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REVIEW

Semaphorins and their receptors in immune cell interactions

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Semaphorins are newcomers to the growing panoply of immunoregulatory proteins. Members of this family were originally identified as proteins that provide axonal guidance cues during neuronal development. However, accumulating evidence indicates that several semaphorins, called 'immune semaphorins', are crucial to various phases of the immune response, from initiation to terminal inflammatory processes. Extensive studies of immune semaphorins have shown not only differences but also parallels in semaphorin functions among physiologically distinct systems, providing unexpected but meaningful insights into the biological activities of this protein family. Here we review the present knowledge of the function of semaphorins and their receptors in the immune system, including the most recent advances in this field.

Since semaphorins were first described in the early 1990s, more than 20 types of these proteins have been identified as guidance factors that assist axon pathfinding during neuronal development[1,2]. Semaphorins have been also shown to have diverse and important functions in other physiological processes, including heart morphogenesis[3–7], vascular growth[4,5,8,9], tumor progression[10–13] and immune cell regulation[14]. In particular, understanding of the immunoregulatory functions of semaphorin family members has advanced considerably over the past several years.

Semaphorins are secreted and membrane-associated proteins characterized by a conserved amino-terminal 'Sema' domain. On the basis of structural elements and amino acid sequence similarity, this diverse group of proteins has been further divided into eight subclasses[15]. Invertebrate semaphorins are grouped into classes I and II, whereas classes III–VII are expressed in vertebrates[2]. In addition, some DNA viruses encode functional semaphorins that are assigned to class VIII (ref. 16). Semaphorins in classes I and IV–VII are membrane associated, whereas those in classes II and III and the viral semaphorins are secreted. Two groups of proteins, plexins and neuropilins, have been identified as the main receptors for semaphorins[17–21]. Most membrane-bound semaphorins directly bind plexins, whereas class III semaphorins require neuropilins as obligate coreceptors. However, it has been suggested that the receptor usage by semaphorins is more complex than previously thought. For example, Sema3E signals independently of neuropilin through plexin-D1 (ref. 8), and Sema7A uses integrin receptors to exert its function in both the nervous and immune systems[22,23]. In addition, two molecules unrelated to plexins and neuropilins, CD72 (ref. 24) and TIM-2 (T cell, immunoglobulin and mucin domain protein 2; ref. 25), functionally interact with class IV semaphorins in the immune system (**Fig. 1** and **Supplementary Table 1** online).

The immune response consists of a series of spatiotemporally well ordered cell-cell interactions, in which immune cells communicate bidirectionally and modulate the functions of interacting cells as well as themselves. So far, studies of the immune semaphorins have shown that several vertebrate and viral semaphorin family members are intimately involved in diverse immune cell interactions. Here we incorporate the present knowledge of immune semaphorins into the context of immune responses.

Sema4D and B cell function

The immune function of semaphorin molecules was first described in B cells through functional analysis of a class IV semaphorin, Sema4D. In the immune system, Sema4D is constitutively expressed on T cells[26,27]. Although B cells have low basal expression of Sema4D, it is upregulated considerably by various stimuli such as lipopolysaccharide and antibody to CD40 (ref. 24). The biological activity of Sema4D in B cells has been confirmed in both human and mouse systems. Transfectants that exogenously express human Sema4D promote the aggregation and survival of human B cells *in vitro*[28]. Recombinant mouse Sema4D and cells expressing Sema4D enhance the CD40- or lipopolysaccharide-induced proliferation and antibody production of mouse B cells[24]. The phenotypes of Sema4D-deficient mice, which have abnormal B cells and altered antibody responses[29], further support the idea that Sema4D is involved in B cell activation in the context of B cell–B cell and B cell–T cell interactions, as discussed below.

The receptor used by Sema4D differs in nonlymphoid versus lymphoid tissues. In the nervous system, Sema4D binds plexin-B1 and exerts chemorepulsive effects on various neurons[21,30,31]. In contrast, the C-type lectin CD72 serves as the functional Sema4D receptor in the

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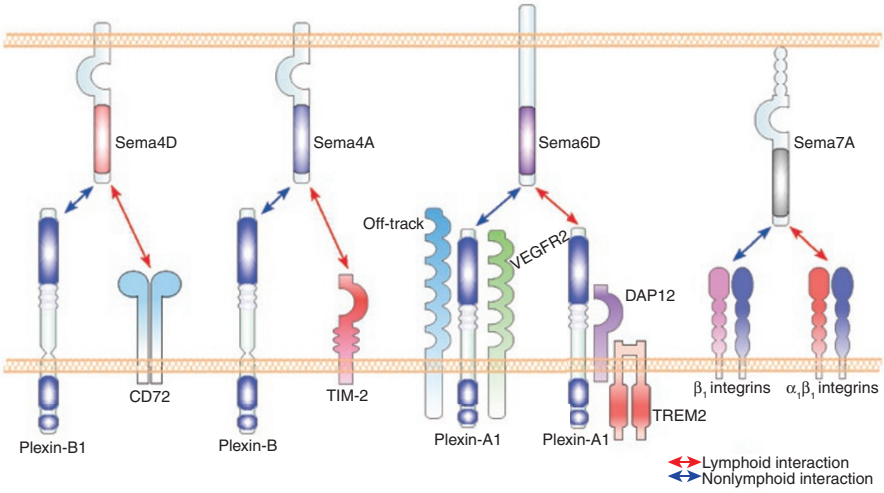

Figure 1 Immune semaphorins and their receptors in lymphoid and nonlymphoid cells. The class IV semaphorin Sema4D binds plexin-B1 and induces chemorepulsive signals in neuronal cells. In the immune system, however, Sema4D uses CD72 as a functional receptor and enhances the activation of B cells and DCs by diminishing inhibitory signals from CD72. Sema4A, another class IV semaphorin, binds TIM-2, and this interaction is critical for T cell activation and differentiation. Although Sema4A might interact with receptors belonging to plexin-B subfamily, the functional importance of these interactions remains unknown. Sema6D exerts different biological activities through plexin-A1, depending on its coreceptors. During chick embryogenesis, plexin-A1 differentially associates with off-track and VEGFR2, and these receptor complexes have distinct functions in heart development. In the immune system, plexin-A1 forms a receptor complex with TREM2 and DAP12 and, after Sema6D binds, this complex transduces signals that stimulate DCs and osteoclasts. The function of the glycosyl phosphatidylinositol-anchored semaphorin Sema7A is mediated through β_1 integrin receptors in both the nervous and immune systems. Sema7A expressed on activated T cells stimulates macrophages through $\alpha_1\beta_1$ integrin to promote inflammatory responses.

immune system[24]. CD72 contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in its cytoplasmic domain and functions as a negative regulator of B cells[32,33]. The regulatory function of CD72 is mediated by the tyrosine phosphatase SHP-1, which is recruited to the phosphorylated ITIMs of CD72. SHP-1 associates with the ITIM regions of several inhibitory receptors, such as CD22 and the KIRs (killer cell immunoglobulin-like receptors), induces dephosphorylation of their signaling proteins and inhibits immune cell activation[34–36]. Several lines of evidence indicate that Sema4D enhances B cell activation by turning off CD72 inhibitory signals. Indeed, both soluble Sema4D[24] and agonistic CD72-specific monoclonal antibodies[32,37] block tyrosine phosphorylation of CD72 and its association with SHP-1 in B cells stimulated with antibody to immunoglobin M. Furthermore, CD72 is constitutively phosphorylated and associated with SHP-1 in Sema4D-deficient B cells, which confers the hyporesponsiveness of these cells[29]. Moreover, Sema4D ligation inhibits the physical association of CD72 with the B cell receptor (BCR) complex[38]. This sequestration of CD72 away from the kinase-rich BCR signalosome might reduce CD72 phosphorylation. Therefore, Sema4D binding induces the dissociation of CD72 from the BCR complex, leading to dephosphorylation of the CD72 ITIM and dissociation of SHP-1 from CD72. This mechanism might be important for controlling the strength of BCR signals, but it remains unknown whether the same mechanism also accounts for the Sema4D-mediated regulation of CD40 or Toll-like receptor 4 signaling.

Several studies of mice with altered signaling molecules 'downstream' of the BCR have shown that changes in the BCR activation threshold affect B cell survival and turnover *in vivo*[39]. Indeed, Sema4D is involved in the homeostatic maintenance of B cell subsets. In young Sema4D-deficient mice, the $CD5^+$ B-1 cell population is smaller, although other B cell subsets seem normal[29]. However, as these mutant mice age, the proportion of $CD21^{hi}CD23^{lo}$ marginal zone B cells gradually increases[38]. Population expansion of marginal zone B cells is often found in mice with defective BCR signaling[39,40], whereas B-1 cell numbers are greater in mice lacking inhibitory receptors, including CD72 (refs. 41–43). This suggests that the requirements for BCR signaling vary among different B cell subsets. Therefore, the higher BCR signaling threshold in Sema4D-deficient mice may promote the development and survival of marginal zone B cells but may be detrimental for the development of B-1 cells. In homeostatic conditions, Sema4D is weakly expressed on resting B cells and might be involved in the maintenance of certain B cell subsets by 'fine tuning' BCR signals. In such circumstances, Sema4D signaling may occur through B cell–B cell interactions (**Fig. 2a**). Notably, the population expansion of marginal zone B cells in Sema4D-deficient mice is accompanied by the development of autoimmunity[38]. A variety of autoantibodies is detectable in the serum of these older mutant mice. Furthermore, leukocytes substantially infiltrate many tissues, including the salivary gland, liver and kidney. Notably, $CD21^{hi}CD23^{lo}$ marginal zone B cells comprise most of the infiltrating cells and are the main producers of autoantibodies.

In addition to a function in controlling B cell homeostasis, the profiles of Sema4D and CD72 expression in lymphocyte populations and the additional phenotypic characteristics of Sema4D-deficient mice suggest that the Sema4D-CD72 interaction is also involved in the development of B cell–mediated immunity. T cell–dependent antibody responses are initiated when B cells encounter helper T cells recognizing the same antigen at the boundary between B cell follicles and T cell zones in secondary lymphoid organs. Here, these cells engage in cognate interactions in which CD40-meditated signaling is indispensable for efficient B cell activation. Sema4D and CD72 are 'preferentially' expressed on T cells and B cells, respectively, and Sema4D enhances CD40-dependent B cell activation through CD72 (ref. 24). Moreover, T cell–dependent antibody responses are considerably impaired in Sema4D-deficient mice[29]. Given such observations, it is conceivable that Sema4D-CD72 interactions are involved in T cell–B cell interactions in the extrafollicular areas (**Fig. 2b**). Some activated B cells migrate into lymphoid follicles and form germinal centers where high-affinity B cells are selected with the provision of T cell–derived help. Sema4D expression in B cells is strongly induced by CD40 stimulation[24], and in fact Sema4D is reported to be expressed on germinal center B cells[28]. It is also noteworthy that Sema4D-deficient mice immunized with T cell–dependent antigens show defective antibody affinity maturation and poor generation of antigen-specific germinal center B cells[29]. Therefore, Sema4D-CD72 interactions might contribute to the population expansion of germinal center B cells through interactions between B cells (**Fig, 2a**) and support the selection of high-affinity B cells by enhancing survival signals from helper T cells (**Fig. 2b**).

Semaphorins and dendritic cell function

Extensive analyses of Sema4D-deficient mice have shown that Sema4D is also important in T cell–mediated immunity. After immunization with protein antigens, $CD4^+$ T cells from the draining lymph nodes of Sema4D-deficient mice show very impaired proliferative responses and cytokine production after antigen restimulation[29,44]. Moreover, Sema4D-deficient mice are resistant to experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG)–derived peptide, a phenotype ascribed to the defective generation of MOG-specific T cells[44]. Conversely, transgenic mice that overexpress soluble Sema4D have enhanced T cell responses[45]. These observations indicate that Sema4D is crucially involved in the initial activation and differentiation of T cells.

T cells are the main Sema4D-producing cells in the immune system. However, Sema4D-deficient T cells respond normally to CD3-specific monoclonal antibodies or mitogens such as concanavalin A[29], which suggests that Sema4D does not act in a T cell–autonomous way. Although it has been proposed that Sema4D functions as a receptor to assist the proliferative activity of human leukemic cells[46], Sema4D seems to act mainly as a ligand in the mouse immune system. Moreover, soluble recombinant Sema4D does not affect T cell activation[29], which suggests that Sema4D has no direct effect on T cells. In contrast, recombinant Sema4D enhances the surface expression of CD80, CD86 and major histocompatibility complex class II molecules on dendritic cells (DCs) and their immunogenicity that was induced by CD40 stimulation[44]. The function of Sema4D in T cell–DC interactions has been addressed with an *in vitro* experimental system[44]. Sema4D-sufficient T cell receptor (TCR)–transgenic $CD4^+$ T cells differentiate normally into cytokine-secreting effector cells even when cultured with antigen and Sema4D-deficient antigen-presenting cells. In contrast, Sema4D-deficient TCR-transgenic T cells fail to differentiate even in the presence of Sema4D-sufficient antigen-presenting cells. Therefore, Sema4D expressed on T cells acts on DCs to promote their activation and maturation, which in turn enhances T cell activation (**Fig. 3a**). Because most stimulatory effects of Sema4D on DCs are reproduced by agonistic CD72-specific monoclonal antibodies[44], DCs as well as B cells seem to use CD72 as their main Sema4D receptor.

Plexins have established functions as semaphorin receptors in the development of the nervous and cardiovascular systems[47]. However, their function in the immune system have not been addressed until recently. Plexin-A1 is one of the gene products induced by the CIITA transcription factor expressed in DCs and is involved in T cell–DC interactions[48]. 'Knockdown' of plexin-A1 by short hairpin RNA impairs the ability of a DC line to activate T cells *in vitro* and *in vivo*. The function of plexin-A1 in DC functions has been further substantiated through the generation and analysis of plexin-A1-deficient mice[49]. These mutant mice are resistant to MOG-induced EAE because of defective generation of MOG-specific T cells. This defect in T cell–mediated immunity is partially attributed to impaired DC function in these mice. Compared with wild-type DCs, plexin-A1-deficient DCs poorly stimulate allogeneic T cells or TCR-transgenic T cells in the presence of cognate antigens. These observations indicate that plexin-A1 expression in DCs is required for the efficient generation of antigen-specific T cells. In addition to its function in DC activation, plexin-A1 is also involved in osteoclast differentiation. Indeed, plexin-A1-deficient mice develop severe osteopetrosis due to decreased bone resorption that is attributed to defective osteoclastogenesis in these mice.

It is well established that plexin-A1 forms a receptor complex with neuropilin-1 to induce chemorepulsive signals of secreted class III semaphorins[2,20]. Additionally, plexin-A1 also serves as a receptor for Sema6D, a class VI transmembrane semaphorin, and contributes to cardiac morphogenesis[6,7]. Sema6D also functions as a ligand for plexin-A1 in the regulation of DC function[49]. Various lymphocyte populations, including T cells, B cells and natural killer cells, have relatively high expression of Sema6D mRNA. Recombinant Sema6D protein stimulates bone marrow–derived DCs to produce cytokines such as interleukin 12 (IL-12) and to increase expression of major histocompatibility complex class II molecules[49]. These activities and binding of Sema6D on DCs are profoundly attenuated in plexin-A1-deficient DCs[49]. Therefore, Sema6D expressed on T cells might stimulate DCs via plexin-A1 during T cell–DC interactions (**Fig. 3a**).

During chick cardiac morphogenesis, plexin-A1 differentially associates with two receptor-type tyrosine kinases, off-track and VEGFR2, and each receptor complex mediates distinct biological activities of Sema6D[6]. However, plexin-A1 on DCs associates with a complex of the receptor TREM2 and the adaptor DAP12 instead of off-track or VEGFR2 (ref.49). DAP12 contains an immunoreceptor tyrosine-based activation motif in its cytoplasmic region, to which Src-like tyrosine kinases, including Zap70 and Syk, are recruited. In natural killer cells, DAP12 forms a complex with Ly49D, CD94-NKG2C and KIR2DS and acts as a signaling adaptor for these natural killer cell–activating receptors[50]. DAP12 also associates with TREM1 or TREM2, although the functions and ligands of these TREM proteins remain unknown[51]. In DCs, DAP12 associates with plexin-A1 indirectly through TREM2 (ref. 49). DCs treated with TREM2-specific small interfering RNA respond poorly to Sema6D stimulation, and

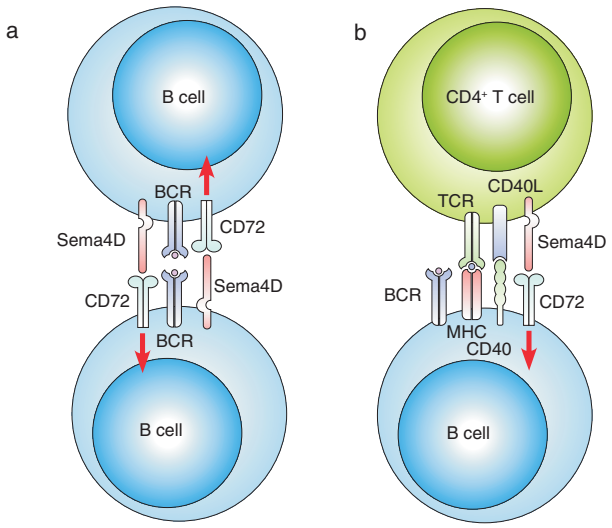

Figure 2 Sema4D in B cell–mediated immunity. (**a**) In homeostatic conditions, Sema4D is weakly expressed on B cells. In this setting, Sema4D-CD72 interactions between B cells might help maintain certain B cell subsets by 'fine tuning' BCR signals. Sema4D expression is strongly upregulated in germinal center B cells. This might enable high-avidity interactions of Sema4D with CD72, promoting the robust population expansion of germinal center B cells. (**b**) During T cell–dependent antibody responses, Sema4D abundantly expressed on $CD4^+$ T cells might be involved in the initial activation of follicular B cells in extrafollicular areas of secondary lymphoid organs by enhancing CD40-mediated signals. After germinal center formation, T cell–derived Sema4D might participate in the interaction between helper T cells and germinal center B cells, in which Sema4D signals might promote the survival of germinal center B cells and support efficient selection of high-affinity B cells. MHC, major histocompatibility complex.

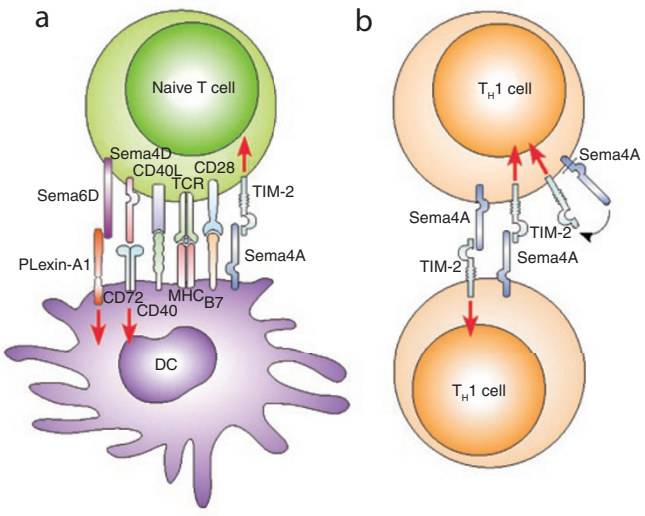

Figure 3 Involvement of semaphorins in T cell activation and differentiation. (**a**) In T cell–DC interactions, semaphorins mediate the reciprocal stimulation of T cells and DCs. The T cell–derived semaphorins Sema4D and Sema6D enhance DC activation and maturation through CD72 and a plexin-A1–TREM2–DAP12 complex, respectively. Conversely, Sema4A expressed on DCs directly stimulates T cells through TIM-2. These semaphorin signals might contribute to the optimal activation of antigen-specific T cells. (**b**) During the differentiation of $CD4^+$ T cells into cytokine-producing effector cells, Sema4A is selectively induced in T_H1 cells. The T_H1 cell–derived Sema4A further promotes T_H1 differentiation, which might be mediated by cognate cellular interactions between T_H1 cells and/or in an autocrine way.

DAP12-deficient DCs have a similar phenotype[49], which indicates that these adaptor molecules mediate Sema6D-induced plexin-A1 signaling.

Semaphorins and T cell activation, differentiation

Another member of the class IV semaphorin subfamily, Sema4A, also helps regulate T cell–mediated immune responses, but unlike Sema4D, Sema4A acts directly on T cells. The expression profile of Sema4A in immune cell populations is unique. Although there is constitutively high expression of Sema4A on the surfaces of all mouse DC subsets[25], its expression on T cells is tightly regulated[52]. After TCR stimulation, T cells transiently upregulate Sema4A within 24 hours, but its expression rapidly decreases thereafter. However, when T cells are stimulated in T helper type 1 (T_H1)–polarizing conditions containing IL-12 and antibody to IL-4, high Sema4A expression is induced and is sustained throughout the culture period. In contrast, stimulation with the T_H2-biasing conditions of IL-4 and antibody to interferon-γ (IFN-γ) induces only transient Sema4A expression. Notably, DC-derived Sema4A and T_H1 cell–derived Sema4A have distinct functions in the development of T cell–mediated immunity, as described below[52].

Recombinant Sema4A protein substantially enhances the proliferation and IL-2 production of naive T cells induced by TCR stimulation[25], which suggests that Sema4A contributes to T cell activation through T cell–DC interactions. Indeed, Sema4A-deficient DCs poorly stimulate allogeneic T cells in mixed lymphocyte culture, despite the fact that Sema4A-deficient DCs mature normally and produce cytokines in response to lipopolysaccharide or agonistic antibodies to CD40 (ref. 52). Consistent with those *in vitro* observations, Sema4A-deficient mice show defective generation of antigen-specific T cells after immunization with various antigens[52]. These observations indicate that Sema4A expressed on DCs is involved in the initial activation of T cells (**Fig. 3a**).

However, Sema4A expression in T cells is required for the differentiation of helper T cells. When cultured in T_H1-inducing conditions, Sema4A-deficient $CD4^+$ T cells fail to differentiate into IFN-γ–producing cells[52]. In contrast, Sema4A-deficient T cells differentiate normally into IL-4-producing cells in T_H2-inducing conditions. The selective defect in the T_H1 differentiation of Sema4A-deficient T cells is associated with lower expression of the IL-12 receptor β2 chain and T-bet, a transcription factor essential for T_H1 development[53]. Notably, the impaired T_H1 differentiation of Sema4A-deficient T cells is fully restored with recombinant Sema4A protein or wild-type T cells[52]. Therefore, Sema4A expressed on T cells might promote T_H1 differentiation through cognate interactions between T cells and/or an autocrine pathway (**Fig. 3b**). Furthermore, the involvement of Sema4A in the regulation of helper T cell differentiation has been confirmed *in vivo*. The generation of IFN-γ-producing antigen-specific T cells is impaired in Sema4A-deficient mice immunized with T_H1-inducing agents such as heat-killed *Propionibacterium acnes*[52]. Conversely, when infected with *Nippostrongylus brasiliensis*, a T_H2-inducing intestinal nematode, Sema4A-deficient mice mount enhanced T_H2 responses relative to those of wild-type mice[52]. Moreover, Sema4A-deficient mice of a T_H2-prone BALB/c strain spontaneously develop atopic dermatitis (unpublished data).

The functions of DC-derived and T cell–derived Sema4A have been delineated in transfer experiments with antigen-pulsed DCs[52]. When Sema4A-deficient DCs are transferred into Sema4A-sufficient mice, antigen-specific T cells show impaired proliferation and IL-2 secretion, but substantial numbers of IFN-γ-producing T cells are generated. In contrast, there is a selective defect in IFN-γ production but not in the proliferation and IL-2 production of antigen-specific T cells in Sema4A-deficient mice receiving Sema4A-sufficient DCs. These *in vivo* observations define distinct functions for Sema4A expressed by two different immune cell subsets: DC-derived Sema4A is essential for T cell priming, and T cell–derived Sema4A is required for T_H1 differentiation.

TIM-2, which belongs to the TIM family[54], was identified as a Sema4A receptor by expression cloning with a mouse T cell cDNA library[25]. Because Sema4A binding induces tyrosine phosphorylation of the cytoplasmic tail of TIM-2, TIM-2 seems to transduce Sema4A signals[25]. Studies have suggested involvement of TIM-2 in the regulation of helper T cell activities. Administration of recombinant TIM-2 protein suppresses the development of EAE in SJL mice immunized with proteolipid protein–derived peptide by inhibiting the generation of T_H1 cells[55]. In a model of airway atopy, TIM-2-deficient mice show exacerbated lung inflammation accompanied by dysregulated T_H2 responses[56]. These findings support the idea that TIM-2 serves as a functional receptor for Sema4A. However, although the main phenotypes of TIM-2- and Sema4A-deficient mice are similar, there are some inconsistencies. For example, T cells from TIM-2-deficient mice but not those from Sema4A-deficient mice show enhanced basal proliferation. Thus, it is likely that Sema4A or TIM-2 has another binding partner in the immune system. Like other class IV semaphorins, Sema4A has the ability to bind several plexin molecules, including the members of the plexin-B subfamily and plexin-D1 (ref. 57), some of which are expressed by T cells (unpublished data). Thus, it is conceivable that Sema4A acts on T cells through these plexin molecules.

In addition to Sema4A, several lines of evidence suggest that a secreted class III semaphorin, Sema3A, also regulates T cell functions. Sema3A is produced by DCs[58] and various types of tumor cells[59]. Treating T cells with recombinant or tumor-derived Sema3A inhibits TCR-mediated proliferation and cytokine production by downregulating mitogen-activated protein kinase signaling cascades, although the receptor system for Sema3A in T cells remains unidentified[58,59]. These observations suggest that Sema3A serves as a negative regulator for T cells in physiological and pathological immune responses. Although there is a report that a class

VII semaphorin, Sema7A, suppresses T cell activation in a cell-autonomous way[60], the observations in that report remain controversial. Instead, Sema7A expressed on T cells has been shown to have a positive regulatory function in the immune system, as described below[23].

Semaphorins and inflammatory responses

In the later phase of T cell–mediated immunity, antigen-specific effector T cells trigger inflammatory responses by activating macrophages in peripheral tissues. This activation of macrophages is promoted by both secreted and cell-associated factors from effector T cells, including IFN-γ and CD40 ligand (CD40L), which leads to pathogen elimination at the site of infection but can also lead to tissue destruction in autoimmune or allergic diseases[61]. A study has shown that Sema7A, a membrane-associated glycosylphosphatidylinositol-linked semaphorin, is important in this process[23].

Sema7A transcripts are detectable in the embryonic nervous system and in adult tissues, including the brain, spinal cord, lung and secondary lymphoid organs[62–64]. Among lymphocyte populations, Sema7A is expressed mainly in activated T cells[23]. Notably, the Sema domain of Sema7A contains an arginine-glycine-aspartate sequence that is a well conserved integrin-binding motif[65]. In accordance with that finding, a neurological study has shown that Sema7A promotes axon outgrowth through β_1 integrin receptors by activating the 'downstream' mitogen-activated protein kinase pathway and contributes to the formation of lateral olfactory tracts[22].

In the immune system, Sema7A expressed on activated T cells stimulates macrophages to produce proinflammatory cytokines through the $\alpha_1\beta_1$ integrin[23]. Thus, integrin-mediated signaling is a common mechanism by which Sema7A functions in both the nervous and immune systems. As a glycosylphosphatidylinositol-anchored protein, Sema7A is recruited to lipid rafts that accumulate at the immunological synapse between T cells and macrophages, where it interacts with $\alpha_1\beta_1$ integrin[23]. So far, $\alpha_1\beta_1$ integrin has been identified as a collagen receptor and as an 'anchor' that interacts with the extracellular matrix to retain effector cells at sites of inflammation[66–69]. Thus, Sema7A interacts with $\alpha_1\beta_1$ integrin in a totally different way than collagens do and might deliver stimulatory signals to macrophages more efficiently through the specialized signaling module.

Sema7A-deficient mice are defective in T cell–mediated immune responses such as contact hypersensitivity and EAE[23]. Detailed *in vivo* analyses have indicated that Sema7A deficiency does not affect the generation of antigen-specific effector T cells and their migration into inflammatory sites. However, Sema7A-deficient T cells fail to induce contact hypersensitivity responses when directly introduced into the antigen-challenged site. Therefore, the interaction of Sema7A with $\alpha_1\beta_1$ integrin is crucial for T cell–mediated macrophage activation at sites of inflammation. It has been reported that Sema7A is involved in the pathogenesis of lung fibrosis induced by transforming growth factor-β1 (ref. 70). In this disease development, transforming growth factor-β1 activates phosphatidylinositol 3-OH kinase and its downstream component protein kinase B (Akt) in a Sema7A-dependent way. Given the importance of the phosphatidylinositol 3-OH kinase–protein kinase B (Akt) pathway in integrin-mediated signaling[65], it is possible that Sema7A is involved in this disease model by means of integrin receptors.

This newly identified immune function of Sema7A provides insight into the mechanism by which effector T cells induce inflammation through macrophage activation. In the conventional model of T cell–mediated inflammation, IFN-γ and CD40L are the most potent effector molecules that promote inflammatory programs in macrophages[61]. However, these factors are synthesized anew in T cells only after antigen recognition on macrophages, a process that requires several hours[61]. Moreover, the induction of CD40 on macrophages requires IFN-γ produced by activated T cells[71]. In contrast, high expression of Sema7A is maintained on effector T cells after they differentiate from naive T cells[23]. Thus, it is conceivable that Sema7A helps initiate inflammatory cascades by stimulating cytokine production by macrophages, and its adhesive interactions with $\alpha_1\beta_1$ integrin support firm contacts between effector T cells and macrophages, ensuring efficient induction and action of IFN-γ and CD40L (**Fig. 4**). In addition to the direct activation of macrophages, effector T cells are also involved in the recruitment of macrophages into inflammatory sites by producing chemokines such as CCL2 (ref. 61). An *in vitro* study has shown that recombinant soluble Sema7A acts as chemoattractant for monocytes with much more potency than canonical chemokines[72], which suggests that Sema7A derived from effector T cells promotes macrophage recruitment to sites of inflammation.

Other semaphorins are also thought to participate in inflammatory immune responses. Indeed, recombinant human class IV semaphorins induce cytokine production from monocytes, although their precise functions in inflammation remain elusive[73]. Furthermore, a vaccinia virus semaphorin, A39R, binds plexin-C1 and induces robust responses in human monocytes, including aggregation and cytokine production[17]. It is possible that infected cells express and secrete this virus semaphorin to enhance inflammatory responses and exacerbate disease by activating the host immune system.

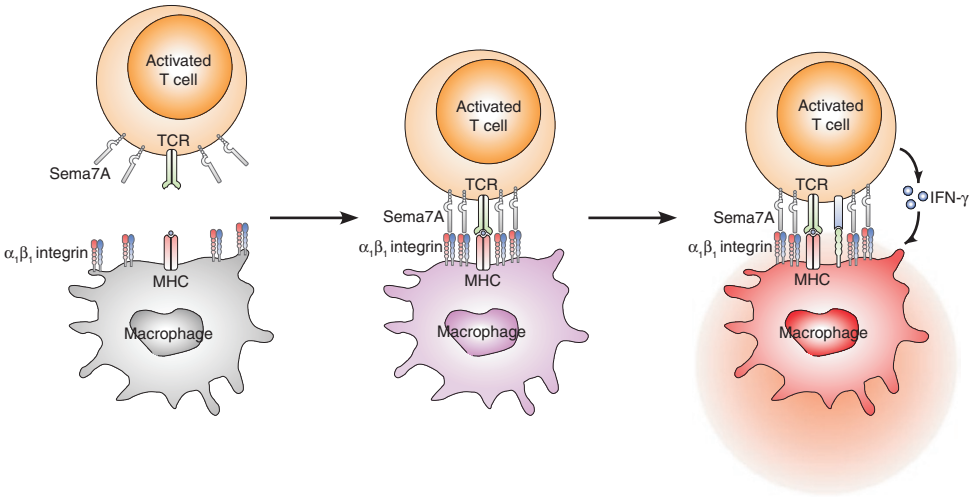

Figure 4 A model for Sema7A function in T cell–mediated inflammation. Sema7A is a glycosylphosphatidylinositol-anchored protein whose expression on T cells is sustained in large amounts once the cells are activated. When tissue-infiltrating effector T cells encounter macrophages, Sema7A on T cells is rapidly redistributed to the immunological synapse along with lipid rafts and induces clustering of $\alpha_1\beta_1$ integrin on macrophages. The Sema7A signals mediated by $\alpha_1\beta_1$ integrin stimulate macrophages to produce proinflammatory cytokines and trigger inflammatory processes at peripheral tissues. The interaction of Sema7A with $\alpha_1\beta_1$ integrin might also promote firm adhesion between T cells and macrophages. Macrophage activation by Sema7A is followed by the action of CD40L and IFN-γ, both of which are induced in effector T cells only after antigen recognition on macrophages and further promote inflammation.

Concluding remarks and future directions

As described above, accumulating evidence has identified the semaphorin family as a new class of immunoregulatory molecules. Although detailed analyses have been made of only a limited number of family members, they actively participate in various aspects of the immune response by several distinct mechanisms. Beyond the basic implications, studies of immune semaphorins have provided valuable insights into therapeutic strategies for autoimmune and allergic diseases, as involvement of semaphorins in the pathogenesis of these immune disorders has been demonstrated in several animal models[23,25,44,49].

However, resolution of an important issue remains elusive. So far, most of the immunological studies of semaphorins have focused on their costimulatory effects on immune cells. In the nervous and cardiovascular systems; however, semaphorins have established functions in regulating cell motility and morphology through receptors belonging to the plexin family[47]. In addition, emerging evidence suggests that there are two convergent mechanisms by which semaphorin-plexin signaling affects these aspects of cellular functions[47]. One is the regulation of actin cytoskeleton through GTPases of the Rho family, such as Rho and Rac, and the other is the modulation of integrin-mediated cell adhesion that involves a Ras-family GTPase, R-Ras. However, the function of semaphorins in regulating the migration and adhesive interaction of immune cells has been poorly elucidated.

Several aspects of immune responses, including the transmigration of leukocytes through vascular endothelium and the formation of immunological synapses, are heavily dependent on cytoskeletal dynamics and integrin activity[74–76]. Thus, it is reasonable that semaphorins might use plexin receptors to regulate the migration and adhesion of immune cells and therefore are involved in these key steps of immune responses. Indeed, there are data supporting that hypothesis. It has been reported that recombinant soluble Sema4D inhibits the migration of B cells, monocytes and immature DCs[77,78]. In DCs, this effect seems to be mediated by plexin-B1, a canonical Sema4D receptor in the nervous system[77]. In addition, the virus-encoded semaphorin A39R suppresses the integrin-mediated adhesion and migration of DCs by means of plexin-C1 (ref. 79). Moreover, Sema6D induces activation of Rac GTPase in DCs[49], which suggests that the Sema6D–plexin-A1 interaction regulates cytoskeletal events in DCs. Because activation of Rac GTPases in DCs is essential for dendrite extensions and contacts with naive T cells[80], it is possible that Sema6D-induced activation of Rac GTPases facilitates the initial scanning of T cells by DCs. In the context of T cell–DC interactions, it is also possible that Sema4A expressed on DCs affects actin-mediated receptor redistribution and integrin activities on the T cell side of the immunological synapse, ensuring optimal T cell activation.

At present there is a surge of innovation in imaging technology for the *in situ* visualization of the immune system. In particular, advances in multiphoton-excited laser-scanning microscopy have allowed real-time imaging of immune cells in living lymphoid tissues[81]. Only a few years after the introduction of this technology, various components of the immune response have been visualized, providing new and valuable insights into fundamental ideas in immunology[82,83]. In combination with conventional experimental methods, this powerful imaging technique could be instrumental in addressing the precise function of semaphorins in regulating immune cell dynamics in a complex physiological milieu. Although studies of immune semaphorins so far have described differences rather than similarities in semaphorin functions among physiologically distinct systems, identifying the unifying principle of semaphorin signaling that operates throughout the body will lead to comprehensive understanding of this interesting family of proteins.

Note: Supplementary information is available on the Nature Immunology website.

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