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Immune Semaphorins: Novel Features of Neural Guidance Molecules

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Abstract
Introduction The immune and nervous system have various common features in the functional characteristics. Both have an intricate network of synaptic connections and an exquisite communication system that enables intercellular signal transduction. They also share a number of messenger molecules such as cytokines and chemical mediators.
Discussion Semaphorins, well-defined axonal guidance molecules in the nervous system, also play critical roles in immune regulation. Various types of semaphorins, including secreted, transmembrane, truncated, and glycosylphosphatidylinositol-anchored forms, function during immune responses. However, some semaphorins utilize receptors in the immune system that are distinct from receptors in the nervous system.
Conclusion This review presents a current overview of 'immune semaphorins' and their receptors, providing insight into the pleiotropic activity of this protein family.

Keywords Semaphorins · semaphorin receptors · immune regulation · autoimmune disease · allergy

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Introduction

The immune response is composed of a series of cell–cell contacts, including the interaction between T cells and antigen-presenting cells (APCs) such as B cells, macrophages, and dendritic cells (DCs). Such cell–cell contact elicits the activation response, which determines clonal expansion and effector function of T cells. The area of cell contact is called an 'immunological synapse', which is structurally similar to the synapse that connects pairs of neurons. There are many similarities between the immune and nervous systems: Both are highly networked systems and share chemical mediators (e.g., prostaglandins) and cytokines (e.g., interleukin 1β (IL-1β), IL-2, tumor necrosis factor alpha (TNFα)) [1].

Semaphorins, true to their name, are axonal guidance factors that function during neural development. Semaphorins were initially identified as chemorepulsive molecules among the neural attractive and repulsive cues in the extracellular environment that guide axon pathfinding [2]. More than 20 types of semaphorins have been identified to date [3]. Semaphorins are currently known to have diverse actions: They can exert repulsive, attractive, or bifunctional effects depending on the biological context in which they are encountered [4]. They are secreted or membrane-associated glycoproteins that have been grouped into eight classes. The semaphorin family carries a long stretch of conserved 'sema domain' in the N terminus. The semaphorins range in size from 400 to 1,000 amino acids depending on additional C-terminal sequence motifs such as immunoglobulin domain, thrombospondin domain, or glycosylphosphatidylinositol (GPI) linkage site. Class I and II semaphorins are found in invertebrates, whereas class III to VII semaphorins are found in vertebrates and class VIII are viral. In vertebrates, proteins in semaphorin classes IV

to VII are membrane-associated, whereas those in class III are secreted. The major semaphorin receptors are plexin family proteins [5, 6]. Plexins are categorized into four groups and also carry sema domains. The plexin intracellular domain shares homology with the GTPase-activating protein domain, indicating that semaphorin-plexin signaling is involved in cellular morphology [7]. Another group of semaphorin receptors, neuropilins (Nrp-1 and Nrp-2), form receptor complexes with plexin-A family members and exclusively binds to class III semaphorins [8].

Semaphorins also play important roles in other biological processes, including cardiac morphogenesis [9], vascular [10, 11] and epithelial growth and invasion [12, 13], tumor progression [14], and immune regulation [15–17]. Interestingly, in organs other than the brain, semaphorin receptor plexins associate with several effector molecules. For example, Off-track (Otk) or vascular endothelial growth factor receptor 2 (VEGFR2) associate with plexin-A1 during cardiac development [18]and Met associates with plexin-B1 in epithelial cells [13]. Notably, in the immune system, some semaphorins use non-plexin receptors, such as CD72 [19] and TIM-2 [20] (see below and Fig. 1). These observations provide insight into the diversity of semaphorin function. In this review, we will present the latest knowledge of semaphorins and their receptors, which are involved in the immune responses.

Sema4D

Sema4D-CD72 Interaction in the Immune System

Sema4D, also known as CD100, is the first semaphorin family member protein identified in the immune system [21, 22]. Sema4D is a 150-kDa cell surface transmembrane protein that forms a homodimer. In the immune system, Sema4D is expressed abundantly in resting T cells but only weakly in resting B cells and APCs [23, 24], and its

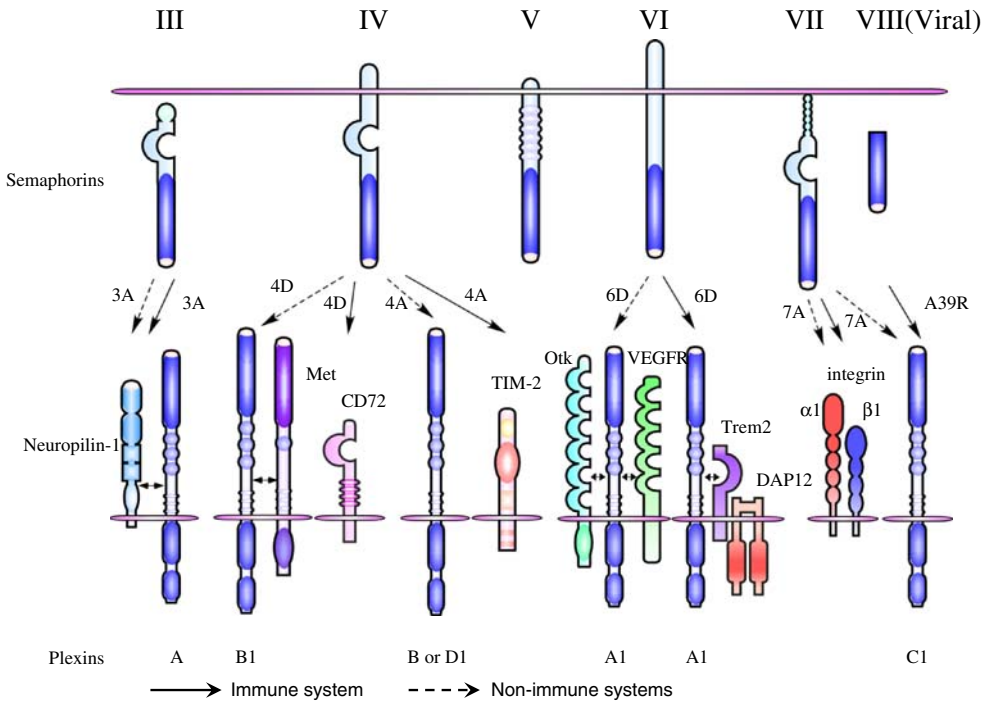

Fig. 1 Representative semaphorins and their multiple receptors. Among the eight classes of semaphorins, class I and II semaphorins are found in invertebrates (not shown in figure) and class III–VII are vertebrate semaphorins. Classes II and III and viral semaphorins are secreted, whereas class IV–VI are transmembrane. Class VII represents GPI-anchored proteins. Sema3A directly binds to Nrp-1, which results in transduction of plexin-A-mediated signals. Although Sema4D binds to plexin-B1 in brain and transduces chemorepulsive signals, plexin-B1 couples with Met in epithelial cells and induces Sema4D-mediated cell outgrowth. In the immune system, Sema4A binds to CD72, which enhances B cell and DC activation. Sema4A recognizes plexin-B and D1 in the non-immune systems but uses TIM-2 as a receptor for T cell activation in the immune system. During cardiogenesis, plexin-A1 associates with Off-track (*Otk*) or VEGFR2 at distinct sites and transduces Sema6D signals. However, plexin-A1 forms a receptor complex with TREM-2-DAP12 in the immune system, which is critical for DC activation and osteoclastogenesis. Sema7A has two types of receptors: $\alpha 1\beta 1$ integrin for macrophage activation and plexin-C1 for inhibition of cell adhesion. Viral semaphorin A39R also recognizes plexin-C1 and modulates dendritic cell function

expression is upregulated upon cellular activation [19]. Cumulative evidence indicates that Sema4D can function as a ligand. Sema4D-transfected B cells promote their aggregation and survival in vitro. In addition, recombinant soluble Sema4D or Sema4D-expressing cells enhance in vivo antibody production as well as in vitro B-cell responses [21, 25].

Two types of proteins, plexin-B1 and CD72, have been identified as Sema4D receptors. The major receptor for Sema4D in the nervous system is plexin-B1. Sema4D binding to plexin-B1 downregulates guanosine triphosphatase (GTPase) activity of R-Ras, a member of the Ras superfamily of small GTP-binding proteins, and induces growth cone collapse in hippocampal neuron [26]. Moreover, plexin-B1 forms a functional receptor complex with Met in epithelial cells and Sema4D binding to plexin-B1 promotes epithelial invasive growth [13]. However, in the immune system, CD72 is the predominant receptor for Sema4D [19]. CD72, a 45-kDa C-type lectin family protein, is constitutively expressed on B cells and APCs. CD72 contains two immunoreceptor tyrosine-based inhibitory motifs in the cytoplasmic domain that recruit the tyrosine phosphatase SHP-1 upon B-cell receptor (BCR) stimulation [27, 28]. SHP-1 associates with many inhibitory receptors, including CD22 and killer cell immunoglobulin-like receptors to inhibit the functions of B cells and natural killer (NK) cells, respectively. B cells from CD72-deficient mice undergo hyper-proliferation and have a rapid calcium response following BCR stimulation compared to B cells from wild-type mice [29]. Therefore, CD72 functions as a negative regulator of B cells.

The molecular mechanisms governing Sema4A-CD72-mediated regulation of BCR signals have been uncovered. Treatment of B cells with soluble Sema4D inhibits phosphorylation of CD72 and its association with SHP-1 [19]. Conversely, CD72 and SHP-1 are constitutively associated in B cells from Sema4D-deficient mice. Stimulation-induced association of CD72 with the BCR complex is inhibited by Sema4D. Finally, Sema4D-deficient B cells are hypo-responsive to BCR stimulation compared to wild-type B cells [25]. Collectively, these results indicate that Sema4D enhances B cell activation by 'turning off' inhibitory CD72 signals [30] (Fig. 2a).

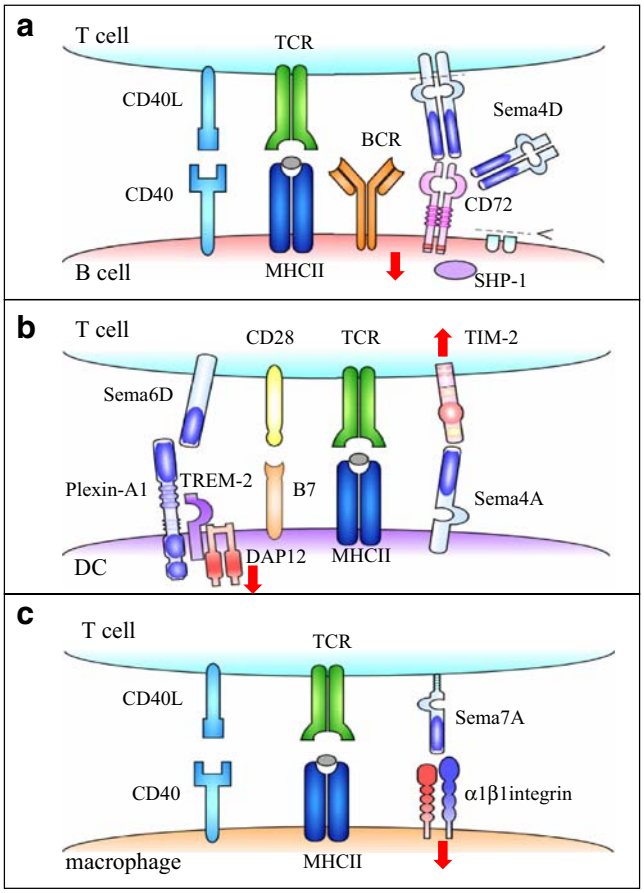

Fig. 2 Semaphorins in immune cell interactions. Semaphorins act at various phase and stage of immune cell interactions. **a** During T-cell-mediated B-cell activation, engagement of CD72 by Sema4D induces dephosphorylation of CD72 and dissociation from SHP-1, which results in enhancement of BCR signals. Sema4D can also be cleaved proteolytically and function as a soluble form in an autocrine/paracrine manner. **b** During T cell–DC interaction, Sema6D on T cells can activate DCs through the plexin-A1-TREM-2-DAP12 receptor complex. Sema4A on DCs binds to TIM-2 and activates T cells. **c** T-cell-mediated inflammatory responses require antigen–MHC class II–TCR interaction and CD40L–CD40 signals. However, the interaction between Sema7A and α1β1 integrin is also critical for activation of inflammatory cells such as macrophages

Sema4D and Immune Homeostasis

Changes in the BCR activation threshold due to alteration of signaling molecules downstream of BCR are thought to affect B-cell survival and turnover [31]. Sema4D is also involved in the homeostatic maintenance of B-cell subsets. In young Sema4D-deficient mice, the population of $CD5^{+}$ B1 cells is significantly reduced, although other B-cell subsets seem normal [25]. However, the proportion of $CD21^{hi}CD23^{lo}$ marginal zone B (MZB) cells in Sema4D-deficient mice gradually increases with advancing age [32]. Expansion of MZB cells is involved in defective BCR signaling, whereas increased B1 cell numbers are observed in mice lacking inhibitory receptors such as CD22 [33] and CD72 [29], suggesting that the requirement for BCR signaling differs among B-cell subsets. Therefore, a higher BCR signaling threshold may promote the development or survival of MZB cells but may be detrimental for the development of B1 cells in Sema4D-deficient mice.

Marginal zone, the region at the interface between lymphoid white pulp and non-lymphoid red pulp in the spleen, has been proposed as a site for sequestration of

autoreactive cells [34]. MZB cells may play a role in homeostasis and tolerance and in host defense and may be important for the induction of autoimmune diseases. Notably, the expansion of MZB cells in aged Sema4D-deficient mice is accompanied by the production of a variety of autoantibodies, including anti-ssDNA, anti-dsDNA, rheumatoid factors, anti-Sjogren's syndrome A, and anti-ribonucleoprotein [32]. Furthermore, these mutant mice exhibit marked perivascular leukocyte infiltration in several tissues, including salivary gland, liver, and kidney, and thickened basement membrane along with the deposition of immunoglobulin G in the kidney glomeruli. Although a limited number of aged CD72-deficient mice exhibit substantial amounts of autoantibodies [35], mice lacking both Sema4D and CD72 show no evidence of autoimmune disease [32]. These observations suggest that breakdown of Sema4D-CD72-mediated B-cell homeostasis may promote the expansion of MZB cells and the development of autoimmune diseases.

Sema4D and T-Cell-Mediated Immunity

As described above, T cells are the major Sema4D-producing cells in the immune system. However, Sema4D-deficient T cells respond normally to in vitro stimulation. Moreover, soluble Sema4D does not affect T-cell activation, suggesting that Sema4D has no direct effect on T cells. In contrast, soluble Sema4D enhances the surface expression of CD80, CD86, and major histocompatibility complex (MHC) class II on DCs [36]. The function of Sema4D in T cell–DC interactions has been addressed in vitro. Sema4D-sufficient $CD4^+$ T cells can differentiate normally into cytokine-secreting effector cells even when cultured with antigen and Sema4D-deficient DCs. In contrast, Sema4D-deficient $CD4^+$ T cells fail to differenciate even in the presence of Sema4D-sufficient DCs. Therefore, Sema4D expressed on T cells acts on DCs to promote their activation and maturation possibly through interaction with CD72, which in turn enhances T-cell activation [37].

The importance of Sema4D in T-cell-mediated immunity has also been verified using the mutant mice. After immunization of Sema4D-deficient mice with protein antigens, proliferative responses of $CD4^+$ T cells from draining lymph nodes are significantly impaired, as is cytokine production after antigen restimulation. Moreover, Sema4D-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG)-derived peptide, a mouse disease model for multiple sclerosis (MS) because generation of MOG-specific T cells is impaired in these mice [36]. Sema4D-deficient mice are also protected from experimental immune complex glomerulonephritis due to reduced T-cell activation and humoral immune responses [38]. These observations indicate that Sema4D is crucially involved in the activation and differentiation of T cells.

Functional Soluble Sema4D Extracellular Domain Fragment

Upon activation, Sema4D is proteolytically cleaved and released from the cell surface, suggestive of an autocrine and/or paracrine mechanism of action [39]. Soluble form of Sema4D released from T and B cells retains biological activity, and a variety of functions has been documented. In vivo antibody responses against T-cell-dependent antigens and generation of antigen-specific T cells are enhanced in transgenic mice expressing a truncated form of Sema4D [40]. It is also noteworthy that a significant amount of soluble Sema4D is detectable in the sera of wild-type mice immunized with a T-cell-dependent antigen and in the sera of an autoimmune-prone MRL/lpr mice, a model of systemic lupus erythematosus [39]. Here, the levels of soluble Sema4D correlate well with titers of antigen-specific antibody or autoantibodies, although soluble Sema4D is undetectable in the sera of non-immunized or normal mice. Interestingly, soluble Sema4D from T cells can also affect neuron and glial cells. In neuroinflammatory diseases such as MS and virus-induced demyelination, inappropriate cross-talk between activated T cells infiltrating the central nervous system (CNS) can sustain the onset and progression of demyelination and axonal degradation. Soluble Sema4D released from activated T cells collapses oligodendrocyte process extensions and triggers neural cell apoptosis. Indeed, high levels of soluble Sema4D in the cerebrospinal fluid are detected in patients suffering with human T lymphotropic virus type 1-induced neuroinflammatory demyelination (TSP/HAM) [41]. These findings suggest that soluble Sema4D is involved in various phases of pathological responses.

Sema4A

Sema4A-Mediated Regulation of T Helper Cell Differentiation

Like Sema4D, Sema4A is also a member of the class IV transmembrane semaphorin subfamily. Sema4A is expressed in a broad range of adult tissues, including brain, lung, spleen, kidney, and testis [20]. The Sema4A expression profile in immune cells is unique. Sema4A is constitutively expressed in all mouse DC subset but is barely detectable in resting or naive T cells. T cell receptor (TCR) stimulation induces transient Sema4A expression within 24 h, but its expression rapidly decreases thereafter.

However, when T cells are cultured under T helper type 1 (Th1)-polarizing conditions, high Sema4A expression is sustained throughout the culture period, whereas Sema4A expression is diminished in Th2-polarizing conditions [42]. Analysis of Sema4A-deficient mice has revealed that DC-derived Sema4A and Th1 cell-derived Sema4A play distinct functional roles in the development of T-cell-mediated immunity, as described below.

When T cells are cultured in Th1-polarizing conditions, Sema4A-deficient naive $CD4^+$ T cells fail to differentiate into interferon gamma (IFN-γ)-producing cells. In contrast, Sema4A-deficient naive cells normally differentiate into IL-4-producing cells under Th2 conditions. This selective defect in Th1 differentiation of Sema4A-deficient T cells is associated with reduced expression of IL-12 receptor β chain and T-bet, a transcription factor essential for Th1 development. Interestingly, normal Th1 differentiation of Sema4A-deficient T cells is fully restored by either the addition of soluble Sema4A protein or coculture with wild-type T cells. Thus, Sema4A on T cells may promote Th1 differentiation through cognate interaction between T cells. Sema4A-mediated regulation of Th cell differentiation has also been confirmed in vivo. The generation of IFN-γ-producing antigen-specific T cells is impaired in Sema4A-deficient mice immunized with Th1-inducing agents such as heat-killed *Propionibacterium acnes*. Conversely, Sema4A-deficient mice mount enhanced Th2 responses when infected with *Nippostrongylus brasiliensis*, a Th2-inducing intestinal nematode [42]. Moreover, it is striking that Sema4A-deficient mice of a Th2-prone BALB/c strain spontaneously develop atopic dermatitis (unpublished data).

Sema4A in DCs

The presence of recombinant soluble Sema4A protein substantially enhances the proliferation and IL-2 production of naive T cells from wild-type mice after TCR stimulation, which suggests that Sema4A contributes to T-cell activation through T cell–DC interactions. Indeed, Sema4A-deficient DCs poorly stimulate allogeneic T cells, despite the fact that Sema4A-deficient DCs mature normally and produce cytokines in response to lipopolysaccharide or anti-CD40. Generation of antigen-specific T cells after immunization with various antigens is consistently defective in Sema4A-deficient mice [42]. These observations indicate that Sema4A expressed on DCs is involved in the initial activation of T cells. Furthermore, when Sema4A-deficient DCs are transferred into Sema4A-sufficient mice, proliferation and IL-2 secretion of antigen-specific T cells are impaired, but substantial numbers of IFN-γ-producing T cells are generated. In contrast, when Sema4A-sufficient DCs are transferred into Sema4A-deficient mice, proliferation and IL-2 secretion are substantial but IFN-γ-production is defective in antigen-specific T cells. Collectively, DC-derived Sema4A is involved in T cell priming, and T cell-derived Sema4A is required for Th1 differentiation.

TIM-2 as a Sema4A Receptor

Thus far, Sema4A receptor in the nervous system has not been identified. However, Sema4A-deficient mice show severe retinal degeneration, suggesting that Sema4A interacts with a specific receptor(s) in the CNS [43]. On the other hand, in the cardiovascular system, plexin-D1 has been identified as a Sema4A receptor and Sema4A–plexin-D1 interaction suppresses angiogenesis [11]. This interaction modulates VEGF-mediated endothelial cell migration and proliferation intracellularly, by suppressing VEGF–VEGFR2-induced activation of Rac1, Akt, and integrins. In the immune system, T cell immunoglobulin and mucin domain-2 (TIM-2), a type I transmembrane protein, has been identified as a Sema4A-binding partner [20]. The TIM gene family consists of eight members (TIMs 1–8, but 5–8 are predictive) in mouse and three members (TIMs 1, 3, and 4) in human. The chromosomal region containing the TIM family is thought to be associated with allergic diseases because the IL-4 cytokine gene cluster, IL-12p40, gene and IL-12 regulator gene are also included in this region. The TIM locus has been positionally cloned by screening congenic mice for the susceptibility in an asthma disease model [44]. Indeed, genetic polymorphisms in both TIM-1 and TIM-3 correlate with susceptibility to asthma in human. There is also much evidence that TIM family proteins are expressed by immune cells and involved in several phases of immune responses [45–47]. Although the TIM-2 gene is not detected in humans, mouse TIM-2 shares great identity with mouse TIM-1 and is thought to be an orthologue of human TIM-1 [48].

Sema4A binding induces tyrosine phosphorylation of the cytoplasmic tail of TIM-2; therefore, TIM-2 seems to transduce Sema4A signals (Fig. 2b) [20]. TIM-2 is expressed on activated $CD4^+$ T cells and is preferentially upregulated during Th2 differentiation. A study using recombinant soluble TIM-2 suggests that TIM-2 plays a role in the regulation of Th2 responses [49]. Administration of soluble TIM-2 during the initiation and early development of an immune response enhances the production of Th2-type cytokines (IL-4 and IL-10) and inhibits Th1 cytokine, IFN-γ. Furthermore, treatment with soluble TIM-2 delays the development of EAE in SJL mice [49]. In TIM-2-deficient mice, lung inflammation is exacerbated in an obalbumin-induced airway hypersensitivity model, accompanied by dysregulated Th2 responses [50]. This phenotype is quite similar to that of Sema4A-deficient mice. Taken together, it is tempting to speculate that Sema4A–TIM-2 interaction negatively regulates of Th2

responses. However, there are some inconsistencies between these mutant mice (e.g., T cells from TIM-2-deficient mice but not from Sema4A-deficient mice show enhanced basal proliferation), which raises the possibility that Sema4A and/or TIM-2 have other binding partners. Indeed, T cells express some plexin-B family members and plexin-D1 to which Sema4A has binding activity [11].

Sema7A

Sema7A and Sema7A Receptors

Unlike other semaphorins, Sema7A/CD108 is a GPI-anchored protein. Sema7A transcripts are abundantly detected in adult tissues, including the brain, spinal cord, lung, testis, thymus and spleen [51, 52]. In the hematopoietic cells, Sema7A is expressed on erythrocytes and is also known as the John–Milton–Hagen human blood group anigen. In the immune system, Sema7A is predominantly expressed in $CD4^+CD8^+$ thymocytes and activated T cells [52].

Plexin-C1 was initially identified as a receptor for Sema7A [53]. Although the signal transduction pathways that transduce semaphoring–plexin-C1 functions remain largely unknown, plexin-C1 activation by Sema7A decreases integrin-mediated cell attachment and spreading [54]. Notably, however, Sema7A contains an arginine-glysine-asparate (RGD) sequence, a well-conserved integrin-binding motif. Indeed, Sema7A binds β1-integrins to induce axon outgrowth and contributes to lateral olfactory tract formation in the nervous system [55]. Furthermore, Sema7A–β1-integrin interactions promote melanocyte adhesion [54]. Therefore, it seems that Sema7A has opposing roles in regulating cell morphology and adhesion by binding different receptors.

Sema7A is an Initiator of Inflammatory Responses

In the immune system, Sema7A expressed by activated T cells stimulates macrophages to produce proinflammatory cytokines through the α1β1 integrin, also known as very late antigen 1 [56]. Recombinant soluble Sema7A stimulates macrophages to release peroxidase and produce proinflammatory cytokine, including IL-1β, IL-6, and TNFα. Furthermore, soluble Sema7A has much greater potency as a monocyte chemoattractant than canonical chemokines [57]. Sema7A induces phosphorylation of focal adhesion kinase, a direct downstream target of integrin signaling. Inflammatory cytokine production is significantly decreased in coculture of Sema7A-deficient T cell and wild-type macrophages, as well as in coculture of wild-type T cells and integrin α1-deficient macrophages [56]. Another Sema7A receptor plexin-C1 is also expressed in macrophages; however, soluble Sema7A-induced proinflammatory cytokine production is unaffected in macrophages from plexin-C1-deficient mice (unpublished data). Therefore, at least for the T cell–macrophage interactions, α1β1 integrin seems to be the predominant receptor for Sema7A.

In the later phase of T-cell-mediated immunity, antigen-specific effector T cells trigger inflammatory responses by activating macrophages in peripheral tissues. Both secreted and cell-associated factors from effector T cells [e.g., IFN-γ and CD40 ligand (CD40L), respectively] promote macrophage activation, which eliminates of pathogen at the infection focus, and can also lead to tissue destruction in autoimmune or allergic diseases. As a GPI-anchored protein, Sema7A is recruited to lipid rafts that accumulate at the immunological synapse between T cell and macrophage. At the lipid raft, Sema7A interacts with α1β1 integrin. Direct immunization of Sema7A-deficient mice and adoptive transfer of antigen-specific Sema7A-deficient T cells fails to induce T-cell-mediated immune responses such as contact hypersensitivity responses and EAE. Therefore, the interaction of Sema7A with α1β1 integrin is crucial for T-cell-mediated macrophage activation at sites of inflammation (Fig. 2c).

Thus far, IFN-γ and CD40L are the most potent T-cell effector molecules for promoting inflammatory responses in macrophages. However, these molecules require de novo synthesis after antigen recognition by macrophages. Furthermore, expression of CD40 on macrophages requires IFN-γ stimulation [58]. Given that Sema7A directly stimulates macrophages to produce proinflammatory cytokines, it is conceivable that Sema7A is involved in T-cell-macrophage concomitant activation and helps to initiate the inflammatory cascade.

Sema6D

Sema6D–plexin-A1 Interaction in Cardiac Development

Plexin-As are receptors for class III and VI semaphorins. Sema3A is a secreted semaphorin that binds to Nrp-1 to form receptor complex with plexin-A1 and transduce a repulsive axon guidance signal. On the other hand, Sema6D directly binds to plexin-A1 and exerts multiple biological effects. Both Sema6D and plexin-A1 are abundantly expressed in embryonic and adult tissues. Accumulating evidence has revealed a unique and complicated mechanism of Sema6D–plexin-A1 interaction during cardiac development. Ectopic Sema6D expression in chick embryo induces ventricular expansion with a decreased density of trabeculae. By contrast, knockdown of Sema6D results in

distorted bending of the cardiac tube. Sema6D inhibits the migration of ventricular endocardial cells but conversely enhances the migration of cells in the conotruncus region. Knockdown of plexin-A1 restores these defects to normal, suggesting that Sema6D–plexin-A1 interaction is crucial for cardiac development. Plexin-A1 forms a receptor complex with VEGFR2 in the conotruncal segment and with Otk (PTK7) in the ventricle segment to exert distinct biological effects [18]. Furthermore, Sema6D–plexin-A1 binding triggers the recruitment of activated tyrosine kinase Abl to the cytoplasmic domain of Sema6D during cardiac ventricle development, suggesting a reverse signaling of Sema6D [59].

Sema6D–plexin-A1 in DCs and Osteoclasts

In the immune system, various lymphocyte populations express Sema6D, including T, B, and NK cells. Plexin-A1 is one of the gene products induced by MHC class II transactivator, a master coactivator of MHC class II genes expressed in DCs, indicating that Sema6D–plexin-A1 engagement might be involved in T cell–DC interaction [60].

Production of IL-12 and expression of MHC class II are increased in DCs stimulated with recombinant soluble Sema6D although not in plexin-A1-deficient DCs. In addition, knockdown of plexin-A1 in DCs decreases the ability to prime T cells. Consistently, T-cell-mediated immunity is severely impaired in plexin-A1-deficient mice. These mice are resistant to MOG-induced EAE because of the defective generation of MOG-specific T cells [61]. These observations suggest that Sema6D on T cells stimulate DCs through plexin-A1, and this interaction is required for the efficient generation of antigen-specific T cells.

Plexin-A1 can associate with a variety of molecules to transduce intracellular signals. Recent study has identified that the triggering receptor expressed on myeloid cell-2 (TREM-2)-DAP12 complex associate with plexin-A1 in DCs and osteoclasts [61]. DAP12, a transmembrane adapter protein well known for its role in transducing activation signals, contains an immunoreceptor tyrosine-based activation motif in its cytoplasmic tail and recruits Src-like tyrosine kinases such as ZAP-70 and Syk. DAP12 is expressed in immune cells, such as NK cells and myeloid cells, and in osteoclasts and oligodendrocytes. Killer activity and ability of T-cell priming ability is decreased in DAP12-deficient NK cells and DCs, respectively [62, 63]. Furthermore, DAP12-deficient mice develop osteopetrosis and hypomyelinosis [64, 65]. Genetic mutations of human DAP12 result in a syndrome characterized by bone cysts and presentile dementia called Nasu–Hakola disease, also known as polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy. Interestingly, plexin-A1-deficient mice also develop severe osteopetrosis due to impaired osteoclastogenesis [61]. The similarity of DAP12-deficient mice and plexin-A1-deficient mice phenotypes indicates that DAP12 might mediate plexin-A1 signaling in both DCs and osteoclasts (Fig. 2b).

Sema6D and Late Phase T-Cell Responses

Recently, O'Connor et al. [66] reported that Sema6D is highly detectable in $CD4^+$ T cells activated for 4 days. Blocking the Sema6D–Sema6D-ligand interaction by monoclonal antibody or recombinant soluble Sema6D protein decreases late-phase proliferation and CD127 induction of $CD4^+$ T cells. Although the interaction partner for Sema6D at the late phase of T cells is unknown, Sema6D on $CD4^+$ T cells may accelerate late phase T-cell responses.

Other Semaphorins and Semaphorin Receptors

Virus-Encoded Semaphorins

Viruses encode proteins in their own genomes that facilitate their transmission. Vaccinia virus semaphorin A39R/SemaVA, which only contains a small truncated extracellular sema domain, binds to plexin-C1 and induces aggregation, cytokine production, and surface expression of ICAM-1 (CD54) in human monocytes [67]. In addition, A39R suppresses integrin-mediated adhesion and migration of DCs and monocytes toward virus-infected cells [68]. A39R also interferes with phagocytosis by DCs [69]. Therefore, viral semaphorins might prevent DCs from acquiring antigens and/or suppress DC migration to lymph nodes to suppress immune cell function and provide a means for viruses to evade immune surveillance by suppressing immune cell functions.

Sema3A and its Immunosuppressive Roles

Sema3A was the first identified vertebrate semaphorin; its function as an axon repellent has been well established. Sema3A directly binds to Nrp-1, which induces activation of plexin-A1 and transduction of axon repulsive signals. Biological activity of Sema3A in the immune system has also been described. Consistent with its chemorepulsive effect on neurons, Sema3A inhibits spontaneous monocyte migration in vitro. Sema3A is expressed in activated DCs, T cells and some tumor cells and suppresses T-cell proliferation by inhibiting actin cytoskeletal reorganization and downregulating mitogen-activated protein kinases signaling [70]. Sema3A stimulation induces Fas transloca-

tion into lipid rafts and sensitizes Fas-mediated apoptosis in leukemic cells [71]. Moreover, Sema3A-deficient T cells exhibit enhanced proliferative responses to anti-CD3 [72]. These observations suggest that Sema3A serves as a negative regulator of T cells through autocrine/paracrine signaling.

Plexin-A4, a Candidate Receptor for Sema3A in the Immune System

Similar to other plexin A members, Plexin-A4 forms a receptor complex with Nrp1 to transduce class III semaphorin-mediated signaling. Plexin-A4 also directly binds to Sema6A [73]. Of the various immune cells, T cells, DCs, and macrophages, but not B and NK cells, express plexin-A4 transcripts. T-cell priming is enhanced, and EAE is exacerbated in plexin-A4-deficient mice. Hyperproliferation and enhanced TCR signals upon anti-CD3 stimulation are observed in plexin-A4-deficient T cells, comparable to that in Sema3A-deficient T cells [72]. Sema6A deficiency does not affect T-cell proliferation (unpublished data); therefore, it is tempting to speculate that Sema3A might be a major ligand for plexin-A4 in the immune system and that system negatively regulates T-cell responses. However, the detailed molecular mechanisms through which plexin-A4 regulates T cells and the relevance of other plexin-A members require further investigation.

Nrp-1/CD304 and Regulatory T Cells

Nrp-1 was originally described as a cell surface glycoprotein that acts as a class III semaphorin receptor, as described above. Nrp-1 is also known as human dendritic cell-specific antigen (blood dendritic cell antigen)-4, a specific plasmacytoid DC marker in humans and was assigned a CD number 304 in 2004. Nrp-1 is detectable in conventional mouse DCs. Tordjman et al. [74] first determined in the immune system that Nrp-1 is expressed in DCs and T cells and is involved in the initiation of primary immune responses. They showed that Nrp-1 localizes into the contact sites between T cells and DCs and proposed that Nrp-1 acts through a homophilic interaction. Later, Nrp-1 was identified as a specific marker for $CD4^{+}CD25^{+}$ regulatory T (Treg) cells [75]. Nrp-1 is part of the group of Foxp3-inducible genes, including CD25, GITR, and CTLA-4 [76]. The biological function of Nrp-1 in Treg cells has recently been described. Sarris et al. [77] revealed that Nrp-1 in Treg cells contributes to the long contact between Treg cells and DCs compared to the contact between naive T cells and DCs. Treg cells make stable contact with DCs that precede the contact of naive T cells with DCs, which might lead to the inhibition of T-cell activation in the steady state. Nrp-1-transduced naive T cells are endowed with the ability to have long interactions with DCs that are comparable to those between Treg cells and DCs [77]. However, whether the long contact is mediated by a homophilic interaction, or by semaphorins and Nrp-1 or Nrp-1-associating molecules such as plexins, remains to be elucidated.

Concluding Remarks

Accumulating evidence reveals that several semaphorins and their receptors have distinct biological activities in various phases of the immune responses ([78–80], see Table 1).

Table 1 Immune Semaphorins and their Functions

Class	Semaphorins	Expression in the immune system	Receptor	Receptor-mediated activity	References
III	3A	T, DC (activated)	Nrp-1-plexin-A1	Monocyte migration↓	[78]
			Nrp-1-plexin-A4?	T cell activation↓	[70–72]
IV	4A	DC, T (activated), Th1	TIM-2	T cell activation↑	[20]
				Th1 differentiation↑(or Th2 ↓)	[42]
	4D	T (high), B, DC (low), platelet, NK	CD72	DC activation↑	[37]
				B cell activation↑	[25, 32]
				NK cell killing activity↑	[80]
				thrombosis↑	[79]
			Plexin-B1	T cell-mediated neuroinflammation↑	[41]
VI	6D	DC, CD4+ T (long-term activated) NK, osteoclast	Plexin-A1	DC activation↑	[61]
				Osteoclastogenesis↑	[61]
			Plexin-A1?	Late phase CD4+ T cell response↑	[66]
VII	7A	CD4+ CD8+ thymocyte, T (activated), NK	α1b1integrin	Macrophage/monocyte activation↑	[56, 57]
				Monocyte migration↑	[57]
VIII (Viral)	A39R		Plexin-C1	DC and monocyte migration↓	[68]
				DC phagocytosis↓	[69]

These semaphorins form a family of immunoregulatory molecules, called 'immune semaphorins'. Lack of semaphorin family proteins results in several immune disorders, including autoimmune diseases, allergy, and congenital bone disease. Alternatively, lack of these proteins induces unresponsiveness to physiological immune responses. Therefore, semaphorin family proteins are at least responsible for the maintenance of immunological homeostasis, based on the sophisticated immune cell communication system. However, several important issues remain to be resolved. Although semaphorins function to regulate cell motility and morphology by activating plexins, most of the immunological studies of semaphorins have only focused on their costimulatory effects. Several functional receptors other than plexins have been identified in the immune system; however, it still remains a possibility that semaphorins exert their functions by affecting cell cytoskeleton. In addition, several lines of evidence indicate that transmembrane semaphorins serve not only as ligands but also as receptors, a process termed bidirectional signaling. This semaphorin-mediated backward signaling may also influence immune cell reactions. Further studies are required to clarify the role of semaphorins in immune cell morphology and dynamics. Finally, understanding of the immune semaphorins should allow pharmacological modulation of their functions leading to potential therapeutic targets for several immune diseases.

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References

1. Steinman L. Elaborate interactions between the immune and nervous systems. Nat Immunol. 2004;5:575–81. doi:10.1038/ni1078.
2. Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell 1993;75:1389–99. doi:10.1016/0092-8674(93)90625-Z.
3. Semaphorin Nomenclature Committee. Unified nomenclature for the semaphorins/collapsins. Cell 1999;97:551–2. doi:10.1016/S0092-8674(00)80766-7.
4. Zhou Y, Gunput RA, Pasterkamp RJ. Semaphorin signaling: progress made and promises ahead. Trends Biochem Sci. 2008;33:161–70. doi:10.1016/j.tibs.2008.01.006.
5. Tamagnone L, Artigiani S, Chen H, He Z, Ming GI, Song H, et al. Plexins are a large family of receptors for transmembrane, secreted, and GPI-anchored semaphorins in vertebrates. Cell 1999;99:71–80. doi:10.1016/S0092-8674(00)80063-X.
6. Winberg ML, Noordermeer JN, Tamagnone L, Comoglio PM, Spriggs MK, Tessier-Lavigne M, et al. Plexin A is a neuronal semaphorin receptor that controls axon guidance. Cell 1998;95:903–16. doi:10.1016/S0092-8674(00)81715-8.
7. Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. Nat Rev Mol Cell Biol. 2005;6:789–800. doi:10.1038/nrm1740.
8. Takahashi T, Fournier A, Nakamura F, Wang LH, Murakami Y, Kalb RG, et al. Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell 1999;99:59–69. doi:10.1016/S0092-8674(00)80062-8.
9. Toyofuku T, Kikutani H. Semaphorin signaling during cardiac development. Adv Exp Med Biol. 2007;600:109–17. doi:10.1007/978-0-387-70956-7_9.
10. Geretti E, Shimizu A, Klagsbrun M. Neuropilin structure governs VEGF and semaphorin binding and regulates angiogenesis. Angiogenesis 2008;11:31–9. doi:10.1007/s10456-008-9097-1.
11. Toyofuku T, Yabuki M, Kamei J, Kamei M, Makino N, Kumanogoh A, et al. Semaphorin-4A, an activator for T-cell-mediated immunity, suppresses angiogenesis via Plexin-D1. EMBO J. 2007;26:1373–84. doi:10.1038/sj.emboj.7601589.
12. Casazza A, Fazzari P, Tamagnone L. Semaphorin signals in cell adhesion and cell migration: functional role and molecular mechanisms. Adv Exp Med Biol. 2007;600:90–108. doi:10.1007/978-0-387-70956-7_8.
13. Giordano S, Corso S, Conrotto P, Artigiani S, Gilestro G, Barberis D, et al. The semaphorin 4D receptor controls invasive growth by coupling with Met. Nat Cell Biol. 2002;4:720–4. doi:10.1038/ncb843.
14. Bielenberg DR, Klagsbrun M. Targeting endothelial and tumor cells with semaphorins. Cancer Metastasis Rev. 2007;26:421–31. doi:10.1007/s10555-007-9097-4.
15. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. Nat Immunol. 2008;9:17–23. doi:10.1038/ni1553.
16. Kikutani H, Suzuki K, Kumanogoh A. Immune semaphorins: increasing members and their diverse roles. Adv Immunol. 2007;93:121–43. doi:10.1016/S0065-2776(06)93003-X.
17. Kikutani H, Kumanogoh A. Semaphorins in interactions between T cells and antigen-presenting cells. Nat Rev Immunol. 2003;3:159–67. doi:10.1038/nri1003.
18. Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Suto F, Kamei J, et al. Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. Genes Dev. 2004;18:435–47. doi:10.1101/gad.1167304.
19. Kumanogoh A, Watanabe C, Lee I, Wang X, Shi W, Araki H, et al. Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity 2000;13:621–31. doi:10.1016/S1074-7613(00)00062-5.
20. Kumanogoh A, Marukawa S, Suzuki K, Takegahara N, Watanabe C, Ch, ng E, et al. Class IV semaphorin Sema4A enhances T-cell activation and interacts with Tim-2. Nature 2002;419:629–33. doi:10.1038/nature01037.
21. Hall KT, Boumsell L, Schultze JL, Boussiotis VA, Dorfman DM, Cardoso AA, et al. Human CD100, a novel leukocyte semaphorin that promotes B-cell aggregation and differentiation. Proc Natl Acad Sci USA. 1996;93:11780–5. doi:10.1073/pnas.93.21.11780.
22. Furuyama T, Inagaki S, Kosugi A, Noda S, Saitoh S, Ogata M, et al. Identification of a novel transmembrane semaphorin expressed on lymphocytes. J Biol Chem. 1996;271:33376–81. doi:10.1074/jbc.271.52.33376.
23. Dorfman DM, Shahsafaei A, Nadler LM, Freeman GJ. The leukocyte semaphorin CD100 is expressed in most T-cell, but few B-cell, non-Hodgkin's lymphomas. Am J Pathol. 1998;153:255–62.
24. Delaire S, Elhabazi A, Bensussan A, Boumsell L. CD100 is a leukocyte semaphorin. Cell Mol Life Sci. 1998;54:1265–76. doi:10.1007/s000180050252.

25. Shi W, Kumanogoh A, Watanabe C, Uchida J, Wang X, Yasui T, et al. The class IV semaphorin CD100 plays nonredundant roles in the immune system: defective B and T cell activation in CD100-deficient mice. Immunity 2000;13:633–42. doi:10.1016/S1074-7613(00)00063-7.
26. Oinuma I, Ishikawa Y, Katoh H, Negishi M. The semaphorin 4D receptor Plexin-B1 is a GTPase activating protein for R-Ras. Science 2004;305:862–5. doi:10.1126/science.1097545.
27. Adachi T, Flaswinkel H, Yakura H, Reth M, Tsubata T. The B cell surface protein CD72 recruits the tyrosine phosphatase SHP-1 upon tyrosine phosphorylation. J Immunol. 1998;160:4662–5.
28. Parnes JR, Pan C. CD72, a negative regulator of B-cell responsiveness. Immunol Rev. 2000;176:75–85. doi:10.1034/j.1600-065X.2000.00608.x.
29. Pan C, Baumgarth N, Parnes JR. CD72-deficient mice reveal nonredundant roles of CD72 in B cell development and activation. Immunity 1999;11:495–506. doi:10.1016/S1074-7613(00)80124-7.
30. Kumanogoh A, Kikutani H. The CD100-CD72 interaction: a novel mechanism of immune regulation. Trends Immunol. 2001;22:670–6. doi:10.1016/S1471-4906(01)02087-7.
31. Niiro H, Clark EA. Regulation of B-cell fate by antigen-receptor signals. Nat Rev Immunol. 2002;2:945–56. doi:10.1038/nri955.
32. Kumanogoh A, Shikina T, Watanabe C, Takegahara N, Suzuki K, Yamamoto M, et al. Requirement for CD100-CD72 interactions in fine-tuning of B-cell antigen receptor signaling and homeostatic maintenance of the B-cell compartment. Int Immunol. 2005;17:1277–82. doi:10.1093/intimm/dxh307.
33. Samardzic T, Marinkovic D, Danzer CP, Gerlach J, Nitschke L, Wirth T. Reduction of marginal zone B cells in CD22-deficient mice. Eur J Immunol. 2002;32:561–7. doi:10.1002/1521-4141(200202)32:2<561::AID-IMMU561>3.0.CO;2-H.
34. Lopes-Carvalho T, Kearney JF. Development and selection of marginal zone B cells. Immunol Rev. 2004;197:192–205. doi:10.1111/j.0105-2896.2004.0112.x.
35. Li DH, Winslow MM, Cao TM, Chen AH, Davis CR, Mellins ED, et al. Modulation of peripheral B cell tolerance by CD72 in a murine model. Arthritis Rheum. 2008;58:3192–204. doi:10.1002/art.23812.
36. Ishida I, Kumanogoh A, Suzuki K, Akahani S, Noda K, Kikutani H. Involvement of CD100, a lymphocyte semaphorin, in the activation of the human immune system via CD72: implications for the regulation of immune and inflammatory responses. Int Immunol. 2003;15:1027–34. doi:10.1093/intimm/dxg098.
37. Kumanogoh A, Suzuki K, Ch'ng E, Watanabe C, Marukawa S, Takegahara N, et al. Requirement for the lymphocyte semaphorin, CD100, in the induction of antigen-specific T cells and the maturation of dendritic cells. J Immunol. 2002;169:1175–81.
38. Li M, O'Sullivan KM, Jones LK, Semple T, Kumanogoh A, Kikutani H, et al. CD100 enhances dendritic cell and CD4+ cell activation leading to pathogenetic humoral responses and immune complex glomerulonephritis. J Immunol. 2006;177:3406–12.
39. Wang X, Kumanogoh A, Watanabe C, Shi W, Yoshida K, Kikutani H. Functional soluble CD100/Sema4D released from activated lymphocytes: possible role in normal and pathologic immune responses. Blood 2001;97:3498–504. doi:10.1182/blood.V97.11.3498.
40. Watanabe C, Kumanogoh A, Shi W, Suzuki K, Yamada S, Okabe M, et al. Enhanced immune responses in transgenic mice expressing a truncated form of the lymphocyte semaphorin CD100. J Immunol. 2001;167:4321–8.
41. Giraudon P, Vincent P, Vuaillat C, Verlaeten O, Cartier L, Marie-Cardine A, et al. Semaphorin CD100 from activated T lymphocytes induces process extension collapse in oligodendrocytes and death of immature neural cells. J Immunol. 2004;172:1246–55.
42. Kumanogoh A, Shikina T, Suzuki K, Uematsu S, Yukawa K, Kashiwamura S, et al. Nonredundant roles of Sema4A in the immune system: defective T cell priming and Th1/Th2 regulation in Sema4A-deficient mice. Immunity 2005;22:305–16. doi:10.1016/j.immuni.2005.01.014.
43. Rice DS, Huang W, Jones HA, Hansen G, Ye GL, Xu N, et al. Severe retinal degeneration associated with disruption of semaphorin 4A. Invest Ophthalmol Vis Sci. 2004;45:2767–77. doi:10.1167/iovs.04-0020.
44. McIntire JJ, Umetsu SE, Akbari O, Potter M, Kuchroo VK, Barsh GS, et al. Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. Nat Immunol. 2001;2:1109–16. doi:10.1038/ni739.
45. Kuchroo VK, Dardalhon V, Xiao S, Anderson AC. New roles for TIM family members in immune regulation. Nat Rev Immunol. 2008;8:577–80. doi:10.1038/nri2366.
46. Meyers JH, Sabatos CA, Chakravarti S, Kuchroo VK. The TIM gene family regulates autoimmune and allergic diseases. Trends Mol Med. 2005;11:362–9. doi:10.1016/j.molmed.2005.06.008.
47. Mizui M, Shikina T, Arase H, Suzuki K, Yasui T, Rennert PD, et al. Bimodal regulation of T cell-mediated immune responses by TIM-4. Int Immunol. 2008;20:695–708. doi:10.1093/intimm/dxn029.
48. Kuchroo VK, Umetsu DT, DeKruyff RH, Freeman GJ. The TIM gene family: emerging roles in immunity and disease. Nat Rev Immunol. 2003;3:454–62. doi:10.1038/nri1111.
49. Chakravarti S, Sabatos CA, Xiao S, Illes Z, Cha EK, Sobel RA, et al. Tim-2 regulates T helper type 2 responses and autoimmunity. J Exp Med. 2005;202:437–44. doi:10.1084/jem.20050308.
50. Rennert PD, Ichimura T, Sizing ID, Bailly V, Li Z, Rennard R, et al. T cell, Ig domain, mucin domain-2 gene-deficient mice reveal a novel mechanism for the regulation of Th2 immune responses and airway inflammation. J Immunol. 2006;177:4311–21.
51. Yamada A, Kubo K, Takeshita T, Harashima N, Kawano K, Mine T, et al. Molecular cloning of a glycosylphosphatidylinositol-anchored molecule CDw108. J Immunol. 1999;162:4094–100.
52. Mine T, Harada K, Matsumoto T, Yamana H, Shirouzu K, Itoh K, et al. CDw108 expression during T-cell development. Tissue Antigens. 2000;55:429–36. doi:10.1034/j.1399-0039.2000.550505.x.
53. Tamagnone L, Comoglio PM. Signalling by semaphorin receptors: cell guidance and beyond. Trends Cell Biol. 2000;10:377–83. doi:10.1016/S0962-8924(00)01816-X.
54. Scott GA, McClelland LA, Fricke AF. Semaphorin 7a promotes spreading and dendricity in human melanocytes through beta1-integrins. J Invest Dermatol. 2008;128:151–61. doi:10.1038/sj.jid.5700974.
55. Pasterkamp RJ, Peschon JJ, Spriggs MK, Kolodkin AL. Semaphorin 7A promotes axon outgrowth through integrins and MAPKs. Nature 2003;424:398–405. doi:10.1038/nature01790.
56. Suzuki K, Okuno T, Yamamoto M, Pasterkamp RJ, Takegahara N, Takamatsu H, et al. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. Nature 2007;446:680–4. doi:10.1038/nature05652.
57. Holmes S, Downs AM, Fosberry A, Hayes PD, Michalovich D, Murdoch P, et al. Sema7A is a potent monocyte stimulator. Scand J Immunol. 2002;56:270–5. doi:10.1046/j.1365-3083.2002.01129.x.
58. Alderson MR, Armitage RJ, Tough TW, Strockbine L, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. J Exp Med. 1993;178:669–74. doi:10.1084/jem.178.2.669.
59. Toyofuku T, Zhang H, Kumanogoh A, Takegahara N, Yabuki M, Harada K, et al. Guidance of myocardial patterning in cardiac development by Sema6D reverse signalling. Nat Cell Biol. 2004;6:1204–11. doi:10.1038/ncb1193.
60. Wong AW, Brickey WJ, Taxman DJ, van Deventer HW, Reed W, Gao JX, et al. CIITA-regulated plexin-A1 affects T-cell-dendritic cell interactions. Nat Immunol. 2003;4:891–8. doi:10.1038/ni960.

61. Takegahara N, Takamatsu H, Toyofuku T, Tsujimura T, Okuno T, Yukawa K, et al. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat Cell Biol. 2006;8:615–22. doi:10.1038/ncb1416.
62. Tomasello E, Desmoulins PO, Chemin K, Guia S, Cremer H, Ortaldo J, et al. Combined natural killer cell and dendritic cell functional deficiency in KARAP/DAP12 loss-of-function mutant mice. Immunity 2000;13:355–64. doi:10.1016/S1074-7613(00)00035-2.
63. Zompi S, Hamerman JA, Ogasawara K, Schweighoffer E, Tybulewicz VL, Di Santo JP, et al. NKG2D triggers cytotoxicity in mouse NK cells lacking DAP12 or Syk family kinases. Nat Immunol. 2003;4:565–72. doi:10.1038/ni930.
64. Bakker AB, Hoek RM, Cerwenka A, Blom B, Lucian L, McNeil T, et al. DAP12-deficient mice fail to develop autoimmunity due to impaired antigen priming. Immunity 2000;13:345–53. doi:10.1016/S1074-7613(00)00034-0.
65. Kaifu T, Nakahara J, Inui M, Mishima K, Momiyama T, Kaji M, et al. Osteopetrosis and thalamic hypomyelinosis with synaptic degeneration in DAP12-deficient mice. J Clin Invest. 2003;111:323–32.
66. O'Connor BP, Eun SY, Ye Z, Zozulya AL, Lich JD, Moore CB, et al. Semaphorin 6D regulates the late phase of CD4+ T cell primary immune responses. Proc Natl Acad Sci USA. 2008;105:13015–20. doi:10.1073/pnas.0803386105.
67. Comeau MR, Johnson R, DuBose RF, Petersen M, Gearing P, VandenBos T, et al. A poxvirus-encoded semaphorin induces cytokine production from monocytes and binds to a novel cellular semaphorin receptor, VESPR. Immunity 1998;8:473–82. doi:10.1016/S1074-7613(00)80552-X.
68. Walzer T, Galibert L, Comeau MR, De Smedt T. Plexin C1 engagement on mouse dendritic cells by viral semaphorin A39R induces actin cytoskeleton rearrangement and inhibits integrin-mediated adhesion and chemokine-induced migration. J Immunol. 2005;174:51–9.
69. Walzer T, Galibert L, De Smedt T. Poxvirus semaphorin A39R inhibits phagocytosis by dendritic cells and neutrophils. Eur J Immunol. 2005;35:391–8. doi:10.1002/eji.200425669.
70. Lepelletier Y, Moura IC, Hadj-Slimane R, Renand A, Fiorentino S, Baude C, et al. Immunosuppressive role of semaphorin-3A on T cell proliferation is mediated by inhibition of actin cytoskeleton reorganization. Eur J Immunol. 2006;36:1782–93. doi:10.1002/eji.200535601.
71. Moretti S, Procopio A, Lazzarini R, Rippo MR, Testa R, Marra M, et al. Semaphorin3A signaling controls Fas (CD95)-mediated apoptosis by promoting Fas translocation into lipid rafts. Blood 2008;111:2290–9. doi:10.1182/blood-2007-06-096529.
72. Yamamoto M, Suzuki K, Okuno T, Ogata T, Takegahara N, Takamatsu H, et al. Plexin-A4 negatively regulates T lymphocyte responses. Int Immunol. 2008;20:413–20. doi:10.1093/intimm/dxn006.
73. Suto F, Tsuboi M, Kamiya H, Mizuno H, Kiyama Y, Komai S, et al. Interactions between plexin-A2, plexin-A4, and semaphorin 6A control lamina-restricted projection of hippocampal mossy fibers. Neuron 2007;53:535–47. doi:10.1016/j.neuron.2007.01.028.
74. Tordjman R, Lepelletier Y, Lemarchandel V, Cambot M, Gaulard P, Hermine O, et al. A neuronal receptor, neuropilin-1, is essential for the initiation of the primary immune response. Nat Immunol. 2002;3:477–82.
75. Bruder D, Probst-Kepper M, Westendorf AM, Geffers R, Beissert S, Loser K, et al. Neuropilin-1: a surface marker of regulatory T cells. Eur J Immunol. 2004;34:623–30. doi:10.1002/eji.200324799.
76. Hill JA, Feuerer M, Tash K, Haxhinasto S, Perez J, Melamed R, et al. Foxp3 transcription-factor-dependent and -independent regulation of the regulatory T cell transcriptional signature. Immunity 2007;27:786–800.
77. Sarris M, Andersen KG, Randow F, Mayr L, Betz AG. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. Immunity 2008;28:402–13. doi:10.1016/j.immuni.2008.01.012.
78. Delaire S, Billard C, Tordjman R, Chedotal A, Elhabazi A, Bensussan A, et al. Biological activity of soluble CD100. II. Soluble CD100, similarly to H-SemaIII, inhibits immune cell migration. J Immunol. 2001;166:4348–54.
79. Zhu L, Bergmeier W, Wu J, Jiang H, Stalker TJ, Cieslak M, et al. Regulated surface expression and shedding support a dual role for semaphorin 4D in platelet responses to vascular injury. Proc Natl Acad Sci USA. 2007;104:1621–6. doi:10.1073/pnas.0606344104.
80. Mizrahi S, Markel G, Porgador A, Bushkin Y, Mandelboim O. CD100 on NK cells enhance IFNgamma secretion and killing of target cells expressing CD72. PLoS One. 2007;2:e818. doi:10.1371/journal.pone.0000818.