Biochemical Pharmacology, Vol. 56, pp. 1539–1547, 1998.

ISSN 0006-2952/98/$–see front matter
PII S0006-2952(98)00213-5

COMMENTARY

Effector Pathways Regulating T Cell Activation

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ABSTRACT. Activation of T lymphocytes is a key event for an efficient response of the immune system. It requires the involvement of the T cell receptor antigen as well as costimulatory molecules such as CD28. Engagement of these receptors through the interaction with a foreign antigen associated with major histocompatibility complex molecules and CD28 counter-receptors B7.1/B7.2, respectively, results in a series of signaling cascades acting in synergy and which culminate in activation of interleukin-2 gene transcription and eventually cell proliferation. Many studies aimed at characterizing these specific effector pathways have been published; however, the actual signaling molecules that transduce activation signals from the cell membrane to the nucleus and that directly regulate interleukin-2 gene transcription are not yet completely defined and remain a matter of debate. In this commentary, we have attempted to analyze the results, which are sometimes diverging if not totally contradictory, characterizing effector pathways that possibly are triggered during T cell activation. BIOCHEM PHARMACOL **56**;12:1539–1547, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. T lymphocyte activation; TcR signaling; CD28 signaling; protein tyrosine kinase; mitogen-activated (MAP) kinases; interleukin-2

Activation of T lymphocytes is an essential event for the efficient response of the immune system. Optimal T cell activation requires at least two stimuli. A primary stimulus is generated upon the interaction of a foreign antigen presented by an APC† in the context of MHC class I or class II molecules, with the TcR; this specific interaction between antigen and TcR is accompanied by interactions between non-polymorphic regions of the class I and class II molecules with CD8 and CD4, respectively, which function as co-receptors. A secondary stimulus is delivered through the interaction of B7.1/B7.2 molecules on the APC with CD28 or CTLA4 present on T lymphocytes. This secondary stimulus appears to be fundamental, since, in its absence, TcR stimulation fails to induce a T cell response and may lead to cellular anergy. These different interactions initiate a series of signals that are transduced from the cell membrane to the nucleus through different specific signaling pathways that regulate cytokine gene transcription. In this commentary, we examine and analyze the different signaling pathways that have been characterized to be possibly triggered during T cell activation.

TCR SIGNALING PATHWAYS

The antigen is presented to the TcR in the context of MHC class I or class II molecules that interact, respectively, with CD8 or CD4 on the T cell membrane. Recognition of an antigen by the TcR leads to activation of numerous downstream intracellular signaling pathways, some of them being implicated in the induction of cytokine gene expression. These signaling cascades have been studied extensively, mostly through the ligation of the TcR/CD3 complex, using monoclonal antibodies.

The TcR is associated with the CD3 cluster, which is composed of invariant subunits γ, δ, and ε, as well as with the ζ:ζ homodimer chains. These chains, which actually represent the transducing signaling complex associated to the TcR, do not possess intrinsic enzymatic activity. However, they display in their cytoplasmic tails motifs termed ITAM [1–5], which are able to be tyrosine phosphorylated and, as a consequence, to recruit PTK. These PTK trigger phosphorylation cascades that transduce the activation signals from the membrane to the nucleus. Rapid phosphorylation of the ITAM by the Src family PTK $p56^{lck}$, which is non-covalently associated with CD4 or CD8, and potentially by $p59^{fyn}$ [6–10], permits TcR association with the Syk family kinase ZAP-70 [11] via two Src homology-2 (SH2) domains [8, 12, 13], leading to phosphorylation of the kinase and its activation [14–16]. These early events appear to be crucial for the triggering of the signaling

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† *Abbreviations:* AP-1, activated protein-1; APC, antigen presenting cell; A-Smase, acidic sphyngomyelinase; CsA, cyclosporin A; ERK, extracellular regulated kinase; IL-2, interleukin-2; ITAM, immunoglobulin family tyrosine-based activation motifs; Itk, IL-2-inducible T cell kinase; JNK, Jun *N*-terminal kinase; MAP, mitogen-activated protein; MEK, MAP kinase kinase; MHC, major histocompatibility complex; NF-AT, nuclear factor of activated T cells; PHA, phytohemagglutinin; PI 3-kinase, phosphoinositide 3-kinase; PKC, protein kinase C; PLCγ1, phospholipase C-γ-1; PTK, protein tyrosine kinase(s); and TcR, T cell receptor.

cascades that stimulate nuclear transcription factors that regulate the production of several cytokines, including IL-2.

IL-2 gene transcription, a key event in T cell activation and proliferation, is regulated by the coordinate action of multiple factors including NF-AT, AP-1, nuclear factor κB (NF-κB), and octamer protein-1 (Oct-1) [17–19]. There are at least two purine-rich binding sites for NF-AT family members in the mouse and human IL-2 promoter [20, 21]; the distal site on the IL-2 promoter is bound with high affinity by a complex formed by NF-AT proteins and an AP-1-type dimer composed of Jun and Fos proteins. The c-Jun homodimers or the c-Jun/c-Fos heterodimers actually stabilize the binding of NF-AT to DNA. Activation of these different transcription factors regulating IL-2 gene transcription results from at least two synergizing transduction pathways triggered at the TcR.

Phosphorylation of the TcR complex allows the association of several "adaptor" molecules, leading to several signaling cascades [22]. One of these cascades involves phosphorylation and activation of PLCγ1 [23–29], which cleaves phosphatidylinositol 4,5-bisphosphate to generate diacylglycerol and inositol 1,4,5-trisphosphate, which, respectively, activate PKC and trigger an increase in the concentration of intracellular calcium. One of the downstream effects of the rapid and sustained increase in $[Ca^{2+}]_i$ is the activation of the calmodulin/calcium-dependent phosphatase calcineurin; this phosphatase regulates IL-2 gene transcription by dephosphorylating the cytoplasmic form of NF-AT ($NF\text{-}AT_p$) and allowing its translocation into the nucleus, and by modulating the synthesis of other transcription factors implicated in complete IL-2 gene transcription ([19] and reviewed in Ref. 30). The involvement of this enzyme in the regulation of cytokine gene transcription is now well documented; the understanding of its mechanism of action actually results from a series of studies aimed at elucidating the effects of the immunosuppressive drugs CsA and FK506 [31–34]. Both drugs form complexes with cytoplasmic proteins known as immunophilins [35, 36], which bind to calcineurin and block its activity and, consequently, the transcription of IL-2 and other cytokine genes. In *in vitro* studies, the effect of this necessary calcium signal for activation of gene transcription factors can be induced by the action of calcium ionophores such as ionomycin.

The second signaling pathway necessary for NF-AT activation involves the low-molecular-weight G protein $p21^{ras}$, which, in quiescent T cells, exists as its "inactive" GDP-bound form and which, upon TcR engagement, rapidly and stably accumulates as its "active" GTP-bound complex (reviewed in Refs. 37–39). It has been shown that phorbol esters that stimulate PKC, as well as expression of a constitutively active mutant of PKC, can mimick $p21^{ras}$ effects on NF-AT [40, 41]. However, it has now been demonstrated that NF-AT induction by phorbol esters or by $p21^{ras}$ occurs through different independent mechanisms [42, 43]. Phorbol esters are, however, commonly used in *in vitro* studies either to stimulate the Ras-mediated pathway, or, in combination with ionomycin, to fully activate NF-AT and IL-2 gene transcription. $p21^{ras}$ Regulation of NF-AT can probably be explained through its effects on AP-1. Activation of AP-1 (reviewed in Ref. 44), which binds the IL-2 promoter [45] both directly and as a component of an NF-AT complex [19, 46], results from increased expression and phosphorylation of Fos and Jun family proteins [47]. It has been established that regulation of c-*fos* gene transcription is mediated by the nuclear factor Elk-1, which is phosphorylated and activated by ras-dependent signal cascades involving the ERK [48] and JNK [48–51]. On the other hand, c-jun is also regulated by JNK for which it appears to be a direct substrate [52]. ERK1/2, JNK, and another pathway involving the kinase p38, which represent parallel kinase cascades initiated at the ras level, are commonly termed MAP kinase pathways [53].

Evidence of the involvement of $p21^{ras}$ in the activation of IL-2 gene transcription was found by several studies; these studies have shown that inhibition of $p21^{ras}$ function through the expression of a dominant negative $p21^{ras}$ mutant prevents the induction of IL-2 gene transcription, while a constitutively active $p21^{ras}$ molecule initiates a signaling pathway that synergizes with calcium/calcineurin-mediated signals to activate the IL-2 gene [38, 54–57]. However, if calcium/calcineurin and the G protein $p21^{ras}$ appear to be key mediators in signal transduction from the TcR to the nucleus, the signaling molecules that lie downstream of $p21^{ras}$ and that are actually involved in cytokine production are not well defined [38, 58] and still remain controversial.

In fibroblasts, signal transduction from $p21^{ras}$ to the nucleus is proposed to involve activation of a MAP/ERK kinase. In human T cells, at least two MAP kinases, ERK-1 and ERK-2, are activated through the Ras pathway in response to occupancy of the TcR [59]. Activation of ERK1/2 is induced by phosphorylation mediated by MEK-1/2 [38, 60, 61], the activity of which is itself regulated through phosphorylation by a MAP kinase kinase kinase; the serine/threonine protein kinase Raf-1, which couples $p21^{ras}$ and interacts with MEK-1 to form a ternary signaling complex [62], appears to be this MAP kinase kinase kinase [63–66]. It has been shown that constitutively active Raf-1 can act in stimulating ERK-2 and, in synergy with calcium signals, in inducing IL-2 gene expression [65, 67]. The $p21^{ras}$/MEK/ERK pathway, which has been shown, in neuronal cells and fibroblasts, to play a crucial role in regulating early gene expression like c-*fos* through the phosphorylation of the transcriptional factor Elk-1 by ERK-2 [48], has been hypothesized to be similarly involved in NF-AT activation and IL-2 gene transcription in T cells. However, if this possibility strongly exists, the involvement of MEK-1/ERK-2 or of alternative signaling mechanisms as the actual links between $p21^{ras}$ and the induction of lymphokine gene expression in T cells is still a matter of debate.

An elegant study [68], using transfected Jurkat cells with constitutively active MEK-1 or Raf-1 mutants, has shown that these active proteins, in the presence of ionomycin, a

calcium ionophore, can stimulate NF-AT activity, leading to the conclusion that, like constitutively active Ras, the activity of Raf-1 or MEK-1 is sufficient to replace the phorbol ester-induced signal required for NF-AT activity. This conclusion was supported by the fact that the expression in Jurkat cells of kinase-attenuated mutants of MEK-1, when coexpressed with constitutively active Raf-1 or Ras, markedly inhibited the ability of the latter active proteins to costimulate NF-AT activity with ionomycin. Moreover, the authors have shown that expression of the ERK specific phosphatase (MKP-1), which blocked the stimulation of ERK-1 and ERK-2 activities induced by phorbol 12-myristate 13-acetate (PMA) treatment, also blocked the ability of constitutively active mutants of MEK-1 or Raf-1 to costimulate with ionomycin NF-AT activity. Altogether, these results represented a clear and convincing demonstration that MEK-1 and ERKs function in conveying stimulatory signals from Raf-1 to the IL-2 gene. However, these results appeared contradictory to a previous report [69] that had demonstrated in a transgenic mouse model that the expression of a kinase-defective mutant of MEK-1 severely impaired the positive selection of thymocytes, but had no effect on thymocyte proliferation stimulated with ionomycin and PMA or on the production of IL-2 by anti-CD3-stimulated splenic T cells. However, as pointed out by the authors, it is unclear whether the splenic T cells in these transgenic mice, which developed in the absence of positive selection, are representative of a normal population of mature T lymphocytes, and it is possible that these cells have developed alternative mechanisms for up-regulating the IL-2 gene independent of the ERK activity.

Another study published almost in parallel [70], which also used MEK-1 mutants (constitutively active or inhibitory), led to a slightly different conclusion. Indeed, in this study, expression of an active form of MEK-1, which resulted in the activation of ERK-2, induced only a marginal activation of NF-AT in the presence of ionomycin, leading to the conclusion that expression of activated MEK is not sufficient to substitute for $p21^{ras}$ for NF-AT induction, i.e. that explanation of $p21^{ras}$ regulation of the transcription factor necessitates the existence of alternative Ras effector pathways. These authors have shown that a dominant negative mutant of Rac-1, another downstream effector of $p21^{ras}$ [71, 72], which had no effect on TcR or $p21^{ras}$ activation of ERK-2 [73, 74], could suppress TcR and $p21^{ras}$ activation of NF-AT and AP-1; these data led to the conclusion of the existence of a Rac-1 controlled signaling pathway operating in parallel with the ERK-2 pathway to mediate induction of the transcription factors. The kinase pathway that regulates the effects of Rac-1 on NF-AT is not yet defined in T cells; by analogy with other models, it is possible that Rac-1, which does not activate ERK-2 in TcR induction, involves other parallel MAP kinase signaling pathways like JNK/SAPK [73–75] or the p38 MAP kinase. It has to be pointed out that activated MEK-1 and activated Rac-1 in combination cannot substitute fully for $p21^{ras}$ for induction of NF-AT; it thus seems likely that other Ras effectors, in addition to Rac-1 and MEK-1, contribute to NF-AT regulation and cytokine gene expression.

These results are lines of evidence that the different Ras effectors function in both parallel and convergent pathways. However, it can be noted that MEK-1 negative mutants do not reduce NF-AT activity to basal level upon induction by TcR or phorbol ester plus ionomycin or by activated Ras, while they totally block ERK activation. Similarly, activation of NF-AT is not inhibited completely by the negative mutant of Rac. This could reflect the existence of not yet characterized signaling pathways that do not involve ERK-2, JNK-1, or p38. It has been shown recently in mast cells that NF-AT activation is not mediated by the "classical" Raf-1/MEK/ERK pathway but involves the Ras effector Rac-1 [76]. Moreover, it has been shown that a serine/threonine kinase, the Cot kinase, which activates the two parallel MAP kinase pathways, the ERK and SAPK cascades, could participate in the regulation of the IL-2 gene transcription [77].

We have shown [78] that stimulation of primary T lymphocytes with the mitogenic lectins PHA or jacalin induces a similarly intense activation of ERK-2, which was accompanied by the expression of IL-2 mRNA; however, when these primary T cells were treated previously with PD098059, a selective inhibitor of MEK [79], ERK-2 activation and IL-2 mRNA expression were totally inhibited in jacalin stimulation but only partially inhibited in PHA-stimulated lymphocytes (even at high doses of the inhibitor). Of course, it cannot be ruled out that PHA triggers a very intense activation that cannot be blocked completely by the inhibitor, but a possibility exists that PHA activates other pathways leading to the phosphorylation of ERK-2 as it has been described in other models [80].

It has to be pointed out that most of the studies aimed at understanding and at characterizing the pathways possibly involved in the activation of transcription factors that regulate cytokine gene transcription have used transformed cell lines (mainly Jurkat cells) transfected with active or inhibitory effector mutants. However, it cannot be excluded that this might be different in primary cells. An interesting study using normal mouse T cells newly activated *in vitro* has shown that individual cytokines can be differently regulated by activation of the MEK/ERK pathway in synergy with a calcium signal [81]. We have shown* in human primary T cells that two different anti-CD3 monoclonal antibodies, both recognizing the ε chain of CD3, can induce IL-2 gene transcription, respectively, with or without activating the Raf-1/MEK-1/ERK-2 pathway. This difference in the two anti-CD3-induced transduction pathways was specifically observed in primary T cells but not in Jurkat cells.

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CD28 COSIGNALING PATHWAYS

In addition to the TcR/CD3 associated signaling cascade, costimulatory signals are required for full T cell activation and proliferation [82, 83]. Indeed, TcR ligation without costimulation can result in functional inactivation [84] and anergy [85–88] and in cell death [89, 90]. The best-characterized costimulatory receptor expressed on a resting T cell is CD28, which, upon interaction with its ligands B7.1 (CD80) and B7.2 (CD86) expressed on the antigen presenting cell, enhances IL-2 synthesis and T cell proliferation (reviewed in Refs. 82, 91, and 92). B7.1 and B7.2 are also recognized by CTLA-4 (reviewed in Ref. 93), a CD28 structurally related molecule to which was devoted an inhibitory role in T cell activation (although this inhibitory role remains controversial and not generally accepted) [94]. Evidence has been found showing that the role of CD28 signaling is actually not to costimulate the initial activation of naive T cells but to sustain the late proliferative response and enhance long-term cell survival [95]; however, in contrast to that, a recent study has demonstrated that proliferation of resting T cells can be induced *in vivo* and *in vitro* through CD28 without engagement of the TcR [96]. Even though costimulatory function of CD28 has become an area of intense investigation, the signal transduction pathways triggered upon its engagement remain poorly understood (for review, see Refs. 91 and 92).

CD28 is a homodimeric glycoprotein expressed on 95% of $CD4^+$ T cells and on approximately 50% of $CD8^+$ lymphocytes [82], and its ligation has been shown to activate PLCγ1 and $[Ca^{2+}]_i$ mobilization [97]. This receptor does not display any obvious intrinsic enzymatic activity. However, it can be phosphorylated on tyrosine residues on its cytoplasmic tail, allowing the binding of different signal transducing molecules such as PI 3-kinase, growth factor-bound protein-2 (Grb-2) from the Grb-2/son of sevenless (Grb-2–SOS) complex, and Itk. The Src family kinases $p56^{lck}$ and $p59^{fyn}$ have also been shown to physically associate with the receptor, and $p56^{lck}$, in particular, demonstrates a remarkable phosphorylating specificity for the $pTyr^{173}$-Met-Asn-Met motif within the CD28 cytoplasmic tail. This phosphorylated motif on Tyr^{173} binds to the SH2 domain of the 85 kDa regulatory subunit of PI 3-kinase [98]. Moreover, a recent result has brought evidence in a transfected insect model [99] that these Src family kinases phosphorylate CD28, allowing the binding of PI 3-kinase, Grb-2, and Itk to the receptor.

Grb-2–SOS, which binds to CD28, appears to be a good candidate to couple CD28 with the $p21^{ras}$ pathway. Indeed, this complex is known to participate directly in $p21^{ras}$ activation in TcR stimulation (reviewed in Ref. 100). However, association of Grb-2–SOS with CD28 was only observed in the presence of extensively cross-linked CD28 mAb [101] and has not been observed upon ligation of CD28 by its physiological ligands. In parallel, if anti-CD28 mAb (CD28.2) was shown to induce an increase in Ras–GTP complexes and in activation of the MAP kinase cascade Raf-1/ERK-2 [102], ligation of CD28 by the natural ligand B7.1 did not detectably activate $p21^{ras}$ [102]. Therefore, it appears that CD28 does have the capacity to couple the $p21^{ras}$ pathway, as demonstrated by the effect of the CD28.2 mAb, but the physiological relevance of this effect is questionable and may not be essential in CD28 costimulation.

In contrast, recruitment and activation of PI 3-kinase seem to be essential in the CD28-induced response (for review, see Refs. 92 and 103). PI 3-kinase is a heterodimer composed of an adaptor subunit (p85) linked to a catalytic domain (p110) that mediates the formation of D-3 phosphoinositide lipids by transferring the terminal phosphate of ATP to the D-3 position of the inositol ring of phosphatidylinositol, phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-bisphosphate, generating PI 3-P, PI 3,4-P_2 and PI 3,4,5-P_3, respectively [104]. PI 3,4-P_2 and PI 3,4,5-P_3 can activate the δ, ε, η [105], and ζ [106] isoforms of the serine/threonine kinase, PKC, as well as the kinase encoded by the proto-oncogene *Akt* [107]. PI 3-kinase may also activate [108] or may be activated by [109] $p21^{ras}$. The possible involvement of PI 3-kinase in CD28 stimulation was mainly studied using the fungal metabolite wortmannin, which irreversibly inhibits PI 3-kinase by covalent interaction with the 110 catalytic site [110]. However, the data obtained from the use of wortmannin have limited value since the studies have yielded discrepant results. Indeed, it has been shown in Jurkat cells that the PI 3-kinase inhibitor can potentiate NF-AT induction [111], whereas other groups were unable to detect any effect of wortmannin in the same cells [112, 113]. These discrepancies led to the idea that PI 3-kinase might have no functional role in CD28-induced IL-2 production [112, 114]. However, it has been observed that wortmannin in nanomolar concentrations did inhibit CD28-mediated costimulation of IL-2 production in primary resting T cells and in human lymphoblasts [111, 115, 116]. This result was actually of major importance since Jurkat cells, being independent of costimulatory signals [91], appear to be a poor relevant model to study CD28-induced costimulation of IL-2 production.

The other CD28 binding protein is Itk, also known as EMT, a Tec family kinase [117] that is expressed in T cells, mast cells, and human NK cell lines [118–122]. CD28 activation of this PTK has been shown to be dependent upon functional Lck [123]. The targets of such a PTK in CD28 signaling are poorly defined, but a recent study has shown that EMT/Itk can phosphorylate all four tyrosines of the CD28 tail, in contrast to Lck, which phosphorylates only tyrosine 173 [124]. Using Itk-deficient mice, Itk has been shown to negatively regulate the amplitude of signaling upon CD28 costimulation [125].

Another pathway triggered upon CD28 ligation includes activation of an A-SMase, which results in the generation of phosphorylcholine and ceramide [126, 127]. Targets of the latter compound include the Ras/Raf pathway [128], JNK [129], and PKCζ [130, 131]. A-SMase is thought to be

sequestered in lysosomes [132] and is sensitive to inhibition by lysosomotropic agents such as chloroquine [133].

The signaling cascade that bridges these early events triggered upon CD28 engagement with the late ones, directly affecting activation of nuclear factors regulating cytokine gene transcription, is still unresolved. As already mentioned, several studies have found evidence that PI 3-kinase may have a direct effect on cytokine gene transcription. Indeed, it has been demonstrated in resting T cells that wortmannin blocks proliferation and IL-2 production in response to CD28 costimulation. However, the fact that the inhibitor has only a limited effect in activated cells and even enhances IL-2 production in Jurkat cells could suggest the existence in activated cells of an alternative CD28 triggered signaling pathway. An elegant study has used T cell blasts stimulated with B7 transfected CHO cells [134]; these cell blasts can be stimulated via the CD28 molecule in the absence of antigenic challenge, and, therefore, allow the distinction between signals triggered by CD28 and those by TcR. The authors have showed that NF-κB generation in CD28 stimulation was sensitive to chloroquine, the inhibitor of A-SMase, but not to wortmannin, while AP-1 generation was inhibited by wortmannin and variably sensitive to chloroquine. These data suggest that AP-1 and NF-κB are direct targets for CD28 signals and that both PI 3-kinase and A-SMase activation are involved in their generation.

Moreover, evidence is accumulating that PI 3-kinase may have direct effects on the regulation of MAP kinase pathways including ERK and JNK [103]. Indeed, *fos* transcription can be activated by a constitutively active p110 mutant of PI 3-kinase [108]; since *fos* activation is Ras dependent, this suggests that PI 3-kinase can modulate the $p21^{ras}$ pathway and, consequently, the ERK cascade. Moreover, Rac-1, which has been shown to regulate the kinase cascade leading to JNK activation [73–75], also appears to be a downstream effector of PI 3-kinase [135]. It is noteworthy that activation of JNK in T cells requires costimulation of TcR and CD28 by their respective antibodies, each stimulus alone resulting in little or no activation of the kinase [136, 137]. This result led to the conclusion that JNK activation could be a nodal event between the TcR-induced signaling pathway and the CD28-mediated costimulation pathway, resulting in IL-2 production. This is strongly supported by data published recently, which have shown that Rac-1 cooperates with Syk tyrosine kinase to synergistically increase JNK activity as well as AP-1 and NF-AT transcriptional activities [75].

CONCLUDING REMARKS

IL-2 synthesis and T cell proliferation result from the activation of several connecting pathways involving PTK, $[Ca^{2+}]_i$, PKC, MAP kinases, and PI 3-kinase triggered at the TcR and through the engagement of costimulatory molecules like CD28. One of the major obstacles in studying these effector pathways in primary T cells is probably to assess the relative contribution of each receptor to specific signaling cascades. Characterization of these pathways results mostly from the use of cell lines (Jurkat cells) transfected with constitutively active or inhibitory mutants. However, Jurkat cells, which are commonly used as a standard in immunologic research, appear to possess a number of unusual features. For example, CsA can uniquely inhibit SAPK or JNK activation in Jurkat cells but not in primary cells or most other cell lines [92]; moreover, IL-2 production, which is blocked in primary T cells by wortmannin, is actually enhanced in Jurkat cells; further, we have shown* in human primary T cells that two different anti-CD3 monoclonal antibodies can induce IL-2 gene transcription, respectively, with or without activating the Raf-1/MEK-1/ERK-2 pathway, and that this difference is not observed in Jurkat cells. This illustrates that Jurkat cells, while a good model for the initial receptor proximal biochemical processes associated with T cell activation, may not be an appropriate model for cytokine gene regulation as it relates to primary human T cells.

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