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Hold on to Your Endothelium: Postarrest Steps of the Leukocyte Adhesion Cascade

In this issue of *Immunity*, two articles provide new evidence for the importance of postadhesion events in neutrophil adhesion strengthening (Zhang et al., 2006) and activation (Hirahashi et al., 2006).

Neutrophils are important for host defense against bacteria, but they also cause severe and sometimes fatal tissue damage in a variety of syndromes caused by ischemia, hypoxia, or noninfectious organ damage. Neutrophils enter most tissues through postcapillary venules, where they attach and roll through selectins and their ligands and then adhere after chemokine- or chemoattractant-mediated activation through integrins. This leukocyte adhesion cascade consisting of rolling and firm adhesion was first demonstrated experimentally in microvascular models and formalized in several influential reviews. Currently, the neutrophil adhesion cascade consists of capture (or tethering), rolling, slow rolling, adhesion, and transmigration (Laudanna and Alon, 2006; Ley et al., 1999). In this issue of *Immunity*, Zhang et al. (2006) suggest that another distinct step should be added (Figure 1): postadhesion, integrin-dependent adhesion strengthening. In an independent study, Hirahashi et al. provide new data that add to our knowledge of adhesion-dependent neutrophil activation (Hirahashi et al., 2006).

Integrins are heterodimeric transmembrane αß heterodimers that have the capability to transduce signals into ("outside-in") and out of ("inside-out") neutrophils. The outside-in signal upon integrin engagement induces cytoskeletal rearrangement and neutrophil responses such as generation of reactive oxygen intermediates, degranulation, cytokine secretion, and ultimately, phagocytosis. This was first recognized when neutrophils from $ß_2$ integrin-deficient patients showed diminished superoxide anion production in response to cytokines (Nathan et al., 1989). The Src-family tyrosine kinase and spleen tyrosin kinase (Syk) are important molecules in the proximal integrin-mediated signaling cascade and have an essential role in the regulation of neutrophil functions. Activation of Syk by integrin ligation requires Src-family kinases; tyrosine phosphorylation of Syk is absent in neutrophils deficient in *Hck*, *Fgr*, and *Lyn*, neutrophil-specific members of the Src kinase family. Hck and Fgr exhibit a concurrent and/or redundant function, whereas the elimination of both kinases leads to a defect in respiratory burst, secondary granule secretion, and spreading after engagement of integrins (Lowell et al., 1996).

Src homology 2 domain-containing leukocyte-specific phosphoprotein of 76 kDa (SLP-76) is an intracellular adaptor molecule that is downstream of Syk kinases and plays a central role in activating signaling intermediates such as the Vav family. The Vav family of guanine nucleotide exchange factors (GEFs) can subsequently activate Rho GTPases (Rho, RhoG, Rac, and Cdc42) by exchanging guanine diphosphate (GDP) for guanine triphosphate (GTP). One linkage between the Rho GTPases and actin regulation is made by members of the Wiskott-Aldrich Syndrome (WAS) protein family. These proteins are characterized by a C-terminal tripartite verprolin homology, central, acidic (VCA) domain that can bind the actin-related protein (Arp 2/3) and thereby influence the initiation of actin polymerization (Burns et al., 2004).

The subject of Zhang et al.'s study is the impaired integrin function of neutrophils in WAS. This is a primary immunodeficiency disease with a high susceptibility for infection due to *WAS* gene mutations that lead to a loss of protein function. Neutrophils from mice with a targeted deletion of the gene encoding Wiskott-Aldrich Syndrome Protein ($Was^{-/-}$) show normal rolling and normal integrin activation and arrest. However, integrin clustering is abnormal, neutrophils show reduced resistance to detachment and abnormal spreading in a flow chamber assay in vitro, and delayed recruitment in vivo (Zhang et al., 2006). Absence of WASp also results in reduced lactoferrin release, a marker of neutrophil degranulation, and decreased tumor necrosis factor-α (TNF-α)-induced superoxide production by neutrophils adherent to fibrinogen or other integrin ligands. These effects are associated with reduced phosphorylation of proline-rich tyrosine kinase (Pyk2), Syk, Cbl, Vav-1, and protein kinase B (PKB) in $Was^{-/-}$ neutrophils. Most of these findings were confirmed in human neutrophils from a WAS patient. Taken together, this paper conclusively demonstrates that WASp is required for adhesion strengthening after arrest and subsequent integrin-dependent activation of other neutrophil functions.

In a second study, Hirahashi et al. (Hirahashi et al., 2006) used an elegant in vivo approach to separate the role of the $ß_2$ integrin, macrophage antigen-1 (Mac-1, also known as $α_M ß_2$ integrin or CD11b-CD18), for neutrophil recruitment versus activation. In the local Shwartzman-like reaction (LSR), which is a complement-dependent inflammatory disease model, Mac-1-deficient mice show neutrophil recruitment similar to that of wild-type littermates, but they show a reduced fibrin and thrombus content and no hemorrhage. This work identifies an important role for Syk in promoting activation of these neutrophil functions by Mac-1 after engagement to complement C3bi. Neutrophils deficient in *Hck* or *Syk* have a phenotype similar to that of neutrophils from Mac-1-deificent mice and exhibit defective elastase release and neutrophil degranulation.

The work by Zhang et al. (Zhang et al., 2006) supports the hypothesis that outside-in signaling via $ß_2$ integrins in neutrophils upon initial arrest leads to adhesion strengthening. This postarrest adhesion may be induced by integrin clustering. Under resting conditions, integrins interact directly with the cytoskeleton through association of their cytoplasmatic tails with actin binding proteins, such as α-actinin, talin, and filamin. These interactions prevent a lateral movement of the integrins. Upon

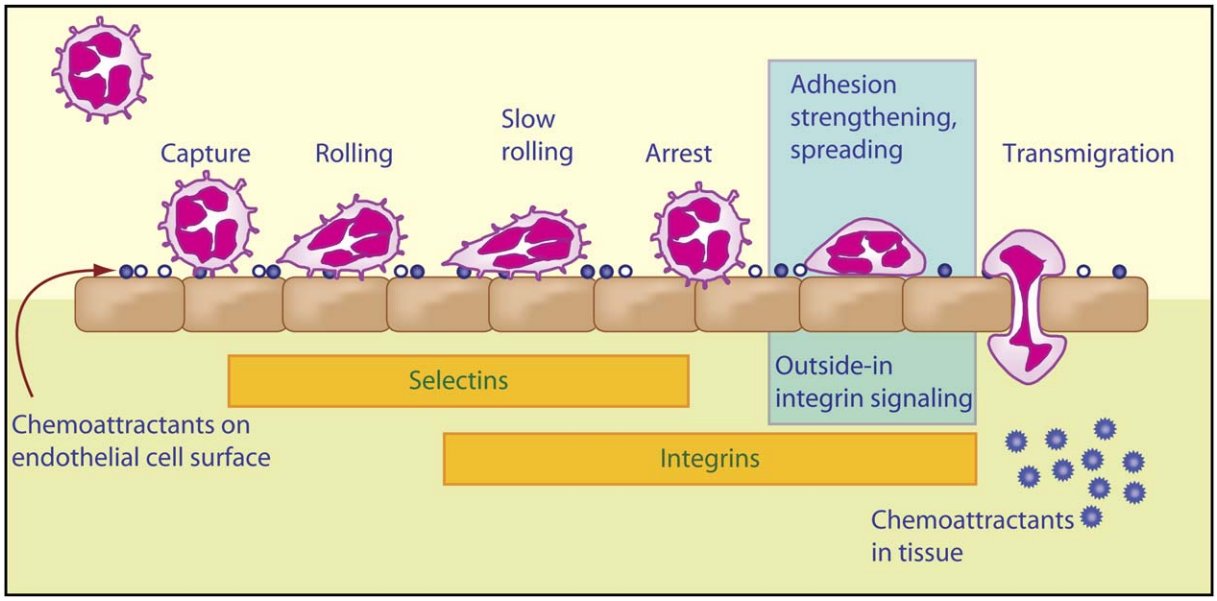

Figure 1. Postarrest Steps in the Leukocyte Adhesion Cascade

Based on new data by Zhang et al. and Hirahashi et al., the adhesion cascade is expanded by a postarrest step (blue box) that involves adhesion strengthening, spreading, and events triggered by outside-in integrin signaling such as degranulation and oxidase assembly. Modified from (Ley et al., 1999).

activation, release from cytoskeletal constraints allows integrin motility, which leads to clustering of these molecules (Kucik et al., 1996). Strengthening of adhesion is maintained through clustering induced by Src-family kinase-dependent signaling from ligand-engaged integrins (Giagulli et al., 2006). The phenotype of the *Was*$^{-/-}$ neutrophils (Zhang et al., 2006) is somewhat reminiscent of *Vav1*$^{-/-}$, *Vav3*$^{-/-}$ neutrophils (Gakidis et al., 2004) or PI3Kγ-deficient neutrophils, both of which adhere normally but then detach rather than remaining adherent. Also, mice lacking the Src kinases Hck and Fgr show much shorter neutrophil adhesion in a static assay (Giagulli et al., 2006). Taken together with the current paper (Zhang et al., 2006), these findings suggest that WASp, Hck, Fgr, Vav-1, Vav-3, and PI3Kγ all are involved in postarrest adhesion strengthening.

The current studies (Hirahashi et al., 2006; Zhang et al., 2006) give important and convincing insights into the downstream signaling of ß$_2$ integrin after engagement to ligands upon adhesion. Src-family kinases, mainly Hck and Syk, mediate signaling upon Mac-1 engagement in myeloid cells. The different Src kinases have partially redundant roles in vivo, confirming earlier in vitro data (Mocsai et al., 2002). The other important finding is the role of WASp in adhesion strengthening. WASp, localized at the leading edge, and the ß$_2$ integrins Mac-1 and lymphocyte-function-associated antigen-1 (LFA-1), localized in the mid regions and the uropod, distribute to different parts of adherent neutrophils under shear flow. This suggests that the effect of WASp on integrin signaling is indirect. One possible explanation is that WASp may regulate cytoskeleton assembly and disassembly, which are required for integrin clustering and subsequent adhesion strengthening. According to this interpretation, Syk is upstream in the signaling pathway, i.e., near the integrin, whereas WASp is further downstream, i.e., engaged with the actin cytoskeleton. However, both are clearly required for postadhesion events in neutrophils. The hierarchical and possibly parallel relationships between these signaling pathways remain to be determined. Because neither Src-family nor Syk kinases are involved in neutrophil migration (Mocsai et al., 2002), further studies are necessary to elucidate the integrin signaling pathways responsible for neutrophil locomotion after they leave the vascular system.

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Selected Reading

Burns, S., Cory, G.O., Vainchenker, W., and Thrasher, A.J. (2004). Blood *104*, 3454–3462.

Gakidis, M.A., Cullere, X., Olson, T., Wilsbacher, J.L., Zhang, B., Moores, S.L., Ley, K., Swat, W., Mayadas, T., and Brugge, J.S. (2004). J. Cell Biol. *166*, 273–282.

Giagulli, C., Ottoboni, L., Caveggion, E., Rossi, B., Lowell, C., Constantin, G., Laudanna, C., and Berton, G. (2006). J. Immunol. *177*, 604–611.

Hirahashi, J., Mekala, D., van Ziffle, J., Xiao, L., Saffaripour, S., Wagner, D.D., Shapiro, S.D., Lowell, C.A., and Mayadas, T.N. (2006). Immunity *25*, this issue, 271–283.

Kucik, D.F., Dustin, M.L., Miller, J.M., and Brown, E.J. (1996). J. Clin. Invest. *97*, 2139–2144.

Laudanna, C., and Alon, R. (2006). Thromb. Haemost. *95*, 5–11.

Ley, K., Brewer, K., and Moton, A. (1999). Microcirculation *6*, 259–265.

Lowell, C.A., Fumagalli, L., and Berton, G. (1996). J. Cell Biol. *133*, 895–910.

Mocsai, A., Zhou, M., Meng, F., Tybulewicz, V.L., and Lowell, C.A. (2002). Immunity *16*, 547–558.

Nathan, C., Srimal, S., Farber, C., Sanchez, E., Kabbash, L., Asch, A., Gailit, J., and Wright, S.D. (1989). J. Cell Biol. *109*, 1341–1349.

Zhang, H., Schaff, U.Y., Green, C.E., Chen, H., Sarantos, M.R., Hu, Y., Wara, D., Simon, S.I., and Lowell, C.A. (2006). Immunity *25*, this issue, 285–295.

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T Helper 2 Cells' Preferred Way to Die

In this issue of *Immunity*, Devadas and colleagues (2006) reveal that granzyme B, long known as a mediator of CD8$^+$ T cell cytotoxicity, has a new role in an internal homeostatic death mechanism that controls the fate of CD4$^+$ T helper 2 cells.

Soon after it was recognized that T lymphocytes have cytotoxic, i.e., cell killing, activity, two forms of cytoxicity were distinguished by whether or not they required calcium (Kägi et al., 1996). The calcium-dependent mechanism involves the release of granules containing perforin and death-inducing serine proteases called granzymes. Calcium-independent death occurs by a completely different mechanism involving an inducible cell-surface protein, called Fas ligand, that is related to tumor necrosis factor (TNF) and specifically binds the Fas receptor (also known as CD95 or APO-1). Thus, there are two distinct killing mechanisms, and both mechanisms play a crucial role in immunity by enabling CD8$^+$ cytolytic T cells or natural killer (NK) cells to destroy virally infected or tumorigenic target cells. These cytolytic mechanisms may also be involved in damaging effects of T cell responses such as in autoimmune disease and graft rejection (Kägi et al., 1996).

However, cell death has another vital role in immunity. T lymphocyte numbers and activity are regulated throughout the course of immune responses. After antigenic stimulation, T cells have a burst of proliferation, which greatly increases the number of specific effector cells to facilitate a strong protective immune response. However, cell numbers must be tightly controlled to avoid the negative consequences of vast numbers of activated T cells, such as autoimmune reactions. Therefore, T lymphocytes have evolved means to downregulate their own numbers by propriocidal or "self-killing" mechanisms that appear to be hard-wired into the biology of these cells (Lenardo et al., 1999). Soon after their discovery, Fas and its ligand were quickly appreciated to have a dual role in both cytotoxic functions and autoregulatory cell death. For example, genetic abnormalities of Fas and its ligand lead to distorted lymphocyte homeostasis, leading to the autoimmune lymphoproliferative syndrome (ALPS) (Lenardo et al., 1999). Nevertheless, the molecular nature of autoregulatory death in different T cell subsets were never fully worked out, and this is where the paper by Devadas et al. and a related work (Sharma et al., 2006) make an important contribution to our understanding of immune regulation.

In the simplest paradigm, naive CD4$^+$ T helper cells (Th0 cells) can respond to specific conditions of Ag stimulation by differentiating into specialized Th1 or Th2 offspring that promote different types of immune responses (see London et al., 1998 and Farrar et al., 2002 for a full delineation of their molecular and functional differences). Interestingly, although both Th1 and Th2 cells undergo autoregulatory cell death, Th1 cells are very sensitive to killing through Fas but Th2 cells are relatively insensitive (Oberg et al., 1997). This puzzling observation has lingered for nearly a decade without a good explanation for how Th2 cells control themselves. Now, Devadas et al. (2006) address this conundrum by demonstrating that granzyme B (GrB), previously implicated in CD8$^+$ T cell and NK cytotoxicity, is crucial for autoregulatory cell death in Th2 cells. Thus, evolution appears to have redeployed the same two cytolytic mechanisms used in immune defense to autoregulate the different subsets of cells by promoting their own suicide under appropriate conditions.

Devadas et al. make a clear case for the importance of GrB in Th2 cell death (Figure 1). They find that the GrB-specific inhibitor zAAD-cmk and a cathepsin C inhibitor (which prevents the processing of GrB) efficiently protect Th2 cells from death, whereas caspase inhibitors have no effect. In contrast, autoregulatory death of Th1 cells can only be blocked by caspase inhibitors, which inhibit key proteolytic events in apoptosis. Devadas et al. provide further insights into the regulation of GrB, which is normally expressed at low amounts in naive CD4$^+$ T cells. They show that T cell receptor (TCR) stimulation upregulates GrB in both Th1 and Th2 cells; however, it is differentially controlled in the two subsets. In Th1 cells, the induced GrB remains sequestered in the lysosomes during cell-death induction as indicated by colocalization with the lysosome-associated membrane protein LAMP-1. In contrast, TCR engagement in Th2 cells leads to the internal release of GrB from cellular granules into the cytosol and nucleus with toxic consequences for the cell. Release of cytotoxic granules is ordinarily vectorial and external when a cytolytic T cell encounters a target cell. The killing proteins are released toward the target cell, and the T cell itself is spared. However, the direct GrB release into the cytoplasm ensures the suicidal demise of Th2 cells. The authors also show that differentially expressed protein inhibitors guide the helper T cells into the two distinct pathways.