Plexin C1 Engagement on Mouse Dendritic Cells by Viral Semaphorin A39R Induces Actin Cytoskeleton Rearrangement and Inhibits Integrin-Mediated Adhesion and Chemokine-Induced Migration

Thierry Walzer, Laurent Galibert, Michael R. Comeau and Thibaut De Smedt


http://www.jimmunol.org/content/174/1/51

**References**
This article **cites 33 articles**, 13 of which you can access for free at:
http://www.jimmunol.org/content/174/1/51.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Plexin C1 Engagement on Mouse Dendritic Cells by Viral Semaphorin A39R Induces Actin Cytoskeleton Rearrangement and Inhibits Integrin-Mediated Adhesion and Chemokine-Induced Migration

Thierry Walzer,1 Laurent Galibert, Michael R. Comeau, and Thibaut De Smedt

The poxvirus A39R protein is a member of the semaphorin family previously reported to bind plexin C1. We show that, in the mouse, plexin C1 is expressed on dendritic cells (DCs) and neutrophils and is the only receptor for A39R on these cells. The biological effects of a recombinant form of A39R were examined in vitro on mouse DCs derived from wild-type or plexin C1−/− mice. A39R binding to plexin C1 on DCs inhibited integrin-mediated adhesion and spreading in vitro. This phenomenon was accompanied by a decrease in integrin signaling, measured by focal adhesion kinase phosphorylation, and a rearrangement of the actin cytoskeleton, without inducing DC maturation or affecting their viability. The A39R effect on DC adhesion was blocked by a specific inhibitor of cofilin phosphorylation, suggesting that the regulation of F-actin turnover by plexin C1 was essential to induce cellular retraction. Furthermore, A39R binding to plexin C1 inhibited chemokine-induced migration of DCs in vitro, suggesting that plexins and semaphorins could be involved in the regulation of leukocyte movement. The Journal of Immunology, 2005, 174: 51–59.

Cytoskeleton is required for cells to move, polarize, change shape, engulf particles, or interact with other cells. Different families of proteins inducing cytoskeleton remodeling have been identified. Among these are semaphorins, which belong to a growing family of soluble and membrane bound proteins that can provide either repellusive or attractive guidance signals on a wide range of neurons (1–5). Developmental defects in several loss of function semaphorin mutants have revealed the crucial importance of these proteins in the guidance or migration of several cell types like neurons or endothelial cells. Semaphorins share a conserved, 500 amino acid residue domain near their amino terminus called the Sema domain. Currently, 25 members have been identified from invertebrates to human (2). A semaphorin homologue called A39R has also been identified as being encoded within the genomes of several poxviruses like vaccinia (6). Two families of semaphorin receptors have been identified: plexins and neuropilins (1–5). Plexins are type I transmembrane proteins that also contain a Sema domain in their extracellular part and an intracellular domain called the SP (sex and plexins) domain (7, 8). They are divided into four subfamilies, plexin A to plexin D, according to the structure of their extracellular domain. Semaphorins signal through direct binding to plexins alone, or in some cases, in combination with neuropilins. The most remarkable consequence of the engagement of plexins by semaphorins in neurons is the local rearrangement of the actin cytoskeleton (3, 5, 9) through the regulation of the activity of small GTPases of the Rho and Rac families (9, 10) and their downstream effector actin-binding protein cofilin (11). Cofilin phosphorylation by Lin-11-Isl-1-Mec-3 kinase in response to semaphorin inhibits cofilin ability to bind and depolymerize pointed ends of F-actin, which inhibits F-actin turnover in neuron growth cones. Moreover, three recent reports indicate that integrin function is regulated by semaphorins (12–14) by an as yet unknown mechanism.

Emerging evidence points also to a role for semaphorins and plexins in the immune system (for a review, see Ref. (15). However, these two families of proteins have been studied independently and no semaphorin-plexin pair has been identified in the immune system. Some semaphorins have been shown to play roles in immune regulation, but they seem to function through unique receptors that are not used in the nervous system. Several plexins are also expressed by leukocytes, but their cellular ligands as well as the consequence of their engagement on cellular physiology and actin-based cytoskeleton in particular are unknown.

We report that plexin C1 is expressed on mouse dendritic cells (DCs)2 and neutrophils and is the only receptor for the viral semaphorin homologue A39R on these cells. We investigated the consequences of plexin C1 engagement by a recombinant form of A39R on mouse bone marrow-derived DCs. We found that in vitro binding of A39R to plexin C1 inhibited integrin-mediated DC adhesion and spread without affecting their maturation or viability. A39R induced a decrease of LPS-induced focal adhesion kinase (FAK) phosphorylation in DCs and the rearrangement of the actin cytoskeleton. Moreover, we found that a synthetic cell-permeable peptide containing a cofilin phosphorylation site suppressed A39R-mediated effects on DC adhesion. Finally, A39R treatment inhibited DC ability to migrate to various chemokines in vitro, indicating a potential role for semaphorins and plexins in the regulation of leukocyte movement.
Materials and Methods

Mice and reagents
C57BL/6 mice were obtained from The Jackson Laboratory and used at the age of 6–12 wk. The generation of plexin C1−/− mice has already been described (12). Plexin C1−/− mice were bred in house. All of the experimental procedures used in this study were approved by a local committee according to federal guidelines. SFHA39R (short-flag-histidine or A39R) was overexpressed from Chinese hamster ovary cells and purified as described (7). All proteins were tested for endotoxin contents using a Limulus assay (Whittaker M.A. Bioproducts) and were shown to be <3 EU/ml. A39R was used at 1 µg/ml in every in vitro assay unless otherwise mentioned. Blocking Abs against β1 integrin (clone HA2/5) and β2 integrin (clone GAME-46) were obtained from BD Immunocytometry Systems and used at 10 µg/ml. Isotype control (hamster IgM and rat IgG1; BD Biosciences) were used. All proteins tested in this study were obtained from Sigma. S3 and RV peptide sequences have been described elsewhere (11). S3 peptide contains the phosphorylation site of cofilin and the cell-permeable sequence motif of penetratin. Cofilin phosphorylation has been shown to be efficiently and specifically inhibited in cells cultured in the presence of this peptide. As a control, the RV peptide containing the same penetratin domain and the reverse sequence of the cofilin phosphorylation domain were used.

DC cultures
Bone marrow cells were isolated by flushing femurs with complete medium supplemented with 2% heat-inactivated FBS (Invitrogen Life Technologies). RBC were lysed. The cells were then resuspended in culture medium containing of McCoy’s medium supplemented with essential and nonessential amino acids, 1 mM L-sodium pyruvate, 2.5 mM L-HEPES buffer (pH 7.4), 2-ME, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.3 mg/ml α-glutamine, and 10% FBS (all medium reagents from Invitrogen Life Technologies). Bone marrow cells were cultured as previously described (16) for 9 days at 10^6 cells/ml in tissue culture flasks (BD Falcon; BD Biosciences Discovery Labware) in culture medium supplemented with 200 ng/ml recombinant human Fms-like tyrosine kinase-3 ligand (Flt3L) (Clontech). Flt3L, DCs were subsequently cultured in IMDM (Invitrogen Life Technologies) supplemented with nonessential amino acids, 1 mM L-sodium pyruvate, 2.5 mM L-HEPES buffer (pH 7.4), 2-ME, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.3 mg/ml α-glutamine, and 10% FBS (all medium reagents from Invitrogen Life Technologies). Bone marrow cultures were used.

Cytokine detection
Levels of IL-1, IL-2, IL-4, IL-6, TNF-α, GM-CSF, IFN-γ, and IL-12p70 in DC culture supernatants were measured using the BeadLyte Mouse Multi-Cytokine Detection System (Upstate Biotechnology) and the LumineX100 plate reader (Luminex) according to the manufacturer’s instructions. Quantification of cytokine levels was performed by regression analysis from a standard curve generated using cytokine standards included in the kit. Only IL-6, TNF-α, and IL-12p70 were detected in DC culture supernatant.

Statistics
All values for p were calculated with the unpaired Student’s t test assuming equal variances.

Results
Plexin C1 is expressed by DCs and neutrophils and is the only receptor for A39R
Plexin C1 was previously identified as a receptor for A39R in human and mouse but its expression has only been partially documented in the immune system (7). We therefore generated an Ab against mouse plexin C1 (see Materials and Methods) that allowed us to measure the expression of plexin C1 on a large panel of immune cells. Little to no expression was found on lymphocytes, NK cells, and macrophages (Fig. 1A). By contrast, neutrophils,
DCs (CD11c+), spleen bone marrow cells (for neutrophils), or peritoneal exudate cells (for macrophages) were stained with various Abs combinations plus an Ab against plexin C1 for flow cytometry. Plexin C1 expression was then determined on the different subsets using the following analyze gates: T cells (CD3+CD19−), B cells (CD19+), NK cells (DX5+CD3−), macrophages (CD11b+F4/80+), neutrophils (GR1+CD11b+), DCs (CD11c−), CD8+ DCs (CD11c+CD8+), CD8+ DCs (CD11c−CD8+), plasmacytoid DCs (pDCs, CD19+CD122+Ly6c+). Flt3L (FL) bone marrow-derived DCs were stained only with the anti-plexin C1 Ab. Results are representative of three experiments. 

FIGURE 1. Plexin C1 is preferentially expressed on DCs and neutrophils and is the only receptor for A39R. A, spleen, bone marrow cells (for neutrophils), or peritoneal exudate cells (for macrophages) were stained with various Abs combinations plus an Ab against plexin C1 for flow cytometry. Plexin C1 expression was then determined on the different subsets using the following analyze gates: T cells (CD3+CD19−), B cells (CD19+), NK cells (DX5+CD3−), macrophages (CD11b+F4/80+), neutrophils (GR1+CD11b+), DCs (CD11c−), CD8+ DCs (CD11c+CD8+), CD8+ DCs (CD11c−CD8+), plasmacytoid DCs (pDCs, CD19+CD122+Ly6c+). Flt3L (FL) bone marrow-derived DCs were stained only with the anti-plexin C1 Ab. Results are representative of three experiments. B, Wild-type and plexin C1−/− bone marrow-derived DCs were either stained with the anti-plexin C1 Ab (left panels) or incubated with A39R and then stained with an Ab against the Flag (right panels). T, T cells; B, B cells; Mø, macrophages; Neutro, neutrophils; pDCs, plasmacytoid dendritic cells; BMDC, bone marrow-derived dendritic cells. Results are representative of three experiments.

Semenaphorins are known to induce axon collapse (1–5), a phenomenon that could involve the regulation of cell adhesion through the modulation of integrin activity (13). We asked whether A39R inhibits integrin-mediated adhesion and inhibits FAK phosphorylation. 

A39R inhibits integrin-mediated adhesion and inhibits FAK phosphorylation

Semenaphorins are known to induce axon collapse (1–5), a phenomenon that could involve the regulation of cell adhesion through the modulation of integrin activity (13). We asked whether A39R could have a similar effect on mouse DCs. To test this hypothesis, we took advantage of the fact that freshly isolated spleen DCs or bone marrow-derived DCs placed in culture transiently adhere to plastic. This adhesion occurs within 2 h and is enhanced by maturation-inducing agents like LPS (Fig. 2A). This adhesion is dynamic as DCs are motile cells that, under in vitro conditions, constantly extend and retract lamellipodia, spreading and stretching their cellular body from one adherence point to another. This makes them often multipolar and from time to time creates long “dendrites” (Fig. 2B and Ref. (17)). Strikingly, when A39R was added at the beginning of the culture together with LPS, it completely prevented the formation of membrane processes by DCs (Fig. 2C) as well as their adhesion to plastic. This effect was dependent on plexin C1 as A39R did not affect plexin C1−/− DCs either cultured in separate wells (Fig. 2, D and E) or in the same wells as wild-type cells (Fig. 2, F and G), wild-type and plexin C1−/− cells discriminated by a prior labeling with green and red dye, respectively). A39R was effective on wild-type DCs at a wide range of concentrations (Fig. 2H), and importantly, without affecting their viability (Fig. 2I). A39R also inhibited DC adhesion when DCs were cultured on extracellular matrix substrates such as fibronectin, (see below) collagen IV, or vitronectin (data not shown), which suggested that A39R-induced signal inhibited DC adhesion mediated by integrins. To explore this possibility, the role of integrins in in vitro DC adhesion on plastic or on a physiologic substrate like fibronectin was studied.

DC adhesion to plastic. β2 integrins confer a general stickiness to polymorphonuclear granulocytes resulting in their strong adhesion to many substrates, including plastic (18). To determine the role of β2 integrins in DC adhesion to plastic, we cultured DCs on plastic, in the presence of blocking anti-β2, or control Abs (isotype control or blocking anti-β1), and measured their adhesion (Fig. 3A, left). Results show that DC adhesion to plastic was dependent on β2
integrins as the addition of anti-β2 Ab reduced DC adhesion to plastic by 60%. By contrast, a blocking anti-β1 Ab had no effect on DC adhesion to plastic and a combination of anti-β2 and anti-β1 Abs had the same effect than the anti-β2 Ab alone.  

DC adhesion to fibronectin. In vitro adhesion of mouse polymorphonuclear or human Langerhans cells to fibronectin occurs through β1 integrin receptors (19). The effect of blocking anti-β2, anti-β1, or control Abs on DC adhesion to fibronectin was measured (Fig. 3A, right). DC adhesion to fibronectin-coated wells was reduced by 40% by the anti-β2 blocking Ab (Fig. 3A, right), showing a residual β2 binding to plastic even in the presence of fibronectin. Anti-β1 Ab alone reduced DC adhesion to fibronectin by 40% as well. A combination of both Abs had an additive effect and reduced DC adhesion by 80%. These results show that in these culture conditions, DC adhesion to fibronectin-coated wells was mediated at 80% by β1 and β2 integrins. 

**Effect of A39R on integrin-mediated DC adhesion.** Results presented in Fig. 3A show that DC adhesion to plastic or fibronectin was reduced by 80% by A39R. In particular, the effect of A39R on DC adhesion to fibronectin was similar than the combined effect of blocking Abs against β1 and β2 integrins. Thus, plexin C1 engagement by A39R inhibited DC adhesion mediated by integrins. These results suggest that integrin function in DCs could be affected following A39R binding to plexin C1. To further test this hypothesis, we measured the effect of A39R on the level of FAK phosphorylation in DCs, as FAK phosphorylation is associated with integrin activation, i.e., cells expressing active integrins contain a high proportion of Y397-phosphorylated FAK (20–24). As shown in Fig. 3B, a high proportion of FAK was phosphorylated on Y397 following a culture in the presence of LPS. By contrast, LPS/A39R-treated DCs displayed a more reduced proportion of phosphorylated FAK. Thus A39R-induced signal inhibits integrin...
signaling as measured by FAK phosphorylation in DCs, further supporting the inhibition of integrin function by A39R.

**A39R induces actin cytoskeleton rearrangement, retraction of membrane processes, and detachment of adherent DCs**

Strong evidence shows that semaphorin binding to plexins induces actin cytoskeleton rearrangement in neurons or cell lines (3, 5, 9), a phenomenon associated with cellular retraction. Therefore, we investigated the effect of A39R on the actin cytoskeleton of DCs. To better visualize this effect, A39R was added to DCs that had been previously left to adhere for 2 h. In these conditions, as monitored by time-lapse microscopy, A39R induced a progressive retraction of adherent DC membrane processes within 10 min of treatment (Fig. 4A). Collapsing DCs displayed multiple spikes rather than the characteristic lamellipodia that progressively disappeared resulting in a round or loosely adherent cell (Fig. 4A). This effect was plexin C1-dependent as plexin C1–/– DC morphology was not altered in response to A39R (data not shown). Detection of F-actin with phalloidin in spread, adherent DCs showed diffuse intracellular pattern and some more intense staining at the edge of the membrane (Fig. 4B, before A39R). Upon treatment with A39R, the collapse of lamellipodia was associated with an increase in the intensity of the F-actin staining at the base of the spikes or within the spikes themselves, consistent with a local reorganization of the actin cytoskeleton (Fig. 4B, after A39R, early, and intermediate stages). At a later stage, F-actin staining was restricted to the perinuclear region of the rounded cell (Fig. 4B, after A39R, final stage). Upon treatment with A39R, FAK phosphorylation on Y397 progressively decreased (Fig. 4C). After 30 min of treatment with A39R, this phosphorylation was barely detectable (Fig. 4C) and DCs were detached (Fig. 4D).

Taken together, these results demonstrate that A39R induces a rearrangement of the actin cytoskeleton, and inhibits FAK phosphorylation in adherent plexin C1 expressing DCs, leading to a retraction of their membrane processes and inhibition of their adhesion.

**A39R effect on DC is blocked by a coflin phosphorylation inhibitor**

Cofilin binds to and depolymerizes pointed ends of F-actin, thereby increasing monomeric actin intracellular concentration leading to an increased F-actin turnover (25, 26). Phosphorylation of cofilin by kinases like Lin-11-Is1-1-Mec-3 kinase dissociates it from F-actin and inhibits its F-actin depolymerization activity. Because Sema3A induces actin cytoskeleton rearrangement through cofilin phosphorylation in neurons (11), we tested the involvement of cofilin phosphorylation in A39R-induced detachment of DCs. DCs were cultured in the presence of S3, a synthetic cell-permeable peptide containing a cofilin phosphorylation site that prevents the phosphorylation of endogenous cofilin and its inactivation (11) or a negative control, scrambled, peptide (RV) before the addition of A39R or PBS, then DC adhesion was measured. As shown in Fig. 5, in the absence of peptide, the addition of A39R induced a detachment of ~60% of DCs. Increasing concentration of RV peptide had no effect on this A39R-induced detachment. By contrast, micromolar concentrations of S3 peptide efficiently inhibited A39R-induced DC detachment, without increasing DC adhesion in the control PBS condition. These results suggest that cofilin phosphorylation is a key downstream event in A39R-induced plexin C1 signaling and further link plexin C1 with the regulation of actin cytoskeleton.

**A39R does not induce maturation of DCs or affects the maturation induced by CpG oligonucleotides**

As DC maturation and activation is associated with changes in adherence and morphology with reorganization of their cytoskeleton (27–29), we tested whether A39R-induced morphological changes were associated with DC maturation. First, DCs were cultured with A39R or medium and MHC class II and costimulatory molecules expression (Fig. 6A) as well as cytokine production (Fig. 6B) was measured. Fig. 6 shows that A39R did not induce DC maturation. Then, we cocultured DCs with or without A39R and stimuli known to induce DC maturation such as a combination of CpG oligonucleotides with CD40L and GM-CSF. Fig. 6 shows that A39R addition did not alter DC maturation induced by these stimuli. Thus, A39R does not induce maturation of DCs or affect the maturation induced by CpG/CD40L/GM-CSF.

**A39R inhibits chemokine-induced DC migration in vitro**

Leukocyte trafficking in homeostatic conditions and their recruitment to inflamed sites are regulated by different classes of chemotactic molecules that regulate leukocyte actin cytoskeleton and their adhesion molecules such as integrins in a coordinated fashion. To investigate the effect of A39R semaphorin on DC migration to chemokines, an in vitro chemotaxis assay was used. Wild-type or plexin C1–/– DCs were placed in the upper chamber of a transwell system and medium or the CCL3 was added to the lower chamber. CCL3 is known to induce immature DC migration to inflamed sites through its binding to the chemokine receptor CCR5. When indicated, A39R, or as a negative control, heat-inactivated A39R were also added to the lower chamber at different concentrations, alone or in combination with CCL3. Results presented in Fig. 7 show that CCL3 induced DC migration to the lower chamber of the transwell system in comparison with medium alone or A39R alone. Strikingly, A39R, but not heat inactivated A39R, inhibited wild-type DC migration to CCL3 in a dose-dependent manner. By contrast, A39R had no effect on plexin C1–/– DC migration induced by CCL3. A similar inhibition of DC
migration by A39R was also observed when the other CCR5 ligands CCL4 and CCL5 or the CXCR4 ligand stromal cell-derived factor-1 were used as chemoattractants (data not shown). Moreover, A39R effect was not directional as it had the same effect when added in the lower chamber with the chemokines than in the upper chamber with the cells, or in both chambers (data not shown). Thus, plexin C1 engagement by A39R semaphorin inhibits chemokine-induced migration of DCs in vitro.

Discussion
An increasing number of reports show semaphorin/plexin expression in immune cells (15). In particular, several semaphorins seem to function in the reciprocal stimulation of T cells and APCs through nonplexin receptors. These semaphorins were thus believed to act through mechanisms that are different from those that are used in the nervous system (15). We show in this study for the first time that semaphorin binding to a plexin can regulate actin cytoskeleton rearrangements as well as integrin function in immune primary cells, in a similar manner than what was described for neurons.

We found that, in the mouse, plexin C1 was expressed mainly on DCs and neutrophils. Very little expression of plexin C1 was measured on B cells, although human plexin C1 was cloned from a B cell line, indicating a possible difference in plexin C1 expression between human and mouse. A39R did not bind to B cells,
peptide, showed a similar phenomenon in Sema3A-treated neurons, suggesting that cofilin phosphorylation is a central event in semaphorin-induced signaling in different cell types. It is, however, unclear how cofilin phosphorylation and the reduction of F-actin turnover may lead to lamellipodia/growth cone retraction. Barberis et al. (14) recently showed that Sema4D induced disassembly of focal adhesions in plexin B1 transfected COS cells. Interestingly, they found that this disassembly occurred before the actin cytoskeleton rearrangement suggesting that integrin inhibition and the resulting loss of adherence was the initial event induced by plexin signaling, causing membrane retraction. It is possible that cofilin phosphorylation occurs as a consequence of the regulation of integrin function by semaphorins. A direct link between plexins and integrins remains however to be identified.

We found that plexin C1 engagement by A39R could prevent chemokine-induced migration of DCs. Similarly, a previous study reported that CD100 and Sema3A semaphorins inhibited chemokine-induced migration of human monocytes in vitro through binding to an unknown receptor (30). This inhibition is not due to an interference with chemokine receptor signaling. Indeed we found that A39R did not alter the calcium response induced by chemokines (data not shown). Instead, the mechanism of A39R-induced inhibition of migration likely involves the inhibition of integrin function that is critical for leukocyte migration. Thus, in a simple model, integrin activity could be the molecular switch used by different guidance molecules either to attract or repel cells. Similarly, following early studies in neurons revealing the repellent activity of many semaphorins on cultured neurons in vitro, it was believed that semaphorins were guiding axons by providing them with “stop” signals in areas where they should not grow. These signals were thought to be complementary to attractant, chemokine signals to guide the growing axons. Recent studies in the field of endothelial cells refined this view. Indeed, Serini et al. (13) showed that autocrine loops of Sema3 chemorepellents exert an essential permissive role in the execution of vasculature remodelling by inhibiting integrin-mediated adhesion of endothelial cells to the extracellular matrix, allowing the de-adhesion necessary for vascular remodelling. They proposed that such a fine-tuning of integrin-mediated adhesion to the extracellular matrix allows a graded control of endothelial cell migration rate and redirection during migration. Thus, semaphorin signals could in fact help cells to

FIGURE 5. A39R effect on DCs is blocked by a cofilin phosphorylation inhibitor. Wild-type bone marrow-derived DCs were cultured in medium supplemented with LPS for 1 h. RV (left) or S3 (right) peptides were then added at the indicated concentrations and DCs were cultured for one more hour. A39R or PBS was then added for 30 min, as indicated. The number of adherent DCs in each well was measured. Results are expressed as the mean percentage ± SD of adherent cells relative to the control condition without A39R and without peptide. Paired bars show that mean adhesions (+) are significantly different (p < 0.001). NS, No statistical difference (p > 0.05).

FIGURE 6. A39R does not induce maturation of DCs or affect the maturation induced by CpG oligonucleotides. Bone marrow-derived DCs were cultured for the indicated times (A) or for 24 h (B) in the indicated conditions. CpG/CD40L/GM-CSF mixture (CpG) is shown in A. CD40, CD86, and I-A expression was then measured by flow cytometry (A) or cytokine secretion was measured in the supernatant (B) as described in Materials and Methods. Results are representative of three experiments. A39R addition had no statistical effect on the parameters measured in A and B.
move along a chemokinetic gradient, provided that both signals are not delivered in an antagonistic manner, by contributing to the dynamic of integrin activity. More experiments using semaphorin and plexin-deficient animals are required to test whether this model could apply to leukocyte migration. In the case of plexin C1, the identification of its cognate cellular ligand will also be a determinant to study its precise function in the regulation of leukocyte movement. Based on in vitro binding studies and homology of A39R with A37R it was suggested that Sema7A is the ligand for plexin C1 (8). However, our own attempts as well as others (31) have failed to show any binding of recombinant Sema7A on primary plexin C1 expressing leukocytes. Moreover, Sema7A has been shown to promote axon outgrowth in neurons in a plexin C1-independent manner (12).

A39R semaphorin is produced by many strains of poxviruses and seems to be secreted at very high amounts by virus-infected cells. A study by Gardner et al. (32) specifically addressed the role of A39R using different recombinant strains of vaccinia virus in a model of intradermal infection in mice. The authors reported that the forced expression of A39R by vaccinia strains that do not normally express it increased the severity and the persistence of skin lesions. It was, however, unclear whether this phenomenon was due to an increased immunopathology or to an increased virulence of the strain. The authors proposed that A39R might have intrinsic proinflammatory properties in this mouse system. This possibility was supported by the observation by Comeau et al. (7) that plexin C1 engagement by A39R induced a modest increase in the production of some proinflammatory cytokines in human monocytes. However, we did not detect any production of IL-1, IL-2, IL-4, IL-6, TNF-α, GM-CSF, IFN-γ and IL-12p70 by various mouse leukocytes (spleen cells, bone marrow cells, DCs, neutrophils, B cells) upon treatment with A39R in vitro (our study and data not shown), suggesting that a proinflammatory activity for A39R in mice is unlikely. Thus, we rather favor the hypothesis that A39R secretion might somehow alter the movement of plexin C1-expressing cells (DCs, monocytes, granulocytes) in the proximity of infected cells. Indeed, the uncontrolled expression of a semaphorin is predicted to have important consequences on the movement of responsive cells based on their activity on integrin function, as illustrated by our in vitro experiments. This effect could benefit the virus by inhibiting the recruitment of immune effector cells at the site of virus infection. Another possibility could be that regulating cell cytoskeleton and shape of neighboring cells could help the virus to infect them, as recently shown for human T cell leukemia virus (33).

In conclusion, we found that A39R binding to plexin C1 on primary mouse DCs induces actin cytoskeleton rearrangement, inhibits integrin-mediated adhesion and FAK phosphorylation, and impairs chemokine-induced migration in vitro. These data identify for the first time semaphorins and plexins as potent regulators of the actin cytoskeleton in the immune system and suggest that they could be involved in the regulation of leukocyte movement.

Acknowledgments
We especially thank Dr. M. K. Spriggs who initiated the work on semaphorins. We also thank Dr. J. Peschon who generated plexin C1-deficient mice, S. Wong Madden for the production of A39R protein, K. Schooley, for technical advice, J. Bradshaw, D. Kaufman, L. Strockbine for technical help, G. Carlton for graphics assistance, and Drs. D. Fitzpatrick, C. Maliszewski, J. Vakili, and A. Youakim for critical reading of the manuscript.

References
laminin and fibronectin occurs through β1 integrin receptors. J. Leukocyte Biol. 51:415.