

"Mechanisms of translocation of CD2 and CD5 to lipid rafts: a role for Lck "

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In T lymphocytes, CD2 and CD5 antigens are involved in the regulation of signals elicited by the TCR/CD3 complex. We have previously demonstrated that CD2 can be found in physical association with CD5 at the surface of T lymphocytes. Moreover, it has been suggested that CD2 can potentiate the CD5 inhibitory effect in thymocyte selection, as demonstrated in the CD2/CD5 double-knockout mouse. The association of CD2 with the tyrosine kinase Lck may account for this effect. Antibody-mediated stimulation of either CD2 or CD5 has been shown to activate the protein kinase Lck, and we hypothesized that this activation would require the targeting of CD2 and CD5 to lipid rafts.

Jurkat cells were stimulated through CD2 or CD5, and targeting of these antigens to membrane lipid rafts was analyzed by Western blotting of cell lysate fractions separated by sucrose gradient. CD5 was massively translocated into lipid rafts upon engagement with different CD5 antibodies, independently of Lck. A significant proportion of CD2 was also targeted to the rafts upon CD5 and CD3 stimulation. However, in J.CaM1 Lck-deficient cells, targeting of CD2 to the rafts was negligible. Reconstitution of cells with functional Lck restored most patterns of CD2 mobilization. Translocation of CD5 induced upon stimulation of other receptors could also be Lck dependent. In Jurkat cells expressing rat CD2, targeting of a significant fraction of CD5 to the rafts could also be accomplished independently by anti-CD2 mAb treatment. However, a cytoplasmic deletion mutant of CD2 devoid of its Lck-binding motifs, or a full-length molecule but expressed on Lck-deficient cells, could not reinstate the effect. Importantly, crosslinking CD5 with CD2 increases the aggregation and polarization of the lipid rafts, as demonstrated by fluorescence microscopy.

Thus, the induced co-translocation of the accessory molecules CD2 and CD5, and the reorganization of lipid rafts is dependent on the enzymatic function of Lck, or in the physical association of Lck with the receptors.

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