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Signal transduction in T cells

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Rapid progress was made during the past year in the delineation of the nature of the initial biochemical events triggered by the T-cell antigen receptor. Antigen-mediated activation of phospholipase C was demonstrated to require protein tyrosine phosphorylation and, most surprising, activation of the Ras family of signal transduction molecules was shown to closely follow stimulation of the T-cell antigen receptor. Major controversy continues over which events are relevant to the various effector functions of T cells.

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Introduction

For the purposes of this review, the term T cell activation refers to the biochemical events that lead to the initiation of interleukin (IL)-2 production after antigen becomes bound to the T-cell antigen receptor (TCR). This represents a period of 2–4 h after stimulation of T cells, which normally reside in the quiescent G0 stage of the cell cycle, and encompasses the expression of many newly transcribed genes [1*]. There are many reasons for studying mechanisms of T-cell activation, such as the rational design of novel pharmacological agents for immunosuppression and understanding some of the mechanisms of leukemogenesis. There is also potential to understand some of the mechanisms required for maintenance of tolerance and the pathological states in which tolerance is broken.

A total understanding of the molecular events of T-cell activation is far from complete. This is partly because of the complex and ill-defined structural composition of the TCR, the fact that T cells do not respond to soluble ligand but only to cell bound ligand, and the realization that both multiple accessory surface receptors and multiple biochemical pathways appear to be activated after T-cell stimulation. This brief review will focus on the highlights of studies published since 1989. Other reviews of signal transduction in T cells that discuss earlier studies [2–4] are available.

The T-cell antigen receptor

Molecular cloning of the TCR was completed this year when the η chain was sequenced and found to be an alternatively spliced form of the ζ chain [5*]. Thus, the TCR consists of the heterodimeric clonotypic α and β-chains, the CD3 γ, δ- and ε-chains, closely related subunits encoded on chromosome 1, and the ζ and η-chains, which are closely related and encoded on a separate chromosome.

Speculation continues about the function of the ζ- and η-chains. The chains were found to be related to the γ-chain of the hetero-trimeric Fc receptor family [6*]. Current hypotheses are that ζ- and η-chains function to couple the TCR to intracellular effector molecules such as protein tyrosine kinases.

Recently, knowledge of yet another level of complexity of the TCR has emerged. The invariant chains of the CD3 complex and ζ dimer complex may be associated in different combinatorial arrays. An example of this is that the ζ chain may consist of complexes containing either ζ-ζ, ζ-η or ζ-Fcy dimers [7**,8**]. In addition, it appears that the TCR can assemble as subtypes containing CD3 γ and ε-chains or CD3 δ and ε-chains [9**,11**]. Thus, the receptor has developed a relatively well understood means of rearrangement of the clonotypic chains to achieve the diversity that is responsible for ligand binding. In addition, the TCR has developed a not yet fully appreciated

Abbreviations

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level of structural diversity of the invariant chains that presumably allows the coupling of the TCR to different signal transduction pathways, possibly at different stages of T-cell differentiation.

Accessory receptors involved in T-cell activation

A central question concerning the role of accessory molecules in T-cell activation is whether the accessory signal enhances the signals provided by the TCR, or whether the signal is distinct from the TCR. Several studies have indicated that the function of the CD28 receptor is distinct from the TCR during the course of T-cell activation. This has been suggested partly because CD28 stimulation was shown to enhance lymphokine production even in the presence of maximal phorbol ester and calcium ionophore stimulation [12]. In addition, CD28 stimulation was shown to increase mRNA levels and secretion of IL-2, interferon (IFN)-γ, granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor (TNF)-α. These lymphokines are all induced by a combination of protein kinase C (PKC) activation provided by phorbol esters, and anti-CD28 stimulation [13]. This means of producing IL-2 is completely resistant to suppression by cyclosporine. In contrast, the major transcriptional stimulation of IL-2 mediated by the TCR or phorbol ester plus calcium ionophore is completely abolished by cyclosporin A.

The primary function of the CD28 receptor appears to be to regulate the amount of lymphokine produced by T cells; interestingly this effect is independent of cell proliferation (DNA synthesis). Lindsten and coworkers [14] found that the primary mechanism by which anti-CD28 augments lymphokine production in mature T cells is by inhibition of the degradation of lymphokine mRNAs. As a result of the stabilization of mRNA, the steady-state levels of lymphokines that have the T helper 1 phenotype increase, leading to enhanced translation and protein secretion. In addition to a primary effect on mRNA stability, costimulation of quiescent T cells with anti-CD3 and anti-CD28 does appear to have a number of secondary effects on T-cell responses. Late after stimulation, IL-2 mRNA levels appear to be enhanced by a CD28-dependent increase in transcription as well as mRNA stability. Studies by Weiss and colleagues [15] have shown this to be the major mechanism by which CD28 stimulation increases lymphokine production in the Jurkat leukemia line. They identified a DNA-binding protein that binds to a site in the IL-2 promoter that is distinct from the previously described sites. Thus, it appears that CD28 may increase lymphokine production by several mechanisms. It remains to be determined whether the binding site identified by Weiss and colleagues is functional in primary T cells or whether it is a phenomenon linked to transformed cells.

One of the most important discoveries last year was the demonstration by Linsley and coworkers [16] that a cell surface ligand for CD28 exists on antigen-presenting cells (APCs). They have obtained convincing evidence that the ligand is the B7/BB1 molecule, an activation antigen expressed primarily on activated B7/B cells that is also a member of the immunoglobulin-gene superfamily. Thus, these results suggest that the potent effects of CD28 monoclonal antibodies (mAbs) observed in vitro may be mediated in vivo by oligomerization of the CD28 receptor by ligand presented by APC. CD28 is clearly the leading candidate to deliver a 'second signal' during T-cell activation by antigen. It is possible that this signal delivered by CD28 determines whether the cell proliferates and matures, or becomes tolerant. For example, T cells that present antigen in the context of the CD28 ligand would be expected to proliferate, whereas T cells that respond to antigen alone would not be expected to proliferate. The fact that CD28 appears during thymic ontogeny and is functional in thymocytes [17,18] also suggests the possibility that CD28 may be involved in positive selection the 'holy grail' of T-cell development. A recent detailed review of the CD28 receptor system is available [19]. Fig. 1 shows several potential roles of the CD28 receptor system in T-cell activation.

Signal transduction cascades

Phospholipase C

The activation of phospholipase C (PLC) has long been recognized as an important event after the binding of antigen to the TCR. The mechanism of PLC activation in T cells appears to be complex. GTP-binding proteins (G proteins) appear able to activate PLC in T cells. For example, direct activation of G proteins by aluminium fluoride causes activation of PLC in T cells [20]. In addition, Goldsmith and coworkers [21] have found that if a known G-protein-coupled receptor is transfected into T cells, PLC activation occurs after the binding of ligand. On the other hand, there is increasing evidence that the primary means of regulating PLC activity in T cells after the binding of antigen, is by protein tyrosine phosphorylation (see below), and thus, the role of G-protein-mediated activation of PLC in T cells remains to be defined. Inokuchi and Imboden [22] performed detailed studies of inositol phospholipid turnover, which suggest that there are multiple potential sites of regulation in the phosphoinositol (PI) cycle.

The protein tyrosine kinase—phospholipase C association

It has been clear for several years that the TCR is coupled to two important signal transduction cascades: PLC and protein tyrosine kinase activation (reviewed in [3]). A major question of T-cell activation has been whether these represent the independent activation of two pathways in parallel, or whether the TCR is primarily coupled to one of the pathways, the other pathway being activated in a serial manner. Using antiphosphotyrosine antibodies to immunoblot lysates of stimulated cells, increased
tyrosine phosphorylation of several substrates was detected seconds after TCR stimulation [23**, 24**]. This rapid onset of tyrosine phosphorylation preceded detection of increased PLC activity, and was unexpected as the time of TCR-chain phosphorylation had been found to be relatively slow, requiring approximately 15 min from TCR stimulation. Furthermore, tyrosine phosphorylation appears to be required for PLC activation as tyrosine kinase inhibitors prevent PLC activation and subsequent events after cellular stimulation [24*, 25*].

The mechanism that couples the TCR to tyrosine phosphorylation remains unclear. Samelson and coworkers [26**] have shown that the fyn kinase can be precipitated with the TCR under certain conditions. The fyn kinase is a member of the src family and is found in a variety of tissues, unlike lck which is primarily expressed in T cells. However, it was recently found that T cells express a uniquely spliced form of fyn kinase [27**]. The role of fyn in T-cell activation remains controversial for several reasons. The stoichiometry of the fyn association with the TCR appears to be low, and to date it has not been shown that stimulation of the TCR activates fyn kinase activity. In contrast, lck has high stoichiometry with the CD4 receptor, and lck kinase activity has been demonstrated to increase after CD4 crosslinking. Finally, it has not yet been shown that the fyn kinase is associated as a result of the TCR in primary T cells, or whether the association is limited to T-cell lines or subtypes. It is likely that multiple tyrosine kinases are required for signal transduction through the TCR, and that both fyn and lck are involved in antigen-stimulated T-cell activation (Fig. 2).

Protein tyrosine phosphatases
The CD45 protein tyrosine phosphatase (PTPase) was shown to be required for antigen and not IL-2 stimulated T-cell proliferation [28**]. Similarly, CD45 is required for the early biochemical events that occur after triggering of the TCR [29**]. Cell lines that lack expression of CD45 retain apparently normal expression of the TCR yet have no detectable activation of PLC or increases in calcium after TCR stimulation. Partial inhibition of tyrosine phosphatases by phenylarsine oxide was shown to augment TCR-mediated signal transduction although higher concentrations of the drug completely inhibited signal transduction by the TCR [30**]. Thus it is likely that the CD45 PTPase is required either to activate or to maintain ac...
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Antigen-presenting cell

MHC

TCR

CD4

CD45

Fyn

Ick

PLC

PKC

ras

MAP2K

Ca²⁺

Fig. 2. The signal transduction cascade involved in antigen-specific T-cell activation. The binding of the T-cell receptor (TCR) and CD4 receptor by antigen plus major histocompatibility complex (MHC) class II antigens activates the associated protein tyrosine kinases, fyn and Ick. The CD45 protein tyrosine phosphatase is required for anti-CD3-induced signal transduction. Fyn and/or Ick lead to phospholipase C (PLC) activation. Protein kinase C (PKC) activation leads to ras, raf-1, and microtubule-associated protein-2 kinase (MAP-2K) activation within 5 min after TCR stimulation. It is not yet known which of these kinase signals are required for interleukin (IL)-2-gene transcription to result.

Protein serine kinases

The product of the c-raf proto-oncogene is a serine/threonine kinase termed raf-1 that is required for growth factor-stimulated proliferation of fibroblasts [32]. Stimulation of the TCR was shown to induce raf-1 kinase activity that was maximal within 5 min of cellular stimulation [33]. Phosphoamino acid analysis showed that TCR-induced raf-1 activation was accompanied by hyperphosphorylation on serine residues, and furthermore, was dependent upon PKC. These results were surprising because previous studies have shown that platelet derived growth factor-induced raf-1 hyperphosphorylation occurs on tyrosine residues. Thus, there appear to have evolved two distinct mechanisms of raf-1 activation, a PKC-dependent form coupled to the TCR and a tyrosine kinase-dependent form that is coupled to tyrosine growth factor receptors. It remains to be determined whether the tyrosine kinase-dependent pathway for raf-1 activation exists in T cells. An attractive possibility would be that growth factor stimulation of T cells by IL-2 or IL-4 involves raf kinase activation via a tyrosine-dependent mechanism, whereas antigen-induced activation is coupled to a distinct, serine kinase-dependent mechanism.

Ras and the T cell

The ras family of G proteins are ubiquitously expressed and are required for control of cell cycle progression. Downward and coworkers [35] demonstrated that ras activation occurs in primary T cells within minutes of TCR stimulation and that this activation is apparently dependent upon PKC activation. This is the first known example of control of ras activity by a cell surface receptor. It remains to be determined whether PKC is responsible for the ras activation. Alternative possibilities are that other
serine/threonine kinases such as raf-1 or MAP-2 kinase subserve this role.

**Signal transduction and effector function**

The above studies have demonstrated a role for protein tyrosine kinases in the initial events of TCR activation. What remains entirely unclear is what role, if any, tyrosine phosphorylated substrates or the second messengers of PLC activation (inositol(1,4,5)P3 and diacylglycerol) have in the nuclear events that lead to initiation of IL-2 gene transcription. Two sides to this controversy have emerged. As mentioned above, Weiss and colleagues [21*] have transfected the Jurkat cell line with the muscarinic M1 receptor. Subsequently they found that stimulation of the muscarinic receptor activates PLC, does not lead to tyrosine kinase activation, and results in substantial secretion of IL-2 [36**]. In the same cells, stimulation of the TCR leads to tyrosine kinase activation, PLC activation and IL-2 production. These results support the conclusion that at least in some cell types, early tyrosine phosphorylation is not required for IL-2 production, and that presumably, activation of PLC and the accompanying cascades is sufficient.

Investigators at the National Institutes of Health [37] have reached a different conclusion regarding the signal transduction pathway that is involved with IL-2 gene expression. They analyzed cell mutants of the 2B4 cell line that did or did not express the ζ and η chains of the TCR, and found examples of cells (η-negative variants) in which activation of PLC was not detectable although normal activation of protein tyrosine kinase and normal IL-2 production was. The explanation for these differences is not immediately evident, and perhaps reflects the fact that different T-cell subtypes use distinct signal transduction pathways to initiate IL-2 gene transcription. Much experimentation will be required to determine the relative importance of these two biochemical pathways under physiological and pathological conditions. This question is of more than academic interest, as the design of specific pharmacological agents to inhibit or stimulate lymphokine production is dependent on precise knowledge of the biochemical events relevant to lymphokine production.

**Conventional and ‘superantigens’**

It has long been known that inbred strains of mice have minor lymphocyte stimulating (Mls) genes that are potent stimulators of CD4 T-cell proliferation. The traditional assay for this effect is the stimulation of T-cell proliferation in mixed lymphocyte cultures of cells from major histocompatibility complex (MHC)-identical strains of mice. It has recently been appreciated that certain bacterial toxins can stimulate T-cell proliferation in a manner that mimics Mls antigens [38**]. T cells appear to recognize Mls and enterotoxins on entire families of the β chain of the TCR, and thus these antigens have been termed ‘super antigens’ because they activate classes of T cells on the basis of the family of the β-chain expressed. T cells in the thymus that encounter endogenous Mls antigens become deleted, whereas mature T cells proliferate and produce IL-2 after Mls or enterotoxin stimulation. O'Rourke and coworkers [39] recently reported an intriguing result that suggests that the biochemical pathways used by ‘conventional’ antigens may differ from that of ‘superantigens’. They found that stimulation of alloreactive T cells with MHC resulted in PLC activation and IL-2 production. In contrast, Mls stimulation resulted in equivalent IL-2 production and no detectable PLC activation. These findings, if confirmed, will be difficult to explain given the current models of the coupling of the TCR to signal transduction cascades. Furthermore, it is not yet known if bacterial superantigens function analogously to endogenous Mls antigens.

**Conclusion**

Understanding of T-cell signal transduction is still far from complete. Future work will undoubtedly uncover more receptor kinases and phosphatases that are involved in T-cell activation. There is increasing evidence to indicate that differential usage of signal transduction mechanisms may occur among T cells in different stages of differentiation. It is possible that improved understanding of signal transduction in thymocytes may yield clues to the means of positive and negative selection that determines the T-cell repertoire. The evidence reviewed herein concerning CD3 heterogeneity indicates an emerging concept of TCR domains, and the role of these domains in signal transduction, if any, remains to be appreciated.

**References and recommended reading**

Papers of special interest, published within the annual period of review, have been highlighted as:
- of interest
- of outstanding interest

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A study demonstrating that the ζ-chain of the TCR can associate with CD3 dimers on natural killer cells, cells that do not express the TCR.


Murine T-cell hybridomas were transfected with the human TCR α-chain. Surprisingly, the expressed TCRs contained both a murine and human TCR α-chain.


The TCRs containing a 40 kD dimer were isolated by immunoprecipitation with anti-TCR antibodies. This dimer was demonstrated to contain the CD3 ε-chain, which implies that the stoichiometry of the TCR is either 1:1:1 by Aluminum Fluoride in Resting T Cell Subsets of Normal and Autoimmune-Poone Inr Mice. *J Immunol* 1989, 143:780-786.


The signal provided by the CD28 receptor was shown to augment IL-2 secretion of T cells stimulated with maximal doses of phorbol ester and calcium ionophore. This was surprising as it was previously thought that the TCR pathway was sufficient to cause maximal IL-2 gene expression.


Evidence that the CD28 receptor stimulates the production of large amounts of IL-2, GM-CSF, TNF-α, INF-γ, and lymphotxin in T cells. Lymphokine production induced by CD28 was resistant to cyclosporin, whereas TCR-induced lymphokine secretion was sensitive, suggesting that CD28 functions in a biochemically distinct manner from the TCR.


CD28 may also increase transcription of IL-2, indicating that the CD28 signal is evident at more than one level. The primary mechanism of the CD28 signal is not yet known.


The type 1 muscarinic receptor was transfected into Jurkat cells. This receptor, found to be coupled to G proteins in other systems, was found to be a potent activator of a PLC. This investigation [20] demonstrates that T cells express PLC that is coupled to G proteins. This is surprising in light of recent data that suggests TCR is coupled to PLC via protein tyrosine kinases.


23. JUNE CH, FLATHER MG, LEDBETTER JA, SAMLSON LE. Increases in Tyrosine Phosphorylation are Detectable before Phos-

Detailed kinetic analysis of the activation of PLC activity and protein tyrosine kinases. Surprisingly, increased kinase activity appeared immediately (within 5 s) after TCR stimulation, whereas a time gap of approximately 15 s occurred before PLC activity could be detected in normal human T cells and the Jurkat T cell line. These findings suggested that a primary event after TCR stimulation is the activation of protein kinase(s)/phosphatases, and called into question the notion that the TCR was coupled primarily to PLC.


See [25*].


This study and that of Mustelin et al. [24*] demonstrated that inhibition of tyrosine kinase activity prevented signal transduction through the TCR.


Mouse T cell clones that lacked CD45, proliferated in response to IL-2, and failed to proliferate after antigen stimulation


The above two studies indicate that expression of CD45 is required for antigen or CD3-induced signal transduction and for cellular proliferation.


A pharmacological inhibitor of protein tyrosine phosphatases, phenylarsine oxide, may augment or inhibit TCR-mediated protein tyrosine kinase activity.

31. COOL DE, TORKS NK, CHARBONNEAU H, WAISH KA, FISCHER EH, KREBS EG. cDNA Isolated from a Human T-Cell Lymphoma Encodes a Member of the Protein Tyrosine Phosphatase Family. Proc Natl Acad Sci USA 1989, 86:5257-5261

A novel protein tyrosine phosphatase is expressed in T cells. The role of this enzyme in T cells remains unknown.


The c-raf protein senne/threonine kinase is activated soon after TCR stimulation.


This study shows that TCR stimulation can increase activity of MAP 2K, and that co stimulation with CD4 augments this activation. Interestingly, both senne and tyrrosine kinase appear to be involved in MAP 2K activation. The relevant substrates for c raf and MAP 2K remain to be identified.


An analysis of ras GTPase activity in human T cells after TCR stimulation. An early event after T cell stimulation is an increase in ras GTPase activity, which appeared to be PKC dependent and to be mediated by inhibition of GAP activity.


This paper shows that a G protein-coupled receptor (muscarinic type 1) when transfected into T cells can stimulate PLC and IL 2 production, in the absence of detectable protein tyrosine kinase activity.


One of a series of papers by this group that demonstrates that IL 2 production can occur without detectable PLC activity. It is not yet clear how to resolve the controversy between this paper and the above study [36*].


The 'superantigen' hypothesis, is summarized here by Marrack and Kappler. There is evidence from many laboratories to indicate that certain bacterial toxins and Ms antigens are recognized by the TCR in a fashion distinct from conventional antigens.


A analysis of PI turnover, IL-2 production and T-cell proliferation after stimulation by alloligens or Ms antigens. Surprisingly, there was no detectable PI turnover after Ms stimulation, although IL-2 production was equivalent. This suggests that the TCR may be able to transduce two distinct signals, and again calls into question the role of PLC activation for IL-2 production.

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