP-1 (Karin, 1995). It was further demonstrated that dominant-negative mutants which block JNK and p38 signaling pathways abrogate the transcriptional activation of the NF-AT *cis*-element which consists of binding sites for AP-1 and NFAT proteins (Matsuda et al., 1998). These results raise the possibility that the immunosuppressive effect of CsA is attributed, at least in part, to the inhibition of JNK and p38 pathways. The presence of two target pathways of CsA in T cells explains the high specificity of its immunosuppressive activity.

In contrast to JNK and p38 signaling pathways, CsA has no effect on the activation of the ERK pathway. Interestingly, CsA failed to block stress-induced activation of JNK and p38 pathways (unpublished observation), indicating that a target(s) of CsA for JNK and p38 activation pathways is involved in a T cell-specific signaling pathway triggered by the TCR and CD28 costimulatory molecule. During T cell activation, JNK and p38 are presumably activated through MKK7 and MKK6, respectively. It was further shown that activation of both MKK7 and MKK6 is also sensitive to CsA (Matsuda et al., 1998). In addition, we found that the activation of MEKK1, a putative activator of MKK7, is inhibited by CsA (unpublished observation). Since it has been suggested that MEKK1 is involved in both NF-κB and JNK pathways (Karin and Delhase, 1998), MEKK1 may mediate the CsA-sensitive NF-κB pathway during T cell activation. Besides MEKK1, MAPKK-Ks such as MLK3 and TAK1 may also participate in the signaling pathway leading to the activation of JNK and/or p38 during T cell activation (Schaeffer and Weber, 1999). However, it is unknown whether these MAPKK-Ks are also activated in a CsA-sensitive manner. As an upstream component(s) of MAPKK-Ks, members of the Rho subfamily of small G-proteins, Rac1 and/or Cdc42, have been proposed (Coso et al., 1995; Jacinto et al., 1998; Reif and Cantrell, 1998). Although it is unknown whether JNK activation is mediated by Rac1 or Cdc42, JNK activation is induced by transfection of a constitutively active form of Rac1 (V12Rac1) in a CsA-insensitive manner (unpublished observation). It has been demonstrated that the transcriptional activity of the IL-2 promoter is augmented by overexpression of Vav1, a guanine nucleotide exchange factor for Rac1 (Collins et al., 1997), and that CsA blocks Vav1 induced transcriptional activation of IL-2 promoter (Wu et al., 1995). These results collectively suggest that CsA inhibits the JNK signaling pathway at a level upstream of Rac1 but affecting Vav1 itself or a downstream molecule(s). On the other hand, JNK activation was unaffected in *vav1*-deficient mice (Costello et al., 1999; Penninger et al., 1999). It is possible that other guanine nucleotide exchange factor(s) for Rac1, such as Vav2 and Dbl, mediate the signaling pathway leading to JNK activation. It is also possible that JNK activation in T cells is mediated by an HPK1 pathway (Kiefer et al., 1996; Anafi et al., 1997; Ling et al., 1999) which is Rac1-independent and that CsA perturbes this pathway (Fig. 1). Identification of a target(s) for CsA-mediated blockade of the JNK and/or p38 pathways will clarify the molecular mechanism leading to JNK activation and uncover a novel therapeutic target(s) for immunosuppression.

FK506 (Tacrolimus), another inhibitor for the calcineurin/NFAT pathway, also blocks the activation of JNK and p38 pathways in T cells (manuscript in preparation). These results strongly suggest the involvement of calcineurin in the signaling pathway leading to JNK and p38 activation. Consistently, Werlen et al. (1998) have reported that activation of calcineurin in combination with PKC-θ causes the activation of JNK. Their conclusion essentially depends on two observations: a constitutively active form of CnA can induce JNK activation in the presence of PMA which activates PKC, and a constitutively active form of PKC-θ can also induce JNK activation with the aid of a calcium ionophore which activates calcineurin. It should be noted, however, that the concentration of PMA used in their paper

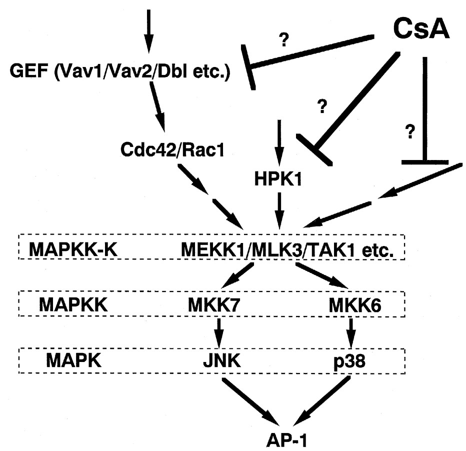

Fig. 1. Potential target sites of CsA for JNK and p38 activation pathways. Guanine nucleotide exchange factors (GEF) for Rac1 and/or Cdc42, such as Vav1, Vav2 and Dbl, are candidates for the target of CsA. Another candidate is an HPK1 pathway, which functions as an activator for MAPKK-Ks such as MLK3 and TAK1. It is also possible that the CsA-sensitive JNK and p38 activation in T cells is mediated by unidentified molecule(s).

(100 nM) is high enough to induce JNK activation without the calcium ionophore, and that JNK activation induced by 100 nM PMA is not inhibitable by 100 ng/ml CsA (unpublished observation), implying that calcineurin is not involved in JNK activation. Furthermore, it has recently been shown that expression of a constitutively active form of PKC-θ alone results in strong activation of JNK (Ghaffari-Tabrizi et al., 1999). Although we do not formally exclude the possible contribution of calcineurin to JNK activation pathway, additional studies will be required before drawing that conclusion.

3. Other effects of CsA

Despite being highly efficacious for prevention of organ transplant rejection, the use of CsA as an immunosuppressant is limited by severe side effects including nephrotoxicity, neurotoxicity, and hepatotoxicity (Kahan, 1989). CsA has been shown to induce the synthesis of TGF-β in vitro and in vivo (Li et al., 1991; Khanna et al., 1994; Wolf et al., 1995; Shihab et al., 1996). Several studies have suggested the involvement of TGF-β in the progression of renal diseases (Klahr et al., 1995; Border and Noble, 1997; Pintavorn and Ballermann, 1997). TGF-β is known to stimulate cells to increase their extracellular matrix (ECM) composition and decreases the production of ECM-degrading proteases, thereby inducing a profibrogenic state (Massagué, 1990). This is in line with the fact that the CsA-induced nephrotoxicity has the characteristics of interstitial fibrosis. Furthermore, it has also been reported that TGF-β produced by CsA administration directly promotes cancer progression (Hojo et al., 1999). The CsA-induced TGF-β synthesis could result from the inhibition of the calcineurin/NFAT pathway, or alternatively, the blockade of the JNK and p38 signaling pathways. However, the precise mechanism remains to be elucidated.

On the other hand, the use of CsA as a pharmacological probe has uncovered novel functions of the calcineurin/NFAT pathway. Studies on HIV-1 showed the critical role of NFAT2 during HIV-1 infection of primary T cells, which can be inhibited by treatment with CsA (Sun et al., 1997; Kinoshita et al., 1998). NFAT3, another member of the NFAT

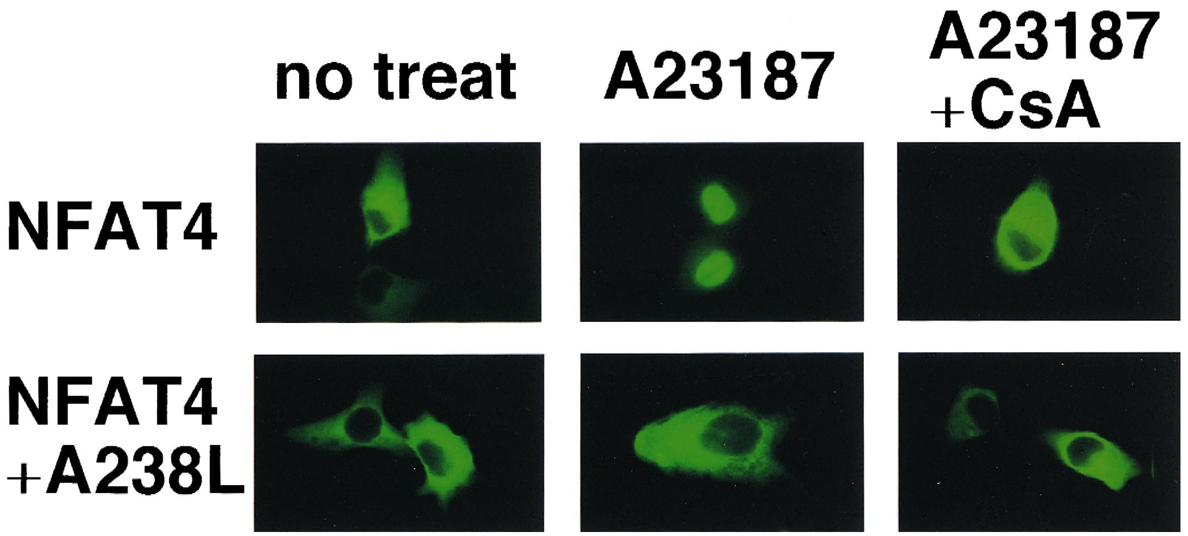

Fig. 2. A238L inhibits the nuclear translocation of NFAT4. Baby hamster kidney cells were transfected with GFP-NFAT4 in the absence (NFAT4) or the presence (NFAT4 + A238L) of A238L. The cells were then treated for 30 min at 37°C with vehicle alone (no treat), calcium ionophore A23187 (A23187), or a combination of A23187 with CsA (A23187 + CsA). The subcellular distribution of GFP-NFAT4 was examined by an immunofluorescence microscopy. Note that co-expression of A238L blocks the nuclear accumulation of GFP-NFAT4 in response to A23187 as observed in the presence of CsA. The concentrations used are 1 μg/ml for A23187 and 100 ng/ml for CsA. The cDNAs for NFAT4 and A238L are kindly provided by F. Shibasaki and L.K. Dixon, respectively.

family, in conjunction with calcineurin was shown to be involved in progression of cardiac hypertrophy. Expression of either constitutively active forms of calcineurin or NFAT3 in mice results in cardiac hypertrophy leading to congestive heart failure and sudden death (Molkentin et al., 1998). Furthermore, in some rat models where cardiac hypertrophy is induced by pathologic stimuli, administration of CsA prevents disease progression (Sussman et al., 1998). These results indicate not only widespread functions of the calcineurin/NFAT pathway in biological events but also novel therapeutic approaches with CsA. It should be noted, however, that the doses of CsA required for prevention of these events are relatively higher than those required for immunosuppression and induction of nephrotoxicity. It is likely that CsA blocks calcineurin/NFAT pathway but not JNK and/or p38 pathways in these cases.

4. Newly identified proteins with putative immunosuppressive activity

Recent studies have identified several proteins which exhibit functions similar to CsA. Sun et al. (1998) identified a novel calcineurin-binding protein named Cabin1, which binds to and inhibits the phosphatase activity of calcineurin. Cabin1 is widely expressed in a variety of tissues and cells, including the spleen and leukocytes. Since Cabin1, when over-expressed, inhibits dephosphorylation of NFAT protein and blocks the transcriptional activation of IL-2 reporter gene during T cell activation, it is suggested that Cabin1 serves as an endogenous inhibitor for calcineurin.

It has also been shown that a viral protein named A238L, derived from African swine fever virus, inhibits NFAT-regulated gene transcription in vivo (Miskin et al., 1998). African swine fever virus inhibits proinflammatory cytokine expression in infected macrophages, whereas a mutant virus lacking A238L fails to block cytokine gene expression. Furthermore, A238L is shown to associate with calcineurin in a yeast two-hybrid system. In addition, A238L blocks the nuclear translocation of NFAT protein induced by the elevation of intracellular calcium concentration, which is presumably mediated by calcineurin activation (Fig. 2). These results indicate that A238L is a viral counterpart of CsA. It will be of interest to ascertain whether A238L blocks the activation of JNK and p38 pathways during T cell activation as observed in CsA-treated T cells.

Further studies on these natural counterparts of CsA could give us better understanding of the mechanisms of action of calcineurin and may provide a better screening system for immunosuppressive drugs acting through one or both of the pathways outlined in this review.

References

Anafi, M., Kiefer, F., Gish, G.D., Mbamalu, G., Iscove, N., Pawson, T., 1997. SH2/SH3 adaptor proteins can link tyrosine kinases to a Ste20-related protein kinase, HPK1. J. Biol. Chem. 272, 27804–27811.

Avruch, J., Zhang, X.F., Kyriakis, J.M., 1994. Raf meets Ras: completing the frame work of a signal transduction pathway. Trends Biochem. Sci. 19, 279–283.

Bierer, B.E., Somers, P.K., Wandless, T.J., Burakoff, S.J., Schreiber, S.L., 1990. Probing immunosuppressant action with a nonnatural immunophilin ligand. Science 250, 556–559.

Blumer, K.J., Johnson, G.L., 1994. Diversity in function and regulation of MAP kinase pathways. Trends Biochem. Sci. 19, 236–240.

Border, W.A., Noble, N.A., 1997. TGF-beta in kidney fibrosis: a target for gene therapy. Kidney Int. 51, 1388–1396.

Borel, J.F., Feurer, C., Gubler, H.V., Stahelin, H., 1976. Biological effect of cyclosporin A: a new anti-lymphocytic agent. Agents Actions 6, 468–475.

Clipstone, N.A., Crabtree, G.R., 1992. Identification of calcineurin as a key signaling enzyme in T-lymphocyte activation. Nature 357, 695–697.

Cohen, P., 1989. The structure and regulation of protein phosphatases. Annu. Rev. Biochem. 58, 453–508.

Collins, T.L., Deckert, M., Altman, A., 1997. Views on Vav. Immunol. Today 18, 221–225.

Coso, O.A., Chiariello, M., Yu, J.C., Teramoto, H., Crespo, P., Xu, N., Miki, T., Gutkind, J.S., 1995. The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. Cell 81, 1137–1146.

Costello, P.S., Walters, A.E., Mee, P.J., Turner, M., Reynolds, L.F., Prisco, A., Sarner, N., Zamoyska, R., Tybulewicz, V.L.J., 1999. The Rho-family GTP exchange factor Vav is a critical transducer of T cell receptor signals to the calcium, ERK, and NF-κB pathways. Proc. Natl. Acad. Sci. U. S. A. 96, 3035–3040.

Crabtree, G.R., 1989. Contingent genetic regulatory events in T-lymphocyte activation. Science 258, 478–480.

Davis, R.J., 1994. MAPKs: new JNK expands the group. Trends Biochem. Sci. 19, 470–473.

Flanagan, W.M., Corthesy, B., Bram, R.J., Crabtree, G.R., 1991.

Nuclear association of a T-cell transcription factor blocked by FK506 and cyclosporin A. Nature 352, 803–807.
Ghaffari-Tabrizi, N., Bauer, B., Villunger, A., Baier-Bitterlich, G., Altman, A., Utermann, G., Uberall, F., Baier, G., 1999. Protein kinase Cθ, a selective upstream regulator of JNK/SAPK and IL-2 promoter activation in Jurkat T cells. Eur. J. Immunol. 29, 132–142.
Granelli-Piperno, A., 1988. In situ hybridization for interleukin 2 and interleukin 2 receptor mRNA in T cells activated in the presence or absence of cyclosporin A. J. Exp. Med. 168, 1649–1658.
Guerini, D., Klee, C.B., 1991. Structural diversity of calcineurin, a Ca^{2+} and calmodulin-stimulated protein phosphatase. Adv. Protein Phosphatases 6, 391–410.
Handschumacher, R.E., Harding, M.W., Rice, J., Drugge, R.J., 1984. Cyclophilin: a specific cytosolic binding protein for cyclosporin A. Science 226, 544–547.
Herold, K.C., Lancki, D.W., Moldwin, R.L., Fitch, F.W., 1986. Immunosuppressive effects of cyclosporin A on cloned T cells. J. Immunol. 136, 1315–1321.
Hojo, M., Morimoto, T., Maluccio, M., Asano, T., Morimoto, K., Lagman, M., Shimbo, T., Suthanthiran, M., 1999. Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 397, 530–534.
Ip, Y.T., Davis, R.J., 1998. Signal transduction by the c-Jun N-terminal kinase (JNK)-from inflammation to development. Curr. Opin. Cell Biol. 10, 205–219.
Jacinto, E., Werlen, G., Karin, M., 1998. Cooperation between Syk and Rac1 leads to synergistic JNK activation in T lymphocytes. Immunity 8, 31–41.
Kahan, B.D., 1989. Drug therapy — cyclosporine. N. Engl. J. Med. 321, 1725–1738.
Karin, M., 1995. The regulation of AP-1 activity by mitogen-activated protein kinases. J. Biol. Chem. 270, 16483–16486.
Karin, M., Delhase, M., 1998. JNK or IKK, AP-1 or NF-κB, which are the targets for MEK kinase 1 action? Proc. Natl. Acad. Sci. U. S. A. 95, 9067–9069.
Khanna, A., Li, B., Stenzel, K.H., Suthanthiran, M., 1994. Regulation of new DNA synthesis in mammalian cells by cyclosporine. Transplantation 57, 577–582.
Kiefer, F., Tibbles, L.A., Anafi, M., Janssen, A., Zanke, B.W., Lassam, N., Pawson, T., Woodgett, J.R., Iscove, N.N., 1996. HPK1, a hematopoietic protein kinase activating the SAPK/JNK pathway. EMBO J. 15, 7013–7025.
Kinoshita, S., Chen, B.K., Kaneshima, H., Nolan, G.P., 1998. Host control of HIV-1 parasitism in T cells by the nuclear factor of activated T cells. Cell 95, 595–604.
Klahr, S., Ishidoya, S., Morrissey, J., 1995. Role of angiotensin II in the tubulointerstitial fibrosis of obstructive nephropathy. Am. J. Kidney Dis. 26, 141–146.
Klee, C.B., Draetta, G.F., Hubbard, M.J., 1988. Calcineurin. Adv. Enzymol. Rel. Areas Mol. Biol. 61, 149–209.
Kronke, M., Leonard, W.J., Depper, J.M., Ayra, S.K., Wong-Staal, F., Gallo, R.C., Waldman, T.A., Greene, W.C., 1984. Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. Proc. Natl. Acad. Sci. U. S. A. 81, 5214–5218.
Lewis, T.S., Shapiro, P.S., Ahn, N.G., 1998. Signal transduction through MAP kinase cascades. Adv. Cancer Res. 74, 49–139.
Li, B., Sehajpal, P., Khanna, A., Vlassara, H., Cerami, A., Suthanthiran, M., 1991. Differential regulation of transforming growth factor-β and interleukin 2 genes in human T cells: demonstration by usage of novel competitor DNA constructs in quantitative polymerase chain reaction. J. Exp. Med. 174, 1259–1264.
Lin, C.S., Boltz, R.C., Siekierka, J.J., Sigal, N.H., 1991. FK506 and cyclosporin A inhibit highly similar signal transduction pathways in human T lymphocytes. Cell. Immunol. 133, 269–284.
Ling, P., Yao, Z., Meyer, C.F., Wang, X.S., Oehrl, W., Feller, S.M., Tan, T.-H., 1999. Interaction of hematopoietic progenitor kinase 1 with adapter proteins Crk and CrkL leads to synergistic activation of c-Jun N-terminal kinase. Mol. Cell. Biol. 19, 1359–1368.
Liu, J., Farmer, J.D., Lane, W.S., Friedman, J., Weissman, I., Schreiber, S.L., 1991. Calcineurin is a common target of cyclophilin–cyclosporin A and FKBP–FK506 complexes. Cell 66, 807–815.
Loh, C., Carew, J.A., Kim, J., Hogan, P.G., Rao, A., 1996. T-cell receptor stimulation elicits an early phase of activation and a later phase of deactivation of the transcription factor NFAT1. Mol. Cell. Biol. 16, 3945–3954.
Marshall, C.J., 1995. Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell 80, 179–185.
Massagué, J., 1990. The transforming growth factor-β family. Annu. Rev. Cell Biol. 6, 597–641.
Matsuda, S., Moriguchi, T., Koyasu, S., Nishida, E., 1998. T lymphocyte activation signals for interleukin-2 production involve activation of MKK6-p38 and MKK7-SAPK/JNK signaling pathways sensitive to cyclosporin A. J. Biol. Chem. 273, 12378–12382.
Mattila, P.S., Ullman, K.S., Fiering, S., Emmel, E.A., McCutcheon, M., Crabtree, G.R., Herzenberg, L.A., 1990. The actions of cyclosporin A and FK506 suggest a novel step in the activation of T lymphocytes. EMBO J. 9, 4425–4433.
Metcalfe, S., Alexander, D., Turner, J., 1994. FK506 and cyclosporin A each inhibit antigen-specific signaling in the T cell line 171 in the absence of a calcium signal. Cell. Immunol. 158, 46–58.
Miskin, J.E., Abrams, C.C., Goatley, L.C., Dixon, L.K., 1998. A viral mechanism for inhibition of the cellular phosphatase calcineurin. Science 281, 562–565.
Molkentin, J.D., Lu, J.-R., Antos, C.L., Markham, B., Richardson, J., Robbins, J., Grant, S.R., Olson, E.N., 1998. A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. Cell 93, 215–228.
Nishida, E., Gotoh, Y., 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem. Sci. 18, 128–131.
Northrop, J.P., Ho, S.N., Chen, L., Thomas, D.J., Timmerman, L.A., Nolan, G.P., Admon, A., Crabtree, G.R., 1994. NF-AT components define a family of transcription factors targeted in T-cell activation. Nature 369, 497–502.

O'Keefe, S.J., Tamura, J., Kincaid, R.L., Tocci, M.J., O'Neill, E.A., 1992. FK-506-and CsA-sensitive activation of the interleukin-2 promoter by calcineurin. Nature 357, 692–694.

Penninger, J.M., Fischer, K.D., Sasaki, T., Kozieradzki, I., Le, J., Tedford, K., Bachmaier, K., Ohashi, P.S., Bachmann, M.F., 1999. The oncogene product Vav is a crucial regulator of primary cytotoxic T cell responses but has no apparent role in CD28-mediated co-stimulation. Eur. J. Immunol. 29, 1709–1718.

Pintavorn, P., Ballermann, B.J., 1997. TGF-beta and the endothelium during immune injury. Kidney Int. 51, 1401–1412.

Rao, A., Luo, C., Hogan, P.G., 1997. Transcription factors of the NF-AT family: regulation and function. Annu. Rev. Immunol. 15, 707–747.

Reif, K., Cantrell, D.A., 1998. Networking Rho family GTPases in lymphocytes. Immunity 8, 395–401.

Rincon, M., Flavell, R.A., 1994. AP-1 transcriptional activity requires both T-cell receptor-mediated and co-stimulatory signals in primary T lymphocytes. EMBO J. 13, 4370–4381.

Robinson, M.J., Cobb, M.H., 1997. Mitogen-activated protein kinase pathways. Curr. Opin. Cell Biol. 9, 180–186.

Schaeffer, H.J., Weber, M.J., 1999. Mitogen-activated protein kinases: specific messages from ubiquitous messengers. Mol. Cell. Biol. 19, 2435–2444.

Schmid, F.X., 1995. Protein folding. Prolyl isomerases join the fold. Curr. Biol. 5, 993–994.

Schreiber, S.L., 1991. Chemistry and biology of the immunophilines and their immunosuppressive ligands. Science 251, 283–287.

Shaw, K.T., Ho, A.M., Raghavan, A., Kim, J., Jain, J., Park, J., Sharma, S., Rao, A., Hogan, P.G., 1995. Immunosuppressive drugs prevent a rapid dephosphorylation of transcription factor NFAT1 in stimulated immune cells. Proc. Natl. Acad. Sci. U. S. A. 92, 11205–11209.

Shenolikar, S., 1994. Protein serine/threonine phosphatases: new avenues for cell regulation. Annu. Rev. Cell Biol. 10, 55–86.

Shibasaki, F., Price, E.R., Milan, D., McKeon, F., 1996. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. Nature 382, 370–373.

Shihab, F.S., Andoh, T.F., Tanner, A.M., Noble, N.A., Border, W.A., Franceschini, N., Bennett, W.M., 1996. Role of transforming growth factor-beta 1 in experimental chronic cyclosporine nephropathy. Kidney Int. 49, 1141–1151.

Sigal, N.H., Dumont, F., Durette, P., Siekierka, J.J., Peterson, L., Rich, D.H., Dunlap, B.E., Staruch, M.J., Melino, M.R., Koprak, S.L., Williams, D., Witzel, B., Pisano, J.M., 1991. Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? J. Exp. Med. 173, 619–628.

Su, B., Jacinto, E., Hibi, M., Kallunki, T., Karin, M., Ben-Neriah, Y., 1994. JNK is involved in signal integration during costimulation of T lymphocytes. Cell 77, 727–736.

Su, B., Karin, M., 1996. Mitogen-activated protein kinase cascades and regulation of gene expression. Curr. Opin. Immunol. 8, 402–411.

Sun, Y., Pinchuk, L.M., Agy, M.B., Clark, E.A., 1997. Nuclear import of HIV-1 DNA in resting CD4+T cells requires a cyclosporin A-sensitive pathway. J. Immunol. 158, 512–517.

Sun, L., Youn, H.-D., Loh, C., Stolow, M., He, W., Liu, J.O., 1998. Cabin1, a negative regulator for calcineurin signaling in T lymphocytes. Immunity 8, 703–711.

Sussman, M.A., Lim, H.W., Gude, N., Taigen, T., Olson, E.N., Robbins, J., Colbert, M.C., Gualberto, A., Wieczorek, D.F., Molkentin, J.D., 1998. Prevention of cardiac hypertrophy in mice by calcineurin inhibition. Science 281, 1690–1693.

Timmerman, L.A., Clipstone, N.A., Ho, S.N., Northrop, J.P., Crabtree, G.R., 1996. Rapid shuttling of NF-AT in discrimination of Ca^{2+} signals and immunosuppression. Nature 383, 837–840.

Waskiewicz, A.J., Cooper, J.A., 1995. Mitogen and stress response pathways: MAPK kinase cascades and phosphatase regulation in mammals and yeast. Curr. Opin. Cell Biol. 7, 798–805.

Werlen, G., Jacinto, E., Xia, Y., Karin, M., 1998. Calcineurin preferentially synergizes with PKC-θ to activate JNK and IL-2 promoter in T lymphocytes. EMBO J. 17, 3101–3111.

Wolf, G., Thaiss, F., Stahl, R., 1995. Cyclosporine stimulates expression of transforming growth factor-beta in renal cells. Possible mechanism of cyclosporines antiproliferative effects. Transplantation 60, 237–241.

Woodgett, J.R., Avruch, J., Kyriakis, J., 1996. The stress activated protein kinase pathway. Cancer Surv. 27, 127–138.

Wu, J., Katzav, S., Weiss, A., 1995. A functional T-cell receptor signaling pathway is required for $p95^{vav}$ activity. Mol. Cell. Biol. 15, 4337–4346.

Zhang, W., Zimmer, G., Chen, J., Ladd, D., Li, E., Alt, F.W., Wiederrecht, G., Cryan, J., O'Neill, E.A., Seidman, C.E., Abba, A.K., Seidman, J.G., 1996. T cell responses in calcineurin Aα-deficient mice. J. Exp. Med. 183, 413–420.