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# LAG3 disrupts the TCR signal by local acidification

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little average change and a high degree of variability between cohort members. It is likely that a lot of this heterogeneity is due to the difficulties of obtaining samples at intervals that would be more informative and consistent. Limiting analysis to T cells in peripheral blood mononuclear cells and not from the site of infection probably also contributes to the variability.

As the pandemic rolls on, it has become possible to study T cell responses in individuals who were infected after vaccination, in so-called breakthrough infections. Given the extraordinary efficacy of the vaccines, it would be predicted that spike responses would dominate even in this scenario, but this is not what has been seen. Instead, there is evidence that the breakthrough infections drive primary responses to non-spike antigens that are equivalent to those seen in unvaccinated subjects. In infected–unvaccinated subjects, CD8<sup>+</sup> cell responses are skewed toward epitopes from the nucleocapsid (N) protein<sup>8</sup>, perhaps due to more efficient processing and presentation of epitopes from the N protein than of those from the spike. In the breakthrough subjects, this advantage seems to hold, and is apparently not outweighed by the advantages that recall of spike-specific memory cells is presumed to enjoy. The broadening of targeted CD8<sup>+</sup> cell specificities in the breakthrough subjects echoes what happens with antibody responses, wherein IgG


antibodies to the SARS-CoV-2 N protein are induced. In other words, vaccination with a single antigen does not suppress immune responses to the full range of SARS-CoV-2 antigens upon breakthrough infections, and this is welcome news.

As the first Omicron wave wanes in much of the world, it is a good time to place what we now know about SARS-CoV-2-specific CD8<sup>+</sup> T cells into context. Most immunologists agree that Omicron successfully evaded whatever protection the vaccines provided against infection with pre-Omicron strains. At the same time, the vaccine-induced protection against severe disease largely held, especially after a third vaccine dose. Protection against disease is often ascribed to adaptive cellular immune responses, but proof that vaccine-induced cellular responses are (or are not) responsible for protection against COVID-19 disease in humans has not been achieved so far. In the case of COVID-19, even proving correlates of protection against disease in humans is challenging, in part as a result of high vaccine efficacy.

So what are the candidates for future movies in the COVID-19 Vaccine Universe? Fans of T cells are probably hoping to see a story focusing on the introduction of additional antigens into current vaccine formulations, such as constructs encoding the nucleocapsid antigen<sup>9</sup>. Waning immunity and the emergence of variants of concern may

even provide fertile ground for testing the efficacy of this path, and the ability to detect an increase in efficacy following a third dose of the current vaccines provides a useful basis for comparison. As a testable scientific hypothesis, its merit is undebatable. In contrast, what remains highly debatable is where that path should lie on our priority list. Strong observational studies of T cell responses in SARS-CoV-2-infected and vaccinated people may be insufficient to push this path upward on the priority list. □

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#### Competing interests

The author declares no competing interests.



## T CELL ACTIVATION

# LAG3 disrupts the TCR signal by local acidification

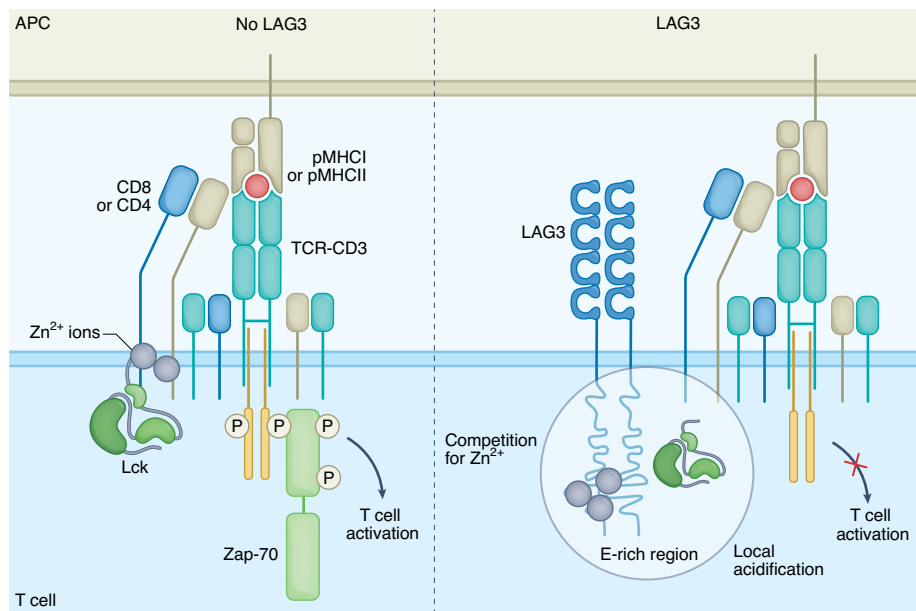
LAG3 interferes with TCR signaling by lowering the pH in the vicinity of the TCR and inducing dissociation of the key signaling kinase Lck from the co-receptors CD8 and CD4.

Claire Hivroz

The transmembrane protein LAG3 (CD223), first cloned in 1990 by Triebel and colleagues<sup>1</sup>, has inhibitory effects on T cell activation. LAG3 is expressed by activated conventional and regulatory CD4<sup>+</sup> and CD8<sup>+</sup> T cells, natural killer (NK) cells and invariant NK T cells, as well as other immune and non-immune

cells. LAG3 is induced by the chronic activation of T cells, and its expression on exhausted tumor-infiltrating lymphocytes is associated with poor prognosis in several cancers. Like PD-1 and CTLA-4, LAG3 is listed as an immune checkpoint inhibitors and is a potent therapeutic target for cancer immunotherapy<sup>2,3</sup>. In the current issue of

*Nature Immunology*, Guy et al. shed some light on the thus far enigmatic molecular mechanisms involved in the LAG3-mediated inhibition of T cell activation<sup>4</sup>. They show that a phylogenetically conserved glutamic-acid-enriched domain in the intracytoplasmic tail of LAG3 is responsible for the inhibitory effects of LAG3 through



**Fig. 1 | Schematic representation of the inhibitory effects of LAG3.** In the absence of LAG3, association of Lck with CD4 or CD8 allows the phosphorylation of ITAMs in the CD3 $\zeta$  complexes. This induces recruitment of the tyrosine kinase ZAP-70 and initiation of the TCR signaling cascade leading to T cell activation. When LAG3 is expressed in T cells, its presence in the vicinity of CD4 or CD8, which does not depend on binding of LAG3 to MHCII, induces the dissociation of Lck from the co-receptors, hampering the signaling cascade and therefore preventing T cell activation. The disruption of the Lck-co-receptor interaction depends on the glutamic acid (E)-rich intracytoplasmic domain of LAG3, which induces acidification of the local microenvironment around CD4 or CD8. The E-rich C-terminal region of LAG3 can also compete with Lck for binding to Zn<sup>2+</sup>, which controls the association of Lck with the co-receptors. APC, antigen-presenting cell.

its ability to locally lower the pH in the vicinity of the T cell receptor (TCR) and induce dissociation of the key signaling kinase Lck from the co-receptors CD8 and CD4, thus preventing the initiation of TCR-induced signaling. These effects do not depend on the binding of LAG3 to major histocompatibility complex class II (MHCII) molecules, one of its known ligands. The authors propose that this LAG3 domain might sequester the Zn<sup>2+</sup> cations essential for the binding of Lck to CD8 or CD4, revealing that LAG3 does not transduce a signal but instead disrupts a signal (Fig. 1).

The *LAG3* gene is located adjacent to the *CD4* gene in most species and probably evolved by gene duplication. The extracellular domains of LAG3 and CD4 share homology, whereas their intracytoplasmic domains have no similarity. Specifically, LAG3 lacks the cysteine motif responsible for the association of CD4 with Lck. Like CD4, LAG3 binds to MHCII and has been reported to selectively recognize stable peptide-MHCII complexes<sup>5</sup>. Yet the inhibitory effects of LAG3 on T cell activation are not due to competition between LAG3 and CD4 for MHCII

binding, as exemplified by the inhibitory effects of LAG3 on CD8<sup>+</sup> T cells.

So far, the mechanisms involved in LAG3 inhibition of T cell activation have remained elusive. Guy et al. now show that CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells purified from *LAG3*<sup>-/-</sup> mice and exposed to activating antibodies to CD3 and CD28 in the absence of MHCII<sup>+</sup> antigen-presenting cells divide more than wild-type T cells do<sup>4</sup>. In addition, *LAG3*<sup>-/-</sup> T cells exhibit increased signaling downstream of the TCR, as indicated by a higher Ca<sup>2+</sup> flux and the phosphorylation of key signaling proteins. These results confirm that LAG3 inhibits T cell activation in the absence of MHCII ligation. However, they do not exclude the possibility that other reported LAG3 ligands that might be present in the cultures, such as FGL1, LSECtin or galectin-3, could be involved. The authors also do not rule out the possibility that binding to MHCII could be involved when LAG3 is not abundant on T cells. These questions could be addressed by analyzing whether the inhibitory effects of LAG3 require its extracellular domain; for example, by expressing chimeric molecules in which the transmembrane and intracellular

domains of LAG3 are associated with an unrelated extracellular domain in *LAG3*<sup>-/-</sup> T cells.

The intracellular region of LAG3, although essential for its inhibitory function, lacks typical inhibitory motifs. However, it contains several phylogenetically conserved amino acid sequences that are not shared with other inhibitory co-receptors: an FSAL sequence in the juxtamembrane region, a KIEELE sequence in the central region, and 10–15 tandem repeats of glutamic acid (E) in the C-terminal region. The KIEELE motif has been reported to be essential for the inhibitory function of LAG3<sup>6</sup>, although this was not reproduced in a different study, which instead demonstrated the importance of the FSAL sequence<sup>7</sup>. Both studies reported a role for the E-rich C-terminal region. Guy et al. now elucidate the role of this region<sup>4</sup>. Using several super-resolution microscopy tools, they show that LAG3 associates with the TCR-CD3 complexes at the immune synapse with a stoichiometry of more than ten LAG3 molecules for one TCR-CD3. This association is observed when T cells form an immune synapse on plates coated with activating antibodies. It would be interesting to investigate whether this association is present at steady state and, if not, what triggers it, and whether it depends on post-translational modification of LAG3, remodeling of the cytoskeleton, recruitment of the intracellular pool of LAG3 or other factors.

Using a cell-free membrane-tethered assay, the authors show that the intracytoplasmic domain of LAG3 interacts closely with the co-receptors CD4 and CD8 and induces their clustering. This is dependent on the E-rich C-terminal region of LAG3 — the region that is also responsible for the dissociation of Lck from the co-receptors. Hence, increased association of Lck with the co-receptors is observed in *LAG3*<sup>-/-</sup> CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and peptides covering the E-rich region of LAG3 displace Lck from preformed CD4-Lck complexes. Thus, by locally inducing the dissociation of Lck from the co-receptors, LAG3 limits the phosphorylation of key substrates, such as the ITAMs of CD3 $\zeta$  complexes and the tyrosine kinase ZAP-70, and interrupts the TCR-induced signaling cascade very early on. Because the TCR-CD3 complexes bind Lck<sup>8</sup> and because LAG3 is more proximal to these complexes than to the co-receptors<sup>4</sup>, LAG3 may also disrupt the association of Lck with the TCR-CD3 complexes. This question deserves exploration, as it could help in designing a chimeric antigen receptor that is insensitive to LAG3 inhibitory signals.

An indication of how the intracytoplasmic domain of LAG3 induces

this dissociation came from the observations that addition of divalent cations, including  $Zn^{2+}$ , blocked the LAG3-induced Lck displacement and that the E-rich C-terminal region of LAG3 can bind  $Zn^{2+}$ , although weakly. Because the association of Lck with CD4 and CD8 depends on  $Zn^{2+}$  (ref. <sup>9</sup>), it seems likely that the LAG3 C-terminal region competes for the  $Zn^{2+}$  when in vicinity of the co-receptors, leading to Lck dissociation and thus to T cell inhibition. The displacement of Lck also relies on local acidification of the membrane at the immune synapse, which depends on the E-rich C-terminal region of LAG3 (Fig. 1). These E-rich intracellular domains control the sorting of proteins from endosomes to lysosomes. Hence, LAG3 may depend on its E-rich domain for its transport to endocytic lysosomal compartments, where it has been described<sup>10</sup>. Recruitment of the low-pH lysosomes containing LAG3 may contribute to the acidification of synaptic membranes and disruption of the association of p56Lck with its co-receptors. In the absence of the E-rich domain, these LAG3-dependent

low-pH lysosomes would not be recruited and the pH in the vicinity of the TCR and CD4 or CD8 would not be altered. It is worth noting that LAG3-dependent local membrane acidification might also modify the reported association of Lck with CD3 $\epsilon$ <sup>8</sup> and LAT<sup>11</sup>. Furthermore, evaluating whether proteins that contain comparable domains have inhibitory effects similar to those of LAG3 may lead to the characterization of new players in the regulation of T cell activation.

Finally, these results are particularly relevant because, despite the lack of functional understanding, LAG3 is a 'hot' target for anti-cancer immunotherapy. It is currently the subject of over 100 ongoing clinical trials, most of them using antagonistic monoclonal antibodies that block the interaction of LAG3 with MHCII<sup>12</sup>. Novel strategies that rely on the discovery reported here may increase the efficiency of LAG3-based immunotherapy. □

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#### Competing interests

The author declares no competing interests.



## INFLAMMASOME

# Dual ligand engagement for noncanonical inflammasome activation

The activation of the noncanonical NLRP3 inflammasome can be elicited by the interaction and interdependent activation of caspase-11 and NLRP3 that follows coincident cytosolic detection of lipopolysaccharide and bacterial mRNA from live Gram-negative bacteria.

Zhang-Hua Yang and Jiahuai Han

Lipopolysaccharide (LPS), also known as endotoxin, is a component of the cell wall and the most studied molecular pattern in Gram-negative bacteria. Murine caspase-11 and human caspase-4 and caspase-5 are cytosolic receptors that detect intracellular LPS and selectively mediate the activation of the noncanonical NLRP3 inflammasome<sup>1,2</sup>. A few mechanisms have been proposed for caspase-11-mediated activation of NLRP3, but these models could not fully explain the activation of noncanonical NLRP3 inflammasome by infection with Gram-negative bacteria<sup>3</sup>. In this issue of *Nature Immunology*, Moretti

et al. show that at least some noncanonical inflammasome activations induced by Gram-negative bacteria require not only LPS but also messenger RNA (mRNA) from the bacteria — with LPS engaging with caspase-11 and the mRNA concurrently binding to NLRP3, potentiating caspase-11 and NLRP3 interaction and activation of NLRP3 inflammasome<sup>4</sup>.

Detection of LPS is central to host defense against infection with Gram-negative bacteria and to the pathogenesis of endotoxemia and sepsis. Extracellular LPS is sensed by Toll-like receptor 4 (TLR4); whereas cytosolic

LPS is detected by caspase-11, which controls the activation of noncanonical inflammasome<sup>2,5,6</sup>. Intracellular LPS binds to and activates caspase-11 directly<sup>2</sup>. The activated caspase-11 cleaves the pore-forming protein gasdermin D (GSDMD)<sup>7–9</sup>, which can lead to a lytic form of programmed necrosis called pyroptosis. The NLRP3 inflammasome can be activated downstream of the activation of caspase-11, and in that case is named noncanonical<sup>1</sup>. The noncanonical NLRP3 inflammasome is widely believed to be a secondary event following the caspase-11-triggered formation of the GSDMD pore<sup>7</sup>. It should