Vukmanović, S., Hogquist, K.A., and Jameson, S.C. (2002). Immunity *17*, 131–142.

Schmitt, T.M., Ragnarsson, G.B., and Greenberg, P.D. (2009). Hum. Gene Ther., in press. Published online October 1, 2009. 10.1089/hum.2009.146.

Tarakhovsky, A., Kanner, S.B., Hombach, J., Ledbetter, J.A., Müller, W., Killeen, N., and Rajewsky, K. (1995). Science *269*, 535–537.

Wong, P., Barton, G.M., Forbush, K.A., and Rudensky, A.Y. (2001). J. Exp. Med. *193*, 1179–1187.

Wucherpfennig, K.W., and Gagnon, E. (2009). Nat. Immunol. *10*, 1143–1144.

Yachi, P.P., Ampudia, J., Gascoigne, N.R., and Zal, T. (2005). Nat. Immunol. *6*, 785–792.

Yin, Y., and Mariuzza, R.A. (2009). Immunity *31*, 849–851.

A Deadly Migration

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CD95 has long been viewed as a death receptor regulating apoptosis. In this issue of *Immunity*, Letellier et al. (2010) tell us a different story, about the capability of CD95L to regulate leukocyte recruitment to sites of inflammation.

It is well established that the signaling pair CD95L-CD95, also known as FASL-FAS, signals to apoptosis in a variety of cellular contexts. This paradigm has been recently challenged by data showing that CD95 may fulfill a range of physiological nonapoptotic functions, including a role as a proinflammatory mediator. In this issue of *Immunity*, Letellier et al. (2010) provide compelling evidence of the capability of CD95 to trigger signaling events leading to leukocyte integrin-dependent adhesion and migration and expand our perception of the role of CD95 in nonapoptotic functions.

CD95 is a prototypical member of the tumor necrosis factor receptor superfamily containing an intracellular death domain in its cytoplasmic tail, which transmits a death signal to the cell (Strasser et al., 2009). CD95 exists as a homotrimer and is activated through binding to trimers of its ligand CD95L (Siegel et al., 2000). In the extrinsic (or death-receptor-regulated) pathway, engagement of the death receptor CD95 by CD95 ligand (CD95L) leads to the activation of caspase 8 by the adaptor protein FADD (FAS-associated death domain) and the formation of the death-inducing signaling complex (DISC) through homologous domain interactions (Bouillet and O'Reilly, 2009). Active caspase 8 then cleaves and activates caspase 3, caspase 6, and caspase 7, which targets vital cellular substrates, thus inducing cell death (Bouillet and O'Reilly, 2009). The expression of CD95L has to be carefully regulated to prevent unintentional killing of healthy cells because CD95 is widely expressed and ligation of CD95 by CD95L potently triggers apoptosis in many cell types (Bouillet and O'Reilly, 2009).

It is largely accepted that several members of the TNF-R family, including some that are classified as death receptors, and their corresponding ligands exert non-apoptotic functions, such as the induction of cellular activation, proliferation, differentiation, or migration. By applying an articulated experimental approach involving both animal models and human subjects, Letellier et al. (2010) investigated the involvement of CD95L as a proinflammatory determinant in spinal cord injury (SCI). The authors showed that injury to the central nervous system (CNS) increases CD95L surface expression on peripheral blood cells in rodents and humans, thus establishing a potential source for CD95L leading to CNS damage. Specifically, neutrophils and macrophages were found to be the major source of CD95L leading to CNS damage. Importantly, neutralization experiments and gene expression analysis showed that CNS damage is dependent on CD95L-mediated proinflammatory activity and not to direct CD95-induced apoptosis of resident neural spinal cells, bringing strong evidence for the role of CD95 in nonapoptotic events. In particular, the authors suggested that myeloid cells infiltrate the lesion site in a CD95L-dependent manner and produce proinflammatory cytokines upon interaction with other immune cells or with CNS-resident cells, leading to the amplification of the immune response. The authors further characterized the activity of CD95L-CD95 on leukocyte function and find a role in regulation of integrin-dependent adhesion and migration. CD95L also regulated cell recruitment in a model of peritonitis, suggesting that its involvement in leukocyte trafficking is not restricted to the CNS. Interestingly, the study by Letellier et al. (2010) suggests that the interaction between leukocyte-expressed CD95 and CD95L, leading to enhanced migration and subsequent tissue destruction, occurs in the periphery before the migration of myeloid cells in the target tissue. It is tempting to speculate that pharmacological modulation of CD95L-CD95 interaction may represent a potential therapeutic approach in some inflammatory diseases.

The study by Letellier et al. (2010) expands our view of death receptors and definitively brings CDC95L-CD95 to the realm of cell trafficking and inflammation. This study also raises a number of

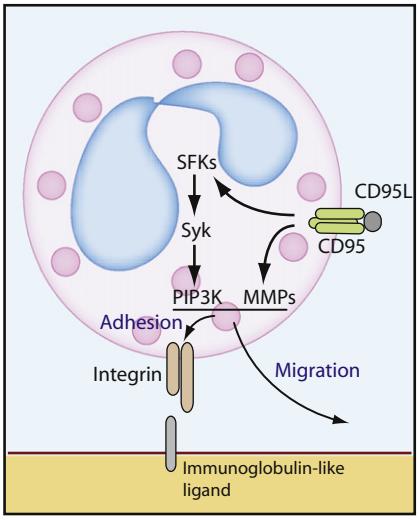

Figure 1. CD95-Triggered Nonapoptotic Signaling Pathway Leading to Adhesion Activation and Migration in Myeloid Cells
Classically, CD95 activates a proapoptotic signaling pathway leading to cell death. The findings by Letellier et al. show that CD95 expressed on myeloid cells (here represented by a polymorphonuclear cell) may trigger a different signaling pathway involving Src-family tyrosine kinases (SFKs), Syk, PIP3K, and MMPs, leading to myeloid cell adhesion to endothelial ligands belonging to the immunoglobulin-like family, such as ICAM-1 or VCAM-1, and recruitment to site of inflammation.

interesting questions for future investigation. For instance, it is not clear how peripheral myeloid cells can "sense" distal tissue damage and upregulate CD95L. Moreover, in the paper by Letellier et al. (2010), a consistent body of results was obtained with soluble CD95L, suggesting that soluble CD95L, by interacting with leukocyte-expressed CD95, is responsible for myeloid cell activation in periphery and enhanced migration to the inflammatory site. However, further studies need to define whether CD95L acts as a real chemoattractant, possibly generating itself a gradient in an autocrine or paracrine fashion, or as a primer for a subsequent chemotactic agonist generated within the damaged tissue. Notably, till recently, although Fas-induced apoptosis was known to require aggregation of preassembled CD95 trimers (Siegel et al., 2000), it was unclear whether membrane-bound CD95L, soluble CD95L, or both cause cell apoptosis. In addition, it was also debated whether either or both of these forms of CD95L have nonapoptotic activities, such as induction of inflammatory responses. Recent work by O'Reilly et al. (2009) sheds light on these uncertainties by demonstrating that membrane-bound CD95L is essential for cytotoxic activity and constitutes the guardian against lymphadenopathy, autoimmunity, and cancer, whereas increased soluble FasL has nonapoptotic proinflammatory effects and appears to promote autoimmunity and tumorigenesis (O'Reilly et al., 2009). Taking into account these recent findings, the data by Letellier et al. (2010) published in this issue suggest that the soluble form of CD95L may represent an agonist generating intracellular signals leading to myeloid cell migration. The emerging picture suggests a role for soluble CD95L in leukocyte migration, as well as in MAP kinase and NF-kB pathway activation, with consequent stimulation of cell proliferation, survival, and cytokine production. However, future studies are required to better characterize the role of soluble versus membrane-bound CD95L during immune responses.

Signal transduction pathways generated by CD95 and leading to apoptosis or nonapoptotic events have been investigated in the last years. From previous studies, performed in vitro and in vivo, FADD, caspase-8, and c-FLIP (components of DISC) are known to link CD95 to nonapoptotic pathways (Peter et al., 2007). Nevertheless, how FADD and caspase-8 are activated during CD95-induced nonapoptotic events and what intracellular nonapoptotic signaling pathways are triggered by caspase-8 are currently unresolved questions (Strasser et al., 2009). The paper by Letellier et al. (2010) investigated the signaling mechanisms triggered by CD95L leading to myeloid cell migration. The authors extended their previous observations showing that CD95L triggers invasion of glioblastoma cells and differentiation of adult neural stem cells via activation of Src tyrosine kinases and PI3K (phosphatidylinositol-3-kinase). They further showed that stimulation of CD95 with soluble CD95L on myeloid cells contributes to migration by triggering Syk tyrosine kinase activity. CD95L-dependent Syk phosphorylation seems to be triggered by Lyn, a member of Src tyrosine kinase family. Moreover, the authors showed that Syk phosphorylation leads to the activation of PI3K, a molecule that has been shown to link G-coupled protein receptors (GPCR) to integrin-dependent adhesion (Constantin et al., 2000). Thus, CD95 death receptor activates migration through a signaling cascade involving Src, Syk, and PI3K and, as previously shown for glioblastoma cells, includes activation of metalloproteinase (MMP)-2 and -9 as well (Letellier et al., 2010) (Figure 1).

The signaling mechanisms described in the paper by Letellier et al. (2010) raise questions about integrin triggering and adhesion. In recent years, the signaling events leading to integrin activation have been the focus of intense investigation. It is now well established that activation of integrin-dependent adhesion should not be considered as just a single phenomenon, but more likely as a group of highly coordinated cellular events globally leading to leukocyte recruitment to the blood vessels (Ley et al., 2007). These events are finely tuned by specific intracellular signaling mechanisms triggered by chemoattractants, each one devoted to controlling specific aspects of the adhesion process (Constantin et al., 2000; Laudanna and Alon, 2006). The most characterized pro-adhesive signaling mechanisms regard the function of Ras and Rho small-GTP binding proteins, namely Rap1 and the recently discovered Rho-module (Bolomini-Vittori et al., 2009). Overall, it appears that integrin activation is controlled by modular signaling networks controlling the various aspects of integrin activation, including constraint removal, affinity, clustering, and postbinding stabilization. In this context, the role of protein tyrosine kinases is still not well defined. Thus, the data presented in this issue by Letellier et al. (2010) clearly highlight the possibility of an involvement of Syk in signaling triggered by proadhesive agonists and possibly leading to Rap1 and rho-small GTPase activation, thus controlling integrin triggering. This relevant aspect needs to be investigated in future studies. Furthermore, it will be interesting to characterize whether CD95L may also act as a direct agonist of integrin affinity triggering, thus suggesting its role in cell arrest under flow, a condition that was not explored by Letellier and colleagues.

Overall, the study by Letellier and colleagues, by linking CD95 (FAS) death receptor to leukocyte trafficking, further emphasizes the relevance of nonapoptotic functions of CD95 and characterizes the signaling machinery controlling CD95 involvement in nonapoptotic events.

These studies potentially provide new targets to treat diseases, such as inflammation and cancer, for which nonapoptotic activities of CD95 are proving important (Peter et al., 2007).

REFERENCES

Bolomini-Vittori, M., Montresor, A., Giagulli, C., Staunton, D., Rossi, B., Martinello, M., Constantin, G., and Laudanna, C. (2009). Nat. Immunol. *10*, 185–194.

Bouillet, P., and O'Reilly, L.A. (2009). Nat. Rev. Immunol. *9*, 514–519.

Constantin, G., Majeed, M., Giagulli, C., Piccio, L., Kim, J.Y., Butcher, E.C., and Laudanna, C. (2000). Immunity *13*, 759–769.

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

Letellier, E., Kumar, S., Sancho-Martinez, I., Krauth, S., Funke-Kaiser, A., Laudenklos, S., Konecki, K., Klussmann, S., Corsini, N.S., Kleber, S., et al. (2010). Immunity *32*, this issue, 240–252.

Ley, K., Laudanna, C., Cybulsky, M.I., and Nourshargh, S. (2007). Nat. Rev. Immunol. *7*, 678–689.

O'Reilly, L.A., Tai, L., Lee, L., Kruse, E.A., Grabow, S., Fairlie, W.D., Haynes, N.M., Tarlinton, D.M., Zhang, J.G., Belz, G.T., et al. (2009). Nature *461*, 659–663.

Peter, M.E., Budd, R.C., Desbarats, J., Hedrick, S.M., Hueber, A.O., Newell, M.K., Owen, L.B., Pope, R.M., Tschopp, J., Wajant, H., et al. (2007). Cell *129*, 447–450.

Siegel, R.M., Frederiksen, J.K., Zacharias, D.A., Chan, F.K., Johnson, M., Lynch, D., Tsien, R.Y., and Lenardo, M.J. (2000). Science *288*, 2354–2357.

Strasser, A., Jost, P.J., and Nagata, S. (2009). Immunity *30*, 180–192.

Dendritic Cell Subsets Digested: RNA Sensing Makes the Difference!

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In this issue of *Immunity*, Luber et al. (2010) report a comprehensive quantitative proteome of in vivo mouse spleen dendritic cell (DC) subsets: a data set of encyclopedic value already revealing that DC subsets exploit different RNA sensors for virus recognition.

Denditic cells (DCs) are key players in the immune response against invading pathogens and provide a direct link between inate and adaptive immune responses. As antigen-presenting cells, DCs are able to recognize, take up, and process pathogens, dying cells, and malignant cells into antigenic fragments that are subsequently presented on major histocompatibility complexes to T cells to initiate adaptive immune responses.

In recent years it has become clear that DCs do not represent one homogeneous population of antigen-presenting cells but rather form a plethora of distinct subtypes, defined by specific cell-surface markers. For some subsets, specialized immune functions have been recognized (Shortman et al., 2009). For example in mice, $CD8\alpha^+$ spleen DCs were recognized as most efficient in cross-presentation of antigens derived from dying cells in MHC class I, a function less well performed by other $CD8\alpha^-$ DC spleen subsets. Although $CD8\alpha$ is not expressed by human DCs, $BDCA3^+$ blood DCs might represent the human counterpart. Furthermore, plasmacytoid DCs, a subset present both in mice and men, are known to master the initiation of antiviral immune responses by secretion of large amounts of type I interferons. However, in spite of all efforts to elucidate the nature of these phenotypically defined subsets, functional differences as well as the relation between subsets in the human and the murine system are still incompletely resolved.

Murine splenic DCs are certainly the most widely studied cells in DC biology. At steady state, two major DC subsets can be distinguished: conventional DCs (cDCs) and plasmacytoid DCs (pDCs). Spleen cDCs can be further subdivided into $CD8\alpha^+CD4^-$ DCs, $CD8\alpha^-CD4^+$ DCs, and $CD8\alpha^-$ $CD4^-$ double negative (DN) DCs. Thus far, mainly comparative microarray studies have been performed in attempts to elucidate functional specializations in these splenic subsets (Robbins et al., 2008). RNA expression, however, does not necessarily predict protein abundance. Transcripts can be translational nonactive, RNA and protein may differ in stability, or protein expression can be controlled by posttranslational modifications rather than at a transcriptional level.

In this issue of *Immunity*, Luber et al. (2010) use their long-standing expertise in proteomics to gain more insight in the differences that exist between these mouse spleen DC subsets. This was not an easy task given that the quantitative comparison of such rare cell populations in vivo by proteomics was until now greatly hampered by the lack of technology and the large costs involved, especially when taking into account the number of biological replicates required for statistical significance. Novel algorithms were developed for label-free