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Differential Requirements for Protein Kinase C-Theta at The Immunological Synapse of Effector Versus Regulatory T Cells and Their Clinical Implications

Noah Isakov*

The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences and the Cancer Research Center, Ben Gurion University of the Negev, P.O.B. 653, Beer Sheva 84105, Israel

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Introduction

T cells play a fundamental role in initiating and mounting effective adaptive immune responses. They are activated by the interaction of their T cell antigen receptors (TCR) with peptide antigen presented on major histocompatibility complex (MHC) molecules on the surface of antigen presenting cells (APC) [1,2]. The $CD4^+$ effector T cells (Teff) are classified into three major lineages, Th1, Th2 and Th17, based on the unique cytokine profile they produce [3]. The nature of the immune response, its specificity, intensity, and duration are intricately regulated by a fourth population of cells, the regulatory T cells (Treg). Treg possess immunosuppressive properties and are essential for maintaining peripheral tolerance, suppression of self-reactive lymphocytes, prevention of autoimmune responses, and restraining immune responses to chronic pathogens and commensals of the gut [4,5].

A productive interaction between a cognate TCR on the surface of a T cell and an MHC-bound peptide antigen on an APC requires a persistent engagement during which the two cells redistribute their receptors and cognate ligands to the interface. This initial step involves the polarization of additional membrane proteins, and recruitment of cytoplasmic molecules to the T cell-APC contact area. The resulting re-structuring of the cell membrane and proteins form a platform for effective signal transduction, and for communication between the cells [2]. This area of interface, termed the immunological synapse (IS), was discovered by Monks and colleagues who noted the occurrence of lateral segregation of proteins at the T cell-APC interface [6,7]. They showed that the TCR/CD3 receptors and protein kinase C theta (PKCθ) concentrate in the center of the contact region in a zone they have termed the central supramolecular activation complex (cSMAC). Other proteins, such as talin and the LFA-1 integrin, concentrate at a more peripheral zone within the IS, termed the peripheral SMAC (pSMAC). Later studies demonstrated the selective recruitment of additional effector molecules to the immunological synapse of activated T cells, including co-receptors (CD2, CD4, CD8 and CD28), tyrosine kinases (Lck and ZAP-70) and adaptor proteins (LAT, Gads, SLP-76) [1,8-11]. While the exact function of the immunological synapse in activated T cells is not fully understood, it may play an important role in stabilizing the engaged TCR and accessory molecules, and delivery of TCR-dependent signals via mechanisms, which may vary from one type of T cell to another.

Human PKCθ was discovered in T cells in 1993 [12] and found to be involved in the regulation of a wide range of T cell functions [13,14]. It is a member of a large family of serine/threonine kinases that contribute to signal transduction networks in almost all cell types. In T cells, PKCθ plays an important role in T cell activation and survival and it links the TCR/CD3-signaling pathway to the activation of NF-κB, AP-1 and NF-AT transcription factors [15-17].

PKCθ received special attention after Monks and colleagues demonstrated that it recruits to the center of the IS in TCR activated T cells [7]. This process, which occurs in a CD28-costimulatory-dependent manner [18-20], is made possible by a proline-rich motif within the PKCθ V3 domain that directly interacts with the CD28 cytoplasmic tail [21]. Furthermore, by phosphorylation of the CARMA1 protein, PKCθ links the CD28 costimulatory receptors to the CARMA1-Bcl10-Malt1 signaling pathway, leading to the activation of the transcription factor, NF-κB [22,23].

The initial observation of PKCθ- and TCR/CD3-residing cSMAC at the interface of cells was made in Th-B cell lymphoma [7]. A similar pattern of protein segregation was observed in Th cells overlaid on artificial supported planar bilayers containing fluorescently labeled MHC and ICAM-1 proteins [24]. An even more symmetric shape of IS, resembling a 'bulls-eye', was observed in $CD8^+$ Tc cells, as well as in natural killer (NK) cells, where a peripheral ring of adhesion molecules surrounded a central region containing the TCR in Tc, and activating or inhibitory receptors in NK cells [25-27], demonstrating that the IS formation is not restricted to Th cells.

In complete contrast to the above mentioned cell types, Zanin-Zhorov and colleagues have found that human peripheral blood Treg responded to signals from an ICAM-1- and anti-CD3 Ab-containing supported planar bilayer, by sequestering PKCθ to the opposite cell pole away from the TCR and IS [23]. The amount of PKCθ observed at the center of the IS was six-fold lower in Treg compared to Teff. A similar reduction in PKCθ content at the Treg's IS was also observed when CD80, the physiological ligand of CD28, was included in the planar bilayer. This is in contrast to the fact that the overall structure of IS in Teff and Treg had a similar symmetry with central clusters

***Corresponding author:** Noah Isakov, The Shraga Segal Department of Microbiology and Immunology, Faculty of Health Sciences, Ben Gurion University of the Negev, P.O.B. 653, Beer Sheva 84105, Israel, Tel: 972-8-647-7267; Fax: 972-8-647-7626; E-mail: noah@bgu.ac.il

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of anti-CD3 Abs surrounded by a ring of ICAM-1, and comparable intensities of phosphorylated ZAP-70 at the IS of both cell types. These findings suggest that discrete localization of PKCθ could drive different functions in distinct T cells subsets.

To test the potential involvement of PKCθ in the suppressive function of Treg, Zanin-Zhorov and her colleagues utilized two complementary approaches to inhibit PKCθ in Treg, either by CD20, a chemical compound that selectively inhibits PKCθ, or PKCθ-specific small interfering RNA (siRNA) that reduced PKCθ expression by ~80%. In both cases, inhibition of PKC augmented the suppressive activity of the Treg, even in the presence of CD28-mediated costimulatory signals [23]. These studies and additional data suggested that TCR-induced activation of PKCθ functions to reduce the ability of Treg to suppress the activation of Teff and ability to respond by proliferation and cytokine secretion.

Because of the important role of $CD4^+CD25^+$ Treg in maintaining immunologic homeostasis and preventing the development of autoimmune diseases, their activity must be regulated by fine-tuned and balanced mechanisms. Recent studies demonstrated a reduced number of Treg and a Th17/Treg imbalance in the peripheral blood of rheumatoid arthritis (RA) patients [28,29]. In addition, Treg isolated from RA patients displayed an anergic phenotype upon stimulation with anti-CD3 and anti-CD28 Abs, and were unable to suppress proinflammatory cytokine secretion by activated T cells [30,31].

RA is a degenerative autoimmune disease characterized by chronic inflammation that may affect different tissues and organs. The pathogenesis in RA patients is driven by proinflammatory cytokine, predominantly TNFα, which binds to the TNFR2 on the surface of Treg [32]. The resulting TNFα-induced signals in Treg of RA patients downregulated mRNA production and protein expression of the transcription factor, Foxp3, a master regulator of the development and function of Treg [30-32].

TNFα treatment of Treg inhibited PKCθ recruitment to the distal pole and promoted its relocalization to the IS, to a position resembling that of PKCθ in the IS of activated Teff [23]. Treatment of RA patients with anti-TNFα Abs (infliximab) restored the suppressive function of the Treg, concomitantly with increasing FOXP3 mRNA production and protein expression. Anti-TNFα Ab treatment also restored the number of peripheral blood Treg in RA patients [30,31]. Furthermore, inhibition of PKCθ by C20 protected Treg from TNF-induced downregulation of their normal activity, prevented the downregulation of Foxp3 expression, and partially restored the suppressive activity of Treg from RA patients [23]. This study indicated that PKC is a critical molecule involved in the mechanism leading to the formation of functionally compromised Treg in RA patients. It therefore raises the possibility that targeting of PKCθ may restore Treg functions and ameliorate disease progression in RA or other autoimmune patients.

The potential critical role of PKCθ in the regulation of Treg functions was further analyzed in an in vivo model of inflammatory colitis in mice. In this study, infusion of C20-treated Treg provided partial protection from colitis and increased the number of Treg in the lymphatic organs, in agreement with previous findings whereby inhibition of PKCθ increased the Treg-mediated suppressive capabilities [23].

The possibility of using PKCθ as a drug target for therapeutic intervention in T-cell-mediated diseases has been previously discussed [33-35], and was based on findings predominantly related to the role of PKCθ in the activation of Teff. The assumptions of this model were based on studies demonstrating that adoptive transferred PKCθ-deficient T cells were less likely to induce a GVH response [36], while PKCθ-deficient mice were more resistant to the induction of experimentally induced autoimmune diseases [37-40].

The recent findings in Treg indicated that PKCθ exhibits proinflammatory effects that are further enhanced by TNFα, which increases the recruitment of PKCθ to the IS of Treg. Inhibition of PKCθ increased the suppressive functions of Treg, enhanced their ability to prevent autoimmune colitis in mice and restored the activity of impaired Treg from RA patients. The role of PKCθ in the regulation of Treg functions makes it an ideal drug target in a range of autoimmune disorders and chronic inflammatory diseases. The fact that inhibition of PKCθ can restore the ability of Treg to suppress Teff functions, such as the secretion of proinflammatory cytokines, may be of significant importance during adoptive immunotherapy for the treatment and/or prevention of diseases such as autoimmunity and graft-versus-host following bone marrow allotransplantation.

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