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.

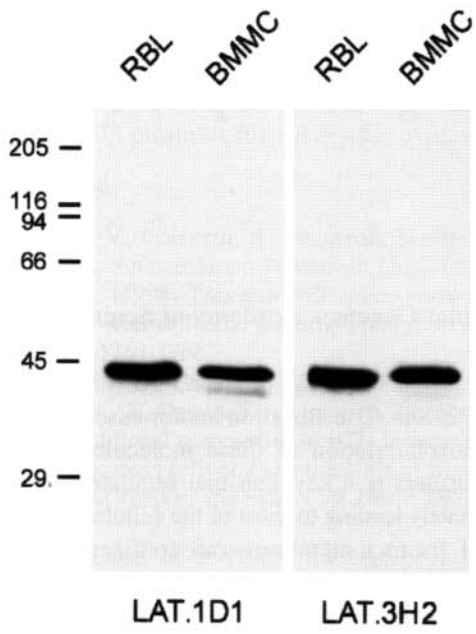

Fig. 1. RBL-2H3 cells (2×10^5) or mouse bone marrow-derived mast cells (BMMC; 3×10^5) were lysed in a lysis buffer (10 mM NaH_2PO_4, pH 7.2, 50 mM NaCl, 50 mM NaF, 1% Triton X-100, 0.5% deoxycholate, 0.1% sodium dodecyl sulphate, 1 mM EDTA, 1 mM Na_3VO_4, 1 mM phenylmethylsulphonylfluoride), resolved by SDS-PAGE under non-reducing conditions and transferred to a nitrocellulose membrane. The membrane was developed with anti-LAT antibodies as indicated, followed by anti-IgG conjugated to horseradish peroxidase. The positions of molecular mass standards are indicated on the left in kDa.

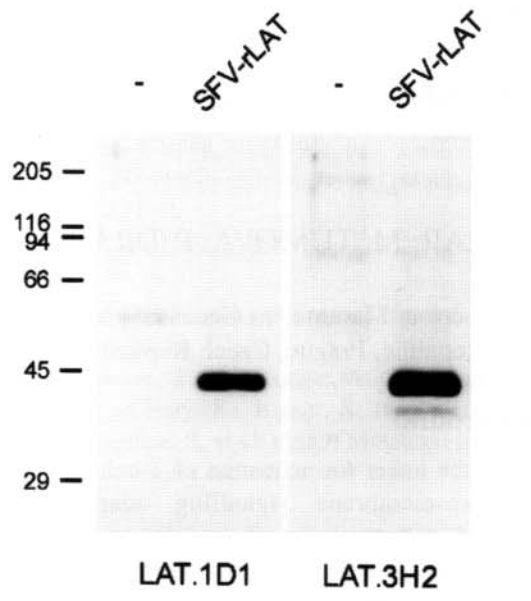

Fig. 2. COS cells were mock-infected (-) or infected with recombinant Semliki Forest Virus containing cDNA of rat *LAT* (SFV-*rLAT*), lysed and analysed by immunoblotting as in Fig. 1. The positions of molecular mass standards are indicated on the left in kDa.

cell lines previously reported to express LAT (data not shown). The reactivity was similar on gels under reducing conditions. The same band was recognized in the light density fractions of the RBL-2H3 mast cell line after sucrose gradient ultracentrifugation (not shown). Both antibodies detected a ~40 kDa protein in lysates of COS cells transiently expressing rat LAT (Fig. 2).

In immunofluorescence assays, the antibodies gave a uniform membrane staining pattern on detergent-permeabilized and paraformaldehyde-fixed RBL cells, but not on non-permeabilized cells as revealed by confocal microscopy (data not shown). The antibodies could also be used for LAT immunoprecipitation under non-denaturing conditions. Since LAT undergoes tyrosine phosphorylation upon MIRR engagement, we tested whether the phosphorylation would affect the reactivity of the antibodies. Fig. 3 shows that the antibodies immunoprecipitated and detected the same amount of LAT from resting as well as antigen-stimulated mast cells, although the phosphorylation of LAT increased dramatically in antigen-stimulated cells. These data indicate that the antibodies can be used for analysis of LAT under various physiological conditions.

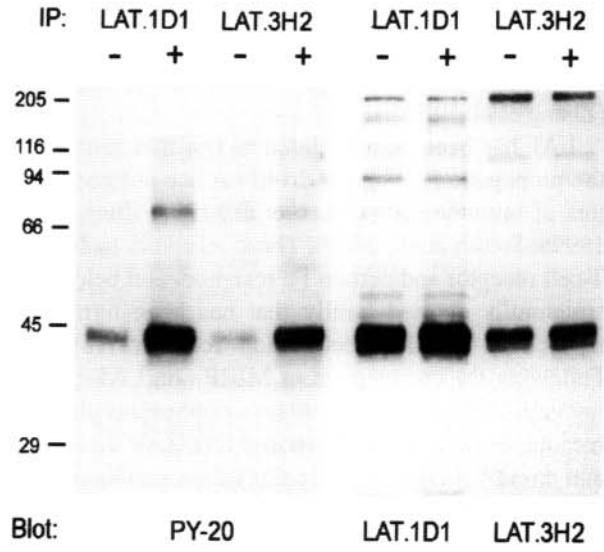

Fig. 3. RBL-2H3 cells (10^7) were sensitized with trinitrophenyl (TNP)-specific IgE and then stimulated (+) or not (–) with 1 μg/ml TNP-bovine serum albumin conjugate. After 5 min at 37°C the cells were lysed as described in Fig. 1 and subjected to immunoprecipitation with anti-LAT antibodies covalently bound to Ultralink Biosupport Medium (Pierce, Rockford, IL). The immunoprecipitates were eluted and analysed by immunoblotting either with anti-phosphotyrosine antibodies (PY-20, Transduction Laboratories, Lexington, KY), or with anti-LAT antibodies (LAT.1D1, LAT.3H2). The positions of molecular mass standards are indicated on the left in kDa.

Properties

Antibodies LAT.1D1 (IgG_{2a}, pI 7.8–8.3) and LAT.3H2 (IgG_1, pI 6.1–6.2) can react with mouse and rat LAT under denaturing as well as non-denaturing conditions.

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