Slit: A Roadblock for Chemotaxis

Aaron Z. Fernandis and Ramesh K. Ganju

(Published 17 July 2001)

A critical component of host immune surveillance is immune cell trafficking. The directional cue to circulating immune cells is provided by an entire superfamily of chemoattractants, cytokines (also known as chemokines) and their receptors (*1*, *2*). Chemokines are highly basic proteins, a feature they share with netrins, which are involved in neuronal movement. Perturbances in chemokine-mediated cell migration are at the core of various pathological states, including tumor metastasis and inflammatory and autoimmune diseases (*1*-*5*).

Although chemokine-induced chemotaxis is critical for immune development, the molecular mechanisms guiding this process have remained unclear (*6*, *7*). There are various "off" and "on" molecular switches controlling immune cell traffic that include extracellular matrix ligands, adhesion receptors, cytoskeletal proteins, and chemoattractants and their receptors (*1*-*4*, *6*, *7*). Recently, another regulatory molecule that modulates leukocyte trafficking was described by Wu *et al*. (*8*). This report provided the first experimental evidence showing that Slit proteins, which guide axonal movement, also modulate chemokine-induced leukocyte migration. These results suggest that there may be similarities in the guidance mechanisms regulating immune and neuronal cell development and movement. Furthermore, this knowledge opens new avenues for manipulation of immune responses and for development of therapies against diseases mediated by chemokines and their receptors, such as inflammatory and autoimmune disorders and infection by human immunodeficiency virus (HIV) (*1*-*5*).

Slit is an ~200 kD secretory protein originally shown to be expressed by neurons and glial cells. This protein, which has been cloned from different model systems, consists of a family of three genes, Slit 1, Slit 2, and Slit 3. Slit proteins contain four leucine rich repeats (LRRs), seven epidermal growth factor

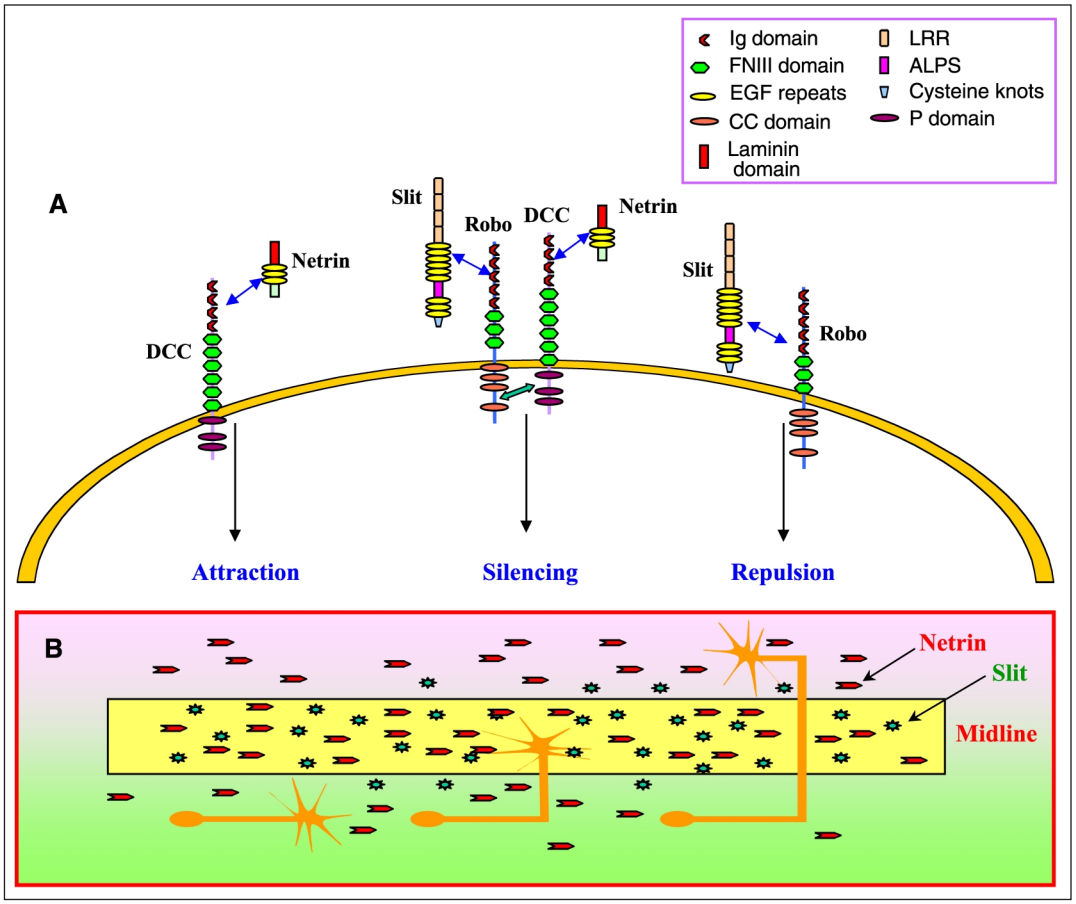

Fig. 1. A schematic representation of the structural (**A**) and functional features of the Slit/Robo and netrin/DCC complexes. Slit/Robo and netrin/DCC regulate the crossing of axons across the midline (**B**). Netrin is an attractant through its receptor, DCC. Slit functions as a silencer of netrin by activating the Robo receptor, which interacts with DCC, thereby inhibiting the effect of the attractant netrin. Upon binding to Robo, Slit also induces axonal repulsion. The legend indicates the various structural domains present in these proteins. CC domains are conserved domains in the Robo receptor cytoplasmic domain; P domains are conserved domains in the DCC receptors.

The authors are at the Division of Experimental Medicine, Beth Israel Deaconess Medical Center, Harvard Institutes of Medicine, 4 Blackfan Circle, Boston, MA 02115, USA. E-mail: rganju@caregroup.harvard.edu

(EGF) repeats, an Agrin-Laminin-Perlecan-Slit spacer (ALPS) domain, and a cysteine knot (Fig. 1A) (*9*, *10*). The molecular target for Slit is a repulsive guidance transmembrane receptor known as the roundabout (Robo) receptor. Robo defines a novel subfamily of immunoglobulin (Ig) superfamily proteins and is highly conserved from fruit flies to mammals. Robo receptors have five Ig repeats and three fibronectin type III (FNIII) repeats in the ectodomain (Fig. 1A) (*10*, *11*). Binding studies have revealed that the NH_2-terminal fragment of Slit, but not the COOH-terminal domain, binds the ectodomain of Robo and is responsible for axon branching and migration (*12*). Furthermore, the LRRs, but not the EGF repeats, of Slit are necessary for binding to Robo (*13*) (Fig. 1A).

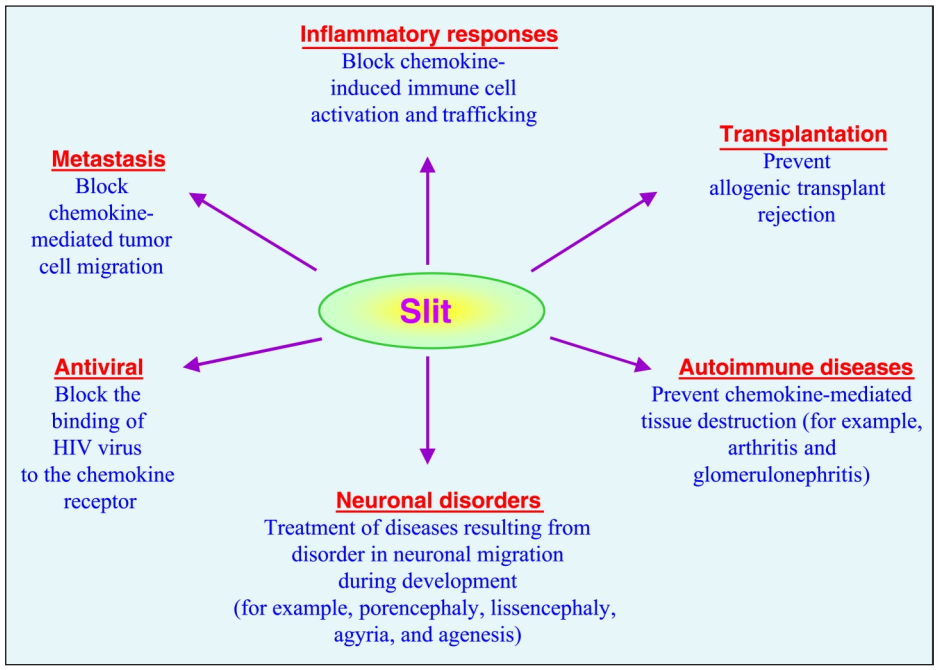

Fig. 2. Potential therapeutic targets of Slit.

In the nervous system, the Slit/Robo receptor complex regulates the growth of axons and their projection to appropriate regions of the brain, and also functions as a repellent preventing axons from crossing non-target areas (*14*). Neuronal migration was realized as early as 1888, but the mechanisms of migration have only recently been deciphered in spinal axons from *Xenopus* embryos (*15*) (Fig. 1). The movement of axons is attracted to the midline by neuronal guidance cues provided by netrins, which mediate attraction by binding to the receptors of the deleted colorectal cancer (DCC) family of guidance receptors (*15*, *16*). The midline is defined by a special group of distinctive cells, which bifurcates the two symmetric halves of the developing central nervous system. Commissural axons cross the midline and project alongside of it, never recrossing. As axons reach the midline, Slit silences the attraction by netrin. Direct physical interaction of the cytoplasmic domain of the Slit receptor, Robo, with the netrin receptor, DCC, mediates the silencing effect of Slit on netrin. Axons do not initially express the Robo receptor on their surfaces. However, upon crossing the midline, Robo proteins are upregulated. In addition to its silencing effect, the Robo receptor, upon binding to Slit, repels axons away from the midline, thus ensuring that they continue on toward their final destination (Fig. 1B). The positioning of the axon depends on the type of Robo receptor expressed. The axons expressing the Robo 1 receptor stay closer to the midline, as compared to axons expressing Robo 2 and Robo 3, which move away from the midline (*17*-*19*).

Slit is a multifunctional signaling molecule in the nervous system: The commissural axons perceive Slit first as a silencer and then as a repellent and later perhaps as a branching and elongation factor. Recently, the role of Slit was extended to the immune system (*8*). Wu *et al*. reported that Slit could act as an inhibitor of chemokine-mediated chemotaxis. Although leukocytes migrate at a much faster rate than do neurons, their studies suggest similarities in the mechanisms regulating both immune and neuronal cell development and movement.

Further, the authors analyzed the presence of the Slit protein and its receptor, Robo, in different tissues, and showed that Slit 1 was specifically expressed in brain, whereas Slit 2 and Slit 3 were expressed in brain as well as kidney, lung, heart, spleen, and lymph nodes. Robo was detected in lymph nodes, thymus, and in neutrophils differentiated from HL-60 cells.

The presence of the Slit protein and its receptor Robo in organs that contribute to immune response suggests that Slit may be involved in the regulation of immune cell chemotaxis. In fact, Slit 2 can inhibit leukocyte chemotaxis mediated by α chemokine, stromal cell derived factor-1α (SDF-1α), and the N-formyl-peptide f-Met-Leu-Phe (fMLP) (*8*). The effects of Slit were mediated by its receptor Robo, because a fragment that contains only the extracellular part of the Robo protein (Robo N) could abolish the inhibitory effects of Slit. Functional interaction between Robo and the cognate receptor of SDF-1α, CXCR4, was sufficient to mediate Slit inhibitory activities. Inhibition of migration was not due to a toxic effect or inhibition of other functions of leukocytes, because no effect was observed on signaling pathways not related to chemotaxis mediated by chemokines.

These findings add a new dimension to our understanding of chemokine-mediated chemotaxis and have opened a new era of potential specific therapeutic interventions for autoimmune, inflammatory, and other diseases (Fig. 2). For example, the chemokine receptors CXCR4 and CCR5 act as coreceptors for HIV (*20*-*22*). Thus, one of the potential targets of Slit could be inhibition of HIV interaction with CXCR4 and CCR5 and a resultant prevention of HIV infection. The work of Wu *et al*. shows that Slit can inhibit SDF-1α induced, CXCR4-mediated chemotaxis (*8*). Therefore, Slit may also be a prime candidate to inhibit breast cancer metastasis, because CXCR4 was recently shown to play a significant role in this process as well (*5*).

In the nervous system, the inhibitory effect on axonal migration induced by Slit is due to the direct interaction between the cytoplasmic domain of the Slit receptor, Robo, and the netrin

receptor, DCC, which has a single transmembrane domain (*23*). However, in the immune system, the Slit/Robo complex inhibits G protein-coupled seven transmembrane chemokine receptor-mediated migration. Therefore, two important questions for the future are, first, to determine how the Slit/Robo complex interacts with chemokine receptors, and second, to characterize signaling events that lead to inhibition of chemotaxis.

References

1. B. J. Rollins, Chemokines, *Blood* **90**, 909-928 (1997).
2. J. G. Cyster, Chemokines and cell migration in secondary lymphoid organs. *Science* **286**, 2098-2102 (1999).
3. B. A. Premack, T. J. Schall, Chemokine receptors: gateway to inflammation and infection. *Nature Med.* **2**, 1174-1178 (1996).
4. T. J. Schall, K. B. Bacon, Chemokines, leukocyte trafficking, and inflammation. *Curr. Opin. Immunol.* **6**, 865-873 (1994).
5. A. Muller, B. Homey, H. Soto, N. Ge, D. Catron, M. E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S. N. Wagner, J. L. Barrera, A. Mohar, E. Verastegui, A. Zlotnik, Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50-56 (2001).
6. R. P. Cherla, R. K. Ganju, Stromal cell-derived factor 1α induced chemotaxis in T cells is mediated by nitric oxide signaling pathways. *J. Immunol.* **166**, 3067-3074 (2001).
7. R. K. Ganju, S. A. Brubaker, J. Meyer, P. Dutt, Y. Yang, S. Qin, W. Newman, J. E. Groopman, The α chemokine, stromal cell derived factor-1α, binds to the transmembrane G-protein-coupled CXCR-4 receptor and activates multiple signal transduction pathways. *J. Biol. Chem.* **273**, 23169-23175 (1998).
8. J. Y. Wu, L. Feng, H. T. Park, N. Havlioglu, L. Wen, H. Tang, K. B. Bacon, Z. Jiang, X. Zhang, Y. Rao, The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors. *Nature* **410**, 948-952 (2001).
9. K. Brose, M. Tessier-Lavigne, Slit proteins: key regulators of axon guidance, axonal branching, and cell migration. *Curr. Opin. Neurobiol.* **10**, 95-102 (2000).
10. A. Chisholm, M. Tessier-Lavigne, Conservation and divergence of axon guidance mechanisms. *Curr. Opin. Neurobiol.* **9**, 603-615 (1999).
11. T. Kidd, K. Brose, K. J. Mitchell, R. D. Fetter, M. Tessier-Lavigne, C. S. Goodman, G. Tear, Roundabout controls axon crossing of the CNS midline and defines a novel subfamily of evolutionarily conserved guidance receptors. *Cell* **92**, 205-215 (1998).
12. J. H. Chen, L. Wen, S. Dupuis, J. Y. Wu, Y. Rao, The N-terminal leucine-rich regions in Slit are sufficient to repel olfactory bulb axons and subventricular zone neurons. *J. Neurosci.* **21**, 1548-1556 (2001).
13. R. Battye, A. Stevens, R. L. Perry, J. R. Jacobs, Repellent signaling by slit requires the leucine-rich repeats. *J. Neurosci.* **21**, 4290-4298 (2001).
14. W. Wu, K. Wong, J. Chen, Z. Jiang, S. Dupuis, J. Y. Wu, Y. Rao, Directional guidance of neuronal migration in the olfactory system by the protein Slit. *Nature* **400**, 331-336 (1999).
15. E. Stein, M. Tessier-Lavigne, Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex. *Science* **291**, 1928-1938 (2001).
16. F. J. Livesey, Netrins and netrin receptors. *Cell. Mol. Life Sci.* **56**, 62-68 (1999).
17. K. Zinn, Q. Sun, Slit branches out: a secreted protein mediates both attractive and repulsive axon guidance. *Cell* **97**, 1-4 (1999).
18. R. J. Giger, A. L. Kolodkin, Silencing the siren: guidance cue hierarchies at the CNS midline. *Cell* **105**, 1-4 (2001).
19. J. H. Simpson, K. S. Bland, R. D. Fetter, C. S. Goodman, Short-range and long-range guidance by Slit and its Robo receptors: a combinatorial code of Robo receptors controls lateral position. *Cell* **103**, 1019-1032 (2000).
20. C. C. Bleul, M. Farzan, H. Choe, C. Parolin, I. Clark-Lewis, J. Sodroski, T. A. Springer, The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* **382**, 829-833 (1996).
21. H. Deng, R. Liu, W. Ellmeier, S. Choe, D. Unutmaz, M. Burkhart, P. Di Marzio, S. Marmon, R. E. Sutton, C. M. Hill, C. B. Davis, S. C. Peiper, T. J. Schall, D. R. Littman, N. R. Landau, Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661-666 (1996).
22. H. Choe, M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, J. Sodroski, The β-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135-1148 (1996).
23. G. J. Bashaw, C. S. Goodman, Chimeric axon guidance receptors: the cytoplasmic domains of slit and netrin receptors specify attraction versus repulsion. *Cell* **97**, 917-926 (1999).