Monoclonal Antibody Register

New Monoclonal Antibodies Recognizing the Adaptor Protein LAT

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Background

LAT, the linker for activation of T cells, is a 36–38 kDa transmembrane signalling adaptor protein expressed exclusively on T lymphocytes, NK cells, mast cells, megakaryocytes and platelets. The molecule consists of a short, 5 amino acid extracellular stretch, and a transmembrane helix followed by a cytoplasmic tail containing 9 conserved tyrosines. Though no modular protein-binding domains have been identified in LAT, several of the tyrosines lie within motifs predicted to bind important downstream signalling molecules (Weber et al, 1998; Zhang et al., 1998a). Due to post-translational palmitoylation of two membrane-proximal cysteins, LAT preferentially sublocalizes to lipid rafts, small regions of the plasma membrane distinct in lipid composition and enriched in signalling molecules (Zhang et al., 1998b).

LAT has been demonstrated to function centrally in the propagation of signals from surface antigen receptors of immune cells (Finco et al., 1998; Zhang et al., 1999a; Saitoh et al., 2000). These receptors include the T-cell receptor and certain Fc receptors and belong to a structurally defined family that has been termed the multichain immune recognition receptors (MIRRs). Following the engagement of MIRRs on LAT-expressing cells, LAT tyrosine residues are phosphorylated by receptor-associated and activated Syk/ZAP-70 kinases, and directly recruit Src homology 2 domain-containing signalling molecules, including PLCγ, Grb2, Gads, Grap, 3BP2, Shb. Translocation and/or susceptibility to further phosphorylation of these molecules and their binding partners is a key step that regulates the pathways ultimately leading to most of the cellular responses. Thus, LAT forms a membrane raft-confined signalling complex, which links the proximal, cell-type specific receptor apparatus with the subsequent, more ubiquitous signalling pathways (reviewed in Wange, 2000). Antigen-driven responses of T cells and mast cells lacking functional LAT are manifestly abrogated (Finco et al., 1998; Zhang et al., 1999b; Saitoh et al., 2000).

Given the essential role of LAT in the development and function of the immune system, it has been proposed that the reduced signalling from antigen receptors under many pathological situations may be related to the impairment of LAT localization and function (Gringhuis et al., 2000; Wange, 2000). Therefore, monoclonal antibodies specifically recognizing LAT are obligatory tools for the investigation of signalling phenotypes of immunocompetent cells under various circumstances.

Production

Hybridoma cell lines producing monoclonal anti-LAT antibodies were obtained after immunization of BALB/c mice with recombinant rat LAT protein, fusion of splenocytes with SP02 myeloma cells, selection, and cloning using standard procedures. The recombinant LAT was generated from the full-length *LAT* cDNA amplified from the rat basophilic leukaemia (RBL) cell line, RBL-2H3, using reverse-transcriptase polymerase chain reaction. The protein was expressed and purified using the pQE expression system (Qiagen, Hilden, Germany). The screening of the hybridoma clones was based on ELISA reactivity of the immunoglobulins with the recombinant protein. Two hybridoma cell lines, LAT.1D1 and LAT.3H2, were isolated.

Specificity

Both cell lines produced antibodies reacting with the recombinant LAT, but not with control recombinant proteins as tested by ELISA screening. The antibodies recognized a band of about 40 kDa on immunoblots with lysates of rat and murine mast cell lines (Fig. 1), but did not react with any similar protein from human

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Abbreviations: BMMC – bone marrow-derived mast cells, MIRR – multichain immune recognition receptor, RBL – rat basophilic leukaemia.