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Review

CD100 is a leukocyte semaphorin

S. Delaire, A. Elhabazi, A. Bensussan and L. Boumsell*

Unité INSERM U448, Faculté de médecine, 8, rue du gal Sarrail, F-94010 Creteil (France),
Fax +33 1 49 81 36 05, e-mail: delair@im3.inserm.fr

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Abstract. CD100 was originally described as an activation molecule on the surface of human T lymphocytes. Its triggering through distinct epitopes leads to different signals of costimulation with phorbol myristate acetate (PMA) or with CD3 and CD2. Interestingly, CD100 was shown to associate with different partner molecules in T cells. First, CD100 can associate with CD45, a key molecule with protein tyrosine phosphatase activity involved in T-cell transduction: this association is physical and has functional consequences for both partners. Second, CD100 interacts in its cytoplasmic domain with a Ser/Thr kinase for which it represents a preferential substrate. Recently, CD100 was identified as a member of the semaphorin gene family. This family comprises approximately 20 structurally related proteins. The first semaphorins were identified in the developing nervous system. Function has been shown for only some of them and involves repulsion during growth cone guidance. Since CD100 was the first semaphorin identified in the immune system, this raises the possibility of the involvement of members of the semaphorin family in other physiological phenomena outside the nervous system.

Key words. Semaphorin; activation molecule; phosphatase; kinase.

The function of the immune system is based on many interactions involving immune cells: these cells interact with one another, with soluble factors and with their environment, the extracellular matrix or nonimmune cells. Furthermore, these interactions are mediated through cell surface molecules that characterize the state of cell activation and differentiation. On T cells, one key complex is CD3/TcR, responsible for antigen recognition; but many other molecules have been identified which are involved in costimulation and/or cell adhesion.

We recently identified CD100 [1], a new cell surface molecule, using two monoclonal antibodies (mAbs), BB18 and BD16, raised in mice immunized with human thymic clones. CD100 is a human 150-kDa, homodimeric, lymphocyte surface antigen: it is expressed on the majority of haemopoietic cells, including B and T lymphocytes and monocytes [2, 3]. Recently, cloning of CD100 [4] permitted classification of the molecule into the semaphorin family [5]. The semaphorins are structurally defined by a conserved 500-amino acid (aa) extracellular domain with 16 conserved cysteines, the so-called Sema domain. Differences among the proteins of the family led to a classification into six groups. CD100 belongs to group IV, which comprises cell surface proteins all containing an immunoglobulin (Ig)-like domain following the Sema domain, and then transmembrane and cytoplasmic domains.

So far, not much is known about the CD100 receptor. However, stimulating through our mAbs as ligands

* Corresponding author.

has identified a role for CD100 in T-cell activation. Interestingly, triggering through the two distinct epitopes did not generate the same activation signals in different classical systems of T-cell stimulation [6, 7]. Because T-cell stimulation through cell surface activation molecules often involves enzymatic activity (such as kinases and phosphatases) for signal transduction, we investigated the molecules associated with CD100 in human T lymphocytes. We discovered that CD100 associates with CD45 [8], a membrane molecule that plays a key role in T-cell activation. More recently, we found that cellular Ser/Thr kinase activity associates with the cytoplasmic domain of CD100 [9, 10].

Since CD100 belongs to a large and phylogenetically conserved family of proteins, it is reasonable to wonder whether all the semaphorins share common functions. Semaphorins were first described in the nervous system [11, 12], where they act as mediators of repulsive growth cone guidance. But it seems that, even for these semaphorins, function is not restricted to the nervous system. Moreover, many other semaphorins have been found whose functions are unknown or completely putative. Studying all these proteins could identify common mechanisms for the action of semaphorins. But it would require identifying their ligands, which so far has only been done for the semaphorins of group III, whose ligands were shown to belong to the neuropilin family [13, 14]. For CD100 as well as for other semaphorins, the ligands are unknown. The fact that CD100 is the only representative of the semaphorin family in the immune system (along with its murine homologue, M-SemaG [15]) provides a great stimulus in the search for CD100 function.

CD100, the first leukocyte semaphorin

CD100 belongs to the semaphorin family

DNA sequence analysis of CD100 complementary DNA (cDNA) revealed a single long open reading frame (ORF) of 2586 bp [4]. A BLAST search of the protein database indicated that the CD100 sequence encodes a novel protein with homology to the semaphorin gene family. Semaphorins form a large family of newly discovered proteins conserved from invertebrates to mammals. They are characterized by a phylogenetically conserved 500-aa Sema domain in their amino terminus, and the first members were shown to be strongly implicated in mediating repulsive and inhibitory neuronal guidance [16]. Neuronal growth cones navigate over long distances along specific pathways to find their targets. Neuronal connections form during embryogenesis when each differentiating neuron sends out an axon (ended by a growth cone) that migrates through the embryonic environment to its synaptic target. Developing axonal projections make very few errors of navigation. This is apparently due to the presence of molecular guidance cues in the environment [16, 17]. These cues belong to different families and mediate four types of effects: contact repulsion and contact attraction, which are short-range cues, or chemorepulsion and chemoattraction, which are long-range cues.

The first diffusible attractants identified were netrins, nondiffusible extracellular matrix molecules [18, 19], closely related to the laminins. Among the molecules that modulate axon growth are members of the immunoglobulin and cadherin superfamilies, receptor protein tyrosine kinases (as the Eph family in vertebrates [20]), receptor protein tyrosine phosphatase and semaphorins.

The first identified semaphorin was found in grasshopper [11], but soon after homologues were found in vertebrates [12]. The semaphorin family contains both transmembrane and secreted proteins. Semaphorins are around 750-aa long, and all share the Sema domain, which contains approximately 500 aa with 15 conserved cysteine residues. The secreted semaphorins have a single Ig-like domain (belonging to the C2 class of Ig domains) and a 70–120-aa carboxy-terminal region. Some secreted semaphorins contain a stretch of highly basic amino acids in this carboxy terminus. Some transmembrane semaphorins contain an Ig domain and an additional 80-aa-stretch carboxy-terminal to the sema domain, a transmembrane domain and an 80–140 residue cytoplasmic tail. The others also contain a short cytoplasmic domain of 45 to 100 residues. These domains share no significant homology to any known proteins and very little similarity to each other. The semaphorins are actually classified into six groups (see fig. 1):

- group I: transmembrane semaphorins of invertebrates without Ig or basic domain; example: G-SemaI
- group II: D-SemaII, a secreted molecule identified in *Drosophila* (invertebrate) which contains an Ig domain but no basic C-terminal region
- group III: chick collapsins and their mammalian homologues; these are secreted molecules with an Ig domain followed by a highly basic carboxy-terminal domain
- group IV: members of this group are transmembrane semaphorins with an Ig domain and a highly variable cytoplasmic domain. CD100 belongs to this group: it shows an amino-terminal signal sequence followed by a Sema domain, an Ig-like domain, a lysine-rich stretch of 104 aa, a hydrophobic transmembrane region and a cytoplasmic tail of 110 aa. CD100 contains 15 of the 16 conserved Sema domain cysteines and 12 potential N-linked glycosylation sites. CD100 is a glycoprotein (see fig. 2A)

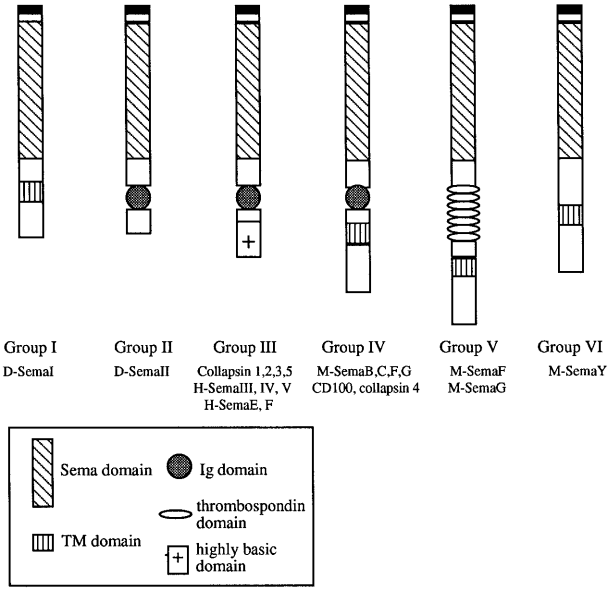

Figure 1. Structure of the semaphorins. Six groups of semaphorins are defined. Each group has structural properties which are represented in the drawings. All the members of the family share a common domain, the Sema domain, which contains 15 cysteine residues. Some domains, such as the Ig domain, are only present in subfamilies. We have provided a few examples of semaphorins which belong to each group. Group III contains only secreted semaphorins. CD100 belongs to group IV. Group V contains two murine proteins, called Sema F and Sema G, that are similar to two other semaphorins of group IV. Group VI was very recently defined.

• group V: two recently identified murine semaphorins that possess seven tandem thrombospondin type 1 motifs
• group VI: one murine transmembrane semaphorin lacking an Ig domain and with structural homology to the semaphorins of group I.

Among the human semaphorins, H-SemaIII is the most closely related to CD100, with which it shares 39% identity in the Sema domain and 33% identity in the Ig-like domain. Recently, a Japanese laboratory identified the mouse homologue to CD100, M-SemaG [15], which is very similar to CD100, especially in the cytoplasmic domains which are identical. Collapsin 4 (which belongs to group IV) appears to be the chicken homologue to CD100/M-SemaG. Among all the identified semaphorins, M-SemaF is the most closely related to CD100/M-SemaG/collapsin 4. The juxtamembrane domain of CD100 is unique and does not show any homology with other known proteins. The cytoplasmic tail contains a consensus site for tyrosine phosphorylation (KPALTGY) and multiple consensus sites for serine phosphorylation, among which five are specific for protein kinase C (PKC) and seven for casein kinase II.

Pattern of expression of CD100

Northern blot analysis of CD100 messenger RNA (mRNA) expression indicated that CD100 is broadly expressed in haematopoietic and nonhaematopoietic tissues. CD100 transcript is easily detectable in peripheral blood lymphocytes (PBL), spleen, thymus and skeletal muscle and is present at lower levels in testes, brain, kidney, small intestine, prostate, heart, placenta, lung and pancreas, but not in colon or liver. Interestingly, the mouse homologue M-SemaG is also well expressed (RNA and protein) in the thymus (strongest level), spleen, brain, kidney, lung, heart and bone marrow, but not in liver.

CD100 cell surface expression was first determined in our laboratory using mAbs [2, 3]. It established that CD100 is widely expressed in the haematopoietic system except on eosinophils and erythrocytes (see fig. 2). CD100 is not detected on immature haematopoietic cells such as CD34 + cells. Expression is weak on thymus cells, granulocytes and monocytes and is the strongest on activated T lymphocytes. On B lymphocytes, expression is intermediate between monocytes and T cells.

CD100 has two forms: a transmembrane isoform and a soluble isoform

Most of the semaphorins exist as soluble or membrane proteins. Originally, we showed that CD100 can exist as two isoforms: the generation of a soluble form does not result from alternative splicing but from proteolytic cleavage. This shedding is provoked by triggering CD100 with one of the mAbs and also after CD45 triggering [8] (see below). Interestingly, Adams et al. [21] recently demonstrated that a murine semaphorin, M-SemD, is converted from proSema to active Sema via proteolytic cleavage by a furinlike protease. The cleavage of a C-terminal propeptide is essential for the semaphorin to acquire its repulsive property. Moreover, the carboxy-terminal propeptide contains a processing signal that is necessary for SemD to be completely active. Different processing sites are identified and contribute differentially to SemD activation. This implies that differential expression and regulation of protease activity would allow cells to regulate the repulsive activity of the semaphorin. Secreted proSemD could be activated by local extracellular proteases or alternatively, cleavage of SemD at one processing site would reduce repulsion, which would allow cells expressing the appropriate protease to go across the repulsive region. This study raises the novel aspect of regulation of repulsive effects, which could involve regulation of protease expression. Such regulation is postulated to be an important process in metastasis.

Metalloproteases are also involved in the shedding of many receptors from the surface of lymphocytes. A

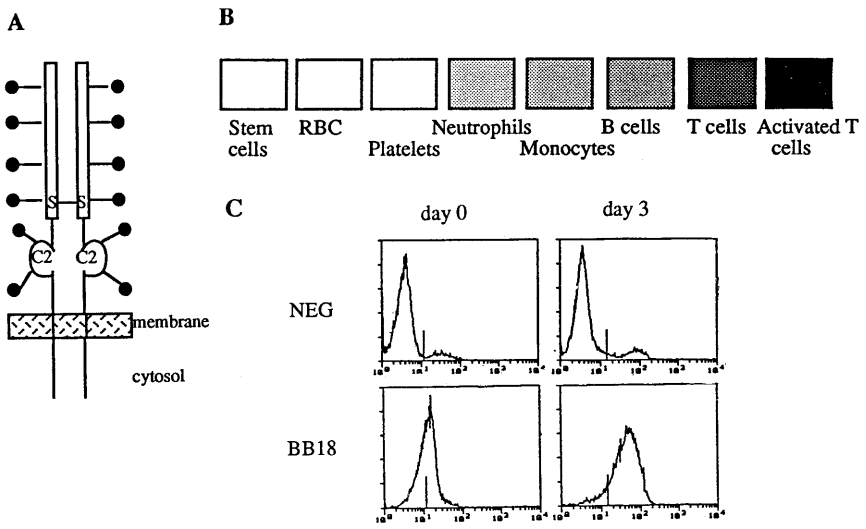

Figure 2. Structure and expression of CD100. (*A*) The structure of CD100 is presented in dimeric form, with the Sema domain followed by an Ig domain and by transmembrane and cytoplasmic regions. (*B*) CD100 shows different expression levels among the haematopoietic cells. There is no expression on stem cells or on red blood cells (RBC) or platelets and the strongest expression can be seen on activated T cells. (*C*) shows the increase of CD100 surface expression on T cells after PHA stimulation (staining with BB18 mAb).

major question regarding generation of soluble isoforms is whether there is a single common proteolytic pathway or many different ways for each molecule. Some data suggest the existence of a single pathway. The first evidence indicates that many molecules are shed after stimulation of cells by the same stimuli as phorbol myristate acetate (PMA) or 12-*o*-tetradecanoylphorbol-13-acetate (TPA), which argues in favour of at most only a few ways. Recently, Arribas et al. [22] showed that very different proteins, such as CD62L, interleukin (IL)6 receptor and pro-tumour growth factor (TGF)β, are cleaved from the surface of Chinese hamster ovary (CHO) cells by a unique proteolytic system. However, the different kinetics of cleavage (from 2 h for CD44 to 24 h for CD71) as well as the diversity of the peptidic sequences of the substrates suggest that different proteolytic systems are involved in shedding of cell surface molecules. Robache-Gallea et al. [23] isolated the protease responsible for tumour necrosis factor (TNF)α cleavage, and it is interesting that this protease seems to be specific for human proTNFα, as it is unable to cleave murine TNFα or other molecules such as IL6 or TNF receptors. Many classes of proteases have been identified, for example the calcium-dependent serine proteases, which are known to cleave prohormones. Furin is a member of this family. Another important class of proteases are the disintegrin proteases, which are characterized by a highly conserved domain, the disintegrin domain [24]. The identification of the protease responsible for TNFα shedding as a member of this family raised great interest in these proteases. The protease involved in CD100 shedding is unknown, as is the site of cleavage. In contrast to M-SemaD, cleavage of CD100 always seems to take place at the cell surface and not during biosynthesis, but there are perhaps multiple sites of action which can regulate the function of CD100.

To date, the function of soluble CD100 is unknown. It may act as an antagonist of the membrane receptor for ligand binding, which would imply that soluble and membrane CD100 have the same ligand. However, it is possible that soluble CD100 has completely different ligands from the membrane isoform and that it mediates the effects of CD100. Interestingly, the generation of soluble CD100 can be induced by triggering the molecule through an mAb against only one epitope. Using another mAb induces downregulation from the cell surface but not by generating a soluble form. In this case, the molecule seems to be internalized. Modulation of CD100 by internalization had already been reported after incubation with a third mAb (F937G2) in a T-cell line, CEM [25]. This mechanism of modulation has never been described for other semaphorins.

CD100 was first characterized as an activation molecule of T lymphocytes

CD100 was defined using five distinct mAbs, two of which (BD16 and BB18) were previously characterized in

our lab. Our aim was to identify new activation molecules of the T cell surface: mice were immunized with activated thymic cell clones [26], and fusion experiments were performed. They allowed us to raise two new mAbs against the same new molecule.

CD100 is an activation molecule, as its expression is greatly enhanced after activation at the surface of T lymphocytes [1] (see fig. 2). CD100 is highly expressed as early as 15 h after activation of peripheral blood mononuclear cells (PBMC) with a CD3 mAb. Its increase parallels that of CD25 during the first days of stimulation but persists as long as 9 days after PHA stimulation.

We used the mAbs to determine the functional role of CD100 in the activation of T lymphocytes. It is interesting that the two mAbs (which were shown to map to different epitopes on the molecule) had different effects [5, 6]. BD16 increased the proliferation induced through CD2 or CD3, whereas BB18 had no effect in such a system. BD16 more precisely potentialized the CD2 response: at day 5 or 6 after stimulation, the proliferation index in the presence of BD16 is still high, whereas with CD2 antibodies alone, it begins to decrease. The effect depends on the presence of accessory cells; in the absence of monocytes, BD16 increases the response induced by CD3, whereas in their presence, BD16 has a negative effect (which does not involve the Fc receptor).

We have searched for a biochemical basis for the role for CD100 in the regulation of T-cell activation. It is intriguing that Shraven et al. [27] described a 150-kDa protein coimmunoprecipitated with CD2 and CD45 on T cells. Furthermore, our results obtained with BD16 in association with CD2 or CD3 were identical to those obtained with a CD45 mAb in the same systems. CD45 is a transmembrane glycoprotein expressed only on haematopoietic cells [28]. CD45 was shown to be crucial in T-cell activation especially by its ability to dephosphorylate Tyr505 in the kinases of the Src family, leading to their activation [29]. So we decided to look for an association between CD100 and CD45. First, we immunoprecipitated CD100 from PHA-activated PBL and showed phosphatase activity associated with CD100. This activity was increased by PHA stimulation, due either to stoichiometric modification in the association or to the increase of CD100 expression after T-cell activation. By Western-blotting CD45 after immunoprecipitating CD100 from stimulated PBL, we proved definitively the physical interaction between CD100 and CD45 [8]. We cannot say whether CD100 and CD45 interact directly or through other proteins. A recent study in mice described an interaction between CD45 and a 30-kDa membrane protein called CD45-AP [30, 31]. It interacts with CD45 via the transmembrane domain and may act as an adaptor between CD45 and other partners. A human homologue of CD45-AP was identified [32], and it would be interesting to determine whether it acts as an adaptor between CD100 and CD45. Moreover, although CD45 was shown to associate with many proteins at the surface of lymphocytes, the interaction domains are not clearly identified, and the role of the extracellular domain of CD45 was unclear. But recently Verhagen et al. [33] have shown that the interaction domain between CD45 and CD2 is in the extracellular region of the two molecules, which implies that the extracellular variable domain of CD45 could be critical in the choice of a ligand. The association between CD4 and CD45 also seemed to be dependent on the specific external domains of the different isoforms [34]. In the case of CD100 and CD45, the site of interaction has still to be determined.

Besides this physical association, CD100 and CD45 seem to form a functional association. First, we observed that triggering CD45 through two distinct epitopes (expressed in all isoforms of CD45) induced a downmodulation of the CD100 molecule, similarly to CD100 mAb BD16. This downmodulation corresponds to the shedding of the CD100 molecule, which generates a soluble form of CD100 also induced by BD16 mAb but not by BB18 mAb. Second, we showed that an antibody against CD45 (called 4.14) induced homotypic aggregation of T cells (without any prior activation). This aggregation was enhanced by the addition of CD100 mAb (see fig. 3). A CD100 mAb alone did not aggregate T cells, indicating that CD100 engagement modifies CD45 function. The role of CD45 in cell adhesion has already been described. CD45 mAbs induce homotypic aggregation of B lymphocytes [35] or activated T lymphocytes [36]. Our CD45 mAb aggregated resting T cells, a result obtained by Lorenz et al. [37] only in the presence of monocytes. CD45 did not modify adhesion through steric effects in its extracellular domain, but it did modulate the phosphorylation status of molecules implied in cell adhesion through its phosphatase activity. Other membrane proteins with phosphatase activity play a role in cell-cell contacts. Unlike CD45, they contain several Ig and FNIII domains which could act directly in cell adhesion [38]. PTP-μ and κ have a special domain called MAM which may be involved in adhesion [39, 40].

The role of CD100 mAbs in T-cell activation has to be defined: Is CD100 a membrane receptor for a ligand expressed in other cells, or is it a substrate of CD45 in the same cell? A possible function of CD100 may be to regulate the dimerization status of CD45: we have previously shown that CD100 exists both as a monomer and a dimer at the cell surface. We still do not know how this phenomenon is regulated, but we can postulate that dimerization of CD100 provokes dimerization of CD45. As the phosphatase activity of CD45 was recently shown to be inhibited when the CD45 intracellular domain is dimerized [41], CD100 may act as a regulator of CD45 activity.

Another aspect of our study focused on the intracellular biochemical signals following CD100 triggering or stimulation. We looked for kinase activity associated with

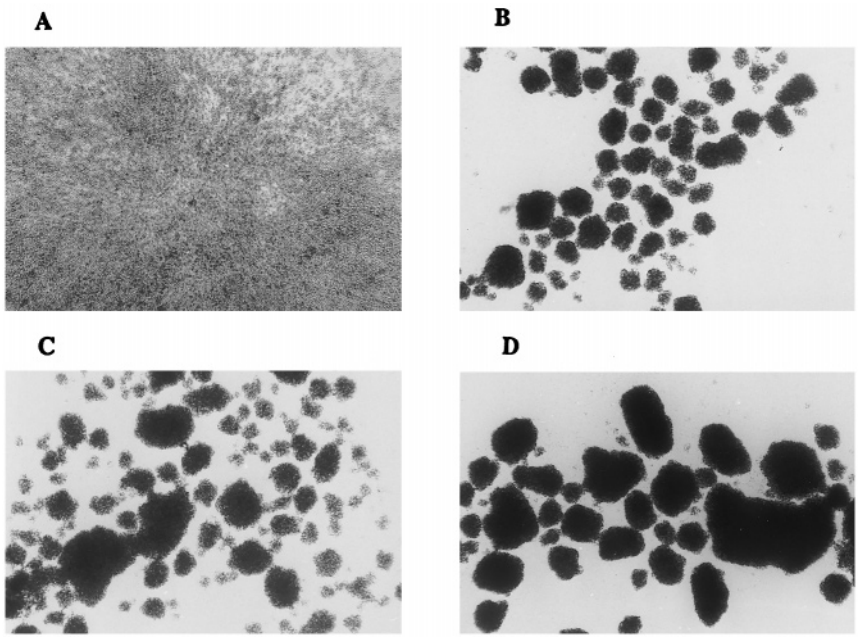

Figure 3. CD100 mAbs modulate CD45 function. Triggering purified T cells (*A*) with a CD45 mAb induces their homotypic aggregation (*B*). This aggregation is greatly increased by the addition of CD100 mAb: BB18 (*C*) or BD16 (*D*).

CD100, since in the sequence we can see a typical site of interaction with Src kinases and multiple consensus sites for serine or threonine phosphorylation. BB18 immunoprecipitated many phosphoproteins from Jurkat cells on Western blot analysis [9]. The major bands identified correspond to two forms of CD100 (150 kDa and 120 kDa) (see fig. 4). A phospho-amino acid analysis showed that the majority of phosphorylated amino acids are serine residues. A few phosphothreonine and no phosphotyrosine residues were detected. It was clear that CD100 associates with Ser/Thr kinase activity that could be detected in different cell types, including T lymphocytes after PHA stimulation. In vitro phosphorylation of CD100 is very low or undetectable in resting T cells. This suggests a link between the activation state and CD100 association with the kinase or an increase in kinase activity after T-cell activation. This association could only be preserved when immunoprecipitates were performed with BB18. More precisely, we showed that triggering CD100 with BD16 dissociates the complex between CD100 and the Ser/Thr kinase activity. CD100 itself is presumably a favourite substrate of the kinase. The nature of the kinase is unclear, as none of the tested inhibitors completely abolished CD100 phosphorylation. Bisindolmaleimide I, a specific inhibitor of PKC, and the Ca^{2+}/calmodulin kinase inhibitor KN93 were ineffective. The most significant inhibition was observed with H89, which inhibits most cellular Ser/Thr kinases.

In the context of our previous results concerning CD100 and CD45 association, it is interesting to note that CD45 is serine-phosphorylated on multiple sites, and these phosphorylations seem to correlate with its enzymatic activity in mouse [42]. It was shown that CD45 is phosphorylated by PKC in vitro [43] and in vivo [44]. Recently, Stover and Walsh described sequential phosphorylations of CD45 in vitro on tyrosine and serine residues [45]. These phosphorylations could lead to an enhancement of the phosphatase activity by up to sevenfold. Regulation of CD45 by the CD100-associated serine kinase is thus quite possible. This is consistent with our previous results showing that mAbs to CD100 and CD45 had similar effects on CD2-triggered PBMC stimulation [6].

The use of mAbs against CD100 allowed us to determine many aspects of the protein. Our two mAbs differentially influence proliferation of T cells as well as the association of CD100 with its different partners. Association with CD45 was greater when BB18 was bound to its epitope, and BD16 epitope ligation induces dissociation of CD100 from its serine kinase. The two mAbs also modulate the molecule differently. The question is whether these very different responses to the different epitopes reflect the existence of different ligands for CD100 which could induce distinct phenomena. Mapping the sites of interaction of the mAbs could help us to answer this question.

It is interesting to note that few equivalent studies have been performed with the other semaphorins to determine their associations with different signalling molecules. Recent data revealed that the cytoplasmic domain of a

new member of the semaphorin family, M-SemaVIb, interacts with c-src [46]. This suggests that transmembrane semaphorins are likely to be receptorstriggering intracellular signalling after interaction with their ligand(s).

Is CD100 functionally related to semaphorins?

Functions of the semaphorins

The first semaphorin was identified in grasshoppers, in a screen to identify surface glycoproteins that are differentially expressed on subsets of axon pathways. It was first named Fasciclin IV [11] and showed dynamic expression on a subset of axon pathways in the developing central nervous system (CNS) and on circumferential bands of epithelial cells in developing limb buds. Embryos cultured with the antibody to FasIV exhibit aberrant formation of the axon pathway of Ti1 pioneer neurons, which make a characteristic turn in one of the bands while extending towards the CNS.

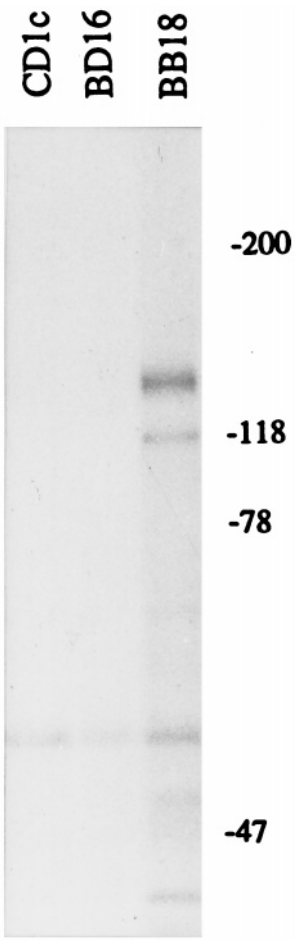

Figure 4. CD100 associates with a serine kinase. In vitro kinase assay performed on immunoprecipitates from Jurkat cells reveals the presence of several phosphorylated proteins in BB18 immunoprecipitate. Two of the proteins (150 kDa and 120 kDa) correspond to CD100. No such band is observed in BD16 immunoprecipitate. The CD1c lane constitutes a negative control.

Screening for homologous sequences resulted in identification of related transmembrane proteins in the beetle *Tribolium* (T-SemaI) and in *Drosophila* (D-SemaI), and of related secreted proteins in *Drosophila* (D-SemaII) and in human tissue (H-SemaIII) [16]. At the same time, biochemical purification of growth cone-collapsing activity from embryonic chick brain membranes led to the identification of a secreted semaphorin, called collapsin-1 (Coll1) [12]. Searches for additional members of the family have identified many new semaphorins, and the human expressed sequence tag (EST) database has turned up several sequences that represent four to nine human proteins with Sema domains. The semaphorins are found in many orders from invertebrates (as well in the nematode *Caenorhabditis elegans* and in insects) to humans: the Sema domain is always present, which suggests that it is an ancient structure which was phylogenetically conserved. Two semaphorins that have truncated Sema domains and are related, even distantly to the semaphorins, have been identified in poxvirus genomes [16]. A truncated semaphorin was also identified in herpesvirus [47]. The presence of these genes in the small genomes of viruses raises the question of the advantage for these intracellular parasites: we can imagine that the presence of a viral semaphorin permits the virus to stay away from the immune cells, which implies a function for semaphorins in the immune system. Very recent work [48] revealed that a poxvirus-encoded semaphorin has an effect on human monocytes, as it induces their homotypic aggregation (and increases CD54 expression) and the production of IL6 and IL8, which are proinflammatory cytokines. However, the role of this semaphorin in viral spreading is unknown.

Several semaphorins act in different ways on axons. Collapsin induces collapse of sensory growth cones: it is likely to function in vivo to steer growth cones. Coll1 exhibits specificity towards a subset of neuronal growth cones [12]. SemaIII acts as a selective chemorepellent for dorsal root ganglion (DRG) afferents whose growth is elicited by nerve growth factor (NGF) but not by NT3 [49]. In the same way, D-SemaII, a secreted *Drosophila* semaphorin, can also function as an inhibitory guidance cue [50]: it seems to be a target-derived signal that inhibits the formation of terminal synaptic arborizations. Thus, it appears that semaphorins can act at different levels of guidance, always with negative effects and with high specificity. Very recently, Yu et al. [51] provided evidence of a requirement for D-SemaI in axonal guidance. This is the first demonstration of the repulsive effect of a transmembrane semaphorin.

But the semaphorins are not only implicated in the neural growth. Behar et al. [52] generated mice mutant in the *SemaIII* gene by homologous recombination. Homozygous-deficient mice are born at ratios lower than

those predicted by Mendelian laws and are indistinguishable from their littermates at birth. The homozygous mice quickly appear weaker and have less milk in their stomachs, and most of them die within the first 3 days. Only a small percentage are viable to adulthood and once adult, they have difficulty maintaining upright posture, are weaker than wild type and finally die due to heart failure.

The brains of the mutant mice are severely reduced in size, but all cortical layers are present; the neuropil is also reduced, and neuronal morphology is altered. Studying the pattern of expression of *SemaIII* revealed that it was expressed in slerotome, dermatome and developing heart. Mutant mice display fusion of cervical bones, partial duplication of ribs and poor alignment of the rib-sternum junction. Moreover, the mice all show a dilated right atrium and an enlarged right ventricle with a hypertrophied wall. The phenotype of these mice reveals that SemaIII plays an important role in several developing tissues which normally express SemaIII. SemaIII could act as a cardiac growth regulator about which little is known.

Two human semaphorins are encoded by genes located in a region often deleted in small-cell lung cancer [53–55]. The region 3p21.3 was often involved in small-cell lung cancer. Screening this region in different pulmonary cell lines led to identification of two new human semaphorins called H-SemaA/V and H-SemaIII/F. The semaphorin A/V was absent in most small-cell lung cancer cases and in 25 out of 42 pulmonary tumour cell lines which were tested. However, effective mutations are very rare events and were found only in 3 cases of cancer out of 40 (compared with 90% of p53 mutation in the same cell lines). No mutation was found in SemaIII/F, which makes the role of these semaphorins as antitumoral genes unclear. Nevertheless, these molecules may be involved in the regulation of cell growth and differentiation.

Using mRNA differential display, Mangasser-Stephan et al. [56] identified two genes whose expression was upregulated in synovial cells in rheumatoid arthritis sites. One of them encoded for the human homologue of M-SemaE and was therefore named H-SemaE. The function of this semaphorin is completely unknown, but recently soluble forms of CD106 and CD62E were implicated in angiogenesis at the rheumatoid sites. It would be interesting to determine whether H-SemaE plays a role in this phenomenon. Interestingly, the same semaphorin was simultaneously identified by a group trying to determine genes involved in multidrug resistance other than the classical multi drug resistance (MDR) genes: *MDR-1* or *MRP* [57]. The authors show that this semaphorin exists as a soluble molecule which may act as an autocrine factor and promote cell death resistance. This could also explain the presence of the same H-SemaE in rheumatoid arthritis, where the irreversible destructive growth of synovial cells is responsible for the disease.

So it appears clear that semaphorins not only participate in the guidance of growth cones. They also seem to play a role in establishing the pattern of different tissues during embryogenesis. They could as well be involved in tumorigenesis and more generally in cell survival. CD100 was shown to promote B-cell survival [4]. Thus, it could have a place in a general model of action in cell survival and motility for semaphorins.

Signal transduction in the target cells

The mechanism of semaphorin action in target cells is not elucidated, because study of transduction pathways implies identification of the receptors. However, a family of proteins, the CRMP (for collapsin response mediator protein) was clearly identified as necessary for collapsin effects. Transfection of the cDNA encoding CRMP62 into *Xenopus* oocytes confers susceptibility to chick Coll1 [58]. More precisely, Coll1 induces an inward current in these cells. The effect of Coll1 is abolished in the presence of pertussis toxin, which suggests the involvement of heterotrimeric G protein in the transduction pathway. Human and murine homologues for CRMP62 were identified. CRMP is homologous to a *C. elegans* gene, *UNC33*, whose mutation induces aberrant neuronal connections and posture troubles, reminiscent of disturbances observed in animals deficient in Sema genes.

Other elements implied in the effects of semaphorins are cytoskeletal elements. After exposure of a growth cone to collapsin, the concentration of actin filaments in the growth cone decreases by depolymerization [59]. Recently, Jin and Strittmatter [60] showed that small G proteins of the Rho family are involved in neuritic retraction and collapse. Injection of active Rac-1 into DRG of chick increases the number of retracting spicules after contact with Coll1. These results are very interesting because of the important role played by Rho proteins in the reorganization of the cytoskeleton. More recently, a study has revealed that collapsin activates antero- and retrograde transport of organites in the axon, confirming the semaphorin-induced reorganization of the cytoskeleton [61].

We actually have no information about transduction events following interaction of CD100 with its ligand. Cytoskeletal reorganization has an important place in migration events in leukocytes. A role for CD100 in such a phenomenon could conceivably use the same pathways as collapsin.

Identification of semaphorin receptors

Using chimera proteins, with the H-SemaIII sequence fused with alkaline phosphatase, two independent laboratories have identified the membrane receptor of this

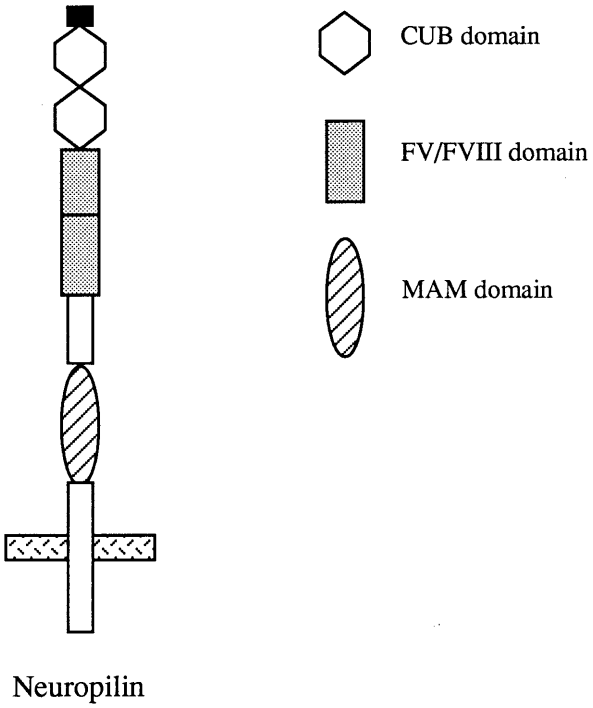

Figure 5. Structure of neuropilin. Neuropilin is a transmembrane molecule which first identified in the *Xenopus* nervous system. It presents several typical domains: two CUB domains which have homology to complement factors C1r/s; two domains with homology to coagulation factors of type V/VIII; and a MAM domain, which had already previously been described in phosphatase and in a protease.

semaphorin [13, 14]. The receptor is neuropilin, a previously described protein. Antibodies against this receptor inhibit DRG growth cone repulsion by cells secreting H-SemaIII. During mouse development, neuropilin is expressed in different populations of neurons known to be responsive to SemaIII (e.g. DRG sensory neurons, trigeminal motor neurons) and in other populations of neurons whose susceptibility to SemaIII remains to be determined. Transgenic mice overexpressing neuropilin [62] and mutant mice missing in SemaIII gene [52] share very similar phenotypes concerning neuronal and nonneuronal tissues (including the cardiovascular system). This suggests that SemaIII and its receptor may participate in morphogenesis of different tissues.

Neuropilin was first identified in *X. laevis* [63], where it acts in the guidance of axons. This molecule has an original structure made up of different modules found in other proteins (fig. 5). There are two CUB domains (homologous with complement subcomponent C1r/C1s domains of the complement system), two domains homologous with C1 and C2 domains of type V/VIII coagulation factors and, in the juxtamembrane region, a domain called MAM which has been defined in receptor tyrosine phosphatase μ as well as in a metalloprotease called meprin α. The cytoplasmic domain is very short. Recently, a second neuropilin was cloned in rat [14], so it seems that neuropilins constitute a family of molecules. Whereas neuropilin-1 and -2 share only 44% identity, their cytoplasmic domains are almost identical, suggesting that these domains are implicated in signal transduction. As these domains do not show any homology to known signalling proteins, it is possible that neuropilin associates with another protein which mediates the signal transduction. It was recently shown that different isoforms of neuropilin-2 are generated by alternative splicing [64]. Neuropilin-2(a) and neuropilin-2(b) share only 10% identity in their cytoplasmic domain, and the generation of these isoforms is developmentally regulated. This raises the possibility that interaction of a semaphorin with its receptor produces different intracellular signals according to the state of development.

Neuropilin-2 binds to SemaE and SemaIV but not to SemaIII [64]. These data confirm that neuropilins may constitute a family of receptors for semaphorins. However, the interaction of neuropilins with semaphorins not belonging to the group III remains to be proven.

Some authors have recently raised the possibility that the receptor for semaphorins may be a multimolecular complex [65]. They have studied the in vitro binding affinities of four distinct collapsins for neuropilin-1 and compared these results with in situ hybridizations. They deduce that neuropilins are only one component of the semaphorin receptor complex and that binding specificity is conferred by other components. In a companion paper, Koppel et al. [66] demonstrate that there are two distinct sites that regulate the function of collapsin, one that mediates the biological response (located in the Sema domain) and is thus responsible for specificity and one that potentiates this response (which is the carboxy-terminal region). Another aspect of their study is that the semaphorin domain is sufficient, when dimerized, for Coll1 activity. It is interesting to note that CD100 also exists in dimeric form, which we postulate to be the active form.

More recently, another study performed on a poxvirus-encoded semaphorin [48] led to the identification of a new semaphorin receptor called VESPR (for virus-encoded semaphorin protein receptor). This receptor was identified on a lymphoid cell line and shows broad expression on haematopoietic cells. Cloning the gene which encodes this receptor revealed that it is a member of the plexin family of molecules [67, 68]. The first plexin was isolated from *Xenopus* nervous tissue and was reported to induce homotypic aggregation [67, 69].

Other plexins were then identified in mouse and in humans. This shows that the receptors for the semaphorins do not belong to a unique family of proteins. Moreover, sequence alignment revealed homology between a portion of the extracellular domain of plexins and a region of the Sema domain in various semaphorins. Thus, this subdomain could be implicated in interactions between proteins of these two distinct families.

Conclusion

CD100 belongs to a subfamily of semaphorins for which no function nor any receptor have yet been identified. Presently, we have described a role for cell surface CD100 in the activation of T lymphocytes. No equivalent studies have been reported for the other semaphorins. The function of transmembrane semaphorins remains to be determined; they could be receptors for different ligands. Determining their association with signalling molecules is thus of great interest. Characterization of the molecule revealed that two distinct epitopes mediate two different types of signals. This may correspond to the existence of at least two distinct ligands for CD100, each of them regulating differentially the association of CD100 with its partners and thus leading to different intracellular signals. The associations of CD100 with CD45 and with a Ser/Thr kinase need to be more precisely defined. The role of the kinase in activation of CD45 needs to be elucidated.
We will continue to look for the function of soluble CD100 and its receptor. Finally, determining a repulsive role of CD100 on lymphoid cell migration would indicate that the immune system development is, like development of the CNS, negatively regulated.

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