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Functional identification of kinases essential for T-cell activation through a genetic suppression screen

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Abstract

Activation of T-cells by antigens initiates a complex series of signal-transduction events that are critical for immune responses. While kinases are key mediators of signal transduction networks, several of which have been well characterized in T-cell activation, the functional roles of other kinases remain poorly defined. To address this deficiency, we developed a genetic screen to survey the functional roles of kinases in antigen mediated T-cell activation. A retroviral library was constructed that expressed genetic suppressor elements (GSEs) comprised of peptides and antisense nucleotides derived from kinase cDNAs including members of the STE, CAMK, AGC, CMGC, RGC, TK, TKL, Atypical, and Lipid kinase groups. The retroviral library was expressed in Jurkat T-cells and analyzed for their effect on T-cell activation as monitored by CD69 expression. Jurkat cells were activated by antigen presenting cells treated with superantigen, and sorted for a CD69 negative phenotype by flow cytometry. We identified 19 protein kinases that were previously implicated in T-cell signaling processes and 12 kinases that were not previously linked to T-cell activation. To further validate our approach, we characterized the role of the protein kinase MAP4K4 that was identified in the screen. siRNA studies showed a role for MAP4K4 in antigen mediated T-cell responses in Jurkat and primary T-cells. In addition, by analyzing multiple promoter elements using reporter assays, we have shown that MAP4K4 is implicated in the activation of the TNF-α promoter. Our results suggest that this methodology could be used to survey the function of the entire kinome in T-cell activation.

Keywords: T-cell activation; MAP4K4; Superantigen; Kinase; Screen; Genetic suppressor element

Abbreviations: GSE, genetic suppressor element; STE, homologs to yeast sterile7, sterile 11, and sterile 20 kinases; CAMK, calcium/calmodulin-dependent-like protein kinases; AGC, containing PAK, PKG, and PKC kinase families; CMGC, containing CDK, MAPK, GSK3, and CLK kinase families; RGC, containing receptor guanylate cyclase kinase family; TK, tyrosine kinase; TLK, tyrosine kinase-like; APC, antigen presenting cell; TCR, T-cell receptor; MHC II, class II major histocompatibility complex; PTK, protein tyrosine kinase; ITAMS, immunoreceptor tyrosine-based activation motifs; SH2, Src homology 2; LAT, linker for activation of T-cells; SAG, superantigen; SEE, *Staphylococcus enterotoxin* E; FACS, fluorescent antibody cell sorting; PMA, 12-myristate 13-acetate; IRES, ribosomal entry site; tNGFR, truncated nerve growth factor; mAb, monoclonal antibody; PE, phycoerythrin; FITC, fluorescein isothiocyanate; APC, allophycocyanin; SDF-1alpha, stromal cell-derived factor 1 alpha

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1. Introduction

T-cell activation initiated by antigens displayed on antigen-presenting cells (APCs) can induce specific T-cell clonal expansion and differentiation, and thereby link innate and adaptive immunity. The activation of a complex network of signal transduction pathways is initiated at the T-cell receptor (TCR), and modulated by costimulatory receptors and integrins [1,2]. Upon TCR engagement of antigen presented by an APC's major histocompatibility complex (MHC), the proximal TCR protein tyrosine kinases (PTK) LCK and FYN are activated and subsequently phosphorylate the immunoreceptor tyrosine-based activation motifs (ITAM) on the cytoplasmic ε, β, γ, and ζ domains of the TCR complex. Phosphorylated ITAMs bind the SH2 (Src homology 2) domains of the PTK ZAP-70, which is phosphorylated and activated by LCK. Activated ZAP-70 phosphorylates the essential adaptor molecules for T-cell activation, LAT (linker for activation of T-cells) and SLP-76. Phosphorylated tyrosine residues on LAT and SLP-76 act as sites for the recruitment of additional adaptor and enzymatic proteins crucial in the activation of the Ras- and Rho-family GTPase signaling networks, calcium mobilization, and cytoskeletal reorganization [2,3]. TCR activated PTKs also regulate inositol phospholipid metabolism, which regulates both intracellular calcium and the activity of serine/threonine kinases, including members of the PKC family and phosphatidyl inositide-3 kinase (PI3K)-controlled serine kinases [1]. The phosphorylation of ITAMs is negatively regulated by the tyrosine phosphatase CD45. Antigen engagement of the TCR excludes phosphatase molecules from the TCR–MHC II complex, leading to LCK activation [1].

TCR interaction with MHC presented antigen on APCs results in an organized structure referred to as the immunological synapse that develops within minutes of signal activation [4]. Synapse formation requires cytoskeletal components, which play critical roles in modulating T-cell-APC contact, early events in T-cell activation, and the assembly of signaling complexes important to subsequent cellular responses [4–6]. The immunological synapse consists of a central supramolecular activation cluster (cSMAC) enriched in TCRs, and a peripheral pSMAC, which contains lymphocyte function associated-1 (LFA-1) and talin [4]. The purpose of the synapse is thought to concentrate receptors and ligands into a localized region, thereby facilitating non-TCR protein–protein interactions, and promoting cross-talk and sustained signaling [7].

T-cells have a pivotal role in regulating pathogen-induced immune responses [8]. While numerous bacterial immunotoxic components activate the innate arm of immunity, superantigens (SAG) activate T-cells of the adaptive arm of immunity [9]. Bacterial SAGs have a wide variation in structure yet share a common mechanism of activity; the binding of class II major histocompatibility complex (MHC-II) alpha-1 domain and/or a zinc-dependent high-affinity site on the MHC beta-1 domain expressed on macrophages and the simultaneous binding to the variable V-beta chain of the T-cell receptor complex [9,10]. SAGs can activate up to 30% of all T-cells at concentrations below 10^{-9} M. In contrast, normal peptide antigens activate T-cells in the range from 0.0001 to 0.001% of the total T-cell population [9]. SAG activated T-cells release substantial quantities of TNF-alpha, followed sequentially by IL-2, IL-6, IL-1, and IFN-gamma [9]. Bacterial SAGs share the characteristic of being among the most potent pyrogens known and all are capable of inducing lethal toxic shock syndrome [9].

Characterization and mapping of protein kinases that regulate acute T-cell activation induced by bactetrial SAGs is crucial for the development of effective immunotherapies. Protein kinases are a common class of enzymes amenable to small compound therapeutic development. Based on the prediction by Manning et al. [11], a total of 518 known and putative protein kinase genes have been identified in the human genome and are categorized based on their conserved catalytic domains. However, the functional study of large sets of gene expression events relevant to the regulation of T-cell physiology presents a daunting challenge. To address the challenge, we developed a reverse genomics screen based on the expression of random fragmented kinase sequences. The approach results in the isolation of kinase sequence fragments or genetic suppressor elements (GSEs) that interfere with T-cell activation by a variety of mechanisms including antisense RNAs and inhibitory peptides. GSE screens have been applied to identify the functional roles for genes essential in HIV infection and replication in T-cells [12,13], and for cell proliferation and apoptosis in cancer cell lines [14–18]. Our screen identified several kinases with novel associations in the regulation of T-cell activation in response to bacterial SAG.

2. Materials and methods

2.1. Cells, antibodies, reagents

The Jurkat (clone E6-1, TIB-152) and Raji (CCL-86) cell lines were purchased from ATCC while the tetracycline repressor expressing Jurkat (T-REx) cell line was purchased from Invitrogen. The amphotropic packaging cell line Clone-3 was derived from the BING cell line obtained from Dr. W. Pear (Rockefeller University, New York), and maintained in DMEM supplemented with 10% FCS at 37 °C in 5% CO_2. All other cell lines were maintained in RPMI supplemented with 10% FCS at 37 °C in 5% CO_2. The anti-mouse IgG1 FITC (BD 349041), anti-mouse IgG1 PE (BD 349043), anti-mouse IgG1 APC (BD 340442), anti-CD3 (OKT3), anti-CD3 FITC, anti-CD19 PE (BD 349209), and anti-CD19 APC (BD 340437) antibodies were purchased from Becton Dickinson. The anti-CD19 FITC (BD Pharmingen 555412), anti-CD28, and anti-CD69 APC (BD Pharmingen 555533) antibodies were purchased from BD Pharmingen. Anti-LAT polyclonal antibody (sc7948) was purchased from Santa Cruz Biotechnology. The anti-NGFR monoclonal antibody was produced