Unraveling Immune Mechanisms of Disease Development

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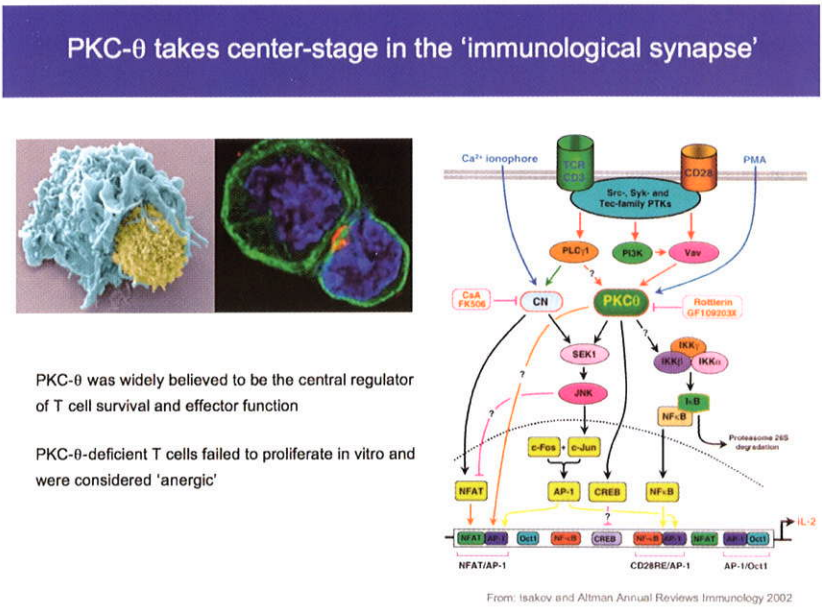
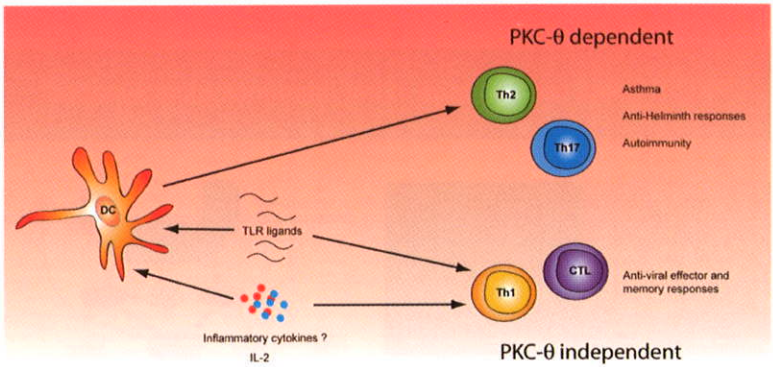
The serine/ threonine-specific protein kinase C-θ (PKCθ) is a core component of the immunological synapse which was shown *in vitro* to play a central role in the activation of T cells following TCR and co-stimulatory molecule stimulation.



In recent years a series of *in vivo* studies have revealed that the situation is far more complex; specifically, PKCθ signaling is differentially required for T-helper type 1, 2, 17 and CD8+ cytotoxic T cell responses. Th2 immune responses against Helminth infection or model allergens were markedly impaired inline with early *in vitro* studies. Surprisingly, Th1 immune responses against the intracellular protozoan *Leishmania major* developed normally, as did antiviral effector/ memory CD4+ and CD8+ T cells and Th1 polarization *in vitro*. Following the recent discovery of IL-17-induced autoimmune diseases, this path of PKCθ-mediated T cell activation was also investigated and found to require intact PKCθ signaling. Taken together, a clear disparity between the nature of T cell differentiation and the requirement for PKCθ signaling became evident.

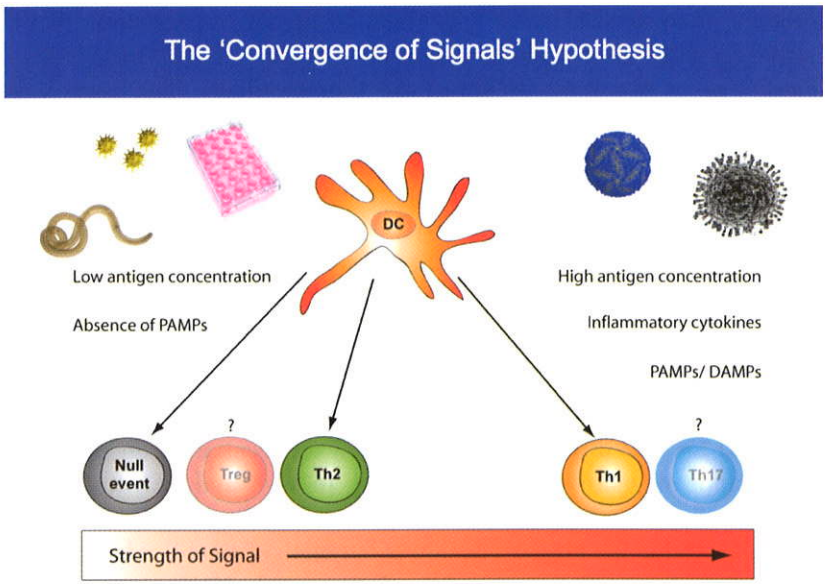
PKC-θ regulates the 'strength of signal' delivered to T cells



PKC-θ is differentially required for Th1, Th2, Th17 and CTL responses *in vivo*. The absence of PKC-θ has a profound effect upon the development of Th2-driven allergic and anti-Helminth responses in addition to Th17-mediated autoimmune disease models. Comparatively, Th1 and CTL responses develop normally in the absence of PKC-θ highlighting the presence of distinct pathways that can overcome its requirement during viral primary and secondary infections. IL-2 can overcome the absence of PKC-θ signaling, and it is plausible other inflammatory cytokines might also function in this manner. TLR ligands such as CpG can act directly upon PKC-θ-deficient T cells to promote proliferation and survival providing a further level of redundancy for the development of Th1 and CTL responses.

We proposed that additional factors present during Th1 and CD8+ T cell responses might bypass the need for PKCθ-signaling. Conversely, the absence of such factors during Th2 stimulating environments could ensure the strict requirement for PKCθ. Possible factors present during Th1 more so than Th2 responses include strong costimulation, high antigen dose, proinflammatory cytokines and bacterial/viral PAMPs. High antigen-load provided by a replicating virus for example, could potentially play a role in overcoming the absence of PKCθ. Evidence

supporting this comes from in vitro proliferation assays, where PKC-θ-deficient T cells were shown to proliferate to a similar degree as wild-type T cells following stimulation with a 10-100 fold higher concentration of antigen. In addition, in vitro co-cultures of T cells and dendritic cells with either high or low dose specific-peptide revealed that high dose peptide cultures induced Th1 differentiation and proliferation in a PKCθ-independent manner, whilst low dose peptide cultures failed to generate Th2 cell. Cytokines also have the potential to bypass the requirement for PKCθ-signaling; addition of IL-2 to *in vitro* cultures restores proliferation of PKCθ-deficient T cells. Recently, we found that the TLR9 ligand, CpG, could directly act upon T cells to induce proliferation and survival factors in a PKCθ-independent manner although the presence of DC-derived inflammatory cytokines was required for appropriate effector T cell differentiation. Thus, there are a number of possible mechanisms by which the immune response might bypass PKCθ signaling, although notably, such factors are generally characteristic of Th1 and CTL, not Th2 responses.



The convergence of multiple signals determines the differentiation of T helper cell subsets. Stimulation of naïve T cells with low concentrations/ low affinity antigen in the absence of PAMPs is considered a 'weak strength of signal' and leads to the development of Th2 cells. A 'strong strength of signal' such as that delivered by high concentrations/ high affinity antigen, the presence of PAMPs and inflammatory cytokines drives the differentiation of Th1 cells. Whilst sufficient experimental data is still lacking, indications suggest that Treg cells develop upon weak TCR stimulus whilst Th17 cells develop upon strong antigen/ PAMP signals and the presence of IL-6 and TGF-β.

Whether the primary function of PKCθ is mediating cell survival, or differentiation per se is not clear. However, our recent data indicates a primary role for PKCθ in cell proliferation and survival as opposed to differentiation. Direct stimulation of PKCθ-deficient T cells by PAMPs supports survival, but full differentiation into Th17 cells requires additional polarizing cytokines irrespective of the antigen. High antigen concentrations in vitro lead to Th1 differentiation, but intermediate/low concentrations of antigen do not lead to polarized Th2 responses in the absence of PKCθ, rather cell death (presumably through insufficient stimuli for cell survival). The Th2 immune response mounted against protein/ alum immunization appears to be heavily reliant upon PKCθ signaling given ex vivo restimulation of cells showed Th2 cell development was at the limit of detection. Comparatively, Th2 responses against the Helminth parasite *Nippostrongylus brasiliensis* were detectible, although substantially reduced. Residual Th2 immunity following Helminth infection may result from as yet unidentified parasite-related signals able to partially bypass PKCθ signaling or components of type 2 immunity (i.e. IL-4, IL-13) derived from non-CD4 T cells such as basophils.

Extensive research of T cell signaling pathways has revealed a general trend whereby many molecules downstream of TCR signaling are essential for Th2 differentiation. Th1 cell differentiation on the other hand, appears more robust, developing in vivo and in vitro in the absence of apparently key signaling factors including PKCθ. We would suggest revising the classic concept of 'strength of signal', which is primarily based upon the nature of the TCR-peptide-MHC interaction. T

helper cell differentiation appears to rely on the presence or absence of multiple signals within which many layers of redundancy are present for Th1 but not Th2 immune responses. The potent ability of PAMPS to drive Th1 differentiation likely reflects the ability of these molecules to promote DC activation leading to increased costimulation, adhesion molecules and proinflammatory cytokines. In keeping with this hypothesis, costimulation through CD28-CD80/86 and upregulation of adhesion molecules including LFA-1/ ICAM increase the strength of engagement between DC and T cells, and lower the TCR threshold required for Th1 differentiation. Thus, such convergence of signals ensures Th1 and CTL development whilst sacrificing the development of Th2 responses.

Implications of this discovery

Pharmaceutical industry

PKC-θ is an ideal target for therapeutic intervention

- Specifically target allergies, some autoimmune disorders without influencing antiviral and antibacterial immunity.

Basic research

PKC-θ directly regulates the 'strength of signal' delivered to T cells

'Danger' signals can be sensed by T cells in addition to antigen presenting cells, providing An additional level of security for antiviral/ bacterial immune responses