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Commentary OMICS International

Stalling Instead of Crawling Allows CD8$^+$ T cells to Cross the Blood-Brain Barrier

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Commentary

In the context of neuroinflammation, circulating T lymphocytes have been shown to extravasate across the blood-brain barrier (BBB) *via* a well explored multistep process [1-4]. Starting with P-selectin glycoprotein-1 (PSGL-1)-mediated T cell rolling on endothelial P-selectin, T cells will slow down on the inflamed BBB and then interact with chemokines or lipid mediators on the surface of the endothelium engaging G-protein coupled receptors (GPCRs) [1,2,5]. GPCR-mediated inside-out-signaling induces affinity maturation of constitutively expressed integrins on the T cell surface which is prerequisite for integrin-mediated arrest on their endothelial ligands from the Ig-superfamily [1,2]. Important interaction partners for T-cell arrest identified between encephalitogenic CD4$^+$ T cells and the inflamed BBB are endothelial VCAM-1 and α4β1-integrin (VLA4) as well as endothelial ICAM-1 and LFA-1 [2,6,7]. Subsequent crawling of CD4$^+$ T cells over long distances against the direction of flow has been demonstrated *in vivo* in the context of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis [8]. *In vitro* studies have shown that interaction of T cell LFA-1 with endothelial ICAM-1 and ICAM-2 is essential for CD4$^+$ T cell crawling [9]. CD8$^+$ T cells have been suggested to be of major importance in the pathogenesis of multiple sclerosis [10]. However, the currently used EAE model is a CD4$^+$ T cell mediated disease model and not suitable to study CD8$^+$ T-cell extravasation across the inflamed BBB *in vivo*.

In this context, our recent study [11] has aimed to improve our understanding of the cellular and molecular mechanisms mediating CD8$^+$ *vs* CD4$^+$ T-cell migration across the BBB in neuroinflammation. Employing *in vitro* live cell imaging in a microfluidic device the multi-step extravasation of activated CD4$^+$ and CD8$^+$ T cells across primary mouse brain endothelial cells (pMBMECs) as an *in vitro* model for the BBB was compared side by side. Compared to CD4$^+$ T cells, CD8$^+$ T cells showed a significantly increased shear resistant arrest on pMBMECs stimulated or not with the proinflammatory cytokines INFγ and TNFα.

Interestingly, the arrest of both T cell subsets was not dependent on Gαi-mediated GPCR-mediated integrin affinity maturation. This is in line with previous studies which have shown that $T_{effector/memory}$ cells express sufficient amounts of high-affinity LFA-1 on their surface which allows for binding to its endothelial ligands in the absence of additional stimuli [2,4,12-14]. Endothelial ICAM-1 and ICAM-2 were of major importance for sufficient T cells arrest of CD8$^+$ T cells, as their absence led to a complete abrogation of enhanced CD8$^+$ T cell arrest compared with CD4$^+$ T cell arrest. This suggests a major involvement of LFA-1 in the process of shear-resistant arrest of CD8$^+$ over CD4$^+$ T cells on pMBMECs. Although CD4 and CD8 T cells were found to express similar cell surface levels of LFA-1 we found CD8 T cells engage higher levels of soluble ICAM-1 suggesting that they display a higher fraction of high affinity LFA-1 on their surface than CD4 T cells (Figure 1).

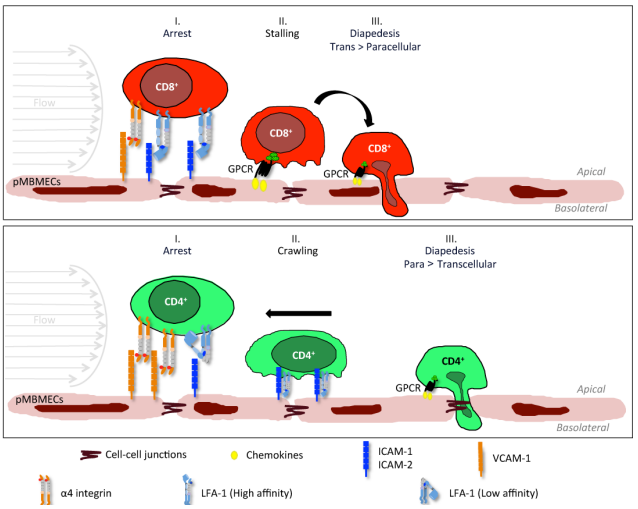

Figure 1: Schematic representation of the pathway of diapedesis of activated CD4$^+$ and CD8$^+$ T lymphocytes across pMBMEC *in vitro* under flow: Side-by-side comparing of the multi-step extravasation of activated CD4$^+$ and CD8$^+$ T cells across the blood-brain barrier (BBB) under flow by *in vitro* live cell imaging identified subset specific mechanisms of interaction. CD8$^+$ T cells arrested in significantly higher numbers, stalled and crossed the BBB preferentially *via* the transcellular route.

CD8$^+$ T cell arrest was followed by a unique behavior, where CD8$^+$ T cells would remain localized within their diameter, but visibly and actively probed the endothelium for sites of diapedesis. We classified this behavior as "stalling" in contrast to the behavior of CD4$^+$ T cells which after initial arrest crawled over the endothelial surface prior to their diapedesis as described before [8,9,15]. In correlation to their stalling behavior, transmigration of CD8$^+$ T cells occurred mostly *via* the transcellular route. In the absence of ICAM-1 and ICAM-2 CD8$^+$ T cells were found to still quite efficiently cross the BBB underscoring that they do not rely as CD4 T cells on ICAM-1 and ICAM-2 mediated crawling for finding a site for diapedesis. Rather the mainly LFA-1/ICAM-1 and ICAM-2 mediated arrest and stalling of CD8$^+$ T cells on the BBB seemed to lead to optimized interactions with endothelial

signaling cascades involved in the formation of ICAM-1 and/or ICAM-2 enriched docking structures that are important for T cell diapedesis [9,15-18].

Taken together our study demonstrated that the cellular and molecular mechanisms involved in the multistep cascade of $CD8^+$ *vs* $CD4^+$ T-cell migration across the BBB shows significant differences. Further investigations are thus desirable in order to discover mechanisms unique to different T cell subsets. This will allow the development of more specific treatments targeting the CNS invasion of destructive T cells without affecting CNS immunosurveillance.

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