# Immune semaphorins: a new area of semaphorin research

**Atsushi Kumanogoh and Hitoshi Kikutani***

Department of Molecular Immunology, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan

*Author for correspondence (e-mail: kikutani@ragtime.biken.osaka-u.ac.jp)

*Journal of Cell Science 116, 3463-3470 © 2003 The Company of Biologists Ltd*
*doi:10.1242/jcs.00674*

## Summary

**The semaphorin family comprises soluble and membrane-bound proteins originally identified as axonal guidance cues that function during neuronal development. Emerging evidence suggests that a subset of semaphorins, called 'immune semaphorins', function in the immune system. The class IV semaphorins Sema4D/CD100 and Sema4A use CD72 and Tim-2, respectively, as receptors during immune responses; these receptors comprise a set distinct from those used by semaphorins in the nervous system. Sema4D/CD100, which is expressed constitutively by T cells, is involved in the activation of B cells and dendritic cells, whereas Sema4A is preferentially expressed on B cells and dendritic cells, and is involved in the activation of T cells. Additionally, increasing evidence suggests that some other semaphorins, including viral-encoded semaphorins, might also play important roles in the immune system.**

Key words: Sema4D/CD100, Sema4A, CD72, Tim-2, Plexin

## Introduction

More than 30 members, identified from viruses to human, make up the semaphorin family (Yu and Kolodkin, 1999; Pasterkamp and Kolodkin, 2003) (Fig. 1). These soluble and membrane-spanning molecules fall into eight subclasses, on the basis of sequence similarity and distinctive structural features (Semaphorin Nomenclature Committee, 1999). The first two semaphorin subclasses, I and II, contain semaphorins identified in invertebrate species. Subclasses III-VII contain vertebrate semaphorins and the viral semaphorins found in certain non-neurotrophic DNA viruses. Several members of the semaphorin family play roles in axonal steering, zonal segregation of axon populations, axonal fasciculation, neuronal polarity, and neuronal cell migration, acting as chemorepellents during neuronal development (Tessier-Lavigne and Goodman, 1996). Recent findings, however, indicate that semaphorins play additional, diverse roles in unrelated processes to axon guidance, including organogenesis, vascularization, angiogenesis, neuronal apoptosis, and neoplastic transformation (Kitsukawa et al., 1995; Behar et al., 1996; Sekido et al., 1996; Soker et al., 1998). It is becoming increasingly clear that several semaphorins are also crucially involved in immune responses (Kikutani and Kumanogoh, 2003). The physiological and pathological significance of these semaphorins in the immune system is not fully understood, but emerging work is describing the biological functions of the immune semaphorins. Here we review these current findings and address several of the controversial areas for future study throughout a broad range of research fields.

## Sema4D/CD100

### Expression

Sema4D is a 150-kDa transmembrane protein of the class IV semaphorin subfamily (Delaire et al., 1998; Kumanogoh and Kikutani, 2001; Kikutani and Kumanogoh, 2003). This semaphorin contains an N-terminal signal sequence followed by a sema domain, an Ig-like domain, a lysine-rich stretch, a hydrophobic transmembrane region, and a cytoplasmic tail (Furuyama et al., 1996; Hall et al., 1996). Both human and mouse Sema4D are proteolytically cleaved from their transmembrane form to generate 120-kDa soluble forms, indicating the existence of two Sema4D isoforms (Herold et al., 1995; Delaire et al., 2001; Elhabazi et al., 2001; Wang et al., 2001) (Fig. 2). Sema4D is expressed at high levels in lymphoid organs, including the spleen, thymus, and lymph nodes, and in non-lymphoid organs, such as the brain, heart, and kidney. In lymphoid organs, Sema4D is abundantly expressed on resting T cells but only weakly expressed on resting B cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). Its expression, however, is upregulated in these cells following treatment with various immunological stimuli. The release of soluble Sema4D from immune cells is also dependent on cell activation.

### Receptor

Two receptor families, the neuropilins and plexins, mediate many semaphorin functions, such as axonal growth cone collapse (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; Comeau et al., 1998; Winberg et al., 1998; Takahashi et al., 1999; Tamagnone et al., 1999). Sema4D uses two receptors, plexin-B1 and CD72, which are expressed in non-lymphoid and lymphoid tissues (Kumanogoh and Kikutani, 2001).

#### Plexin-B1

Plexin-B1, expressed at high levels in the fetal brain and kidney

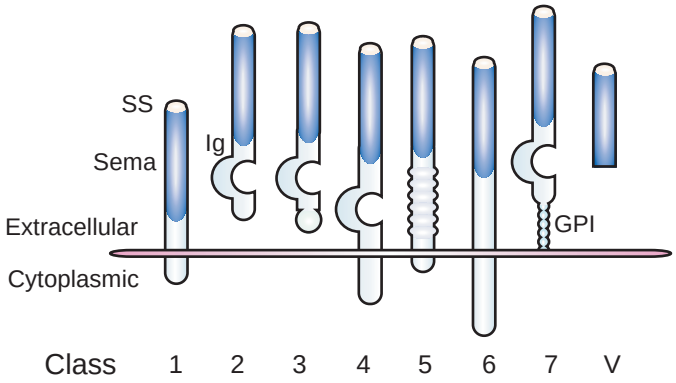


**Fig. 1.** Structure of the semaphorin family. The semaphorin family contains a large number of phylogenetically conserved secreted and transmembrane proteins. The members have been divided into eight classes based on structural features, one of which includes a unique set of viral molecules. All members of the semaphorin family share a common sema domain. Abbreviations: GPI, glycosylphosphatidylinositol-anchored; Ig, immunoglobulin-like domain; Sema, Sema domain; SS, signal sequence.

(Maestrini et al., 1996), demonstrates a high affinity ($K_d$=~1×10$^{-9}$ M) for Sema4D (Tamagnone et al., 1999; Kumanogoh et al., 2000). Human Sema4D stimulation enhances the interaction of the plexin-B1 cytoplasmic region with the small GTPase Rac1 (Vikis et al., 2000). Binding of Sema4D to plexin-B1 also activates the Rho guanine-nucleotide-exchange factor PDZ-RhoGEF/LARG, leading to RhoA activation (Swiercz et al., 2002). In human epithelial cells, plexin-B1 forms a functional receptor complex with Met, the tyrosine kinase receptor for scatter factor-1/hepatocyte growth factor receptor (Giordano et al., 2002). Binding of Sema4D to plexin-B1 stimulates the intrinsic tyrosine kinase activity of Met, leading to the phosphorylation of both the receptor and the Met substrate Gab1 (Fig. 3). Interestingly, Artigiani et al. have recently reported that the extracellular domain of plexin-B1 can be cleaved and modulates the effects of Sema4D (Artigiani et al., 2003). However, it still remains to be determined whether Sema4D can signal through plexin-B1 in the immune system.

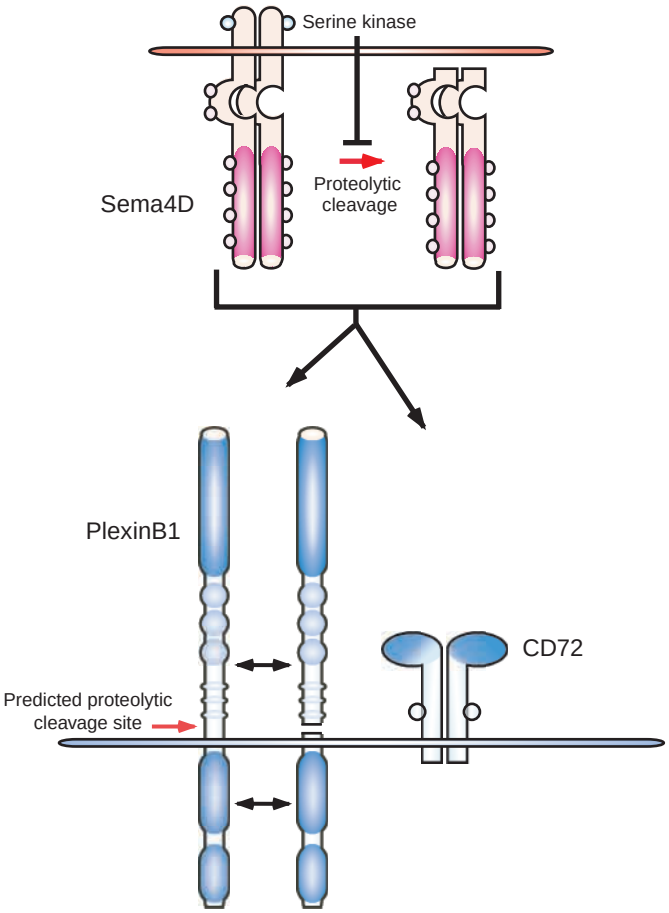


**Fig. 2.** Sema4D uses two different receptors. Sema4D is a member of the class IV semaphorin subfamily. Several consensus sites for serine phosphorylation exist in the cytoplasmic domain. Although Sema4D is a transmembrane-type semaphorin, it can be proteolytically cleaved from the surface to produce a soluble form. Serine kinase activities associated with the cytoplasmic region of Sema4D are implicating in the regulation of Sema4D proteolytic cleavage. Sema4D uses two receptors, CD72 and plexin-B1. Sema4D exerts its effects on immune cells, such as B cells and DCs, through CD72, whereas it induces growth cone collapse and epithelial cell invasive growth through plexin-B1 and plexin-B1/Met, respectively. Of note, the extracellular region of plexin-B1 is also cleaved (Artigiani et al., 2003).

## CD72

Sema4D utilizes CD72 as its major receptor in lymphoid tissues (Kumanogoh et al., 2000; Kumanogoh and Kikutani, 2001; Kikutani and Kumanogoh, 2003; Suzuki et al., 2003). CD72 expression is detectable throughout B cell differentiation from early progenitors to mature B cells (Nakayama et al., 1989; Von Hoegen et al., 1990; Gordon, 1994). Expression is downregulated, however, upon terminal differentiation into plasma cells. CD72 is expressed by professional APCs, such as macrophages and DCs (Tutt Landolfi and Parnes, 1997; Kumanogoh et al., 2002b). Several lines of evidence demonstrate that CD72 is the lymphocyte receptor for Sema4D (Kumanogoh et al., 2000): recombinant soluble Sema4D binds specifically to CD72-expressing transfectants; antibodies specific for CD72 block the binding of soluble Sema4D to B cells; and soluble mouse Sema4D fails to stimulate CD72-deficient B cells, owing to a lack of surface binding. A direct protein-protein interaction between mouse recombinant soluble Sema4D and CD72 can be detected by surface plasmon resonance. CD72, a member of the C-type lectin family, is thought to function as a negative regulator of B cell responses by recruiting SHP-1, a tyrosine phophatase, to an immunoreceptor tyrosine-based inhibitory motif (ITIM) in its cytoplasmic region (Adachi et al., 1998). In support of this, B cells from CD72-deficient mice are hyper-proliferative in response to various stimuli (Pan et al., 1999). Both agonistic anti-mouse CD72 monoclonal antibodies and soluble mouse Sema4D protein can induce dephosphorylation of mouse CD72, facilitating the dissociation of SHP-1 from CD72 (Wu et al., 1998; Kumanogoh et al., 2000). Thus, it appears that Sema4D binding turns off inhibitory signaling by CD72 (Kumanogoh et al., 2000; Kumanogoh and Kikutani, 2001) (Fig. 3).

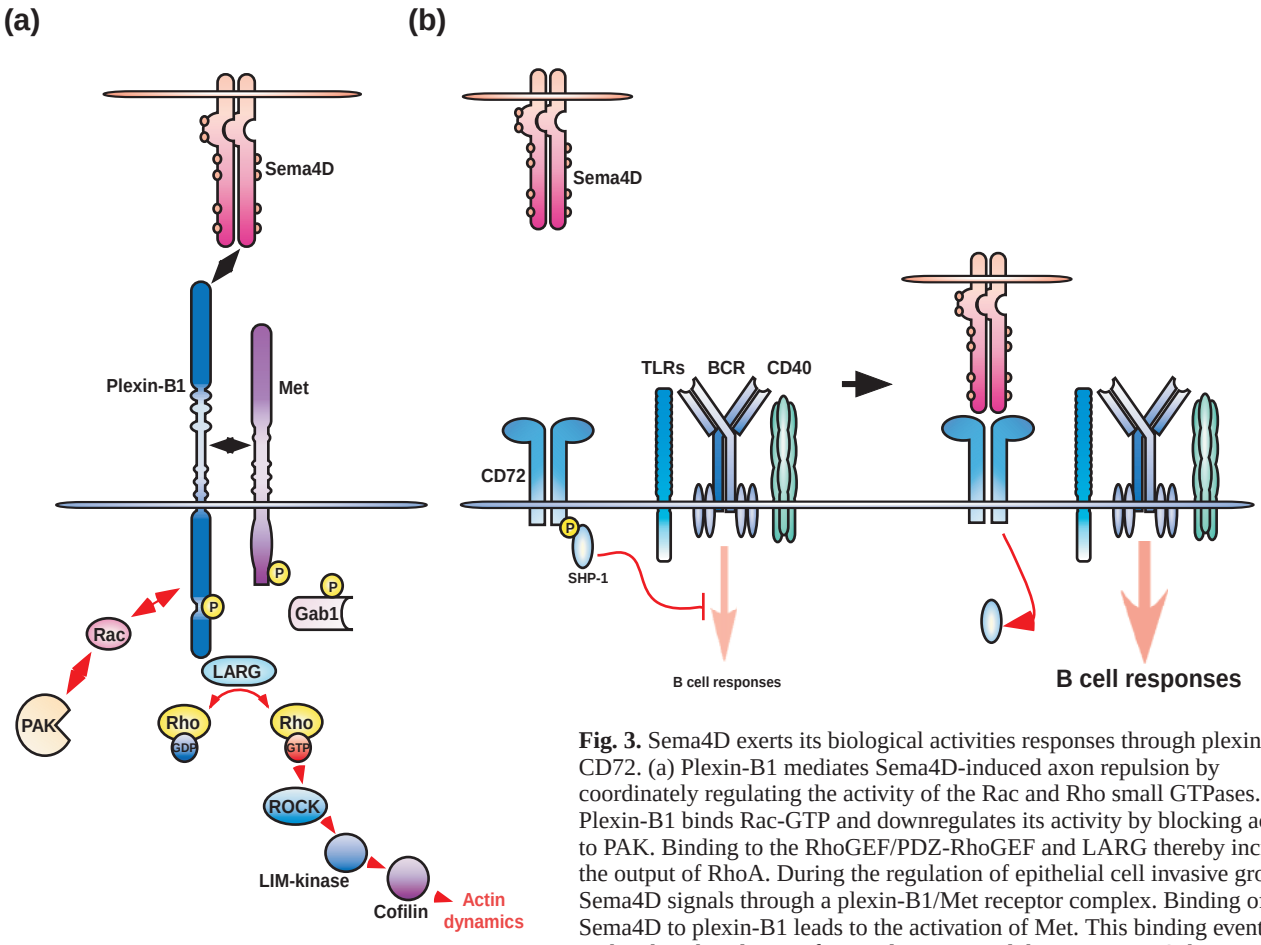


**Fig. 3.** Sema4D exerts its biological activities responses through plexin-B1 or CD72. (a) Plexin-B1 mediates Sema4D-induced axon repulsion by coordinately regulating the activity of the Rac and Rho small GTPases. Plexin-B1 binds Rac-GTP and downregulates its activity by blocking access to PAK. Binding to the RhoGEF/PDZ-RhoGEF and LARG thereby increases the output of RhoA. During the regulation of epithelial cell invasive growth, Sema4D signals through a plexin-B1/Met receptor complex. Binding of Sema4D to plexin-B1 leads to the activation of Met. This binding event results in the phosphorylation of Met, plexin-B1 and the Met target Gab1. (b) Sema4D turns off the negative signaling of CD72. Signals from the BCR, CD40, and TLR4 are homeostatically regulated by Sema4D-CD72 interactions. In the absence of Sema4D signaling, SHP-1 is associated with the ITIM of CD72. SHP-1 induces tyrosine dephosphorylation and the inactivation of several signaling effectors, including syk and lyn. Binding of Sema4D to CD72 induces the dephosphorylation of the CD72 ITIMs, resulting in the dissociation of SHP-1 from CD72.

## Biological activities and physiological roles of Sema4D

The expression patterns of Sema4D and its high-affinity receptor, plexin-B1, imply a role in axonal guidance. In *Drosophila*, plexin-B controls the axonal guidance of certain motor neurons by enhancing Rho signaling (Hu et al., 2001). Sema4D binding to plexin-B1 results in RhoA activation by regulating PDZ-RhoGEF/LARG, the GEF responsible for Sema4D-induced growth cone collapse in primary hippocampal neurons (Swiercz et al., 2002). In addition, Sema4D triggers the invasive growth of epithelial cells, including cell-cell dissociation, anchorage-independent growth, and branching morphogenesis by binding to the plexin-B1–Met complex (Giordano et al., 2002). The physiological relevance of the above biological activities of Sema4D remains to be clarified, because no apparent phenotypes in non-lymphoid organs have yet been observed in Sema4D-deficient mice (Shi et al., 2000). Some of Sema4D functions might be of course compensated for by other semaphorin molecules in those mice.

A number of biological activities of Sema4D in the immune system have been reported, and some of these have been confirmed by analysis of Sema4D-deficient mice (Shi et al., 2000). Sema4D-expressing CHO cell transfectants promote the in vitro aggregation and survival of B cells (Hall et al., 1996). Human Sema4D stimulation also induces shedding of CD23 (a low affinity receptor for IgE, which is used as an activation marker for B cells) from the surface of B cells (Hall et al., 1996). Sema4D-expressing CHO cell transfectants or the addition of soluble recombinant mouse Sema4D can enhance CD40-induced proliferation and immunoglobulin production in mouse B cells (Kumanogoh et al., 2000). Similarly, B cell responses to CD40 or LPS stimulation in vitro and humoral immune responses against T-cell-dependent antigens in vivo are impaired in Sema4D-deficient mice (Shi et al., 2000). Sema4D also plays a role in the activation and maturation of DCs (Kumanogoh et al., 2002b): soluble recombinant Sema4D can enhance CD40-induced DC maturation, as measured by upregulation of CD40 and CD80, and enhanced production of interleukin 12. In Sema4D-deficeint mice, T cell priming, in which DCs play a central role, is severely impaired (Shi et al., 2000). Sema4D also seems to play a role in monocytes and macrophages: soluble human Sema4D inhibits the spontaneous and MCP-3-induced migration of freshly isolated monocytes and monocytic cell lines (Delaire et al., 2001). We have also

demonstrated that both recombinant soluble human Sema4D and agonistic anti-huma- CD72 monoclonal antibodies induce the production of proinflammatory cytokines, such as IL-6 and IL-8, by human monocytes (Ishida et al., 2003). Collectively, these findings suggest that Sema4D has a crucial function in a broad range of tissues.

By contrast, some reports suggest a possible function for Sema4D as an immune receptor. Antibody crosslinking of human Sema4D enhances T cell proliferation in the presence of submitogenic doses of either monoclonal antibodies against CD3 (a component of the T cell receptor complex) or monoclonal antibodies against CD2 [a T cell surface antigen that interacts with LFA-3 (CD58)] (Bougeret et al., 1992; Herold et al., 1995). In human T cells, Sema4D is associated with an unidentified serine/threonine kinase and a protein tyrosine phosphatase (PTP), CD45 (Elhabazi et al., 1997; Herold et al., 1996). A switch at the terminal stage of B cell differentiation in the type of PTP activity associated with human Sema4D has also been reported (Billard et al., 2000).

A *Drosophila* transmembrane semaphorin, Sema-1a, plays a role in axon guidance, acting through the plexin-A receptor (Winberg et al., 1998). However, it might also have a bi-directional role as both a ligand and a receptor in central synapse formation in *Drosophila* (Yu et al., 1998). This paradigm supports the plausibility of a bi-directional function for Sema4D. In what situations might Sema4D function as a receptor? Transfectants expressing human plexin-B1 sustain the proliferation of normal and leukemic $CD5^+$ B cells, both of which express human Sema4D (Granziero et al., 2003). These data suggest that human Sema4D can function as a receptor for human plexin-B1. It remains to be clarified whether the cytoplasmic region of Sema4D is involved in physiological or pathological roles during the growth of normal or leukemic $CD5^+$ B cells, respectively, in vivo.

## Sema4A

### Expression

Sema4A, a class IV semaphorin originally identified as semB (Puschel et al., 1995), possesses a structure similar to that of Sema4D. Its expression gradually increases during embryonic development, and Sema4A mRNA is detectable in the brain, spleen, lung, kidney, and testes of adults (Kumanogoh et al., 2002a). Sema4A is expressed by bone-marrow-derived and splenic DCs as well as by B cells, but not by resting T cells. No differences in Sema4A expression are observable between $CD8\alpha^+$ (lymphoid origin) and $CD8\alpha^-$ (myeloid origin) DCs. Sema4A is upregulated following B cell stimulation with anti-CD40 monoclonal antibodies. Expression of Sema4A becomes detectable upon stimulation of T cells with anti-CD3 monoclonal antibodies (Kumanogoh et al., 2002a). This expression pattern of Sema4A contrasts that of Sema4D, in which expression is preferentially seen on T cells.

### Tim-2 as a Sema4A receptor

Whereas Sema4D binds plexin-B1, the binding of Sema4A to plexins remains to be investigated. In the immune system, Sema4A-binding partners exist on the surface of activated T cells but not on B cells and DCs. The binding of Sema4A to the cell surface of a subset of T cell lines, such as EL-4 cells, is also observed. Expression cloning using human IgG1-Fc portion-conjugated Sema4A (Sema4A-Fc) revealed that the Sema4A receptor is a member of the T cell, immunoglobulin and mucin domain proteins (TIM) family, Tim-2 (Kumanogoh et al., 2002a) (Fig. 4) – a 305-residue transmembrane protein. Sema4A-Fc binds COS7 cells transfected with Tim-2 cDNA in a specific manner (Kumanogoh et al., 2002a) and stimulation of cells with Sema4A induces Tim-2 tyrosine phosphorylation.

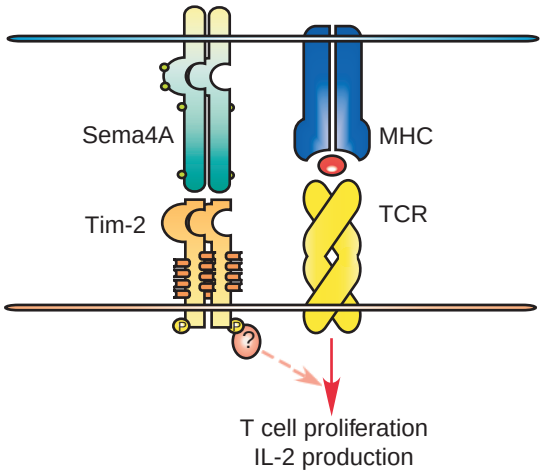


**Fig. 4.** Involvement of Sema4A in T cell activation through Tim-2. Sema4A, preferentially expressed on DCs and B cells, is a class IV transmembrane-type semaphorin family member. Tim-2, a member of the Tim protein family, possesses an Ig-like domain, a mucin domain, a transmembrane region, and a cytoplasmic region containing a consensus tyrosine phosphorylation site. The mucin domain has multiple putative sites for *O*-linked glycosylation, while the Ig domain has several sites for putative *N*-linked glycosylation. Following T cell activation through TCR ligation, Tim-2 expression on T cells interacts with Sema4A, resulting in enhanced T cell activation via tyrosine phosphorylation of Tim-2, acting through an unknown signaling pathway.

The Tim family of proteins is expressed on T cells and characterized by a conserved set of immunoglobulin and mucin domains. McIntire et al. originally identified a locus conferring susceptibility to mouse allergen-induced airway hypersensitivity, which they dubbed T cell and airway phenotype regulator (*Tapr*) (McIntire et al., 2001). Subsequent positional cloning revealed this locus to contain a new family of genes, the *Tims*. There are remarkable numbers of Tim-1 sequence polymorphisms within both *Tapr* and human *Tim-1* (hHAVcr-1) (McIntire et al., 2001). The human homologue of Tim-2 has not yet been identified. Kuchroo and colleagues independently identified Tim-3 in a screen for Th1 (a functional T cell subset that preferentially produces IFN-γ but not IL-4 and plays crucial roles in cell-mediated immunity)-reactive monoclonal antibodies as a Th1 cell-specific surface protein (Monney et al., 2002). Administration of anti-Tim-3 monoclonal antibodies promotes the development of EAE, a Th1-dependent autoimmune disease. This result suggests that Tim-3 is involved in the interactions between Th1 cells and macrophages, which results in the expansion and activation of macrophages. The fact that both Tim-1 and Tim-3 play a role in T-cell-mediated immune responses underscores the functional relevance of Tim-2, a member of the Tim family, in

T cell function. Although the natural ligands of Tim-1 and Tim-3 have not been identified, the known Tim protein ligands, including Sema4A, are likely to be regulatory molecules influencing the activation and differentiation of T cells.

### Activities

Although recombinant soluble Sema4A does not appear to stimulate B cells and DCs, Sema4A can provide T cell costimulation (Kumanogoh et al., 2002a). The addition of recombinant soluble Sema4A enhances T cell proliferation and IL-2 production following stimulation with anti-CD3 monoclonal antibodies. Soluble Sema4A promotes the induction of either Th1-cell-mediated IFN-γ production or Th2-cell-mediated IL-4 production, depending on the respective culture conditions. In addition, soluble Sema4A enhances mixed lymphocyte reactions (MLRs) between allogeneic T cells and DCs. Anti-Sema4A can block the MLRs, which indicates that Sema4A functions in T cell activation by influencing stimulatory interactions between T cells and DCs.

The role of Sema4A in immune responses in vivo has been clarified by use of soluble Sema4A and anti-Sema4A monoclonal antibodies (Kumanogoh et al., 2002a). Soluble Sema4A significantly enhances the generation of antigen-specific T cells. In contrast, administration of anti-Sema4A monoclonal antibodies blocks antigen-specific T cell priming. Treatment of mice with anti-Sema4A monoclonal antibodies inhibits the development of EAE induced by myelin oligodendrocyte glycoprotein (MOG)-peptide administration. Delayed administration of soluble Sema4A or anti-Sema4A antibodies does not affect the generation of antigen-specific T cells, which suggests that Sema4A acts early in T cell activation in vivo (Kumanogoh et al., 2002a; Kikutani and Kumanogoh, 2003).

## Transmembrane forms versus soluble forms

The functions of semaphorins as axonal chemorepellents have been well characterized in the case of the secreted class III subclass members (Tessier-Lavigne and Goodman, 1996). Five of the seven semaphorin subclasses, however, are transmembrane semaphorins. *Drosophila* Sema-1a, a transmembrane semaphorin, functions in axonal guidance in vivo (Raper, 2000). Whether transmembrane semaphorins exert their biological activities through direct contact between ligand-expressing cells and target cells or function remotely as soluble molecules is not clear. Some transmembrane semaphorins, such as Sema4D, can be released from the cell surface by proteolytic cleavage. The generation of soluble Sema4D is highly regulated (Wang et al., 2001); release from primary T and B cells is strictly dependent on a proteolytic cascade controlled by cellular activation. A significant amount of soluble Sema4D is detectable in the sera of mice immunized with a T-cell-dependent antigen, as well as in the sera of autoimmunity-prone MRL/lpr mice. The levels of soluble Sema4D correlate well with the titers of antigen-specific antibodies or auto-antibodies; soluble Sema4D is undetectable in the sera of unimmunized normal mice (Wang et al., 2001). The profiles of soluble Sema4D expression in vivo thus suggest that soluble Sema4D has important immunological activities. Transmembrane Sema4D, however, also appears to be active. Sema4D-expressing CHO cell transfectants promote B cell proliferation and immunoglobulin production (Hall et al., 1996; Kumanogoh et al., 2000). Thus, transmembrane Sema4D might function as a transmembrane ligand and also exist as a reservoir for soluble Sema4D production. If so, proteolytic cleavage of Sema4D would be a critical step regulating its function in the immune and nervous systems.

How is proteolytic cleavage of the transmembrane form regulated? The cytoplasmic region of Sema4D appears to be necessary for this regulation. A serine kinase activity is associated with the cytoplasmic region of human Sema4D (Elhabazi et al., 1997) (Fig. 2). Staurosporine (a cell-permeable, broad specificity inhibitor of serine kinases) enhances the release of soluble Sema4D (Elhabazi et al., 2001), which suggests that serine phosphorylation is involved in the regulation of Sema4D cleavage. Despite weak cell surface expression, high levels of soluble Sema4D are detectable in the sera of transgenic mice expressing a truncated Sema4D lacking the cytoplasmic region (Watanabe et al., 2001). Thus, the cytoplasmic region of Sema4D is necessary for proper regulation of soluble form generation. Further studies will be required to determine whether these findings, particularly the regulation of soluble Sema4D production, are applicable to other transmembrane semaphorins.

## Other semaphorins that play potential roles in the immune system

### Virus-encoded semaphorins

Viruses encode proteins that can function as immune modulators. Such proteins modulate the host immune response to facilitate the infectious process or support viral transmission. Kolodkin et al. discovered an open reading frame, A39R, related to the semaphorin family in the genome of vaccinia virus, a member of the poxvirus family (Kolodkin et al., 1993). A39R induces robust responses in human monocytes, including cell aggregation, induction of proinflammatory cytokines (e.g. IL-6 and IL-8), and upregulation of the monocyte cell surface marker CD54 (ICAM-1) (Comeau et al., 1998), which suggests it affects the immune responses of vaccinia-infected hosts. In addition, Ensser and Fleckenstein have reported that another semaphorin, AHVsema, is encoded by the alcelaphine herpesvirus (AHV) (Ensser and Fleckenstein, 1995).

Comeau et al. (Comeau et al., 1998) have found that both of these viral semaphorins, A39R and AHVsema, bind to the cellular receptor VESPR/CD232/plexin-C1. A39R-induced induction of inflammatory cytokines is abrogated by a blocking antibody against VESPR/CD232/plexin-C1 (Comeau et al., 1998). These findings suggest a pathogenic role for viral semaphorins.

### Sema7A

Sema7A (also known as Sema-K1 or CD108) is a molecule homologus to AHVsema. It was originally discovered as the John-Milton-Hagen human blood group antigen. Sema7A is a GPI-anchored cell surface glycoprotein preferentially expressed on activated lymphocytes and thymocytes (Xu et al., 1998; Yamada et al., 1999). Sema7A specifically binds to VESPR/CD232/plexin-C1 (Tamagnone et al., 1999), which

confirms that this semaphorin is a cellular counterpart of AHVsema. Recombinant soluble Sema7A protein exhibits activities against monocytes similar to those of AHVsema, including induction of the production of inflammatory cytokines such as TNF-α, IL-6 and IL-8. In addition, Sema7A demonstrates chemotactic attraction for monocytes (Holmes et al., 2002), which substantiates its role in inflammatory responses. Thus, the viral semaphorins may have an immunomodulatory effect by mimicking Sema7A. It will therefore be important to determine the physiological importance of interactions between Sema7A and VESPR/CD232/plexin-C1.

### Class III semaphorin: Sema3A in immune cell migration

Sema3A is the human homologue of collapsin-1, the first identified vertebrate semaphorin (Kolodkin et al., 1993). The function of Sema3A as an axonal guidance factor has been extensively studied in the neurobiology field. Delaire et al. reported that Sema3A also plays a role in regulating monocyte migration, Sema3A inhibiting spontaneous monocytic cell migration in a transwell assay (Delaire et al., 2001). Neuropilin-1, the known receptor of Sema3A, is not expressed on the target immune cells, however, which suggests that the inhibitory effect of Sema3A on immune-cell migration is mediated by receptors different from those previously known to bind within the nervous system (Delaire et al., 2001).

## Why is the receptor system so complicated?

Two receptor families, plexins and neuropilins, have been implicated in mediating the functions of semaphorins in axon guidance (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; Comeau et al., 1998; Winberg et al., 1998; Takahashi et al., 1999; Tamagnone et al., 1999). Vertebrate class III secreted semaphorins use neuropilins as ligand-binding receptor components, which form the receptor complex with plexins (Takahashi et al., 1999; Pasterkamp and Kolodkin, 2003). Semaphorins of invertebrates, and transmembrane and GPI-anchored semaphorins in vertebrates interact directly with plexins (Yu and Kolodkin, 1999). Viral-encoded semaphorins, A39R and AHVsema, and Sema7A, interact directly with plexin-C1, inducing activation of monocytes. Why, then, do Sema4D and Sema4A exert their functions in the immune system through CD72 and Tim-2, respectively, molecules that have no structural relationship with neuropilins and plexins. Although it is currently not known whether other semaphorins expressed in the immune system also use distinct receptor systems, the receptor systems used by Sema4D and Sema4A do not fit well into our current understanding of semaphorin receptor systems. Because Sema4D can use plexin-B1 in addition to CD72, Sema4A might also function through neuropilins or plexins during development. Sema4D and Sema4A might therefore have acquired new functions in the immune system during evolution through the use of new receptors. Such pleiotropic gene function represents an efficient way to use limited genetic resources.

The semaphorin receptor system is more complex than previously thought. Several novel components besides plexins and neuropilins are emerging, aiding our understanding of the complexity of the system. Off-track, a protein similar to receptor tyrosine kinases that contains a catalytically inactive kinase domain, associates with *Drosophila melanogaster* PlexinA to mediate Sema1a repulsive functions (Winberg et al., 2001). L1, a cell adhesion molecule in the immunoglobulin superfamily, plays a role in repulsive responses to the class III semaphorin Sema3A as part of a neuropilin-plexin receptor complex (Castellani et al., 2002). Moreover, as mentioned above, plexin-B1 forms a receptor complex with Met, whose intrinsic kinase activity is required for promoting epithelial cell invasive growth in response to Sema4D (Giordano et al., 2002). These recent findings suggest that many semaphorin receptors exist as large holoreceptor complexes that mediate ligand binding and signal transduction. The pairing of receptor components can also have diverse biological functions in a broad range of tissues. It will be crucial to carefully re-evaluate the composition of several semaphorin receptors formerly thought to contain one or a few different proteins, including CD72 and Tim-2. More comprehensive studies on receptor usage, including the presence or absence of additional receptor components in both lymphoid and non-lymphoid tissues, will help delineate the pleiotropic effects of the multiple semaphorin-receptor systems.

## Are semaphorin interactions clinically relevant?

Given that semaphorins are emerging as immunological mediators, it is worth considering the clinical relevance of immune semaphorins and their ability to manipulate pathological immune responses. Novel in vivo findings suggest the potential use of Sema4D and Sema4A to enhance host immunity against pathogens. Administration of recombinant soluble Sema4D accelerates antigen-specific antibody production and antigen-specific T cell generation (Kumanogoh et al., 2000; Shi et al., 2000), as does the administration of recombinant soluble Sema4A (Kumanogoh et al., 2002a). Stimulation of antigen-pulsed DCs with recombinant mouse Sema4D in combination with anti-CD40 monoclonal antibodies significantly augments in vivo immunogenicity, resulting in enhanced generation of antigen-specific effector and memory T cells (Kumanogoh et al., 2002b). Thus, administration of Sema4D, Sema4A, or agonistic reagents that stimulate CD72 or Tim-2, in conjunction with conventional immunization may enhance immune responses, particularly those generated against infectious agents by vaccines that are weakly immunogenic. Detailed investigation of the effects of immune semaphorins in both humans and experimental animals is required, however, prior to these studies. The combination of immune semaphorin stimulation with vaccination or vigorous antibiotic therapy might be beneficial for promoting immunity against various pathogens.

In contrast, inhibition of immune semaphorins may prove to be a therapeutic approach for the control of certain immune disorders. Sema4D-deficient mice are resistant to EAE induced following administration of MOG-derived peptides, owing to the impaired generation of MOG-reactive T cells (Kumanogoh et al., 2002b). Sema4A blockade also suppresses the development of EAE (Kumanogoh et al., 2002a), which suggests that these molecules serve as potential targets in the treatment of autoimmunity. In addition, an antibody against VESPR/CD232/plexin-C1 inhibits viral semaphorin-induced

induction of inflammatory cytokines by monocytes (Comeau et al., 1998). Delaire and colleagues recently reported that soluble Sema3A and Sema4D inhibits both spontaneous and MCP-3-induced monocytic cell migration (Delaire et al., 2001). They might thus prove to be useful clinical anti-inflammatory agents. The manipulation of immune semaphorin function, through either reinforcement or inhibition by recombinant proteins and specific antibodies or drugs, is a crucial area for future clinical studies.

## Further questions

Recent studies of Sema4D and Sema4A have uncovered crucial roles of these semaphorins in the immune system (Kikutani and Kumanogoh, 2003). However, many questions regarding interpretation of the biological significance of this protein family still remain. For instance, an intriguing issue is whether Sema4D and Sema4A can also exert their functions through CD72 and Tim-2, respectively, in non-lymphoid organs, including the nervous system. Furthermore, neuropilins or plexins might be involved in the immunological functions of these semaphorins. More comprehensive studies on their receptor usage in both lymphoid and non-lymphoid tissues will address these questions. In addition, recent studies, including work on gene-targeted mice, sometimes show results that contradict previous notions of pairing of semaphorin receptor and co-receptor systems (Cheng et al., 2001; Suto et al., 2003). Note that identification of semaphorin receptors and co-receptors has often been based only on binding analysis data or non-physiological functional assays (for instance, COS cell contraction assays). It is, therefore, crucial that we carefully re-evaluate the semaphorin receptor systems.

Semaphorins other than Sema4D and Sema4A also appear to function in immunity, representing a new family of immunoregulatory molecules. Because the studies with the 'immune semaphorins' suggest that these molecules function in autoimmune diseases and viral infections, other semaphorins may prove to be viable targets for research into therapies for immune disorders and infectious diseases. Future studies clarifying the biological functions of semaphorins in the immune system should help establish a new paradigm of cell-cell communication through semaphorin networks.

We thank K. Kubota for excellent secretarial assistance. We also thank N. Takegahara for excellent artistry in the creation of figures. This study was supported by research grants from the Ministry of Education, Culture, Science, and Technology of Japan to A.K. and H.K.

## References

**Adachi, T., Flaswinkel, H., Yakura, H., Reth, M. and Tsubata, T.** (1998). The B cell surface protein CD72 recruits the tyrosine phosphatase SHP-1 upon tyrosine phosphorylation. *J. Immunol.* **160**, 4662-4665.

**Artigiani, S., Barberis, D., Fazzari, P., Longati, P., Angelini, P., van de Loo, J. W., Comoglio, P. M. and Tamagnone, L.** (2003). Functional regulation of semaphorin receptors by proprotein convertases. *J. Biol. Chem.* **278**, 10094-10101.

**Behar, O., Golden, J. A., Mashimo, H., Schoen, F. J. and Fishman, M. C.** (1996). Semaphorin III is needed for normal patterning and growth of nerves, bones and heart. *Nature* **383**, 525-528.

**Billard, C., Delaire, S., Raffoux, E., Bensussan, A. and Boumsell, L.** (2000). Switch in the protein tyrosine phosphatase associated with human CD100 semaphorin at terminal B-cell differentiation stage. *Blood* **95**, 965-972.

**Bougeret, C., Mansur, I. G., Dastot, H., Schmid, M., Mahouy, G., Bensussan, A. and Boumsell, L.** (1992). Increased surface expression of a newly identified 150-kDa dimer early after human T lymphocyte activation. *J. Immunol.* **148**, 318-323.

**Castellani, V., de Angelis, E., Kenwrick, S. and Rougon, G.** (2002). Cis and trans interactions of L1 with neuropilin-1 control axonal responses to semaphorin 3A. *EMBO J.* **21**, 6348-6357.

**Cheng, H.-J., Bargi, A., Yaron, A., Stein, E., Pleasure, S. J. and Tessier-Lavigne, M.** (2001). Plexin-A3 mediates semaphorin signaling and regulates the development of hippocampal axonal projections. *Neuron* **32**, 249-263.

**Comeau, M. R., Johnson, R., DuBose, R. F., Petersen, M., Gearing, P., VandenBos, T., Park, L., Farrah, T., Buller, R. M., Cohen, J. I. et al.** (1998). A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR. *Immunity* **8**, 473-482.

**Delaire, S., Elhabazi, A., Bensussan, A. and Boumsell, L.** (1998). CD100 is a leukocyte semaphorin. *Cell Mol. Life Sci.* **54**, 1265-1276.

**Delaire, S., Billard, C., Tordjman, R., Chedotal, A., Elhabazi, A., Bensussan, A. and Boumsell, L.** (2001). Biological activity of soluble CD100. II. Soluble CD100, similarly to H-SemaIII, inhibits immune cell migration. *J. Immunol.* **166**, 4348-4354.

**Elhabazi, A., Lang, V., Herold, C., Freeman, G. J., Bensussan, A., Boumsell, L. and Bismuth, G.** (1997). The human semaphorin-like leukocyte cell surface molecule CD100 associates with a serine kinase activity. *J. Biol. Chem.* **272**, 23515-23520.

**Elhabazi, A., Delaire, S., Bensussan, A., Boumsell, L. and Bismuth, G.** (2001). Biological activity of soluble CD100. I. The extracellular region of CD100 is released from the surface of T lymphocytes by regulated proteolysis. *J. Immunol.* **166**, 4341-4347.

**Ensser, A. and Fleckenstein, B.** (1995). Alcelaphine herpesvirus type 1 has a semaphorin-like gene. *J. Gen. Virol.* **76**, 1063-1067.

**Furuyama, T., Inagaki, S., Kosugi, A., Noda, S., Saitoh, S., Ogata, M., Iwahashi, Y., Miyazaki, N., Hamaoka, T. and Tohyama, M.** (1996). Identification of a novel transmembrane semaphorin expressed on lymphocytes. *J. Biol. Chem.* **271**, 33376-33381.

**Giordano, S., Corso, S., Conrotto, P., Artigiani, S., Gilestro, G., Barberis, D., Tamagnone, L. and Comoglio, P. M.** (2002). The semaphorin 4D receptor controls invasive growth by coupling with Met. *Nat. Cell Biol.* **4**, 720-724.

**Gordon, J.** (1994). B-cell signalling via the C-type lectins CD23 and CD72. *Immunol. Today* **15**, 411-417.

**Granziero, L., Circosta, P., Scielzo, C., Frisaldi, E., Stella, S., Geuna, M., Giordano, S., Ghia, P. and Caligaris-Cappio, F.** (2003). CD100/Plexin-B1 interactions sustain proliferation and survival of normal and leukemic CD5+ B lymphocytes. *Blood* **101**, 1962-1969.

**Hall, K. T., Boumsell, L., Schultze, J. L., Boussiotis, V. A., Dorfman, D. M., Cardoso, A. A., Bensussan, A., Nadler, L. M. and Freeman, G. J.** (1996). Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation. *Proc. Natl. Acad. Sci. USA* **93**, 11780-11785.

**He, Z. and Tessier-Lavigne, M.** (1997). Neuropilin is a receptor for the axonal chemorepellent Semaphorin III. *Cell* **90**, 739-751.

**Herold, C., Bismuth, G., Bensussan, A. and Boumsell, L.** (1995). Activation signals are delivered through two distinct epitopes of CD100, a unique 150 kDa human lymphocyte surface structure previously defined by BB18 mAb. *Int. Immunol.* **7**, 1-8.

**Herold, C., Elhabazi, A., Bismuth, G., Bensussan, A. and Boumsell, L.** (1996). CD100 is associated with CD45 at the surface of human T lymphocytes. Role in T cell homotypic adhesion. *J. Immunol.* **157**, 5262-5268.

**Holmes, S., Downs, A., Fosberry, A., Hayes, P., Michalovich, D., Murdoch, P., Moores, K., Fox, J., Deen, K., Pettman, G. et al.** (2002). Sema7A is a Potent Monocyte Stimulator. *Scand. J. Immunol.* **56**, 270-275.

**Hu, H., Marton, T. F. and Goodman, C. S.** (2001). Plexin B mediates axon guidance in Drosophila by simultaneously inhibiting active Rac and enhancing RhoA signaling. *Neuron* **32**, 39-51.

**Ishida, I., Kumanogoh, A., Suzuki, K., Akahani, S., Noda, K. and Kikutani, H.** (2003). Involvement of CD100, a lymphocyte semaphorin, in the activation of human immune system via CD72: implications for the regulation of immune and inflammatory responses. *Int. Immunol.* (in press).

**Kikutani, H. and Kumanogoh, A.** (2003). Semaphorins in interactions between T cells and antigen-presenting cells. *Nat. Rev. Immunol.* **3**, 159-167.

**Kitsukawa, T., Shimono, A., Kawakami, A., Kondoh, H. and Fujisawa, H.** (1995). Overexpression of a membrane protein, neuropilin, in chimeric mice causes anomalies in the cardiovascular system, nervous system and limbs. *Development* **121**, 4309-4318.

**Kolodkin, A. L., Matthes, D. J. and Goodman, C. S.** (1993). The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. *Cell* **75**, 1389-1399.

**Kolodkin, A. L., Levengood, D. V., Rowe, E. G., Tai, Y. T., Giger, R. J. and Ginty, D. D.** (1997). Neuropilin is a semaphorin III receptor. *Cell* **90**, 753-762.

**Kumanogoh, A. and Kikutani, H.** (2001). The CD100-CD72 interaction: a novel mechanism of immune regulation. *Trends Immunol.* **22**, 670-676.

**Kumanogoh, A., Watanabe, C., Lee, I., Wang, X., Shi, W., Araki, H., Hirata, H., Iwahori, K., Uchida, J., Yasui, T. et al.** (2000). Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. *Immunity* **13**, 621-631.

**Kumanogoh, A., Marukawa, S., Suzuki, K., Takegahara, N., Watanabe, C., Ch'ng, E., Ishida, I., Fujimura, H., Sakoda, S., Yoshida, K. et al.** (2002a). Class IV semaphorin Sema4A enhances T-cell activation and interacts with Tim-2. *Nature* **419**, 629-633.

**Kumanogoh, A., Suzuki, K., Ch'ng, E., Watanabe, C., Marukawa, S., Takegahara, N., Ishida, I., Sato, T., Habu, S., Yoshida, K. et al.** (2002b). Requirement for the lymphocyte semaphorin, CD100, in the induction of antigen-specific T cells and the maturation of dendritic cells. *J. Immunol.* **169**, 1175-1181.

**Maestrini, E., Tamagnone, L., Longati, P., Cremona, O., Bione, S., Tamanini, F., Neel, B. G., Toniolo, D. and Comoglio, P. M.** (1996). A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. *Proc. Natl. Acad. Sci. USA* **93**, 674-678.

**McIntire, J. J., Umetsu, S. E., Akbari, O., Potter, M., Kuchroo, V. K., Barsh, G. S., Freeman, G. J., Umetsu, D. T. and DeKruyff, R. H.** (2001). Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nat. Immunol.* **2**, 1109-1116.

**Monney, L., Sabatos, C. A., Gaglia, J. L., Ryu, A., Waldner, H., Chernova, T., Manning, S., Greenfield, E. A., Coyle, A. J., Sobel, R. A. et al.** (2002). Th1-specific cell surface protein Tim-3 regulates macrophage activation and severity of an autoimmune disease. *Nature* **415**, 536-541.

**Nakayama, E., von Hoegen, I. and Parnes, J. R.** (1989). Sequence of the Lyb-2 B-cell differentiation antigen defines a gene superfamily of receptors with inverted membrane orientation. *Proc. Natl. Acad. Sci. USA* **86**, 1352-1356.

**Pan, C., Baumgarth, N. and Parnes, J. R.** (1999). CD72-deficient mice reveal nonredundant roles of CD72 in B-cell develoment and activation. *Immunity* **11**, 495-506.

**Pasterkamp, R. J. and Kolodkin, A. L.** (2003). Semaphorin junction: making tracks toward neural connectivity. *Curr. Opin. Neurobiol.* **13**, 79-89.

**Puschel, A. W., Adams, R. H. and Betz, H.** (1995). Murine semaphorin D/collapsin is a member of a diverse gene family and creates domains inhibitory for axonal extension. *Neuron* **14**, 941-948.

**Raper, J. A.** (2000). Semaphorins and their receptors in vertebrates and invertebrates. *Curr. Opin. Neurobiol.* **10**, 88-94.

**Sekido, Y., Bader, S., Latif, F., Chen, J. Y., Duh, F. M., Wei, M. H., Albanesi, J. P., Lee, C. C., Lerman, M. I. and Minna, J. D.** (1996). Human semaphorins A(V) and IV reside in the 3p21.3 small cell lung cancer deletion region and demonstrate distinct expression patterns. *Proc. Natl. Acad. Sci. USA* **93**, 4120-4125.

**Semaphorin Nomenclature Committee** (1999). Unified nomenclature for the semaphorins/collapsins. *Cell* **97**, 551-552.

**Shi, W., Kumanogoh, A., Watanabe, C., Uchida, J., Wang, X., Yasui, T., Yukawa, K., Ikawa, M., Okabe, M., Parnes, J. R. et al.** (2000). The class IV semaphorin CD100 plays nonredundant roles in the immune system: defective B and T cell activation in CD100-deficient mice. *Immunity* **13**, 633-642.

**Soker, S., Takashima, S., Miao, H. Q., Neufeld, G. and Klagsbrun, M.** (1998). Neuropilin-1 is expressed by endothelial and tumor cells as an isoform-specific receptor for vascular endothelial growth factor. *Cell* **92**, 735-745.

**Suto, F., Murakami, Y., Nakamura, F., Goshima, Y. and Fujisawa, H.** (2003). Identification and characterization of a novel mouse plexin, plexin-A4. *Mech. Dev.* **120**, 385-396.

**Suzuki, K., Kumanogoh, A. and Kikutani, H.** (2003). CD100/Sema4D, a lymphocyte semaphorin involved in the regulation of humoral and cellular immune responses. *Cytokine Growth Factor Rev.* **14**, 17-24.

**Swiercz, J. M., Kuner, R., Behrens, J. and Offermanns, S.** (2002). Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology. *Neuron* **35**, 51-63.

**Takahashi, T., Fournier, A., Nakamura, F., Wang, L. H., Murakami, Y., Kalb, R. G., Fujisawa, H. and Strittmatter, S. M.** (1999). Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. *Cell* **99**, 59-69.

**Tamagnone, L., Artigiani, S., Chen, H., He, Z., Ming, G. I., Song, H., Chedotal, A., Winberg, M. L., Goodman, C. S., Poo, M. et al.** (1999). Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. *Cell* **99**, 71-80.

**Tessier-Lavigne, M. and Goodman, S. C.** (1996). The molecular biology of axon guidance. *Science* **274**, 1123-1133.

**Tutt Landolfi, M. and Parnes, J. R.** (1997). CD72 workshop panel report. In *Leukocyte Typing VI* (ed. T. Kishimoto, H. Kikutani, A. E. G. Kr. Von dem Borne et al.), pp. 162-164. New York: Garland Publishing.

**Vikis, H. G., Li, W., He, Z. and Guan, K. L.** (2000). The semaphorin receptor plexin-B1 specifically interacts with active Rac in a ligand-dependent manner. *Proc. Natl. Acad. Sci. USA* **97**, 12457-12462.

**Von Hoegen, I., Nakayama, E. and Parnes, J. R.** (1990). Identification of a human protein homologous to the mouse Lyb-2 B cell differentiation antigen and sequence of the corresponding cDNA. *J. Immunol.* **144**, 4870-4877.

**Wang, X., Kumanogoh, A., Watanabe, C., Shi, W., Yoshida, K. and Kikutani, H.** (2001). Functional soluble CD100/Sema4D released from activated lymphocytes: possible role in normal and pathologic immune responses. *Blood* **97**, 3498-3504.

**Watanabe, C., Kumanogoh, A., Shi, W., Suzuki, K., Yamada, S., Okabe, M., Yoshida, K. and Kikutani, H.** (2001). Enhanced immune responses in transgenic mice expressing a truncated form of the lymphocyte semaphorin cd100. *J. Immunol.* **167**, 4321-4328.

**Winberg, M. L., Noordermeer, J. N., Tamagnone, L., Comoglio, P. M., Spriggs, M. K., Tessier-Lavigne, M. and Goodman, C. S.** (1998). Plexin A is a neuronal semaphorin receptor that controls axon guidance. *Cell* **95**, 903-916.

**Winberg, M. L., Tamagnone, L., Bai, J., Comoglio, P. M., Montell, D. and Goodman, C. S.** (2001). The transmembrane protein off-track associates with plexins and functions downstream of semaphorin signaling during axon guidance. *Neuron* **32**, 53-62.

**Wu, Y., Nadler, M. J., Brennan, L. A., Gish, G. D., Timms, J. F., Fusaki, N., Jongstra-Bilen, J., Tada, N., Pawson, T., Wither, J. et al.** (1998). The B-cell transmembrane protein CD72 binds to and is an in vivo substrate of the protein tyrosine phosphatase SHP-1. *Curr. Biol.* **8**, 1009-1017.

**Xu, X., Ng, S., Wu, Z. L., Nguyen, D., Homburger, S., Seidel-Dugan, C., Ebens, A. and Luo, Y.** (1998). Human semaphorin K1 is glycosylphosphatidylinositol-linked and defines a new subfamily of viral-related semaphorins. *J. Biol. Chem.* **273**, 22428-22434.

**Yamada, A., Kubo, K., Takeshita, T., Harashima, N., Kawano, K., Mine, T., Sagawa, K., Sugamura, K. and Itoh, K.** (1999). Molecular cloning of a glycosylphosphatidylinositol-anchored molecule CDw108. *J. Immunol.* **162**, 4094-4100.

**Yu, H. H. and Kolodkin, A. L.** (1999). Semaphorin signaling: a little less per-plexin. *Neuron* **22**, 11-14.

**Yu, H. H., Araj, H. H., Ralls, S. A. and Kolodkin, A. L.** (1998). The transmembrane Semaphorin Sema I is required in Drosophila for embryonic motor and CNS axon guidance. *Neuron* **20**, 207-220.