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Editorial

Editorial: **Nck has a knack for T cell differentiation**

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Introduction

Nck is a ubiquitous SH2/SH3 adaptor protein that acts as a scaffold, connecting receptor tyrosine kinases in the plasma membrane with cytoplasmic signaling proteins involved in rearrangement of the actin cytoskeleton. Therefore, Nck is an important player in actin-guided axonal growth in neurons and in kidney podocyte mobility [1]. However, Nck also mediates the activation of signaling pathways that directly result in transcriptional regulation of gene expression. Indeed, proteins recruited by Nck may activate JNK and ERK MAPK pathways, as well as Rac1/Cdc42 pathways [2]. In B cells, Nck has been associated with PI3K pathway activation by binding and recruiting the adaptor protein BCAP to a phosphorylated tyrosine in the Igα subunit of the BCR [3]. Antigen-induced binding of Nck to phospho-Igα depends on the SH2 domain of Nck. In contrast, in the context of T cells, Nck is recruited to a unique, proline-rich sequence in the cytoplasmic domain of the CD3ε subunit of the TCR, which is absent in other TCR subunits and the BCR [4, 5] (**Fig. 1**).

The mechanistic differences between Nck recruitment to BCRs vs. TCRs described above highlight a nick in TCR signaling models. What molecular entity could be playing the part of BCAP in T cells potentially to mediate TCR-driven PI3K activation through Nck? This question indeed hinges on the assumption that similar to its behavior in B cells, Nck must also participate in the activation of the PI3K/Akt pathway in T cells. In a recent paper, published in this issue of the *Journal of Leukocyte Biology*, Lu et al. [6] present strong evidence in favor of this working model. With the use of a conditional knockout mouse line with null and reduced expression of both Nck genes (Nck1 and Nck2) in T cells, termed Nck.T$^{-/-}$, Lu et al. [6] show that Nck is required for the assembly of a humoral response to T-dependent antigens. Alterations in this antigen response seem to emanate from defects in a specific type of differentiated CD4$^+$ T cells, namely, Tfh cells, which are essential in assisting B cells at germinal centers, as they express important ligands for costimulatory receptors in B cells, including CD40 ligand or ICOS, and secrete cytokines, such as IL-21. These molecular entities are all required for Ig class-switch and affinity maturation in B cells.

A priori, the Tfh defect in Nck.T$^{-/-}$ mice could originate from a deficiency in differentiation of naive CD4$^+$ T cells into Tfh. Nck has indeed been found essential for T cell proliferation in response to weak antigens, i.e., antigens with low affinity for the TCR, but not in response to strong antigens [7]. Interestingly, this behavior correlated with a previous study, in T cells bearing a mutation in the proline-rich sequence of CD3ε, which therefore, are unable to recruit Nck to the TCR. This mutation also gave rise to defective proliferation in response to weak, although not to strong, antigens [8], suggesting that Nck is placed immediately downstream of the TCR in response to weak antigens. As the authors discuss, this differential participation in weak vs. strong antigen TCR signaling places the spotlight on Nck as a target for the treatment of autoimmune diseases, such as lupus erythematosus, which involves the production of antibodies against autoantigens [9]. Previous studies described that strong and weak antigens also differentially promote CD4 T cell differentiation to Th1 or Tfh [10]. Therefore, it would have been highly consistent with preexisting data to establish a correlation between the differential requirement of Nck for T cell activation by weak vs. strong antigens and CD4 T cell differentiation into Tfh cells. However, the authors show that in CD4$^+$CXCR5$^+$ T cells from Nck.T$^{-/-}$ mice (where CXCR5 is a marker of Tfh), expression of the defining transcription factor of Tfh differentiation (Bcl6) is not affected compared with their wild-type counterparts. Therefore, Nck seems not to be required for Tfh differentiation. Rather, the authors find that Tfh cells from Nck.T$^{-/-}$ mice are highly susceptible to apoptosis, which they link to a deficient activation of the crucial PI3K effector, Akt. This brings us back to the initial question of how Nck influences PI3K activation in T cells. As mentioned above, Nck in B cells links the adaptor BCAP and the BCR. However, the effect

Abbreviations: BCAP = B cell adaptor for PI3K, Nck = noncatalytic region of tyrosine kinase adaptor protein 1, Rac1 = Ras-related C3 botulinum toxin substrate 1, SH2/3 = Src homology 2/3, Tfh = follicular Th

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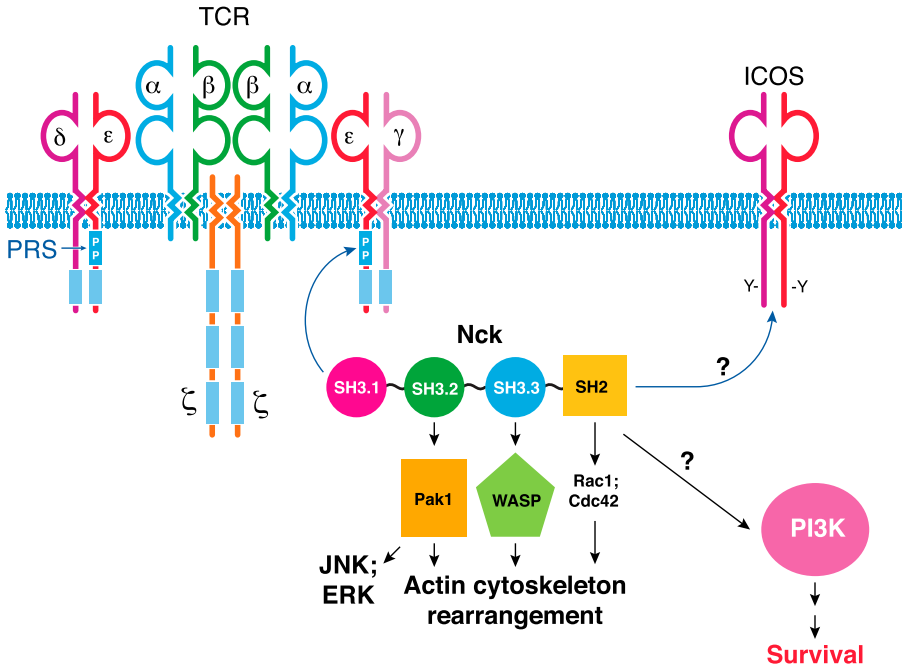

Figure 1. Missing links in the role of Nck in T cell differentiation and survival. Nck connects membrane receptors with intracellular pathways involved in remodeling of the actin cytoskeleton, cell activation, and cell survival. In T cells, Nck is recruited to a proline-rich sequence (PRS) in the cytoplasmic tail of CD3ε, allowing for recruitment of downstream effectors, such as p21 protein (Cdc42/Rac)-activated kinase 1 (Pak1), Wiskott-Aldrich syndrome protein (WASP), and many others, to the TCR. In B cells, Nck binds the adaptor BCAP, hence bringing about activation of the PI3K pathway under the direct control of the BCR. In T cells, such a connection between Nck and the PI3K pathway is missing. The results of Lu et al. [6] indicate that Nck plays an important role in Tfh cell survival by a PI3K-dependent mechanism. However, the identity of the membrane receptor that activates this pathway is still unknown. The TCR is a known candidate, although costimulatory receptors that activate the PI3K pathway and therefore, are important for Tfh differentiation and survival, such as ICOS, might potentially direct the pathway.

of Nck deficiency on Akt activation is stronger than that of BCAP deficiency. This suggests that Nck could stand downstream, not only of the BCR but also of other receptors on B cells, such as CD19, which strongly influence PI3K/Akt activity [1]. Likewise, in Tfh cells, PI3K appears downstream of other important receptors, such as Notch [11, 12], and of costimulatory receptors, such as ICOS. Indeed, the transcription factor forkhead box protein O1, a PI3K/Akt effector, must first become inactivated by ICOS signaling to allow initial Tfh differentiation, although it requires activation at a later stage to reach complete germinal center Tfh differentiation [13]. Hence, Nck deficiency in T cells from Nck.T$^{-/-}$ mice does not provide direct clues concerning the nature of the receptors involved in PI3K signaling. Therefore, the results of Lu et al. [6] leave the possibility open for considering Nck as a signaling coordinator that acts through multiple membrane receptors, ultimately resulting in activation of the PI3K pathway in B cells and T cells.

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