

RESEARCH HIGHLIGHT



Sabotaging TCR signaling—LAG3 interferes with the CD3ε–LCK interaction

Gina J. Fiala¹ and Wolfgang W. Schamel^{1,2}✉

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T cell activation occurs when the TCR–CD3 complex binds pMHC, leading to recruitment of the kinase LCK to CD3ε, which triggers CD3 phosphorylation by LCK and downstream signaling. The inhibitory receptor LAG3 dampens TCR–CD3 signaling and T cell responses; two recent studies clarified its inhibitory mechanisms and therapeutic applications in cancer and autoimmunity.

T cells are activated and mount an immune response when the T cell antigen receptor (TCR–CD3) binds to its ligand, peptide presented by MHC molecule (pMHC). This binding exposes the CD3ε's basic-rich sequence (BRS) and receptor-kinase (RK) motif, which subsequently recruit the kinase LCK to promote CD3 phosphorylation and downstream signaling (Fig. 1a, b).^{1,2} The binding of CD3ε to LCK might occur in the form of phase-separated condensates.³

T cell activation can be suppressed by the inhibitory receptor LAG3, which is expressed on activated, including exhausted and autoreactive, T cells. Upon binding to its ligand MHC class II (MHCII), LAG3 dampens T cell activation.⁴ LAG3 has gained interest due to two potential applications in the clinics. Firstly, in immune checkpoint therapy against cancer, the immune response is enhanced by blocking key inhibitory receptors on T cells known as immune checkpoints. This checkpoint blockade mostly targets the inhibitory receptors PD-1 and CTLA-4. Recently, a first LAG3-blocking antibody received FDA approval for the treatment of melanoma and is used in combination with a PD-1 inhibitor.⁵ Secondly, in autoimmune diseases, unwanted T cell activation causes tissue damage. In this context, LAG3 activation might inhibit pathogenic autoreactive T cells, thus diminishing autoimmunity. While LAG3 has emerged as a promising therapeutic target, the mechanisms underlying its suppressive function have remained poorly understood. Two recent complementary studies published in *Cell* shed light on LAG3's signaling mechanisms.

Since LAG3 does not contain classical inhibitory motifs in its cytoplasmic tail, Jiang et al.⁶ tested in the May issue of *Cell* whether ligand engagement would modify LAG3's cytoplasmic tail. To this end, the authors analyzed LAG3 by mass spectrometry upon TCR–CD3 and LAG3 stimulation with pMHCII. They discovered that lysine 498 (K498), located within the conserved KIEELE motif, is ubiquitinated by the E3 ligases c-Cbl and Cbl-b (Fig. 1). In LAG3, a BRS, known to bind to membranes, is located adjacent to the FSALE motif, a region required for LAG3's inhibitory function. In the non-ubiquitinated state, the BRS and

FSALE motifs are inserted into the hydrophobic interior of the plasma membrane, rendering them inaccessible (Fig. 1b). K498 ubiquitination disrupts this interaction, exposing the FSALE motif and enabling LAG3 to exert its inhibitory effect (Fig. 1c). In support of this, mutation of K498 to arginine (K498R) prevented ubiquitination and impaired LAG3's inhibitory function in vitro. Although a K498R mutation alone had limited effect on tumor growth in vivo, it significantly enhanced tumor control in combination with PD-1 blockade in mice. Similarly, Jiang et al. provide evidence that in human patients, the combined expression of LAG3 and CBL could serve as a prognostic factor to identify patients with poor outcome prognoses but likely to respond to LAG3 blockade therapy.

pMHCII binding to LAG3 unleashes LAG3's inhibitory capacity. Since pMHCII binds simultaneously to TCR–CD3 and LAG3, it might induce recruitment of LAG3 to the TCR–CD3 (Fig. 1c). Indeed, it was shown previously that LAG3 is closely associated with TCR–CD3.⁷ By using an engineered system in which proximity between TCR–CD3 and LAG3 can be induced by the small molecule rapalog, Du et al.⁸ show in the June issue of *Cell* that inducing proximity between LAG3 and TCR–CD3 enhanced the LAG3-mediated inhibition of TCR–CD3 signaling. This proximity was required for the LAG3's FSALE and EP motifs to form condensates with CD3ε using purified proteins in vitro and inhibiting the CD3ε–LCK condensates (Fig. 1c). Indeed, using a bead-based binding assay, the authors show that LAG3 inhibits the LCK–CD3ε interaction. LAG3 could form condensates with doubly phosphorylated CD3ε, thus reducing or inhibiting already ongoing TCR–CD3 signaling.

In an old model of TCR activation, it was the co-receptor CD4 that brought LCK into vicinity of the TCR–CD3. In line with this, it was found that LAG3 might inhibit this mechanism.⁹ However, Du et al. show that neither CD4 nor the CD4–LCK interaction was required for LAG3's inhibitory function.⁸ This is in line with the new allosteric models of TCR–CD3 activation,¹⁰ in which CD3ε recruits LCK.¹

Finally, Du et al. developed a bispecific, engineered antibody that binds simultaneously to TCR–CD3 and LAG3 and bypassed the requirement for their simultaneous pMHCII binding.⁸ This reagent could suppress the activation of CD4⁺ and CD8⁺ T cells that express LAG3, making it a potential drug for pathogenic autoreactive T cells in autoimmune diseases. As a proof of concept, the reagent could alleviate autoimmune symptoms in

¹Signalling Research Centres BIOSS and CIBSS, and Department of Immunology, Institute of Biology III, Faculty of Biology, University of Freiburg, Freiburg im Breisgau, Germany.

²Centre for Chronic Immunodeficiency (CCI), and Centre for Cell and Gene Therapy Freiburg (CGF), Medical Centre Freiburg and Faculty of Medicine, University of Freiburg, Freiburg im Breisgau, Germany. ✉email: wolfgang.schamel@biologie.uni-freiburg.de

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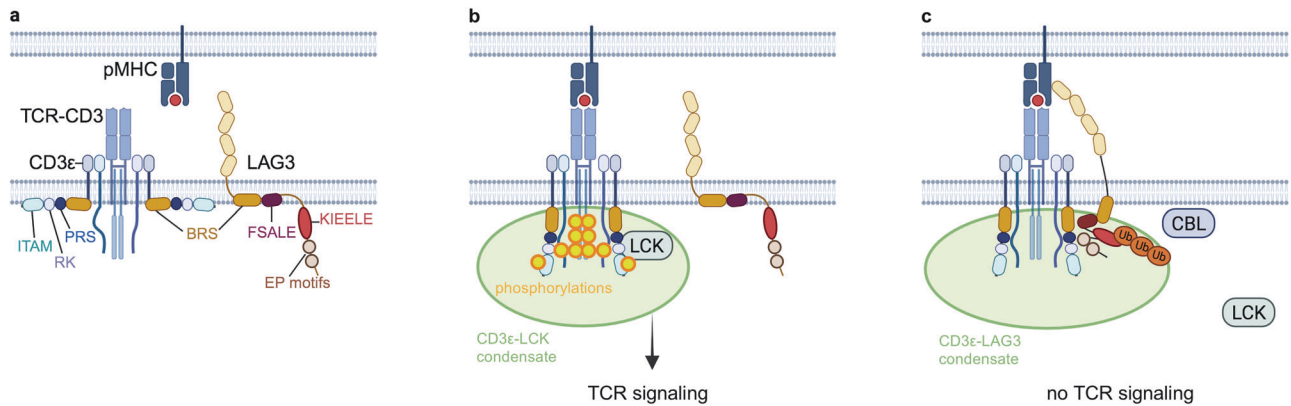


Fig. 1 The molecular mechanism of how LAG3 inhibits TCR-CD3 signaling. **a** In the resting state, CD3 ϵ signaling motifs are not accessible for binding to LCK. Likewise, the BRS and FSALE motifs of LAG3 are shielded within the membrane. **b** Upon pMHC binding, the TCR-CD3 undergoes conformational changes, exposing the BRS and RK motifs, which then bind to LCK. TCR-CD3-LCK condensates form, allowing CD3 phosphorylation and downstream signaling. **c** Simultaneous binding to pMHCII brings TCR-CD3 into vicinity of LAG3 and induces KIEELE motif ubiquitination by CBL. Ubiquitination detaches the FSALE and EP motifs from the membrane allowing them to form condensates with CD3 ϵ , thereby dissolving the CD3 ϵ -LCK condensates. This inhibits TCR-CD3 signaling. Created with BioRender.com.

several mouse models for autoimmune diseases, such as type I diabetes and multiple sclerosis.

In conclusion, uncovering details of the LAG3 inhibitory mechanisms on TCR-CD3 signaling has opened new ideas to treat cancer and autoimmune diseases.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Wolfgang W. Schamel.

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