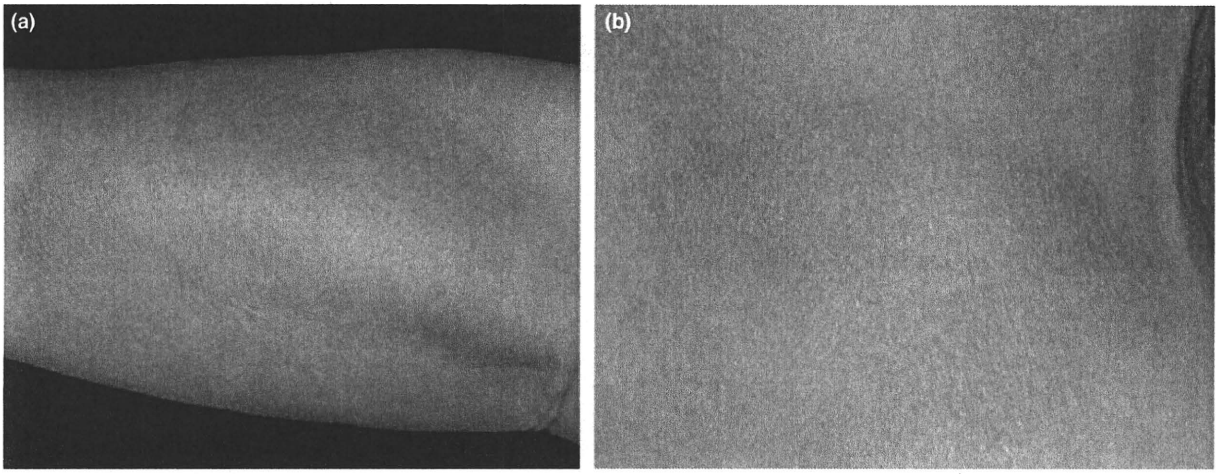

Figure 1. Erythematous plaques on the forearm (a) and neck (b).

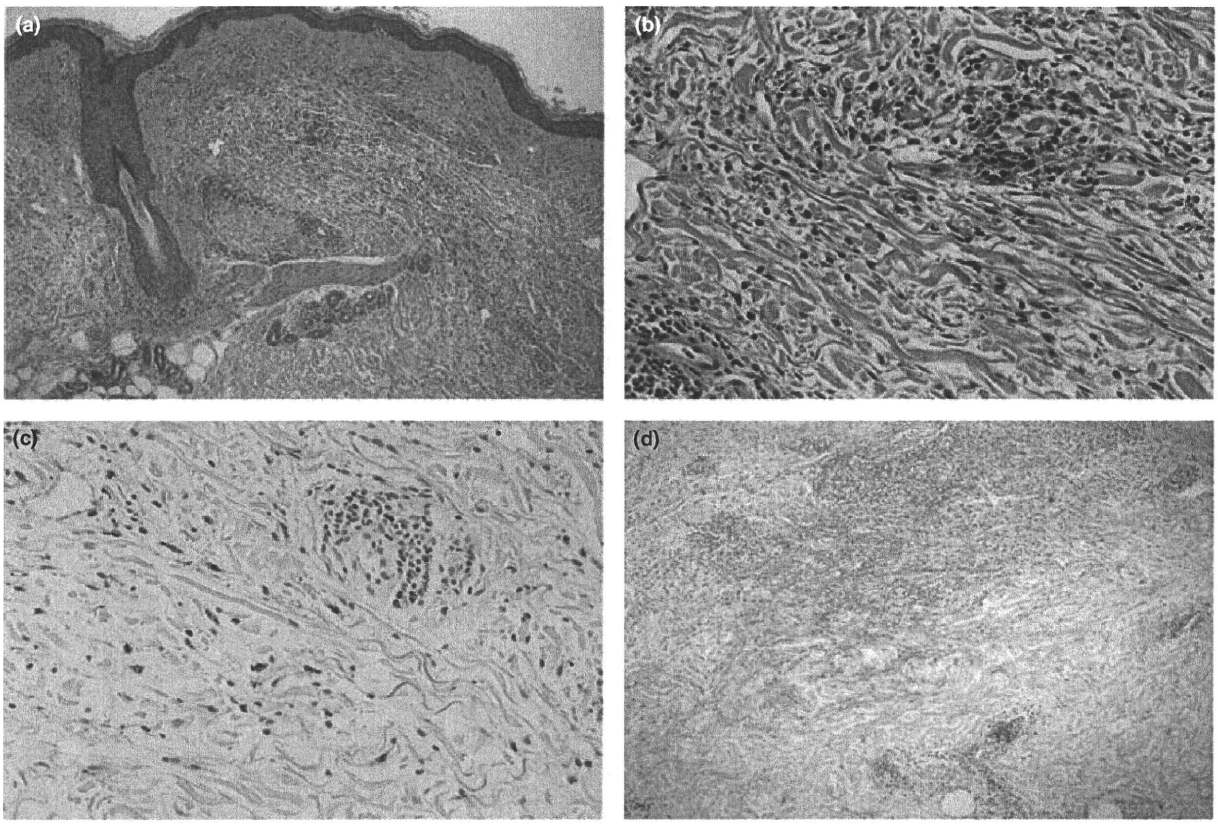

Figure 2. Dermal interstitial lymphocytic infiltrate with CD68-positive histiocytes. (a) Infiltration of inflammatory cells in dermal interstitium. (b) Granulomatous infiltration with foci of collagen degeneration. (c) Infiltration of CD68-positive histiocytes in dermal interstitium. (d) Colloidal iron stain-positive mucin deposition in reticular dermis.

absent. Chest X-ray was also within normal limits. These findings excluded the possibility of sarcoidosis. For diagnosing SS, we performed the Schirmer test, Saxon test and lip biopsy. The Schirmer test was negative (right 25 mm, left 28 mm) and the Saxon test was positive (1.5 mL/2 min). Histopathological

examination of the lip biopsy sample showed focal lymphocytic sialadenitis, defined as the presence of dense aggregates of more than 50 lymphocytes in the periductal areas. These laboratory and clinical findings fulfilled the American–European Consensus Group criteria for diagnosing SS. A skin biopsy taken from the erythematous region of the left forearm revealed dermal interstitial lymphocytic infiltration with CD68 (KP1)-positive histiocytes around the deposit of colloidal iron stain-positive mucin in the reticular dermis (Fig. 2). These histopathological findings were consistent with GA. The erythematous plaques showed no change after discontinuing all the oral medications for a month, indicating that the skin symptoms were not a drug-related adverse reaction. Topical corticosteroid (0.05% betamethasone butyrate propionate ointment) was effective, and the plaques resolved in 2 weeks, but reappeared soon after discontinuing the treatment.

DISCUSSION

Interstitial granulomatous dermatitis (IGD) is a new disorder described by Ackerman *et al.*,[3] and is usually associated with arthritis caused by rheumatoid disease. It has the characteristic histological finding of interstitial histiocytic infiltration: interstitial histiocytes infiltrate the reticular dermis and severe collagen degeneration is observed. Neutrophils and eosinophils are most apparent in the collagen degeneration zones and mucin is usually scant or absent.[4] In our patient, however, skin biopsy revealed histiocytes and mucin deposition in the perivascular and interstitial dermis, indicating interstitial-type GA and excluding IGD as a differential diagnosis. GA is histologically classified into three groups: interstitial (mononuclear infiltrative), palisade and epithelioid.[5] It is frequently associated with diabetes mellitus, malignant diseases and infectious diseases. A recent report shows that GA is also associated with autoimmune diseases, including systemic sclerosis and dermatomyositis.[6] Further, Gal *et al.*[7] described patients with both sarcoidosis and SS. These facts indicate that granulomatous diseases and autoimmune diseases, including SS, are closely related.

In conclusion, GA should be recognized as a cutaneous manifestation associated with autoimmune diseases, including SS, and the presence of GA should make SS a differential diagnosis.

REFERENCES

1 Dafni UG, Tzioufas AG, Staikos P, Skopouli FN, Moutsopoulos HM. Prevalence of Sjogren's syndrome in a closed rural community. *Ann Rheum Dis* 1997; **56**: 521–525.
2 Soy M, Piskin S. Cutaneous findings in patients with primary Sjogren's syndrome. *Clin Rheumatol* 2007; **26**: 1350–1352.
3 Ackerman A, Guo Y, Vitale P, Vossaert K. *Clues to Diagnosis in Dermatopathology*. American Society of Clinical Pathologists Press, Chicago 1993; 309–312.
4 Tomasini C, Pippione M. Interstitial granulomatous dermatitis with plaques. *J Am Acad Dermatol* 2002; **46**: 892–899.
5 Umbert P, Winkelmann RK. Histologic, ultrastructural and histochemical studies of granuloma annulare. *Arch Dermatol* 1977; **113**: 1681–1686.
6 Imamura S, Ohnishi R, Kawasaki Y, Yoshida M. Long-standing solitary granuloma annulare on the palm of a patient with dermatomyositis. *J Dermatol* 2008; **35**: 304–305.
7 Gal I, Kovacs J, Zeher M. Case series: coexistence of Sjogren's syndrome and sarcoidosis. *J Rheumatol* 2000; **27**: 2507–2510.

most information immediately after diagnosis. After the completion of treatment the contacts of the patients with their doctor will diminish,[3] while the information need of the melanoma survivor may still exist. Patients diagnosed and treated shortly before the completion of the questionnaire might have a clearer picture of the information they received and therefore reported to have received more information.

Younger and more highly educated melanoma patients are more likely to be actively involved in decision-making processes and ask more questions. Older patients are reported to be less interested in detailed information and leave the provision of details up to the doctor.[9] Likewise, doctors can be prejudiced against older patients. Furthermore, older and less educated patients may have more difficulties processing and remembering information they receive and may compensate for their reduced cognitive capacity by asking fewer questions to their specialist.[9]

The observed variation in information satisfaction levels between patients treated in different hospitals can be explained by the variation in patient-centred information provision, which is strongly related to information recall and understanding. Our finding that only 25% of the melanoma survivors wanted more information could be explained by the increasing internet use of melanoma patients.[10] When patients are not satisfied with the information received from their health care provider, they will search for additional information on the internet.

Health care providers often have limited time and resources to provide the information that melanoma patients require. With growing evidence that well-informed patients are more satisfied with their care, and do better clinically, efforts are needed to improve the information provision to melanoma patients. Exploration of the patients' personal information needs must lead to a more patient-tailored approach of informing melanoma patients. A good opportunity would be the implementation of a survivorship care plan, which aims at providing a cancer survivor with a summary of their course of treatment, management of late effects, and strategies for health promotion.

Acknowledgments

The present research was supported by the Dutch Cancer Society (UVT 2009-4349). We thank all patients and their dermatologists for their participation in the study.

O. Husson*†
C. Holterhues‡
F. Mols*†
T. Nijsten‡
L.V. van de Poll-Franse*†

*CoRPS – Center of Research on Psychology in Somatic diseases, Department of Medical Psychology and Neuropsychology, Tilburg University, 5000 LE Tilburg, the Netherlands
†Comprehensive Cancer Centre South (CCCS), Eindhoven Cancer Registry, Eindhoven, the Netherlands
‡Department of Dermatology, Erasmus Medical Centre Rotterdam, Rotterdam, the Netherlands
E-mail: O.Husson@uvt.nl

References

1 Francken AB, Shaw HM, Thompson JF. Detection of second primary cutaneous melanomas. *Eur J Surg Oncol* 2008; **34**:587–92.
2 Davies NJ, Kinman G, Thomas RJ, Bailey T. Information satisfaction in breast and prostate cancer patients: implications for quality of life. *Psychooncology* 2008; **17**:1048–52.
3 McInnes DK, Cleary PD, Stein KD *et al.* Perceptions of cancer-related information among cancer survivors: a report from the American Cancer Society's Studies of Cancer Survivors. *Cancer* 2008; **113**:1471–9.
4 Harrison D, Young JM, Price MA *et al.* What are the unmet supportive care needs of people with cancer? A systematic review. *Support Care Cancer* (in press).
5 Snyder CF, Dy SM, Hendricks DE *et al.* Asking the right questions: investigating needs assessments and health-related quality-of-life questionnaires for use in oncology clinical practice. *Support Care Cancer* 2007; **15**:1075–85.
6 Schofield PE, Beeney LJ, Thompson JF *et al.* Hearing the bad news of a cancer diagnosis: the Australian melanoma patient's perspective. *Ann Oncol* 2001; **12**:365–71.
7 Mols F, Holterhues C, Nijsten T, van de Poll-Franse LV. Personality is associated with health status and impact of cancer among melanoma survivors. *Eur J Cancer* 2010; **46**:573–80.
8 Arraras JI, Kuljanic-Vlasic K, Bjordal K *et al.* EORTC QLQ-INFO26: a questionnaire to assess information given to cancer patients a preliminary analysis in eight countries. *Psychooncology* 2007; **16**:249–54.
9 Jansen J, van Weert J, van Dulmen S *et al.* Patient education about treatment in cancer care: an overview of the literature on older patients' needs. *Cancer Nurs* 2007; **30**:251–60.
10 van de Poll-Franse LV, van Eenbergen MC. Internet use by cancer survivors: current use and future wishes. *Support Care Cancer* 2008; **16**:1189–95.

Key words: *cancer survivors, information provision, information satisfaction, melanoma*

Conflicts of interest: none declared.

Cutaneous symptoms in a patient with cardiofaciocutaneous syndrome and increased ERK phosphorylation in skin fibroblasts

DOI: 10.1111/j.1365-2133.2010.09875.x

Madam, Cardio-facio-cutaneous (CFC) syndrome, which is characterized by distinctive facial features, mental retardation and heart defects, was first described in 1986.[1] Patients have ectodermal features including sparse, curly hair with sparse eyebrows and eyelashes, hyperkeratosis, keratosis pilaris, ichthyosis, naevi and haemangioma.[2,3] They also present with craniofacial features that are similar to Noonan syndrome, including macrocephaly, broad forehead, bitem-

poral narrowing, hypoplasia of the supraorbital ridges, short nose with depressed nasal bridge, a high-arched palate, and low-set, posteriorly rotated ears with prominent helices.[3] Patients with CFC syndrome share many phenotypic features with patients with Noonan, LEOPARD (multiple lentigines, electrocardiographic conduction abnormalities, ocular hypertelorism, pulmonary stenosis, abnormal genitalia, retardation of growth and sensorineural deafness) and Costello syndromes, leading to the theory that the pathogenesis of these syndromes may be interdependent. Since the identification of pathological mutations in *KRAS*, *BRAF* and mitogen-activated protein kinase kinase 1 and 2 (*MAP2K1*/*MAP2K2*) in patients with CFC syndrome[4,5] in 2006, the number of patients diagnosed with CFC syndrome has increased dramatically and phenotypic diagnostic criteria are now under reconsideration.[6]

Here we describe a *MAP2K2* mutation in a patient with typical CFC ectodermal phenotype with scattered naevi over the whole body.

The patient was a 13-year-old Japanese boy. He was delivered by caesarean section at 34 weeks 5 days gestation. His parents were healthy and nonrelated. His birth weight was 2788 g, body length was 44·3 cm and head circumference was 35 cm. His mother experienced polyhydramnios during pregnancy. He showed mild developmental delay, walking at 1·8 years of age, and mild mental retardation (DQ50). No abnormalities were observed on electroencephalogram, electrocardiogram or magnetic resonance imaging of the brain. Echocardiography at age 13 years showed mild mitral regurgitation and aortic regurgitation. His ejection fraction was 70%. Cardiomyopathy and pulmonary stenosis were not found.

He presented with dark brown pigmented spots which gradually increased in number with age. Physical examination revealed multiple brown to black spots of 1–2 mm in size over the whole body. There was also a darker brown macule of 3 cm in diameter (Fig. 1a–f). He had sparse, curly hair, and lacked eyebrows and eyelashes. He also showed follicular keratosis and hyperkeratosis of the sole, and sacral lesions. His forehead was high with bitemporal constriction, and supraorbital ridges were hypoplastic. He also had pigeon breast, right exotropia, and relative microcephaly (weight 40·1 kg, height 154·4 cm, head circumference 59 cm). Histologically, the

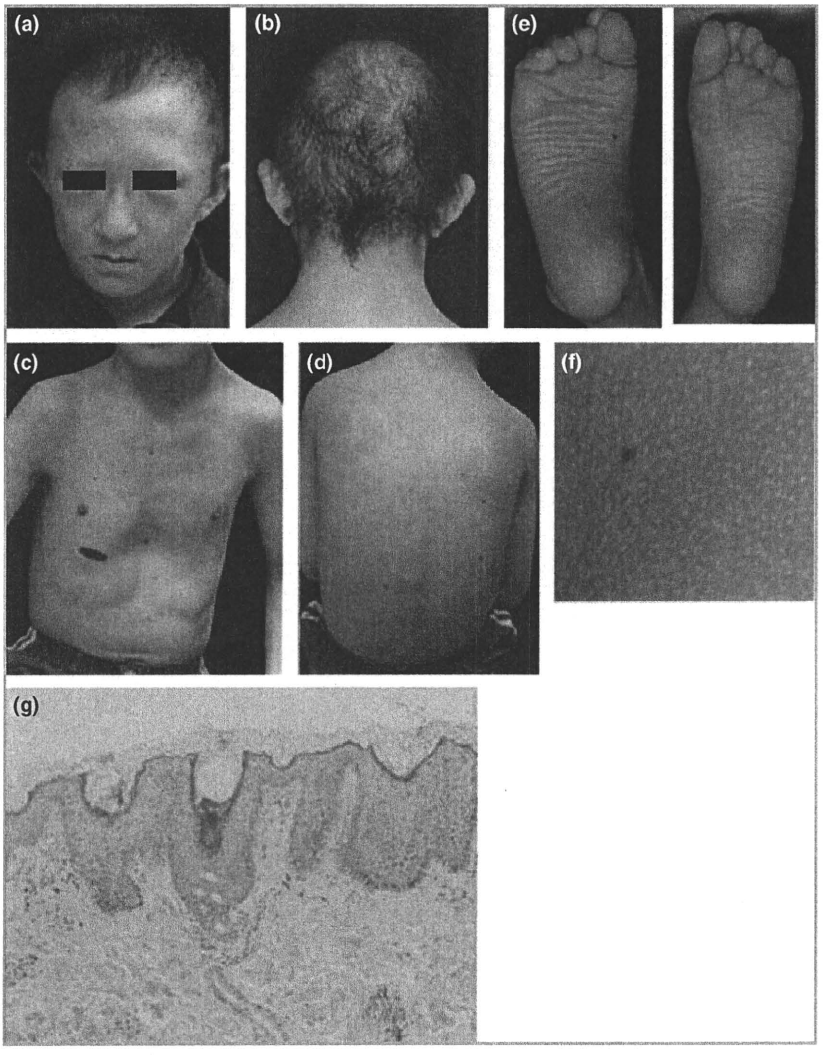

Fig 1. Clinical features of the patient. (a, b) Multiple dark brown naevi were scattered on the face. His hair was sparse and curly, and he lacked eyebrows and eyelashes. His forehead was high and the supraorbital ridges were hypoplastic. (c, d) Multiple dark brown naevi were also scattered on the chest, the abdomen, and the back. A dark brown macule was seen on the chest. (e) Hyperkeratosis and naevus of the sole. (f) Follicular keratosis of the back skin. (g) Histology of naevus. Follicular keratosis was also observed.

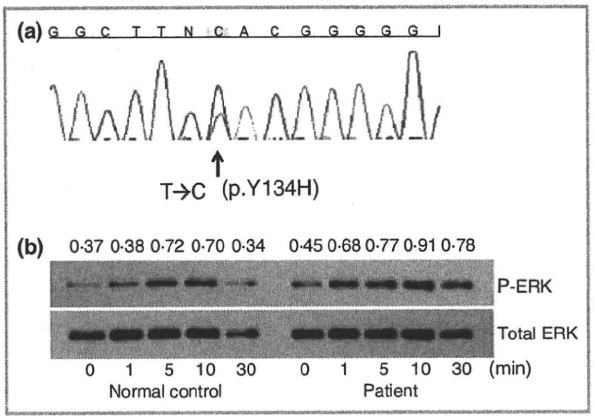

Fig 2. Identification of a mutation in *MAP2K2* and ERK activation of patient and normal control fibroblasts. (a) Heterozygous c.400T>C (p.Y134H) mutation was found in exon 3 of *MAP2K2*. (b) ERK phosphorylation was induced by adding 50 ng mL^{-1} of epidermal growth factor, and cells were harvested at the indicated time point. ERK phosphorylation was enhanced in the patient's fibroblasts. The numbers indicate the relative ratio of phosphorylated ERK (P-ERK) to total ERK.

number of melanocytes in the basal layer was elevated, and keratinocytes were hyperpigmented. Pigmentary incontinence and melanophages were observed in the papillary dermis. Follicles were plugged with compacted orthokeratin (Fig. 1g). A diagnosis of CFC syndrome was considered due to these clinical findings and ectodermal features. The patient's parents gave their written informed consent as the legal guardians for genetic testing, and direct sequencing of the coding regions of *BRAF*, *KRAS* and *MAP2K1/2* was performed to confirm the diagnosis.

Mutation analysis of *BRAF* and *MAP2K1/2* revealed a heterozygous single nucleotide substitution in *MAP2K2* (c.400T>C, p.Y134H) (Fig. 2a), thereby confirming the diagnosis of CFC syndrome.

To examine whether this mutation alters RAS/MAPK pathway activation, primary fibroblasts were taken from biopsied lesions and ERK phosphorylation was induced by adding 50 ng mL^{-1} of epidermal growth factor to the cultured medium. ERK phosphorylation in patient skin fibroblasts was more prolonged than in control skin fibroblasts taken from a healthy volunteer (Fig. 2b). Although it has been reported that three other MEK mutants-transfected human embryonic kidney 293T cells were more active than wild-type MEK in stimulating ERK phosphorylation,[4] it is interesting that this phenomenon was observed in skin fibroblasts taken from our patient. This activated RAS/MAPK pathway may contribute to the cutaneous phenotype of this patient.

Mutation analysis of patients clinically diagnosed with CFC syndrome revealed that 35–47% had *BRAF* mutations, 7·1–10% had *MAP2K1* mutations, 5·9–10% had *MAP2K2* mutations, and 2·5–5·9% had *KRAS* mutations.[6] The mutation found in our patient was also reported in another case report, although the other clinical features of this patient were not described.[6] Congenital heart defects seem to be less common in *MAP2K1/2* mutation-positive patients than in *BRAF* mutation-positive patients.[7] Our patient also lacked congenital heart defects.

Our patient presented with scattered pigmented spots which gradually increased in number as he aged. Hyperpigmentation and/or naevi are found in 27·3% of patients with CFC syndrome.[6] Histological analysis of a pigmented spot in our patient revealed it to be lentigo simplex. There is no previous report that describes histological findings in pigmented spots of CFC. On the other hand, it is reported that both lentigo simplex and melanocytic naevus exist in LEOPARD syndrome.[8]

As the syndromes caused by germline mutations in genes that encode components of the RAS/MAPK pathway such as Noonan, CFC, Costello and LEOPARD syndromes show overlapping phenotypic features, it is now suggested that these syndromes should be considered as a group of 'RAS/MAPK syndromes'[2] or 'RASopathies'.[9,10]

Here we have reported a case of CFC syndrome with scattered pigmented spots and overactivated RAS signalling in skin fibroblasts. As CFC and other syndromes caused by mutations of the RAS/MAPK pathway present many dermatological features, it is important for dermatologists to be aware of these syndromes.

Department of Dermatology,
**Department of Pediatrics and*
†Research Institute for Microbial Disease,
Osaka University Graduate School of Medicine,
Suita, Osaka 565-0871, Japan
E-mail: mterao@derma.med.osaka-u.ac.jp

M. Terao
N. Sakai*
S. Higashiyama†
Y. Kotobuki
A. Tanemura
M. Wataya-Kaneda
M. Yutsudo†
K. Ozono*
I. Katayama

References

1 Reynolds JF, Neri G, Herrmann JP *et al.* New multiple congenital anomalies/mental retardation syndrome with cardio-facio-cutaneous involvement – the CFC syndrome. *Am J Med Genet* 1986; **25**:413–27.
2 Aoki Y, Niihori T, Narumi Y *et al.* The RAS/MAPK syndromes: novel roles of the RAS pathway in human genetic disorders. *Hum Mutat* 2008; **29**:992–1006.
3 Tidyman WE, Rauen KA. Noonan, Costello and cardio-facio-cutaneous syndromes: dysregulation of the Ras-MAPK pathway. *Expert Rev Mol Med* 2008; **10**:e37.
4 Rodriguez-Viciana P, Tetsu O, Tidyman WE *et al.* Germline mutations in genes within the MAPK pathway cause cardio-facio-cutaneous syndrome. *Science* 2006; **311**:1287–90.
5 Niihori T, Aoki Y, Narumi Y *et al.* Germline *KRAS* and *BRAF* mutations in cardio-facio-cutaneous syndrome. *Nat Genet* 2006; **38**:294–6.
6 Schulz AL, Albrecht B, Arici C *et al.* Mutation and phenotypic spectrum in patients with cardio-facio-cutaneous and Costello syndrome. *Clin Genet* 2008; **73**:62–70.
7 Dentici ML, Sarkozy A, Pantaleoni F *et al.* Spectrum of *MEK1* and *MEK2* gene mutations in cardio-facio-cutaneous syndrome and

genotype–phenotype correlations. Eur J Hum Genet 2009; 17:733–40.
8 Rodriguez-Bujaldon A, Vazquez-Bayo C, Jimenez-Puya R et al. LEOPARD syndrome: what are café noir spots? Pediatr Dermatol 2008; 25:444–8.
9 Rauen KA, Schoyer L, McCormick F et al. Proceedings from the 2009 genetic syndromes of the Ras/MAPK pathway: from bedside to bench and back. Am J Med Genet A 2010; 152A:4–24.
10 Tidyman WE, Rauen KA. The RASopathies: developmental syndromes of Ras/MAPK pathway dysregulation. Curr Opin Genet Dev 2009; 19:230–6.

Key words: cardio-facio-cutaneous syndrome, LEOPARD syndrome, MAPK, RAS, RASopathies.

Conflicts of interest: none declared.

Topical methyl aminolaevulinate–photodynamic therapy in erosive facial mycosis fungoides

DOI: 10.1111/j.1365-2133.2010.09898.x

MADAM, Photodynamic therapy (PDT) is a recently introduced therapeutic modality that has proved effective in some subsets of nonmelanoma skin cancers and their precursors.[1] According to preliminary records, it might also be useful in patients with mycosis fungoides (MF), particularly for patch and plaque stages.[2] In this perspective, we report a patient with specific erosive facial lesions resistant to conventional therapies and successfully treated with PDT using methyl aminolaevulinate (MAL) cream.

An 87-year-old man was referred for patch and plaque stage MF of 10 years duration, previously treated with topical steroids, psoralen plus ultraviolet A (PUVA) and retinoid plus PUVA, with a partial and transient benefit. The occurrence of new, erosive lesions affecting the eyelids, the cheeks and the forehead impairing his quality of life and an increasing number of body plaques prompted treatment with methotrexate at an initial weekly dosage of 15 mg. However, this treatment showed poor efficacy especially on facial erosions that progressively worsened, involving the whole upper face and resulting in bilateral ectropion (Fig. 1a). Four months after its introduction, methotrexate was replaced by bexarotene 600 mg daily, with a significant effect on extrafacial lesions. However, because of persistent disease activity on the face only, PDT was considered as an additional treatment. Accordingly, 3 months after starting the rexinoid, the patient was given two sessions of MAL-PDT within 4 weeks on the left cheek only, with 20% MAL cream (Metvix®; Galderma, Lausanne, Switzerland) under occlusive dressing for 3 h followed by 10 min of irradiation delivered by an Aktilite (Galderma) lamp (635 nm, 37 J cm^{-2}, 86 mW cm^{-2}). Side-effects were restricted to the treated areas and consisted of moderate burning sensations during exposures, discrete to moderate erythema and oedema followed by erosion and crust formation, with complete healing over the following 2–3 weeks. After this initial period, cutaneous lesions progressively improved on the treated area with reduced erythema and infiltration, disappearance of scaling and erosions and reduction of ectropion (Fig. 1b). The right side, primarily used as an internal control of PDT-specific therapeutic action, progressively improved as well, possibly owing to the systemic treatment, but significantly later, more slowly and to a much lesser extent compared with the left side. Subsequently, topical MAL-PDT was repeated four times at intervals of 2 months on the left side and applied to the right cheek as well with a good and sustained final result on both sides. Additionally, an eroded and infiltrated plaque of the right forehead was treated with nine courses of radiotherapy (accelerated electrons, 27 Gy) with a good response. Unfortunately, the patient died of unrelated causes 19 months after the first course of PDT.

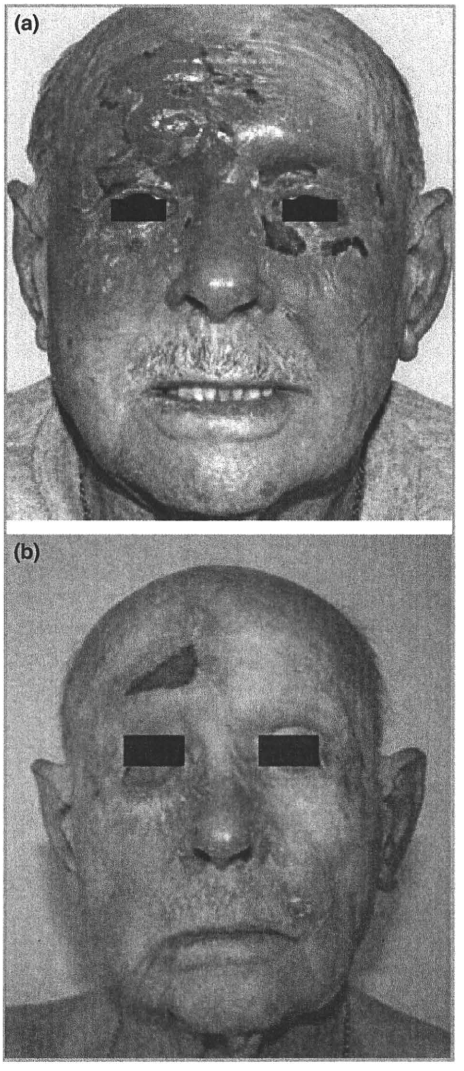

Fig 1. (a) Mycosis fungoides facial lesions before methyl aminolaevulinate–photodynamic therapy (MAL-PDT). (b) Clinical appearance at 4 months after two sessions of MAL-PDT on the left side and radiotherapy on the right side of the forehead.

Published in final edited form as:
Nat Immunol. 2010 July ; 11(7): 594–600. doi:10.1038/ni.1885.

Semaphorins guide the entry of dendritic cells into the lymphatics by activating myosin II

Hyota Takamatsu[1,2], **Noriko Takegahara**[1,2], **Yukinobu Nakagawa**[1,2,3], **Michio Tomura**[4], **Masahiko Taniguchi**[5], **Roland H. Friedel**[6], **Helen Rayburn**[7], **Marc Tessier-Lavigne**[8], **Yutaka Yoshida**[9], **Tatsusada Okuno**[1,2], **Masayuki Mizui**[2,10], **Sujin Kang**[1,2], **Satoshi Nojima**[1,2,11], **Tohru Tsujimura**[12], **Yuji Nakatsuji**[13], **Ichiro Katayama**[3], **Toshihiko Toyofuku**[1,2], **Hitoshi Kikutani**[2,10], and **Atsushi Kumanogoh**[1,2,*]

[1] Department of Immunopathology, Research Institute for Microbial Diseases, Osaka, University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan

[2] World Premier International Immunology Frontier Research Center, Osaka University, 3-1 Ymada-oka, Suita, Osaka 565-0871, Japan

[3] Department of Dermatology, Osaka University Graduate School of Medicine, 2-2, Yamad-oka, Suita, Osaka 565-0871, Japan

[4] Laboratory for Cell Function and Dynamics, Advanced Technology Development Center, Brain Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

[5] Department of Biochemistry, Cancer Research Institute, Sapporo Medical University School of Medicine, S-1, W-17, Chuo-ku, Sapporo, Hokkaido 060-8556, Japan

[6] Institute of Developmental Genetics, Helmholtz Center Munich, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany

[7] Department of Developmental Biology, Stanford University, 318 Campus Drive, Stanford, California 94305, USA

[8] Division of Research, Genentech, Inc., 1 DNA Way, South San Francisco, California 94080, USA

[9] Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA

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

[11] Department of Pathology, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

[12] Department of Pathology, Hyogo College of Medicine, 1-1 Mukogawa-cho, Nishinomiya, Hyogo 663-8501, Japan

*To whom correspondence should be addressed. kumanogo@ragtime.biken.osaka-u.ac.jp.

AUTHOR CONTRIBUTIONS

A.K. and H.T. designed the study and wrote the manuscript. H.T. performed most experiments and analysed the data with N.Y., T.O., M.M., S.J. and S.N.. N.T. produced Sema6D$^{-/-}$ mice, recombinant Sema6D protein and anti-Sema6D antibody. M.T. performed two-photon microscopic experiments. M.T. produced Sema3A$^{-/-}$ mice. R.H.F., H.R. and M. TL. produced Sema6C$^{-/-}$ mice. Y.Y. produced anti-plexin-A1 antibody. T.T. performed histological analysis. Y.N., I.K., T.T. and H.K. provided critical collaborative suggestion to promote this study.

[13] Department of Neurology, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

Abstract

Recirculation of leukocytes is essential for proper immune responses. However, the molecular mechanisms that regulate leukocyte entry into the lymphatics remain unclear. Here we show that plexin-A1, a primary receptor component for class III and class VI semaphorins, is crucially involved in the entry of dendritic cells (DCs) into the lymphatics. Additionally, we show that Sema3A, but not Sema6C or Sema6D, is required for DC transmigration, and that Sema3A produced by the lymphatics promotes actomyosin contraction at the trailing edge of migrating DCs. These findings not only demonstrate that semaphorin-signals are involved in DC trafficking but also provide a novel mechanism that induces actomyosin contraction as these cells pass through narrow gaps.

INTRODUCTION

Dendritic cells (DCs) are highly mobile antigen-presenting cells that use a coordinated trafficking system to ensure that they are present in the right place and at the right time to induce proper immune responses [1]. Before foreign antigens (Ag) encounter, DCs reside as sentinels in the peripheral tissues. However, after Ag exposure, DCs migrate from the peripheral tissues to lymphoid organs, where they activate T cells. To exit the periphery, DCs must migrate towards and enter the initial lymphatics [2,3]. This is an active process that involves chemokines and adhesion molecules [4–8]. Transmission electron microscopic analyses have shown that DCs must undergo dramatic morphological changes in order to pass through endothelial junctions [9,10]. Non-muscle myosin II is required to propel DC cellular bodies forward and pass through narrow gaps [11]. However, it is still unknown how DCs interact with the lymphatic endothelium during transmigration and whether the lymphatics, in turn, control and affect DC mobilization to coordinate their cytoskeletal dynamics, including contraction and adhesion disassembly.

Semaphorins were initially identified as axonal guidance cues that determine the direction and migration of neurons during neurogenesis [12]. In addition, they have diverse and important functions in other physiological processes, including heart development [13], vascular growth [14], tumor progression [15,16] and immune responses [17]. Several semaphorins exhibit co-stimulatory molecule-like activities that promote the activation of B cells [18], T cells [19], DCs [20] and macrophages [21] through cell-cell interactions. In the nervous system, semaphorins regulate cell motility and morphology through interaction with receptors of the plexin family [22]. A similar role for semaphorins in regulating the migration of immune cells was proposed. Among the plexin family members, plexin-A1 (official gene symbol, Plxna1) represents the primary receptor for class III and class VI semaphorins. Together with ligand-binding neuropilins (Nrps) plexin-A1 transduces repulsive axon guidance signals for soluble class III semaphorins[23]. Independently of Nrps, plexin-A1 functions in neurogenesis and cardiogenesis as a receptor for class VI transmembrane semaphorins Sema6C and Sema6D[13,24]. In the immune system, plexin-A1 is specifically expressed in DCs and it may facilitate DC-mediated T-cell stimulation [25]. Plexin-A1-deficient (*Plxna1*$^{-/-}$) mice have severe defects in Ag-specific T-cell responses [20]. However, it is still unclear whether semaphorins physiologically regulate immune cell migration through plexin receptors.

Here we demonstrate that plexin-A1 has a crucial role in DC trafficking, particularly during entry into lymphatics. Specifically, we show that Sema3A produced in the lymphatics functions as a ligand for the plexin-A1-Nrp-1 receptor complex expressed by DCs. Furthermore, we demonstrate that Sema3A acts on the rear side of DCs, where plexin-A1 is

localized. Sema3A induces phosphorylation of the myosin light chain (MLC) to promote actomyosin contraction, resulting in increased DC transmigration as these cells pass through narrow gaps.

RESULTS

Plxna1$^{-/-}$ mice have impaired DC trafficking to the draining LNs

Antigen-specific T cells generation is severely impaired in *Plxna1*$^{-/-}$ mice [20]. However, it is unclear how plexin-A1 is involved in Ag-specific T cell priming. To investigate these mechanisms in greater detail, we activated OT-II T cells [26] in wild-type and *Plxna1*$^{-/-}$ mice. CFSE-labeled OT-II T-cells proliferated extensively after OVA peptides and CFA were subcutaneously administered to wild-type recipient mice (Fig. 1a). In contrast, Ag-specific proliferative responses were markedly reduced when OT-II T cells were transferred into *Plxna1*$^{-/-}$ recipient mice (Fig. 1a), confirming the importance of plexin-A1 in Ag-specific T-cell responses (Supplementary Fig. 1a).

Ag-specific T cells are primed in the draining lymph nodes (LNs) via encounters with Ag-pulsed DCs [27]. To examine the impact of *Plxna1*$^{-/-}$ DCs on DC-T cell interactions in the draining LNs, we injected CMTMR-labeled OVA-pulsed DCs derived from wild-type or *Plxna1*$^{-/-}$ mice into the footpads of wild-type recipient mice and analyzed the popliteal LNs by two-photon microscopy. When wild-type DCs were injected into the footpads, CMTMR-labeled DCs were abundantly distributed and localized throughout the T cell area of the draining LNs (Fig.1b **upper**). By contrast, when *Plxna1*$^{-/-}$ DCs were injected, DCs were only minimally observed in the draining LNs of recipient mice (Fig. 1b lower), indicating that *Plxna1*$^{-/-}$ DCs had impaired migratory capabilities. To quantitatively compare the *in vivo* migratory activities of wild-type and *Plxna1*$^{-/-}$ DCs, we injected CFSE-labeled DCs into the footpads of wild-type mice and examined their arrival in the draining LNs. Compared to wild-type DCs, *Plxna1*$^{-/-}$ DCs exhibited impaired migratory activities (Fig. 1c, Supplementary Fig. 1b). The migration of endogenous DCs was analyzed under steady-state conditions. The number of CD11c^{+}MHC II^{hi} and CD11c^{+}CD4^{-}CD8^{-} migratory DC subsets in the skin-draining LNs [6,28] was reduced in *Plxna1*$^{-/-}$ mice (Supplementary Fig. 1c). Because the expression of plexin-A1 is increased during DC maturation (Supplementary Fig. 2b), we investigated endogenous DC trafficking to the draining LNs under inflammatory conditions. FITC was epicutaneously applied to an area of skin that drains to the brachial LNs and the number of FITC-positive DCs in the draining LNs was determined [29,30]. Significantly reduced number of FITC-positive DCs accumulated in brachial LNs in *Plxna1*$^{-/-}$ mice compared to wild-type mice (Fig. 1d). Collectively, these findings indicate that plexin-A1 is critically involved in DC trafficking and imply that impaired DC migration is the primary reason for defective T cell priming in *Plxna1*$^{-/-}$ mice.

Plxna1$^{-/-}$ DCs exhibit impaired transmigration

To determine which step during DCs trafficking from he peripheral tissues to the lymphatics[3] requires plexin-A1, we studied Ag uptake, interstitial migration toward the lymphatics in response to chemokines, and transmigration across the lymphatics in greater detail (Supplementary Fig. 3). We did not find differences between wild-type and *Plxna1*$^{-/-}$ DCs in Ag uptake (Fig. 2a). In addition, there were no differences in the ability of DCs to migrate in response to CCL19, CCL21 or CXCL12 in transwell assays (Fig. 2b) or to sense direction in a chemokine gradient assay (Fig. 2c, Supplementary Movie 1). Consistent with these findings, CCR7 and CXCR4 were comparably expressed in wild-type and *Plxna1*$^{-/-}$ DCs (Fig. 2d). These results indicate that plexin-A1 is not required for Ag uptake or chemokine responsiveness during migration.

To assess the roles of plexin-A1 in the transmigration process *in vivo*, we adoptively transferred wild-type or *Plxna1*$^{-/-}$ CFSE-labeled DCs into the dermis of oxazolone-treated mice and assessed the fate of emigrating DCs [7]. Large numbers of *Plxna1*$^{-/-}$ DCs were retained along the LYVE-1-positive lymphatics in the dermis of recipient mice 24 h after adoptive transfer (Fig. 3a), a behavior not observed with wild-type DC, indicating that *Plxna1*$^{-/-}$ DCs have impaired transmigration across the lymphatics.

By time-lapse imaging we investigated whether plexin-A1 deficiency in DCs affected the initial contacts between DCs and lymphatic epithelial cells (ECs). Wild-type DCs interacted with ECs at the cell-cell junctions of lymphatic ECs and transmigrated across the ECs (Fig. 3b upper, Supplementary Movie 2). Although *Plxna1*$^{-/-}$ DCs actively moved, extended their dendrites and contacted the lymphatic ECs to the same degree as the wild-type DCs, they did not transmigrate across the lymphatic ECs (Fig. 3b lower, Supplementary Movie 2), To confirm this observation, we added CFSE-labeled DCs onto monolayers of lymphatic ECs stained with the F-actin marker phalloidin and observed these cells by confocal Z-stack imaging. Wild-type DCs were observed from the top to the bottom of the ECs. In contrast, although *Plxna1*$^{-/-}$ DCs were able to attach to the lymphatic ECs, they could not transmigrate across these cells (Fig. 3c, Supplementary Movie 3). Additionally, *Plxna1*$^{-/-}$ DCs showed significantly impaired chemokine-induced transmigration across EC monolayers in transwell experiments (Fig. 3d). Taken together, these results strongly suggest that plexin-A1 plays an important role in the transmigration of DCs across the lymphatics.

Sema3A is responsible for plexin-A1-dependent DC trafficking

Plexin-A1 is a receptor component for two types of semaphorins: a secreted class III semaphorin, Sema3A, and class VI transmembrane semaphorins, Sema6C and Sema6D (Supplementary Fig. 2a). Notably, Sema3A, Sema6C and Sema6D are all expressed in the lymphatic ECs (Supplementary Fig. 2b). To determine which interaction was responsible for the defects in *Plxna1*$^{-/-}$ DCs, we adoptively transferred DCs from wild-type mice into *Sema3A*$^{-/-}$ [31], *Sema6C*$^{-/-}$ (Supplementary Fig. 4) and *Sema6D*$^{-/-}$ (Supplementary Fig. 5) mice. DCs from wild-type mice exhibited impaired migration to the draining LNs when transferred into *Sema3A*$^{-/-}$ recipient mice (Fig. 4a). However, there were no defects in migration of wild-type DCs in *Sema6C*$^{-/-}$ or *Sema6D*$^{-/-}$ recipient mice (Fig. 4a). These results suggest that Sema3A in the lymphatics is indispensable for DC trafficking. Consistently, the Sema3A receptor component, Nrp1, was expressed in DCs (Supplementary Fig. 2b). In addition, DCs from *Nrp1*$^{sema-}$ knock-in mice [32], in which the Sema3A-binding sites are defective, showed impaired trafficking to the draining LNs when transferred into wild-type recipient mice (Fig. 4b left). We confirmed these findings by performing *in vitro* transmigration assays in a lymphatic EC monolayer and found that DCs from *Nrp1*$^{sema-}$ knock-in mice displayed impaired transmigration (Fig. 4b right), similar to that of DCs from *Plxna1*$^{-/-}$ mice (Fig. 3d). Although DCs express Sema6D (Supplementary Fig. 2b), DCs from *Sema6D*$^{-/-}$ mice did not show any defects (data not shown). These results imply that Nrp1 expression in DCs is required for DC trafficking. Consistent with these results, *Sema3A*$^{-/-}$ and *Nrp1*$^{sema-}$ knock-in mice had defects in T-cell priming (Fig. 4c) that were comparable to *Plxna1*$^{-/-}$ mice (Supplementary Fig. 1a), whereas neither *Sema6C*$^{-/-}$ nor *Sema6D*$^{-/-}$ mice had any defects in Ag-specific T-cell priming (Fig. 4c). Collectively, these results indicate that Sema3A, but not Sema6C or Sema6D, is a functional ligand for plexin-A1 during DC trafficking into the LNs.

Sema3A acts on the rear side of DCs

Sema3A was identified as a chemorepellent factor that guides the direction of neurons [12]., while here we show it promote DC trafficking. Therefore, we hypothesized that cell polarity

generated by chemokines during DC migration is critical for the effects induced by Sema3A. To determine the mechanism by which Sema3A regulates DC migration, we performed chemotaxis assays by adding Sema3A to the lower or upper chambers of transwells in the absence or presence of chemokines. In the absence of chemokines, Sema3A did not show an effect on spontaneous DC migration. By contrast, in the presence of chemokines, DC chemotaxis was enhanced in transwell assays when Sema3A was added to the upper chamber, where Sema3A acted on the rear side of DCs, but not to the lower chamber (Fig. 5a). In two-dimensional directional migration assays evaluated by EZ-TAXIScan, Sema3A increased the motilities and velocities of DCs when it was applied against a chemokine gradient (Fig. 5b, Supplementary Movie 4). Consistent with these findings, plexin-A1 localized at the trailing edge but not at the leading edge of migrating DCs, where actin polymerization was readily observed (Fig. 5c,d, Supplementary Movie 5). These results suggest that the effects of Sema3A depend on the polarity of migrating DCs.

Sema3A induces actomyosin contraction through myosin II activity

Myosin II, which is regulated by phosphorylation of MLC [33] and ROCK, is involved in DC trafficking [11]. The localization of these molecules resembles that of plexin-A1 (Fig. 5c, Supplementary Movie 5) [11]. Myosin II is believed to be required to squeeze the cell body and induce actomyosin contraction when cells pass through narrow gaps or constricted areas [11]. In addition, myosin II is implicated in Sema3A-mediated axon retraction [34,35]. Considering these findings, we investigated whether Sema3A affects the function of myosin II in DCs during their mobilization. As expected, MLC phosphorylation was enhanced in DCs that were stimulated with recombinant Sema3A (Fig. 6a), and this effect was not observed in *Plxna1*$^{-/-}$ DCs (Supplementary Fig. 6), indicating that Sema3A promotes MLC phosphorylation through plexin-A1. In 3D collagen matrices, which have been used to model passage through constricted areas *in vitro*, Sema3A increased the velocities of DCs and the motile DC fraction. (Fig. 6b, Supplementary Movie 6) and significantly enhanced DC transmigration, and this effect was abolished in *Plxna1*$^{-/-}$ DCs (Fig. 6c). In addition, Sema3A-induced DC transmigration in collagen matrices (Fig. 6d **left**) or across lymphatic EC monolayers (Fig. 6d **right**) was abolished when the DCs were treated with either a myosin II inhibitor, blebbistatin, or a ROCK inhibitor, Y-27632. Collectively, these results suggest that Sema3A induces actomyosin contraction in DCs during plexin-A1-mediated transmigration (Supplementary Fig. 7).

DISCUSSION

Although semaphorins were originally identified as axonal guidance cues that regulate cell motility and morphology, accumulating evidence indicates that they also function as immune-regulatory molecules. To date, most functional studies on semaphorins and their receptors have focused on their co-stimulatory effects on immune cells [17]. Although the nervous and immune systems have considerable crosstalk and overlap in their molecular repertories and machineries [36], it is still unknown whether semaphorins function as guidance cues that physiologically regulate immune cell movement. Here we demonstrate that semaphorin signals are crucial for DC trafficking, particularly for the entry of DCs into the lymphatics. Furthermore, we highlighted a novel mechanism for DC transmigration across the lymphatics, in which Sema3A promotes actomyosin contraction at the trailing edge of migrating DCs so they can pass through narrow gaps.

To exit peripheral tissues, DCs must migrate towards and enter the initial lymphatics. Although it has been assumed that the migration of DCs into the lymphatics is an indolent process, we provide evidence that Sema3A expressed in lymphatics is crucially involved in DC transmigration. Furthermore, we found that plexin-A1 localizes at the trailing edge of migrating DCs, which is responsible for actomyosin contraction. Numerous factors,

including chemokines [6,8], inflammatory molecules [5,37,38] and adhesion molecules [7], have been reported to participate in DC transmigration. However, in our study, neither chemokine responsiveness nor the expression of chemokine receptors was affected in the absence of plexin-A1. The adhesion of DCs to extracellular matrix (ECM) proteins or to lymphatic ECs was comparable between wild-type and *Plxna1*$^{-/-}$ DCs and the expression levels of integrins were similar between wild-type and *Plxna1*$^{-/-}$ DCs (data not shown). In addition, there were no differences in the secretion of pro-MMP9 and TNF-α between wild-type and *Plxna1*$^{-/-}$ DCs (data not shown). Thus, these results not only indicate that DC transmigration is regulated by active mechanisms but also provide a novel mechanism for this process.

Plexin-A1 is a primary receptor component not only for soluble semaphorin Sema3A, in association with the Nrp1 receptor, but also for transmembrane-type semaphorins Sema6C and Sema6D. We here determine that Sema3A produced in the lymphatics functions as a ligand for the plexin-A1-Nrp1 receptor complex expressed in DCs, indicating that the Sema3A-Nrp1-plexin-A1 pathways play important roles in DC migration by mediating interactions between DCs and lymphatic ECs. However, it is still unclear why Sema3A, but not Sema6C or Sema6D, is involved in this process. One possible explanation is that signaling downstream plexin-A1 is modified by the presence of Nrp1 in the receptor. Alternatively, differential effect may be due to the fact that Sema3A is a soluble protein. In contrast, Sema6C and Sema6D are membrane-bound semaphorins, which may prevent them from functioning at critical interaction sites between transmigrating DCs and ECs.

Members of the Rho family of small GTPases, Rac1, Cdc42 and RhoA, regulate cell movement by altering actin assembly, adhesion and actomyosin contraction [39]. Among these molecules, Rac1 is required to generate actin-rich lamellipodial protrusions and integrin-mediated adhesion [40]. In contrast, RhoA activates ROCK and subsequently activates non-muscle myosin II, which promotes an actomyosin contractile force [41]. Mesenchymal cell movement depends on integrin-mediated traction forces. In contrast, amoeboid cell movement, particularly in three-dimensional environments does not require integrins,. Instead, ROCK and myosin II- dependent contraction, is crucially required for passage through narrow gaps [11]. Consistent with this, we found that Sema3A promoted actomyosin contraction by inducing MLC phosphorylation. Furthermore, this effect was attenuated by blocking ROCK activity, indicating that the Sema3A-induced effects on myosin II activity require RhoA-ROCK-mediated signals. In neurons, Sema3A induces the local translation of RhoA in neuronal dendrites [42], and siRNA-mediated knockdown of RhoA blocked Sema3A-mediated growth cone collapse [43]. In addition, Sema3A induces MLC phosphorylation, and inhibition of myosin II activity blocks Sema3A-mediated axon retraction [34,35]. These findings indicate that Sema3A-mediated signals promote actomyosin contractile force through RhoA-dependent myosin II activation in both immune and neuronal cells.

DCs have to pass through different environments. In tissues such as fibroblastic reticular tissues and inner vessel walls, DCs use integrin-mediated attachment and contractile force for cell movement. By contrast, in constricted areas, DCs use myosin II-mediated actomyosin contractile force to move forward because such tissues confine and mechanically anchor cell bodies [44,45]. During DC transmigration, at least three sequential mechanisms may be required. First, DCs have to form a lamellipodial protrusion at the leading edge in response to chemokines, which are the driving signal for forward movement. Second, DCs must contract and squeeze their bodies by actomyosin contraction to pass through narrow gaps. Third, after DCs are exposed to the lumenal side, the trailing edge has to detach from ECs in order for these cells to enter the circulation. Here, we show that Sema3A acts on the rear side of DCs through plexin-A1 to promote DC migration. These findings indicate that Sema3A-mediated signals are involved not only in actomyosin

contraction but also in disassembling adhesive components at the trailing edge during DC transmigration. Indeed, myosin II promotes a traction stress that facilitates detachment at the trailing edge [46,47], which suggests that detachment can be induced by Sema3A-mediated actomyosin contraction. On the other hand, Sema3A can inhibit integrin-mediated adhesion by inducing the sequestration of phosphatidylinositol phosphate kinase type I isoform PIPKIγ661 from talin, a major component of focal adhesion [48]. In this context, it is plausible that Sema3A plays dual or integral roles in regulating actomyosin contraction and adhesion disassembly at the trailing edge during the course of DC transmigration.

In conclusion, our study not only shows the importance of Sema3A-mediated signal in DC trafficking, particularly in the passage through the lymphatics, but also provides a novel mechanism that promotes actomyosin contraction at the trailing edge of migrating cells. Since semaphorins are also expressed in vascular endothelial cells [14,16], it is plausible that they play a role in leukocyte extravasation or cancer metastasis. Additional detailed studies are required to gain insight into these mechanisms which have the potential to regulate immune reactions in order to treat autoimmune, allergic and infectious diseases and inhibit cancer metastasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank D. D. Ginty and A. L. Kolodkin for the Neuropilin-1 mutant mice, and T. Yazawa for technical support. This study was supported by research grants from JSPS Research Fellowships for Young Scientists (H.T.), NIH grant RO1NS065048 (Y.Y.), the Ministry of Education, Culture, Sports, Science and Technology of Japan, grants-in-aid from the Ministry of Health, Labour and Welfare, the program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (A.K.), the Target Protein Research Program of the Japan Science and Technology Agency (T.T and A.K.), Uehara memorial foundation (A.K.) and Takeda Scientific Foundation (T.T. N. T. and A.K.). The authors have no conflicting financial interests.

References

1. Steinman RM, Banchereau J. Taking dendritic cells into medicine. Nature 2007;449:419–26. [PubMed: 17898760]
2. Randolph GJ, Angeli V, Swartz MA. Dendritic-cell trafficking to lymph nodes through lymphatic vessels. Nat Rev Immunol 2005;5:617–28. [PubMed: 16056255]
3. Alvarez D, Vollmann EH, von Andrian UH. Mechanisms and consequences of dendritic cell migration. Immunity 2008;29:325–42. [PubMed: 18799141]
4. Forster R, et al. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. Cell 1999;99:23–33. [PubMed: 10520991]
5. MartIn-Fontecha A, et al. Regulation of dendritic cell migration to the draining lymph node: impact on T lymphocyte traffic and priming. J Exp Med 2003;198:615–21. [PubMed: 12925677]
6. Ohl L, et al. CCR7 governs skin dendritic cell migration under inflammatory and steady-state conditions. Immunity 2004;21:279–88. [PubMed: 15308107]
7. Johnson LA, et al. An inflammation-induced mechanism for leukocyte transmigration across lymphatic vessel endothelium. J Exp Med 2006;203:2763–77. [PubMed: 17116732]
8. Kabashima K, et al. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. Am J Pathol 2007;171:1249–57. [PubMed: 17823289]
9. Stoitzner P, Pfaller K, Stossel H, Romani N. A close-up view of migrating Langerhans cells in the skin. J Invest Dermatol 2002;118:117–25. [PubMed: 11851884]
10. Baluk P, et al. Functionally specialized junctions between endothelial cells of lymphatic vessels. J Exp Med 2007;204:2349–62. [PubMed: 17846148]

11. Lammermann T, et al. Rapid leukocyte migration by integrin-independent flowing and squeezing. Nature 2008;453:51–5. [PubMed: 18451854]
12. Kolodkin AL, Matthes DJ, Goodman CS. The semaphorin genes encode a family of transmembrane and secreted growth cone guidance molecules. Cell 1993;75:1389–99. [PubMed: 8269517]
13. Toyofuku T, et al. Dual roles of Sema6D in cardiac morphogenesis through region-specific association of its receptor, Plexin-A1, with off-track and vascular endothelial growth factor receptor type 2. Genes Dev 2004;18:435–47. [PubMed: 14977921]
14. Serini G, et al. Class 3 semaphorins control vascular morphogenesis by inhibiting integrin function. Nature 2003;424:391–7. [PubMed: 12879061]
15. Neufeld G, Kessler O. The semaphorins: versatile regulators of tumour progression and tumour angiogenesis. Nat Rev Cancer 2008;8:632–45. [PubMed: 18580951]
16. Capparuccia L, Tamagnone L. Semaphorin signaling in cancer cells and in cells of the tumor microenvironment--two sides of a coin. J Cell Sci 2009;122:1723–36. [PubMed: 19461072]
17. Suzuki K, Kumanogoh A, Kikutani H. Semaphorins and their receptors in immune cell interactions. Nat Immunol 2008;9:17–23. [PubMed: 18087252]
18. Kumanogoh A, et al. Identification of CD72 as a lymphocyte receptor for the class IV semaphorin CD100: a novel mechanism for regulating B cell signaling. Immunity 2000;13:621–31. [PubMed: 11114375]
19. Kumanogoh A, et al. Nonredundant roles of Sema4A in the immune system: defective T cell priming and Th1/Th2 regulation in Sema4A-deficient mice. Immunity 2005;22:305–16. [PubMed: 15780988]
20. Takegahara N, et al. Plexin-A1 and its interaction with DAP12 in immune responses and bone homeostasis. Nat Cell Biol 2006;8:615–22. [PubMed: 16715077]
21. Suzuki K, et al. Semaphorin 7A initiates T-cell-mediated inflammatory responses through alpha1beta1 integrin. Nature 2007;446:680–4. [PubMed: 17377534]
22. Kruger RP, Aurandt J, Guan KL. Semaphorins command cells to move. Nat Rev Mol Cell Biol 2005;6:789–800. [PubMed: 16314868]
23. Takahashi T, et al. Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors. Cell 1999;99:59–69. [PubMed: 10520994]
24. Yoshida Y, Han B, Mendelsohn M, Jessell TM. PlexinA1 signaling directs the segregation of proprioceptive sensory axons in the developing spinal cord. Neuron 2006;52:775–88. [PubMed: 17145500]
25. Wong AW, et al. CIITA-regulated plexin-A1 affects T-cell-dendritic cell interactions. Nat Immunol 2003;4:891–8. [PubMed: 12910265]
26. Barnden MJ, Allison J, Heath WR, Carbone FR. Defective TCR expression in transgenic mice constructed using cDNA-based alpha- and beta-chain genes under the control of heterologous regulatory elements. Immunol Cell Biol 1998;76:34–40. [PubMed: 9553774]
27. Mempel TR, Henrickson SE, Von Andrian UH. T-cell priming by dendritic cells in lymph nodes occurs in three distinct phases. Nature 2004;427:154–9. [PubMed: 14712275]
28. Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol 2002;2:151–61. [PubMed: 11913066]
29. Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. J Exp Med 1987;166:1654–67. [PubMed: 3119761]
30. Angeli V, et al. B cell-driven lymphangiogenesis in inflamed lymph nodes enhances dendritic cell mobilization. Immunity 2006;24:203–15. [PubMed: 16473832]
31. Taniguchi M, et al. Disruption of semaphorin III/D gene causes severe abnormality in peripheral nerve projection. Neuron 1997;19:519–30. [PubMed: 9331345]
32. Gu C, et al. Neuropilin-1 conveys semaphorin and VEGF signaling during neural and cardiovascular development. Dev Cell 2003;5:45–57. [PubMed: 12852851]
33. Clark K, Langeslag M, Figdor CG, van Leeuwen FN. Myosin II and mechanotransduction: a balancing act. Trends Cell Biol 2007;17:178–86. [PubMed: 17320396]

34. Gallo G. RhoA-kinase coordinates F-actin organization and myosin II activity during semaphorin-3A-induced axon retraction. J Cell Sci 2006;119:3413–23. [PubMed: 16899819]
35. Brown JA, Wysolmerski RB, Bridgman PC. Dorsal root ganglion neurons react to semaphorin 3A application through a biphasic response that requires multiple myosin II isoforms. Mol Biol Cell 2009;20:1167–79. [PubMed: 19109430]
36. Boulanger LM. Immune proteins in brain development and synaptic plasticity. Neuron 2009;64:93–109. [PubMed: 19840552]
37. Cumberbatch M, Kimber I. Tumour necrosis factor-alpha is required for accumulation of dendritic cells in draining lymph nodes and for optimal contact sensitization. Immunology 1995;84:31–5. [PubMed: 7890303]
38. Ratzinger G, et al. Matrix metalloproteinases 9 and 2 are necessary for the migration of Langerhans cells and dermal dendritic cells from human and murine skin. J Immunol 2002;168:4361–71. [PubMed: 11970978]
39. Lauffenburger DA, Horwitz AF. Cell migration: a physically integrated molecular process. Cell 1996;84:359–69. [PubMed: 8608589]
40. Chhabra ES, Higgs HN. The many faces of actin: matching assembly factors with cellular structures. Nat Cell Biol 2007;9:1110–21. [PubMed: 17909522]
41. Vicente-Manzanares M, Ma X, Adelstein RS, Horwitz AR. Non-muscle myosin II takes centre stage in cell adhesion and migration. Nat Rev Mol Cell Biol 2009;10:778–90. [PubMed: 19851336]
42. Wu KY, et al. Local translation of RhoA regulates growth cone collapse. Nature 2005;436:1020–4. [PubMed: 16107849]
43. Hengst U, Jaffrey SR. Function and translational regulation of mRNA in developing axons. Semin Cell Dev Biol 2007;18:209–15. [PubMed: 17478114]
44. Friedl P, Weigelin B. Interstitial leukocyte migration and immune function. Nat Immunol 2008;9:960–9. [PubMed: 18711433]
45. Lammermann T, Sixt M. Mechanical modes of 'amoeboid' cell migration. Curr Opin Cell Biol 2009;21:636–44. [PubMed: 19523798]
46. Lombardi ML, Knecht DA, Dembo M, Lee J. Traction force microscopy in Dictyostelium reveals distinct roles for myosin II motor and actin-crosslinking activity in polarized cell movement. J Cell Sci 2007;120:1624–34. [PubMed: 17452624]
47. Smith LA, Aranda-Espinoza H, Haun JB, Dembo M, Hammer DA. Neutrophil traction stresses are concentrated in the uropod during migration. Biophys J 2007;92:L58–60. [PubMed: 17218464]
48. Toyofuku T, et al. FARP2 triggers signals for Sema3A-mediated axonal repulsion. Nat Neurosci 2005;8:1712–9. [PubMed: 16286926]

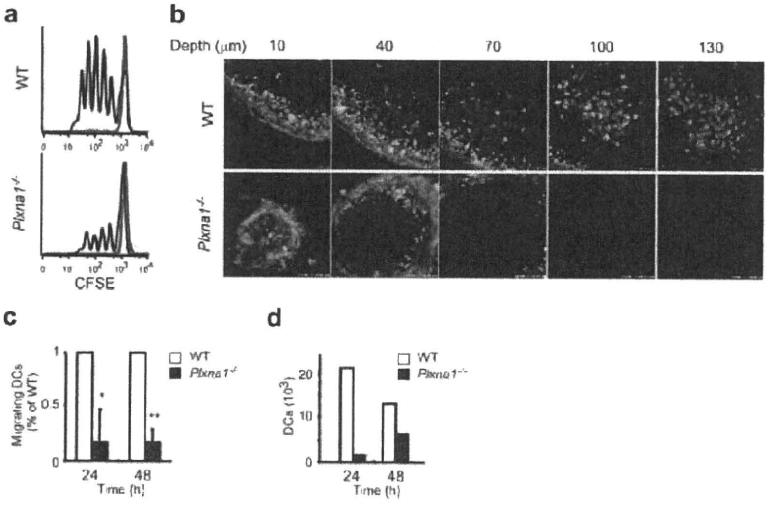

Figure 1. *Plxna1*$^{-/-}$ mice show impaired T-cell responses due to defects in DC migration into the LNs

(**a**) CFSE dilution by CD4^{+} OT-II T cells intravenously transferred into wild-type or *Plxna1*$^{-/-}$ mice, subcutaneously injected in the footpads with OVA peptides in CFA. Ag-specific T cell responses were assayed in the draining LNs (black lines) or non-draining LNs (red lines). The data are representative of three independent experiments. (**b**) Two-photon microscopy imaging of wild-type or *Plxna1*$^{-/-}$ CMTMR-labeled BMDCs (orange) injected into the footpads of wild-type recipient mice that also received CMFDA-labeled CD4^{+} OT-II T-cells (green). DC trafficking into the popliteal LNs was observed 24 hours post injection (**c**) Numbers of wild-type or *Plxna1*$^{-/-}$ DCs trafficking into the popliteal LNs of wild-type recipient mice following foodpad injections. Donor BMDCs were CFSE-labeled and the following calculation was used: (% of input cells) = (total cell number) x (% of CFSE^{+} cells)/(input cell number). Mean ± SD, *p<0.01, **p<0.001, by Mann-Whitney's U test. (**d**) Absolute numbers of endogenous DCs isolated at the indicated time points the brachial LNs of wild-type and *Plxna1*$^{-/-}$ mice after epicutanous administration of FITC-isomer to the shoulder skin.

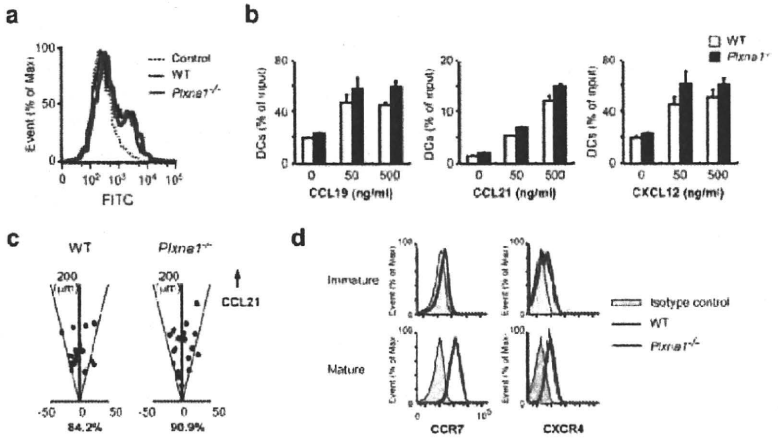

Figure 2. FITC-dextran uptake and responses to chemokines are not affected in *Plxna1*$^{-/-}$ DCs

(**a**) FITC-dextran uptake by wild-type and *Plxna1*$^{-/-}$ BMDCs at 37°C for 30 min. Cells cultured with FITC-dextran on ice were used as controls. (**b**) Chemotaxis of wild-type and *Plxna1*$^{-/-}$ DCs toward CCL19, CCL21 or CXCL12 gradients in transwell migration assays (pore size: 5 μm). (**c**) Directional sensing in wild-type or *Plxna1*$^{-/-}$ DCs in response to a CCL21 gradient in a Zigmond chamber. Scatter plots show the position of wild-type and *Plxna1*$^{-/-}$ DCs relative to their original positions after 60 min of chemokine gradient. The percentages of cells that ended up within a 30° arc facing the CCL21 source are shown. (**d**) CCR7 and CXCR4 expression in wild-type and *Plxna1*$^{-/-}$ DCs.

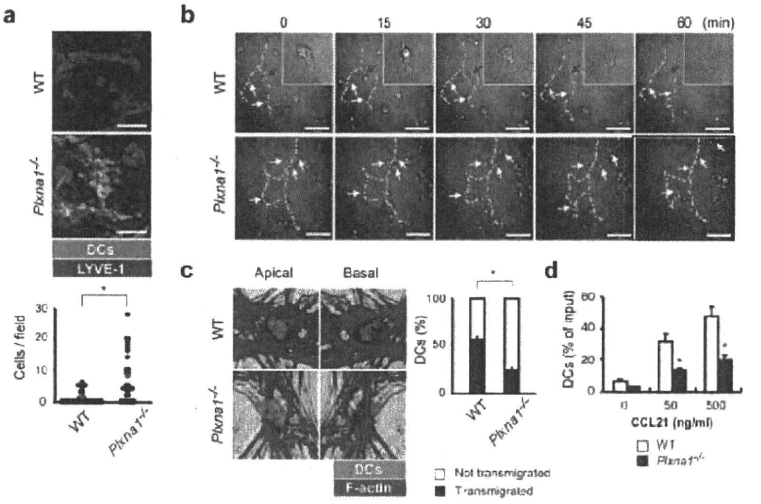

Figure 3. *Plxna1*$^{-/-}$ DCs exhibit impaired transmigration across the lymphatics

(**a**) Upper: Confocal Z-stack imaging of wild-type or *Plxna1*$^{-/-}$ CFSE-labeled BMDCs (green) intradermally injected into the ear tissues of oxazolone-sensitized mice. Whole-mount staining was performed 24 h post transfer using biotinylated anti-LYVE-1 with streptavidin-Cy3 (red). Scale bars, 50 μm (upper). Lower: Quantification of the number of retained DCs in the fields. Red circles indicate the mean number of cells. *p<0.001, by Mann-Whitney's U test. (**b**) Transmigration of wild-type and *Plxna1*$^{-/-}$ BMDCs across a lymphatic EC monolayer. Interactions between DCs and the lymphatic ECs were recorded every 30 sec by a time-lapse video microscope. The yellow dotted lines show the cellular junctions of the ECs. White arrows indicate DCs that were contacting the lymphatic ECs. Red arrows indicate the transmigration process that was observed in wild-type DCs. Scale bars, 50 μm. (**c**) Left: Confocal microscopy of wild-type and *Plxna1*$^{-/-}$ CFSE-labeled DCs added to EC monolayers, incubated for 45 min, fixed, and then stained with Alexa 546-conjugated phalloidin. Images were obtained with an optical section separation (Z-interval) of 0.22 μm. Right: Quantification of DC transmigration determined by confocal microscopy and displayed as percentage of transmigrated DCs relative to the total number of DCs. Mean ± SD. *p<0.001, by Student's t-test. (**d**) Chemotaxis of wild-type or *Plxna1*$^{-/-}$ DC across transwells (pore size: 5 μm) layered with lymphatic EC in response to a CCL21 gradient. Mean ± SD. *p<0.001, by Student's t test.

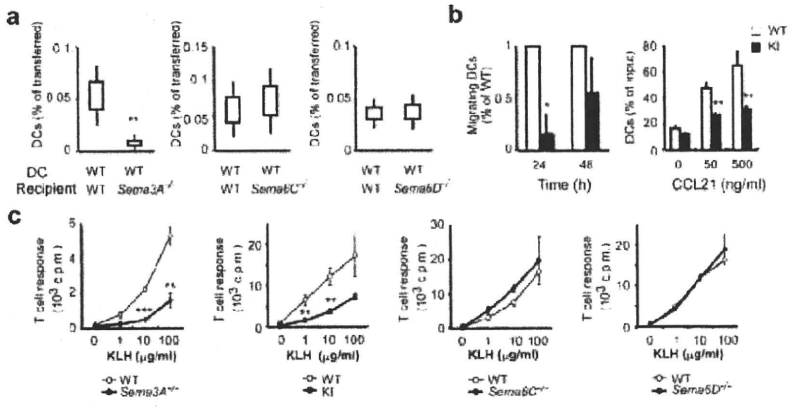

Figure 4. Sema3A-Nrp1-plexin-A1 interactions are responsible for DC trafficking

(**a**) Wild-type DC trafficking in the lymphatics after adoptive transferred into wild-type and *Sema3A*$^{-/-}$, *Sema6C*$^{-/-}$ or *Sema6D*$^{-/-}$ mice. Data are pooled from three independent experiments. Standard error ± 95% confidence interval, $^{**}p<0.01$, by Mann-Whitney's *U* test. (**b**) Left: DCs trafficking into the lymphatics following adoptive transferred of wild-type and *Nrp1*$^{sema-}$-knock-in (KI) DCs into wild-type recipients. Mean ± SE, $^{*}p<0.05$, by Mann-Whitney's *U* test. Right: Chemotaxis of wild-type and *Nrp1*$^{sema-}$-knock-in (KI) DCs through transwells (pore size: 5 μm) layered with lymphatic ECs in response to a CCL21 gradient. Mean ± SD, $^{**}p<0.01$, by Student's *t* test. (**d**) *In vitro* CD4^{+} T cell proliferation in response to KLH following KLH in CFA immunization of *Sema3A*$^{-/-}$, *Nrp1*$^{sema-}$-knock-in (KI), *Sema6C*$^{-/-}$, *Sema6D*$^{-/-}$ and wild-type mice. Mean ± SD. $^{**}p<0.01$, $^{***}p<0.001$, by Student's *t* test.

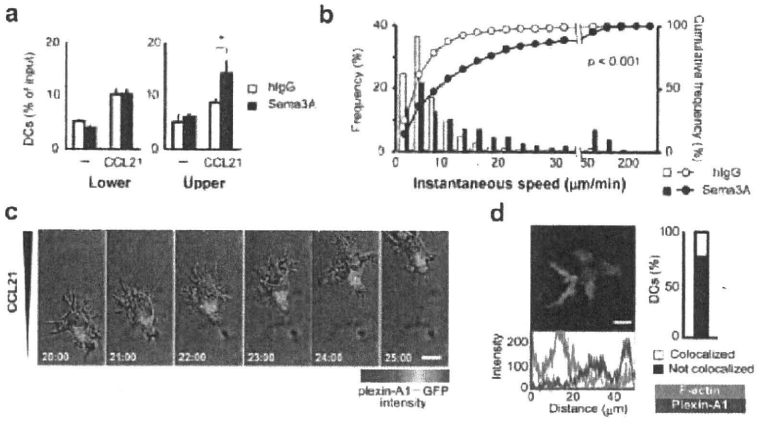

Figure 5. Sema3A acts on the rear side of DCs

(**a**) Chemotaxis of DCs in the presence of human IgG or recombinant Sema3A proteins in the lower (left) or upper (right) chambers of transwells, while CCL21 was absent or present in the lower chambers. Mean ± SD. *$p<0.01$, by Student's t test. (**b**) DC velocities in two-dimensional DC chemotaxis assays using EZ-TAXIScan in which Sema3A or human IgG was added to the opposite side of the CCL21. The frequency distribution (bar chart) and cumulative frequency distribution (line chart) of the instantaneous speed were determined. $p<0.001$, by Mann-Whitney's U test. Data are representative of three independent experiments. (**c**) Confocal time-lapse video-microscopy of plexin-A1-EGFP expressing BMDCs treated with LPS, suspended in type I collagen gels and placed into a Zigmond chamber with chemokine gradients. DC locomotion was examined at 1-min intervals. (**d**) Left: Confocal Z-stack imaging showing localization and intensity of plexin-A1 (anti-plexin-A1 polyclonal antibody plus anti-rabbit IgG-Cy3-red) and F-actin (Alexa 488-conjugated phalloidin-green) in DC. Scale bars, 10 μm. Right: Percentage of cells with no co-localization of signals. Data are representative of three independent experiments.