

Fueling the fire: Src family kinases drive inflammation

The Src family kinases (SFK) Hck, Fgr, and Lyn are the main SFKs in neutrophils and monocytes. In this issue, in a tour-de-force study of multiple disease models (autoantibody-induced arthritis [K/B×N serum-transfer], skin inflammation induced by systemic administration of collagen VII-specific antibodies, and reverse passive Arthus reaction), Kovacs et al. show that triple knockout (TKO) mice lacking Fgr, Lyn, and Hck are completely protected from myeloid cell infiltration and inflammation. Surprisingly, this is not due to a cell-intrinsic migration defect, but more likely due to defective production of inflammatory cytokines and chemokines by myeloid cells in the inflamed tissue.

Hck, Fgr, and Lyn are involved in integrin and selectin ligand signaling pathways in leukocytes. If the adhesion receptor signaling functions were critical for *in vivo* inflammation, one would expect that neutrophils from TKO mice could not enter the inflammatory site. However, in mixed bone marrow chimeras reconstituted with TKO and wild-type bone marrow, both TKO and wild-type neutrophils enter the sites of inflammation, suggesting that the effect of knocking out all three SFKs is not cell autonomous. So, the wild-type neutrophils provide something that the TKO neutrophils cannot provide. This finding rules out an important role for the SFKs in adhesion receptor signaling pathways, at least as far as leukocyte recruitment is concerned.

What then do the SFKs actually do *in vivo*? It seems that they are required to set up the proper inflammatory environment. In the TKO mice, the induction of the key inflammatory cytokine IL-1 β is defective, as is induction of the chemokines CXCL1, CCL2, and CXCL2 and the 5-lipoxygenase (5-LO) product leukotriene LTB₄. In the arthritis model, this seems to be caused by defective signaling through Fc receptor γ chain (FcR γ), which is known to signal via SFKs. Specifically, the authors showed that FcR γ phosphorylation was defective; a GST fusion protein of the tandem SH2 domains of spleen tyrosine kinase Syk, a downstream target of SFKs, pulled down FcR γ from lysates of immune complex-activated wild-type but not TKO neutrophils. The importance of this pathway is further supported by the finding that mice lacking FcR γ phenocopy the TKO mice in the serum arthritis model. Although the FcR γ signaling defect may explain the observations in the arthritis model, it is not clear whether this observation translates to the skin inflammation and reverse Arthus models.

The fact that expression of SFKs in some of the recruited myeloid cells was sufficient to restore inflammation suggests that SFKs support amplification mechanisms that are defective in mice reconstituted with SFK TKO bone marrow alone. A very good candidate for this amplifier is LTB₄, a lipid mediator known to enhance neutrophil accumulation, migration, and mediator production. This is testable, because mice lacking 5-LO or BLT1, the pertinent receptor for LTB₄, are available. Both BLT1 and 5-LO are known targets for pharmacologic modulation of inflammation, but their clinical use has been quite limited so far.

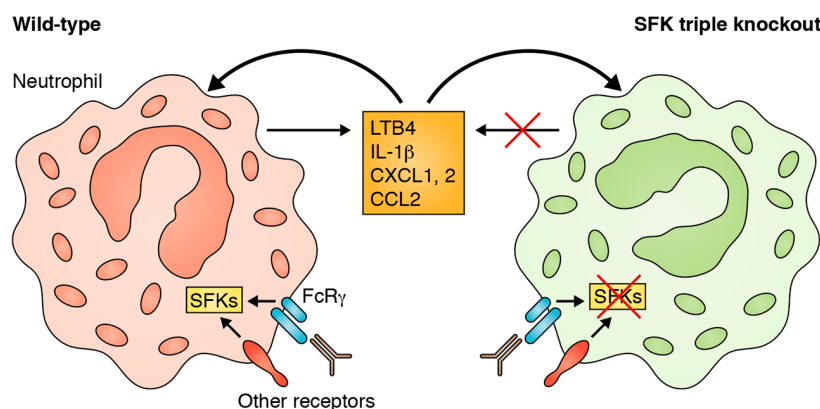
However, there may be some redundancy between LTB₄ and other inflammatory mediators controlled by SFKs; future work will be needed to identify such candidate mediators.

SFK inhibitors have potential as antiinflammatory agents. However, since SFKs are also involved in many other processes, significant side effects must be expected. The SFK inhibitor dasatinib is used to treat chronic myeloid leukemia and can cause anemia, bleeding, and other side effects. SFK inhibitors specific for the myeloid SFKs may have a more favorable side effect profile.

Kovacs, M., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20132496>.



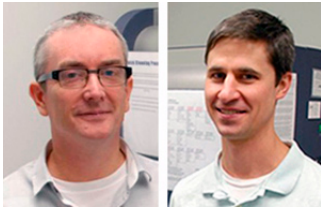
Insight from
Klaus Ley



In response to FcR ligation, wild-type neutrophils (left) produce inflammatory mediators (box) that have paracrine effects even on SFK-TKO neutrophils (right), which themselves cannot produce these mediators.

Klaus Ley, La Jolla Institute for Allergy & Immunology: klaus@liai.org

Parasite uses host NFAT to co-opt immune response



Insight from Christopher Hunter (left) and David Christian

Intracellular pathogens promote their survival by targeting host signaling pathways that either turn off antimicrobial activities or activate events that support their replication and/or survival of the host cell. The parasite *Toxoplasma gondii* introduces an array of proteins from specialized organelles (rhoptries and dense granules) into infected cells, and a small number of these proteins have been shown to modify host signaling, including STAT and NF- κ B pathways.

In this issue, Ma et al. identify a novel role for GRA6 (a dense granule protein) in activating the transcription factor NFAT4 in the host cell. This activity is mediated through the interaction of the C-terminal domain of GRA6 with the host protein calcium modulating ligand (CAMLG). The authors show

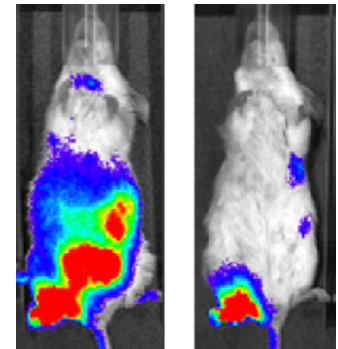
that the parasite needs GRA6 for full virulence in mice, as shown by parasite burdens measured by luminescence imaging (pictured). At the local site of infection, GRA6 activation of NFAT4 is required for chemokine production and recruitment of monocytes and neutrophils which, when infected, promote parasite dissemination.

These findings may explain why certain *T. gondii* strains with polymorphisms in the C terminus of GRA6 are less virulent in mice. As *T. gondii* infects a wide variety of hosts, these polymorphisms could reflect specialization of different parasite strains for different hosts.

Ma et al. link *T. gondii* virulence to NFAT for the first time and add GRA6 to the small number of rhoptry and dense granule proteins known to modulate host signal transduction. However, there are hundreds of parasite proteins introduced into host cells whose functions are not known. With the new technical advances in parasite and host genetics, it should now be possible to systematically delete each of these secreted proteins in multiple parasite strains and use these mutants to define their impact on different host responses.

Ma, J. S., et al. 2014. *J. Exp. Med.* <http://dx.doi.org/10.1084/jem.20131272>.

Christopher Hunter and David Christian, University of Pennsylvania: chunter@vet.upenn.edu and dac2@vet.upenn.edu



Bioluminescent imaging reveals reduced dissemination of GRA6 knockout parasites (right) compared with wild-type (left).