inflammation on pathogenesis and whether therapeutic intervention to inhibit NLRP3 is ultimately beneficial to the host.

1. Hoffman, H.M., Mueller, J.L., Broide, D.H., Wanderer, A.A. & Kolodner, R.D. *Nat. Genet.* **29**, 301–305 (2001).
2. Agostini, L. *et al. Immunity* **20**, 319–325 (2004).
3. Hornung, V. *et al. Nat. Immunol.* **9**, 847–856 (2008).
4. Halle, A. *et al. Nat. Immunol.* **9**, 857–865 (2008).
5. Jones, J.D.G. & Dangl, J.L. *Nature* **444**, 323–329 (2006).
6. Dostert, C. *et al. Science* **320**, 674–677 (2008).
7. Cassel, S.L. *et al. Proc. Natl. Acad. Sci.* **105**, 9035–9040 (2008).
8. Li, H., Willingham, S.B., Ting, J.P.Y. & Re, F. *J. Immunol.* **181**, 17–21 (2008).
9. Eisenbarth, S.C. & Colegio, O.R. *Nature* **453**, 1122–1126 (2008).
10. Fujisawa, A. *et al. Blood* **109**, 2903–2911 (2007).
11. Willingham, S.B. *et al. Cell Host Microbe* **2**, 147–159 (2007).
12. Shao, F. *et al. Science* **301**, 1230–1233 (2003).
13. Kanneganti, T. *et al. Immunity* **26**, 433–443 (2007).

Distinct functions for HS1 in chemosensory versus adhesive signaling

Peter Beemiller & Matthew F Krummel

Host immunity requires cytotoxic lymphocytes that are able to move toward their targets but are also able to stop after identifying target cells and then establish stable cell-cell contact. A new study shows that separate phosphorylation sites in HS1, an actin cytoskeleton–remodeling factor, can regulate both processes.

Many lymphocytes must balance continuous host surveillance through motility, with adhesion and target cell recognition mediated by the activation of immune receptors. Lymphocytes, such as natural killer (NK) cells in search of virus-infected target cells and T cells seeking antigen-presenting cells bearing agonist peptides, must coordinate signaling pathways that allow movement toward target cells with pathways that induce the arrest and killing of target cells or activation of effector lymphocytes. Both cellular migration and immune receptor activation are dependent on the actin cytoskeleton, a flexible, dynamic network of fibers that cells use not only to apply mechanical force but also to organize and modulate signaling pathways. Activation of lymphocyte effector functions is also often mediated by actin-driven assembly of cell-cell synapses, such as the lytic synapse of NK cells or the immune synapse that activates T cells; stabilization of these interactions requires the activation of integrins, such as the β_2 integrin LFA-1 in the effector lymphocyte plasma membrane, and concomitant signaling to actin cytoskeletal regulators[1,2]. Although it has been apparent for some time that immune receptor and motility signaling must converge to cooperatively regulate the actin cytoskeleton, factors that might serve as such a central 'switch' have not been clearly identified. In this issue of *Nature Immunology*, Butler, Kastendieck and Cooper show that both adhesive and chemotactic signaling converge on the actin cytoskeleton–remodeling factor HS1, with distinct HS1 phosphorylation sites regulating each process[3].

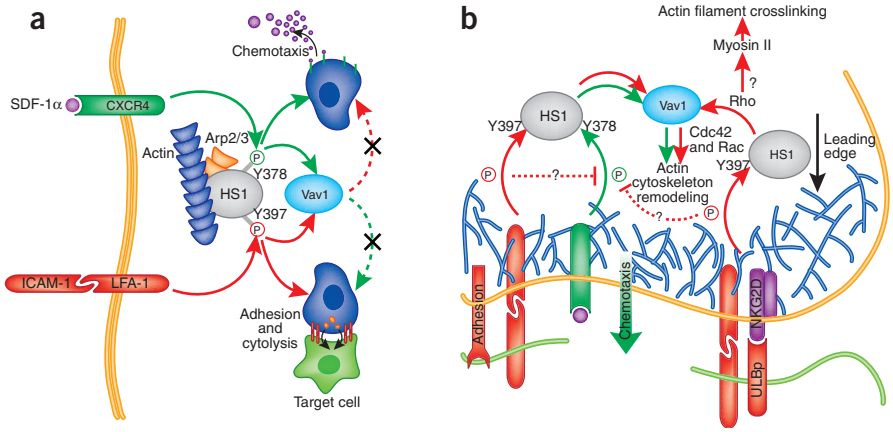

Figure 1 Distinct HS1-mediated signaling paths converge at Vav1 through HS1. (**a**) Adhesion receptor signals induce phosphorylation of HS1 at Tyr397, which leads to Vav activation and adhesion (red solid arrows), but not chemotaxis (red dashed arrow). Chemokine receptor signals induce phosphorylation of HS1 at Tyr378, which also induces Vav phosphorylation but triggers chemotaxis (green solid arrows), not adhesion (green dashed arrow). (**b**) Both chemotaxis and adhesion lead to the assembly of a branched actin filament network (blue). However, the two signals are not redundant. Phosphorylation of HS1 at Tyr397 may downregulate pathways mediated by HS1 phosphorylated at Tyr378 (left, dashed arrow), leading to migration arrest. Alternatively, a separate population of HS1 molecules phosphorylated exclusively at Tyr397 may induce migration arrest by initially inducing actin filament crosslinking and inhibiting Cdc42 and Rac (right, dashed arrow); subsequent reactivation of Cdc42- and Rac-mediated cytoskeleton remodeling might then facilitate adhesion. ULBp, UL16-binding protein.

HS1 is a member of the cortactin family of proteins, which are Src kinase substrates that contribute to the assembly of a branched actin network by binding nascent filaments and the actin nucleation–promoting factor Arp2/3 complex[4]. HS1 has been linked to the regulation of immune synapse formation in T lymphocytes through its ability to regulate actin assembly through the Rho-family GTPase guanine nucleotide–exchange factor Vav1 (ref. 5). Consistent with that finding, Butler and colleagues now demonstrate that HS1 is also necessary for formation of the lytic synapse and cytotoxic killing[3]. In doing so, they note that HS1 tyrosine residues are phosphorylated in response to the ligation of a variety of receptors, including the integrin ligand ICAM-1, the chemokine receptor CXCR5, and NKG2D, the NK cell–activating receptor responsible for initiating target killing. Combining knockdown of HS1 mediated by short hairpin RNA in NK cells with ectopic expression of HS1 molecules bearing phenylalanine substitutions at Tyr397 or Tyr378 (Y397F or Y378F, respectively), Butler and colleagues go on to examine the distinct functions of these two tyrosine residues, which are involved in

Peter Beemiller and Matthew F. Krummel are in the Department of Pathology, University of California San Francisco, San Francisco, California 94143, USA.
e-mail: matthew.krummel@ucsf.edu

Vav1-dependent actin polymerization during the formation of immune synapses[5].

This approach proves useful, as it allows direct demonstration that phosphorylation of Tyr397 but not of Tyr378 is critical in adhesion to ICAM-1-bearing substrates. This specific requirement for HS1 is not applicable to all substrates, as loss of HS1 does not impair adhesion to fibronectin-coated surfaces, a β_1 integrin–mediated process that is more likely relevant to adhesion to the extracellular matrix than to target cells. Mechanistically, phosphorylation of HS1 at Tyr397 is needed to facilitate the binding of β_2 integrins to Vav1 and cytohesin-1, a critical regulator of LFA-1 activation[6]. Butler and colleagues find that these interactions cannot be 'rescued' by expression of the Y397F HS1 mutant, regardless of whether the Tyr378 site is intact, which emphasizes the specificity of signaling through distinct HS1 phosphorylation sites.

In contrast to Tyr397, Tyr378 seems to be partially dispensable for target killing, which suggests that this phosphorylated tyrosine residue of HS1 could be involved in other actin cytoskeleton–dependent signaling processes. Aware that defective target lysis induced by HS1 knockdown could be partially attributed to a defect in NK cell motility, Butler and colleagues undertake studies in which they relieve NK cells of the requirement to move to target cells. In conditions that allow the NK cells to find their target cells without migrating, the Y378F mutant but not Y397F mutant restores the partial defect in killing noted in the standard killing assays requiring NK cells to move to their targets. Butler and colleagues hypothesize that Tyr378 is specifically involved in chemotaxis before arrival at target cells and assembly of lytic synapses. Indeed, NK cells expressing the Y378F HS1 mutant in place of endogenous wild-type HS1 show impaired chemotaxis induced by the chemokine SDF1-α. This defect does not seem to be due to a general defect in motility, as loss of HS1 activity does not induce a defect in 'haptotaxis', an indicator of migration induced by a gradient of adhesion substrates. Consistent with the proposition that Tyr378 is required for sensing chemoattractants, cells expressing the Y378F HS1 mutant show modest defects in the phosphorylation of phosphatidylinositol-3-OH kinase, the kinase PAK and Vav1 in response to SDF1-α. As substitution of Tyr397 has little effect on chemotactic signaling, this suggests distinct functions for each of these HS1 tyrosine residues (**Fig. 1a**).

Perhaps not unexpectedly, given the involvement of LFA-1–ICAM-1 interactions in mediating the formation of stable lytic synapses, when NK cells are presented with beads coated with ICAM-1 and the NKG2D ligand UL16-binding protein, phosphorylation of Tyr397 proves essential for activation of 'downstream' signaling[3]. This observation does not seem to reflect a requirement for integrin binding, as the most proximal event in NKG2D signaling, phosphorylation of the DAP10 adaptor protein, does not depend on HS1 phosphorylation. However, the association of Vav1 with DAP10 is completely dependent on HS1 phosphorylation. Notably, whereas overall activation of phosphatidylinositol-3-OH kinase is not noticeably lower in cells subjected to HS1 knockdown, the association of phosphatidylinositol-3-OH kinase with DAP10 is partially impaired by loss of HS1 or by Tyr397 substitution. This may indicate involvement of HS1 in linking specific receptor signaling with key mediators of cytoskeleton remodeling.

Although HS1 phosphorylation regulates several 'downstream' signaling molecules, Vav1 phosphorylation is particularly notable. Vav proteins are members of the DH family of Rho-family guanine nucleotide–exchange factors that activate Cdc42, Rac and Rho GTPases[7], which in turn can modulate Arp2/3-mediated assembly of the actin network[8]. Butler and colleagues find that Vav1 activation after integrin ligation or during SDF-1α-mediated chemotaxis can only be restored by HS1 molecules that can be phosphorylated at Tyr397 or Tyr378, respectively (**Fig. 1a**). Consistent with those results, defects in Cdc42 and Rac GTPase activation can only be restored by the Y397F HS1 mutant (for chemokine-induced activation) or the Y378F HS1 mutant (during integrin-mediated activation). However, it is not clear which Rho-family GTPases use Vav proteins for nucleotide exchange during different receptor-mediated processes in different cell types[9,10], and it is important to note that Vav proteins probably accomplish functions independently of their nucleotide-exchange activities[11]. Notably, activation of Cdc42 and Rac 'downstream' of the Y378F HS1 mutant during chemotaxis is accompanied by deactivation of RhoA, but Rho deactivation seems to be regulated independently of HS1 tyrosine phosphorylation. As Rho is known to be activated by T cell receptor crosslinking[12], and Rho versus Cdc42 and Rac leading-edge activities are generally antagonistic[13,14], reactivation of Rho through an immune receptor after an encounter with a target cell may provide an HS1-independent 'shutoff switch' for chemotaxis (possibly through myosin II–based crosslinking of actin filaments[15]; **Fig. 1b**). Subsequent reactivation of Cdc42 and Rac through LFA-1 and NKG2D signaling could then facilitate remodeling of the cytoskeleton for stable adhesion to targets and for killing. Such a system could help immune effector cells remodel the microarchitecture of the actin cytoskeleton to properly interpret contradictory signals stimulating continued migration versus halting and tightly coupling to the target cell.

More generally, the use of an immunoblot 'bulk method' to analyze what is certainly a spatiotemporal regulation cannot provide insight into whether separate HS1 populations are used for different functions. Analysis of other residues of HS1, particularly in the Src homology 3 domain, could identify interactions with distinct signaling complexes that are specifically activated to execute chemotactic, adhesion or lymphocyte effector functions. It would be useful to determine whether cells expressing a collection of HS1 proteins with substitutions at either Tyr378 or Tyr397 have lytic synapse defects in response to ICAM-1 and NKG2D. If two types of HS1 molecules participate at different times and places, this combination might fully restore cytotoxicity and chemotaxis, despite the fact that neither mutant molecule alone would have done so. Similarly, in an NK cell undergoing chemoaxis, does an encounter with a target cell require dephosphorylation of Tyr378 to stabilize coupling with the target cell, or does phosphorylation of Tyr397 override signaling initiated through phosphorylation of Tyr378? That is, although Butler and colleagues show that the functions of HS1 in chemotaxis and cytolysis are distinct, are they mutually exclusive?

Certainly, it is important for cells to assemble appropriate cytoskeletal structures for chemotaxis versus adhesion and killing functions, and this process probably requires considerable remodeling before a stable synapse can be established. The generation of distinct signaling pathways could prevent cells from being 'distracted' by faint signals generated by opposing extracellular cues. Regardless of the answers to these additional questions, the work of Butler, Kastendieck and Cooper emphasizes HS1 as a potential 'master switch' with the ability to 'interpret' divergent cues from the extracellular environment.

1. Barber, D.F., Faure, M. & Long, E.O. *J. Immunol.* **173**, 3653–3659 (2004).
2. Perez, O.D. *et al. Nat. Immunol.* **4**, 1083–1092 (2003).
3. Butler, B. *et al. Nat. Immunol.* **9**, 887–897 (2008).
4. Weaver, A.M. *et al. Curr. Biol.* **11**, 370–374 (2001).
5. Gomez, T.S. *et al. Immunity* **24**, 741–752 (2006).
6. Kolanus, W. *et al. Cell* **86**, 233–242 (1996).
7. Crespo, P., Schuebel, K.E., Ostrom, A.A., Gutkind, J.S. & Bustelo, X.R. *Nature* **385**, 169–172 (1997).
8. Stradal, T.E.B. & Scita, G. *Curr. Opin. Cell Biol.* **18**, 4–10 (2006).
9. Zeng, R. *et al. J. Immunol.* **171**, 1360–1368 (2003).
10. Patel, J.C., Hall, A. & Caron, E. *Mol. Biol. Cell* **13**, 1215–1226 (2002).
11. del Pozo, M.A. *et al. J. Immunol.* **170**, 41–47 (2003).
12. Borroto, A. *et al. Eur. J. Immunol.* **30**, 3403–3410 (2000).
13. Van Keymeulen, A. *et al. J. Cell Biol.* **174**, 437–445 (2006).
14. Xu, J. *et al. Cell* **114**, 201–214 (2003).
15. Pestonjamasp, K.N. *et al. Blood* **108**, 2814–2820 (2006).