INTERVIEW about

PKC and Erk in CTL degranulation

with

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What are PKC and ERK?

Hanne L. Ostergaard: Protein kinase C (PKC) is not a single protein, but rather a family of serine threonine kinases involved in numerous cellular processes in essentially all cell types. Different PKC family members can be categorized as classical or conventional (cPKC α β γ), novel (nPKC δ ε η θ), or atypical. The classical isozymes require both the lipid signaling molecule diacylglycerol and calcium for optimal activity whereas novel isozymes require only diacylglycerol for activation. The atypical are regulated independently of both diacylglycerol and calcium. The nPKCθ is of particular interest because it specifically localizes to the center of the immunological synapse between a T cell and antigen presenting cell (1). It was generally accepted that this unique localization to the contact point bestowed an essential function for PKCθ, making it a topic of numerous studies related T cell activation.

Activation of PKC by diacylglycerol can be mimicked by the phorbol ester TPA. T cells can be activated with TPA in combination with calcium ionophores that increase intracellular calcium concentrations. As PKC were known to be the only TPA stimulated enzymes, it was assumed that all activity stimulated by TPA was attributable to PKC. This view, however, was radically challenged by the discovery of the RasGRP family of Ras exchangers and examination of their function in T cells (2-4). These exchangers serve to activate the Ras family of G-proteins, which in turn activates a signaling cascade resulting in the downstream activation of the important serine threonine kinase ERK. Treatment of T cells with TPA induces the recruitment of these exchangers to the membrane where they activate Ras resulting in ERK activation. The discovery of the RasGRP family and demonstration that ERK could be activated by TPA through these exchangers brought to question many pathways that were initially attributed to PKC.

Is PKC required for ERK activation in T cells?

Hanne L. Ostergaard: A number of studies suggested that PKC regulates ERK activation in T cells. However, after the discovery of RasGRP, the role of PKC in ERK activation, particularly based on TPA stimulation, was largely discounted. Furthermore, early studies on PKCθ suggested that this kinase could regulate the Ras pathway, however this view was brought into question when it was shown that PKCθ deficient mice appear to have intact ERK kinase signaling (5,6). Nevertheless, dismissing a contribution of PKC to ERK activation may have been premature, as it was recently shown that PKC, including PKCθ, can phosphorylate RasGRP, suggesting that PKC may indeed influence activation of the Ras pathway (4,7-9).

Our studies with cytotoxic T lymphocytes (CTL) have revealed that the regulation of ERK activation may be considerably more complex in T cells than initially anticipated and that the requirement for PKC varies depending on how the T cells are stimulated (10). When T cells are stimulated with crosslinked anti-CD3, cPKC regulates ERK downstream of Ras. In contrast, when cells are stimulated with plate-bound anti-CD3, a stimulus that leads to more sustained signals and functional activation in the form of CTL degranulation of cytolytic molecules, there is a requirement for nPKC upstream of Ras, consistent with a role in phosphorylating RasGRP. These additional studies implicate multiple pathways in which PKC can regulate ERK activation in T cells.

Is PKC required for CTL degranulation?

Hanne L. Ostergaard: Numerous studies have shown that broad specificity PKC inhibitors block CTL degranulation. Pardo et al showed that cPKC is required for degranulation (11), which is in agreement with our results (10). However, a requirement for nPKC is more controversial. In one study, the nPKC inhibitor Rottlerin did not inhibit degranulation (11). However, we found Rottlerin to be problematic because of its off-target effects and CTL toxicity. Unfortunately, there are no nPKC specific inhibitors so it is difficult to test this question directly. Furthermore, CTL derived from PKCθ knockout mice have perfectly normal degranulation, however there is clearly altered expression of other PKC to potentially compensate for the absence of PKCθ (10). Recently, it was shown that PKCθ may enhance degranulation but it is not absolutely required for degranulation (12). Our studies examining PKC leading to ERK activation may provide some insight into the potential roles of PKC in degranulation. We have shown that ERK is required for optimal CTL degranulation (13). Our studies suggest that nPKC, but not cPKC, is important for regulating ERK function stimulated with plate-bound anti-CD3 (10). However, we know that cPKC are still required for degranulation (10,11). We posit that nPKC are required for ERK activation leading to degranulation whereas cPKC is required for an ERK-independent aspect of degranulation. Knockout mice and inhibitors have provided limited insight into PKC function during degranulation because of redundancy and potential compensation by other PKC family members. Knocking down PKC expression, individually or in combination, using RNA interference in transient systems may be informative as this can be achieved quickly, perhaps prior to compensatory expression of other PKC family members. It is clear that additional studies are required to unravel the many potential contributions of PKC family members to CTL degranulation.

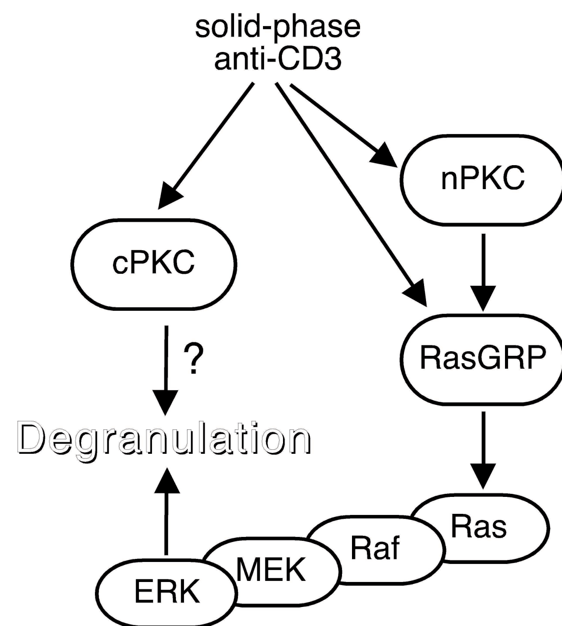

Figure: **Proposed contribution of PKC to CTL degranulation.**

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