[S2-2] [4/17/2003(Thur) 14:40-15:20/Maple Hall]

Adaptor Proteins in T Cells Regulate IL-2

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T cell activation is initited by the interaction of T cells with antigen-presenting cells (APCs) in the context of peptide antigen. Initial conjugates are formed by binding between lymphocyte-associated antigen-1 (LFA-1, also known as CD11a-CD18) and intercellular adhesion molecule-1 (ICAM-1), or CD2 and LFA-3, or other pairs of interactive proteins. Whithin minutes of contacting APCs, T cells undergo actin-marked polymerization at the contact site, providing a potential assembly point for signaling proteins. Conjugate formation involves a rearrangement of receptors, comprising a central supramolecular activation cluster (cSMAC) enriched with T cell receptor (TCR), CD2 and CD28, surrounded by a peripheral SMAC (pSMAC) enriched with LFA-1. The relevance of alterations in receptor topography to the generation of intracellular signals is suggested by the presenc of tyrosine phosphorylation and plecstrin homology (PH) domain binding sites outside the rearranged receptors.

Several adaptors that integrate signals from surface receptors in immune cells have been identified, including linker for activation of T cell (LAT), Grb2-related adaptor downstream of Shc (Gads), SH2 domain-containing leukocyte protein of 76 kDa (SLP-76) and FYN-T-binding protein (FYB) or adhesion and degranulation-promoting adaptor protein (ADAP, previously known as SLP-76-associated protein). LAT and SLP-76 are needed for pre-TCR and mature TCR signalling, as well as for platelet and mast cell function, and ADAP binds to the SH2 domain in SLP76. ADAP has two isoforms of 120 and 130kDa, which differ by an insert of 46 amino acids. Both isoforms have binding sites for the SH2 domains of SLP-76 and FYN-T, two putative nuclear localization sites, an internal SH3 domain, a binding site for an Enabled/vasodilator-stimulated phosphoprotein (VASP) homology 1 (EVH1) domain,and a C-terminal SH3-like domain. ADAP cooperates with its binding partners FYN-T and SLP-76 to up-regulate the transcription of interleukin 2 (IL-2), whereas T cells from ADAP-deficient mice show impaired LFA-1-mediated adhesion and peripheral T cell function. Thus ADAP

specifically modulates both clustering of beta-1 and beta-2 integrins and adhesion on T cells and basophils. By contrast, the adaptor Wiscott-Aldrich syndrome protein (WASP) selectively modulates TCR capping, and VAV regulates both TCR- and LFA-1-mediated adhesion.

In turn ADAP binds to another hematopoietic specific T cell adaptor protein, Src kinase-associated phosphoprotein of 55 kDa (SKAP-55; encoded by SCAPI). The association was initially detected in a two-hybrid screen using ADAP as a bait and in coprecipitation studies. SKAP-55 has a unique N-terminal region, followed by a PH domain and a C-terminal SH3 domain, and is related to a widely expressed homolog known as SKAP-55-related or SKAP-55 homolog. Binding can occur in two directions: the SH3 domain of SKAP-55 binds to a proline-rich region in ADAP, and the SH3 domain of ADAP binds to a tyrosine-based RKxxYxxY motif in SKAP-55. SKAP-55 interacts with CD45 and up-regulates the activity of p59fyn and mitogen-activated protein kinase. Even though these connections to other proteins have been identified, a clear role for SKAP-55 in regulating T cells has not been described as yet.

Despite the importance of T cell-APC conjugate formation in determining the T cell response to foreign antigen, VAV is the only scaffold to have been implicated directly in regulating this event. Here we show that SKAP-55 can potently increase conjugate formation between T cells and APCs and enhance integrin-mediated adhesion to fibronectin and ICAM-1. The enhanced conjugation is comparable to that induced by ADAP and is abrogated by deletion of the SH3 domain of SKAP-55. We also show that SKAP-55 colocalizes with F-actin at the T-cell-APC synapse and enhances the clustering of LFA-1 on the surface of cells. Conjugate formation induces the translocation of SKAP-55 to membrane rafts, a process that is regulated by both LFA-1 and TCR ligation, resulting in up-regulation of IL-2 transcription. Taken together, these finding show that SKAP-55 functions in integrin-mediated adhesion and T cell-APC conjugate formation and therefore identify a mechanism by which SKAP-55 modulates T cell responses to antigen.