Sphingosine Kinase-Dependent Migration of Immature Dendritic Cells in Response to Neurotoxic Prion Protein Fragment

Nicole C. Kaneider,¹ Arthur Kaser,² Stefan Dunzendorfer,¹ Herbert Tilg,² and Christian J. Wiedermann^{1*}

Division of General Internal Medicine¹ and Division of Gastroenterology,² Department of Internal Medicine, University of Innsbruck, A-6020 Innsbruck, Austria

Received 9 October 2002/Accepted 5 February 2003

The concept that circulating dendritic cells mediate neuroinvasion in transmissible spongiform encephalopathies received strong support from recent observations that prion protein is expressed in myeloid dendritic cells. We observed that prion protein fragment 106-126 is a chemoattractant for monocyte-derived immature but not mature dendritic cells. Signaling events in chemotaxis involved enzymes downstream of G_q protein and were inhibited by blockade of sphingosine kinase, suggesting transactivation of sphingosine-1-phosphate-dependent cell motility by prion protein.

Prion glycoproteins may become unprecedented infectious pathogens that cause a group of invariably fatal neurodegenerative diseases by novel mechanisms (27, 29). New-variant Creutzfeldt-Jakob disease [(v)CJD] and scrapie, known as transmissible spongiform encephalopathies, are typically initiated by exposure to the causative agent and early prion replication in lymphoid tissues (20). (v)CJD has raised concerns that bovine spongiform encephalopathies might be communicable to humans by dietary exposure (4, 28).

Gut M-cell-dependent transepithelial uptake of dietary prion protein is followed by transcytosis directly to intraepithelial pockets, where key players of the immune system, including dendritic cells (DCs), are located (11). DCs are also able to open the tight junctions between epithelial cells, send dendrites outside the epithelium, and directly sample pathogens in an M-cell-independent way (30). The details of the mechanism by which infective prions are transferred from the gastrointestinal tract to the nervous system are unknown. It is important to understand how central lymphoid organs and peripheral neurons become exposed to infective prion protein (PrP^{sc}).

Evidence suggests that circulating blood cells may have a role in enteric prion infection. Results from animal models have emphasized the fact that infective material can be isolated from the cell fraction of spleen soon after the ingestion of PrP^{sc} (19), whereas in mice, bone marrow-derived myeloid cells have been shown to be required for its propagation and spread (2). It was shown previously that cellular prion protein (PrP^c) is strongly expressed in myeloid DCs, which may act as carrier cells for the spread and circulation of the abnormal isoform PrP^{sc} (3). In the absence of prion disease, high levels of expression of PrP^c in human spleen occur principally on myeloid DCs immediately adjacent to the white pulp, whereas follicular DCs do not strongly express PrP^c; myeloid DCs are found in the red pulp of the spleen, and cells migrate into its lymphoid

areas after receiving a maturation stimulus (3). Moreover, DCs can be found in the peripheral and central nervous system (9, 25). Here we report on the chemotaxis of immature DCs and arrest of mature DCs by a synthetic peptide corresponding to residues 106 to 126 of human PrP (PrP₁₀₆₋₁₂₆). Signal transduction mechanisms that may be involved in directed migration of monocyte-derived DCs toward PrP₁₀₆₋₁₂₆ are described.

PrP₁₀₆₋₁₂₆, which is toxic to neurons, increases chemotaxis, oxygen free radical release, and intracellular calcium concentration in neutrophils and monocytes (5). To determine whether PrP₁₀₆₋₁₂₆ is a chemoattractant of monocyte-derived DCs (17), chemotaxis experiments in modified multiwell Boyden chambers (Neuroprobe, Gaithersburg, Md.) using nitrocellulose micropore filters (Sartorius, Göttingen, Germany) were performed as previously described (6). DCs were prepared as described previously (6, 7, 17, 18). Distinction between mature and immature DCs was made by fluorescence-activated cell sorting analyses (Fig. 1).

Immature DCs migrated for 4 h toward PrP₁₀₆₋₁₂₆ (Bachem, Bubendorf, Switzerland) in a concentration-dependent manner, whereas PrP₁₀₆₋₁₂₆ was not chemotactic for mature DCs (Fig. 2). Maximum chemotactic activity of PrP₁₀₆₋₁₂₆ for immature DCs was seen at concentrations of 0.1 to 10 nmol/liter and was comparable in its potency to that of RANTES [20 ng/ml] (Peprotech, London, United Kingdom). As a control, chemotaxis toward scrambled PrP₁₀₆₋₁₂₆ and PrP₁₁₈₋₁₃₅ was monitored. Neither the scrambled form nor PrP₁₁₈₋₁₃₅ exerted chemotactic effects on immature DCs (Fig. 2). Checkerboard analysis revealed that the migration of immature DCs toward PrP₁₀₆₋₁₂₆ is true concentration gradient-dependent chemotaxis (Table 1). The influence of PrP₁₀₆₋₁₂₆ on 6Ckine-induced chemotaxis of mature DCs was tested. Combination of 6Ckine (1 µg/ml) with PrP₁₀₆₋₁₂₆ (10 fM to 1 µM) in the lower wells of the chemotaxis chamber deactivated mature DC migration. The mean distance of random migration was 45 ± 5.2 µm, the mean 6Ckine-induced migration was 100 ± 8.4 µm, and the mean when 6Ckine was combined with PrP₁₀₆₋₁₂₆ was 78 ± 6.3 µm ($P < 0.05$). The effects of PrP₁₀₆₋₁₂₆ and 6Ckine alone are shown in Fig. 1.

Tyrphostin-23 (a tyrosine kinase inhibitor), bisindolylmale-

* Corresponding author. Mailing address: Department of Internal Medicine, University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. Phone: 43-512-5044180. Fax: 43-512-504674180. E-mail: Christian.Wiedermann@uibk.ac.at.

FIG. 1. Cytofluorometric analysis of DC surface phenotype. A total of 5×10^5 DCs were washed in phosphate-buffered saline–2% fetal calf serum and resuspended in a solution containing 250 μg of human immunoglobulin G per ml, phosphate-buffered saline, and 2% fetal calf serum. After pelleting, DCs were incubated alternatively with 10 μg of anti-CD80 per ml or anti-HLA-DR monoclonal antibodies and the respective isotype-matched control immunoglobulins. After a washing in phosphate-buffered saline–2% fetal calf serum, a 1:40 dilution of fluorescein isothiocyanate–anti-mouse immunoglobulin G in phosphate-buffered saline–2% fetal calf serum was incubated for 30 min at 4°C. Cells were immediately analyzed on a FACScan. Analysis was performed with CellQuest software (BD Biosciences, Mountain View, Calif.).

imide (GFX; a protein kinase C inhibitor), wortmannin (WTN; a phospholipase 3 inhibitor), rolipram (a phosphodiesterase inhibitor; Sigma Chemical Co., St. Louis, Mo.), and dimethylsphingosine (DMS; a sphingosine kinase inhibitor) were used

for blocking signaling enzymes; pertussis toxin (PTX) and cholera toxin (CTX; Sigma Chemical Co.) were used for testing involvement of G_i/G_o protein and G_s protein, respectively. Patterns of migration toward other DC attractants, including

FIG. 2. PrP induces chemotaxis in immature DCs and lacks an effect on mature DCs. DCs were allowed to migrate toward various concentrations of PrP₁₀₆₋₁₂₆ for 4 h in modified multiwell Boyden chambers with micropore nitrocellulose filters. The mean random migration distances were $40 \pm 6.2 \mu\text{m}$ for immature and $49 \pm 8.3 \mu\text{m}$ for mature DCs, respectively. The chemotaxis index is the ratio between directed and random migration. Statistical analysis was done by the Mann-Whitney U test after Kruskal-Wallis analysis; *, $P < 0.05$, $n = 6$. Random migration of mature DCs was inhibited (#, $P < 0.05$, $n = 6$). RANTES and 6CKine were used as positive controls for immature and mature DCs, respectively.

TABLE 1. Checkerboard analysis of effects of concentration gradients of PrP₁₀₆₋₁₂₆ on immature DC migration^a

PrP ₁₀₆₋₁₂₆ concn (mol/liter) in lower compartment	Chemotaxis index (mean $\pm$ SEM) ^b for PrP ₁₀₆₋₁₂₆ concn (mol/liter) in upper compartment			
	Medium	10 ⁻¹⁰	10 ⁻⁸	10 ⁻⁶
Medium	1.000 $\pm$ 0.000	1.025 $\pm$ 0.053	1.084 $\pm$ 0.086	0.943 $\pm$ 0.192
10 ⁻¹⁰	1.884 $\pm$ 0.138*	0.963 $\pm$ 0.052	1.036 $\pm$ 0.137	0.691 $\pm$ 0.099*
10 ⁻⁸	2.211 $\pm$ 0.106*	2.411 $\pm$ 0.132*	0.951 $\pm$ 0.090	1.014 $\pm$ 0.142
10 ⁻⁶	2.613 $\pm$ 0.098*	2.273 $\pm$ 0.175*	1.712 $\pm$ 0.161*	0.663 $\pm$ 0.081*

^a Directed migration of DCs to PrP₁₀₆₋₁₂₆ was tested by checkerboard analysis. Cells were allowed to migrate to various concentration gradients of PrP₁₀₆₋₁₂₆ for 4 h. Migration depth was quantified by microscopy.

^b Ratio between directed and undirected migration of cells. The mean distance of migration to medium in the upper and lower chambers was 36 $\pm$ 4.2 μ m. Statistical analysis was done with the Mann-Whitney U test after Kruskal-Wallis analysis of variance ($P < 0.001$); $n = 3$. *, $P < 0.05$ compared with the value obtained for medium in the upper and lower chambers. Only groups within the matrix for which chemotaxis was extensive enough to distinguish between chemokinesis and chemotaxis were compared statistically by the Mann-Whitney U test.

N-formyl-methionyl-phenylalanine (fMLP; Sigma Chemical Co.) and substance P (Neosystem, Strasbourg, France), were compared with that of PrP₁₀₆₋₁₂₆. Signaling studies revealed that PrP₁₀₆₋₁₂₆-induced chemotaxis is sensitive to GFX, tyrphostin-23, rolipram, WTN, and PTX. This signaling pattern mimicked that of substance P, another peptide attractant of DCs (8), but differed from that of fMLP. CTX did not affect chemotaxis to any of them (Table 2).

Activation of protein kinase C leads to phosphorylation of sphingosine kinase and sphingosine-1-phosphate production (24), which induces G-protein-dependent cell migration (16). Sphingosine-1-phosphate-induced chemotaxis of DCs has been reported recently (14). As PrP₁₀₆₋₁₂₆-induced migration of DCs was inhibited by the inhibitor of protein kinase C and by blockade of G_i/G₀ (Table 2), effects of the sphingosine kinase inhibitor DMS (Biomol, Plymouth Meeting, Pa.) on PrP₁₀₆₋₁₂₆-induced chemotaxis were tested. Migration of DCs toward optimal concentrations of PrP₁₀₆₋₁₂₆ was inhibited in a concentration-dependent manner by pretreatment with DMS, whereas chemotaxis of immature DCs toward fMLP was not inhibited (Fig. 3).

Results of this in vitro study demonstrate that PrP₁₀₆₋₁₂₆ induces migration of monocyte-derived immature DCs in a dose-dependent manner. In tissues of animals infected by transmissible spongiform encephalopathy agent and in humans suffering from (v)CJD, PrP^{Sc} is particularly concentrated in follicular DCs, whereas high levels of PrP^c are present in myeloid DCs (3), which are ontologically and functionally distinct from follicular DCs (15). Myeloid DCs are derived from bone marrow precursor cells or from monocytes and their precursors

and are readily identified within circulating cell populations. In the spleen, myeloid DCs are found in the red pulp and immediately adjacent to the white pulp. The cells migrate into the lymphoid areas, where they are powerful mediators of T-cell activation. Given the close anatomic and functional connection of myeloid DCs with lymphoid follicles, these results raise the possibility that migration of myeloid DCs toward prion protein may play a role in the propagation of PrP^{Sc} in humans (3). It has been observed that injection of prion-infected DCs induced scrapie without accumulation of prions in the spleen in a model of RAG-1 knockout mice, indicating that DCs can propagate prions from the periphery to the central nervous system in the absence of any additional lymphoid element (1). Migratory bone marrow-derived DCs, entering the intestinal wall from blood, sample antigens from the gut lumen and carry them to the lymph nodes. Huang et al. showed that DCs acquire PrP^{Sc} in vitro and transport intestinally administered PrP^{Sc} directly into lymphoid tissue in vivo, suggesting that DCs are a cellular bridge between the gut lumen and the lymphoid transmissible spongiform encephalopathy replication machinery (13).

In the present study, it was observed that the prion protein fragment is able to induce a chemotactic response in immature DCs and to arrest mature DCs, suggesting that DC trafficking may directly depend on the infective agent. DCs might be attracted toward high concentrations of prion protein, as are found in the gut epithelial venules or splenic lymphoid follicles, where uptake and accumulation of pathogens take place as a prerequisite of prion spread. When stimuli are received as a

TABLE 2. Effects of signaling enzyme blockers on DC chemotaxis^a

Treatment	Chemotaxis index ^b (mean $\pm$ SEM)		
	PrP ₁₀₆₋₁₂₆ (10 nmol/liter)	Substance P (1 nmol/liter)	fMLP (10 nmol/liter)
Medium	1.477 $\pm$ 0.208	1.529 $\pm$ 0.221	1.820 $\pm$ 0.048
GFX (500 nmol/liter)	1.112 $\pm$ 0.031*	0.723 $\pm$ 0.092*	1.654 $\pm$ 0.135 (NS)
Rolipram (10 μ mol/liter)	1.034 $\pm$ 0.180*	1.029 $\pm$ 0.106*	1.816 $\pm$ 0.064 (NS)
Tyr-23 (10 ng/ml)	0.889 $\pm$ 0.209*	1.058 $\pm$ 0.098*	1.685 $\pm$ 0.028*
WTN (10 nmol/liter)	1.111 $\pm$ 0.043*	1.049 $\pm$ 0.243*	1.859 $\pm$ 0.069 (NS)
CTX (1 nmol/liter)	1.636 $\pm$ 0.244 (NS)	1.729 $\pm$ 0.172 (NS)	1.918 $\pm$ 0.064 (NS)
PTX (1 nmol/liter)	1.074 $\pm$ 0.080*	1.227 $\pm$ 0.033*	1.268 $\pm$ 0.037*

^a DCs were incubated with GFX, rolipram, tyrphostin-23 (Tyr-23), WTN, CTX, and PTX for 20 min. Chemotaxis toward PrP₁₀₆₋₁₂₆, substance P, and fMLP was tested after a washing using modified multiwell Boyden chambers.

^b Ratio between directed and random migration. The mean random migration distance was 54 $\pm$ 0.05 μ m. Statistical analysis was done by the Mann-Whitney U test after Kruskal-Wallis analysis. *, $P < 0.05$, $n = 3$. NS, not significant.

FIG. 3. DMS inhibits PrP₁₀₆₋₁₂₆-induced chemotaxis. DCs were incubated with DMS for 20 min. Chemotaxis toward PrP₁₀₆₋₁₂₆ and fMLP was tested after a washing by using modified multiwell Boyden chambers. Mean random migration was $52 \pm 7.5 \mu\text{m}$. The chemotaxis index is the ratio between directed and random migration. Statistical analysis was done by the Mann-Whitney U test after Kruskal-Wallis analysis; *, $P < 0.05$, $n = 3$.

result of pathogen uptake, maturation might occur and DCs might subsequently be arrested at the sites of pathogen uptake.

We further observed an inhibition of migration after pretreatment of cells with DMS, which was used as specific inhibitor of sphingosine kinase. Previously it was suggested for monocytes that PrP₁₀₆₋₁₂₆ might use the formyl peptide-like receptor 1, a pattern recognition seven transmembrane receptor for chemotaxis also expressed in DCs (12). This receptor is coupled to G proteins and may be sufficient for migration induction (22). The observation that PrP₁₀₆₋₁₂₆-induced chemotaxis is sphingosine kinase dependent suggests that transactivation of chemotaxis toward sphingosine-1-phosphate, a lysophospholipid that activates cytoskeletal remodeling and motility via endothelial-differentiation-gene receptors (EDG) (21), is also involved in prion protein fragment-dependent effects in DCs (23, 24, 26, 31). Immature and mature DCs express the mRNA for different sphingosine-1-phosphate receptors (EDG-1, -3, -5, and -6), where sphingosine-1-phosphate induces further chemotaxis (14). Activation of sphingosine kinase and production of sphingosine-1-phosphate increases intracellular Ca^{2+} concentrations, an effect known to take place in PrP₁₀₆₋₁₂₆-induced apoptosis of several cell lines (10). These observations strengthen the concept that migration of antigen-presenting cells toward high concentrations of PrP₁₀₆₋₁₂₆ involves sphingosine kinase.

Since in dietary prion disease, PrP^{Sc} is incorporated via the lymphatic system of the gut, migration of circulating DCs toward high concentrations of prion protein and their accumulation for prion uptake may be important pathophysiological mechanisms that involve a chemotactic lysophospholipid. Interference with sphingosine kinase-dependent pathways might become a novel pharmaceutical target for preventing prion-dependent DC trafficking and the associated spread of infection.

REFERENCES

- Aucouturier, P., F. Geissmann, D. Damotte, G. P. Saborio, H. C. Meeker, R. Kascasak, R. Kascasak, R. I. Carp, and T. Wisniewski. 2001. Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. *J. Clin. Invest.* **108**:703–708.
- Blattler, T., S. Brandner, A. J. Raeber, M. A. Klein, T. Voigtlander, C. Weissmann, and A. Aguzzi. 1997. PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* **389**:69–73.
- Burthum, J., B. Urban, A. Pain, and D. J. Roberts. 2001. The normal cellular prion protein is strongly expressed by myeloid dendritic cells. *Blood* **98**:3733–3738.
- Chesebro, B. 1997. Human TSE disease—viral or protein only? *Nat. Med.* **3**:491–492.
- Diomedea, L., S. Sozzani, W. Luini, M. Algeri, L. De Gioia, R. Chiesa, P. M. Lievens, O. Bugiani, G. Forloni, F. Tagliavini, and M. Salmona. 1996. Activation effects of a prion protein fragment [PrP-(106–126)] on human leukocytes. *Biochem. J.* **320**:563–570.
- Dunzendorfer, S., A. Kaser, C. Meierhofer, H. Tilg, and C. J. Wiedermann. 2000. Dendritic cell migration in different micropore filter assays. *Immunol. Lett.* **71**:5–11.
- Dunzendorfer, S., A. Kaser, C. Meierhofer, H. Tilg, and C. J. Wiedermann. 2001. Cutting edge: peripheral neurotrophins attract immature and arrest mature blood-derived dendritic cells. *J. Immunol.* **166**:2167–2172.
- Dunzendorfer, S., and C. J. Wiedermann. 2001. Neurotrophins and the immune system: focus on dendritic cells. *Crit. Rev. Immunol.* **21**:523–557.
- Fischer, H. G., and G. Reichmann. 2001. Brain dendritic cells and macrophages/microglia in central nervous system inflammation. *J. Immunol.* **166**:2717–2726.
- Florio, T., S. Thellung, C. Amico, M. Robello, M. Salmona, O. Bugiani, F. Tagliavini, G. Forloni, and G. Schettini. 1998. Prion protein fragment 106–126 induces apoptotic cell death and impairment of L-type voltage-sensitive calcium channel activity in the GH3 cell line. *J. Neurosci. Res.* **54**:341–352.
- Heppner, F. L., A. D. Christ, M. A. Klein, M. Prinz, M. Fried, J. P. Kraehenbuhl, and A. Aguzzi. 2001. Transendothelial prion transport by M cells. *Nat. Med.* **7**:976–977.
- Hollopeter, G., H. M. Jantzen, D. Vincent, G. Li, L. England, V. Ramakrishnan, R. B. Yang, P. Nurdan, A. Nurdan, D. Julius, and P. B. Conley. 2001. Identification of the platelet ADP receptor targeted by antithrombotic drugs. *Nature* **409**:202–207.
- Huang, F. P., C. F. Farquhar, N. A. Mabbott, M. E. Bruce, and G. G. MacPherson. 2002. Migrating intestinal dendritic cells transport PrP^{Sc} from the gut. *J. Gen. Virol.* **83**:267–271.
- Idzko, M., E. Panther, S. Corinti, A. Morelli, D. Ferrari, Y. Herouy, S. Dichmann, M. Mockenhaupt, P. Gebicke-Haerter, F. Di Virgilio, G. Girolomoni, and J. Norgauer. 2002. Sphingosine 1-phosphate induces chemotaxis of immature and modulates cytokine-release in mature human dendritic cells for emergence of Th2 immune responses. *FASEB J.* **16**:625–627.
- Jimenez-Huete, A., P. M. Lievens, R. Vidal, P. Piccardo, B. Ghetti, F. Tagliavini, B. Frangione, and F. Prelli. 1998. Endogenous proteolytic cleavage of normal and disease-associated isoforms of the human prion protein in neural and non-neural tissues. *Am. J. Pathol.* **153**:1561–1572.
- Kaneider, N., A. Djanani, R. Fischer-Colbrie, and C. Wiedermann. 2002. Sphingosine kinase-dependent directional migration of leukocytes in response to phorbol ester. *Biochem. Biophys. Res. Commun.* **297**:806.
- Kaser, A., S. Dunzendorfer, F. A. Offner, O. Ludwiczek, B. Enrich, R. O. Koch, W. W. Cruikshank, C. J. Wiedermann, and H. Tilg. 2000. B lymphocyte-derived IL-16 attracts dendritic cells and Th cells. *J. Immunol.* **165**:2474–2480.
- Kaser, A., S. Dunzendorfer, F. A. Offner, T. Ryan, A. Schwabegger, W. W. Cruikshank, C. J. Wiedermann, and H. Tilg. 1999. A role for IL-16 in the cross-talk between dendritic cells and T cells. *J. Immunol.* **163**:3232–3238.
- Kimberlin, R. H., and C. A. Walker. 1989. The role of the spleen in the neuroinvasion of scrapie in mice. *Virus Res.* **12**:201–211.
- Klein, M. A., R. Frigg, A. J. Raeber, E. Flechsig, I. Hegyi, R. M. Zinkernagel, C. Weissmann, and A. Aguzzi. 1998. PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nat. Med.* **4**:1429–1433.
- Kluk, M. J., and T. Hla. 2001. Role of the sphingosine 1-phosphate receptor EDG-1 in vascular smooth muscle cell proliferation and migration. *Circ. Res.* **89**:496–502.
- Le, Y., J. J. Oppenheim, and J. M. Wang. 2001. Pleiotropic roles of formyl peptide receptors. *Cytokine Growth Factor Rev.* **12**:91–105.
- Lee, M. J., S. Thangada, J. H. Paik, G. P. Sapkota, N. Ancellin, S. S. Chae, M. Wu, M. Morales-Ruiz, W. C. Sessa, D. R. Alessi, and T. Hla. 2001. Akt-mediated phosphorylation of the G protein-coupled receptor EDG-1 is required for endothelial cell chemotaxis. *Mol. Cell* **8**:693–704.
- Mazurek, N., T. Megidish, S. Hakomori, and Y. Igarashi. 1994. Regulatory effect of phorbol esters on sphingosine kinase in BALB/C 3T3 fibroblasts (variant A31): demonstration of cell type-specific response—a preliminary note. *Biochem. Biophys. Res. Commun.* **198**:1–9.
- McWilliam, A. S., S. Napoli, A. M. Marsh, F. L. Pemper, D. J. Nelson, C. L. Pimm, P. A. Stumbles, T. N. Wells, and P. G. Holt. 1996. Dendritic cells are recruited into the airway epithelium during the inflammatory response to a broad spectrum of stimuli. *J. Exp. Med.* **184**:2429–2432.
- Paik, J. H., S. Chae, M. J. Lee, S. Thangada, and T. Hla. 2001. Sphingosine

- 1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of $\alpha_v\beta_3$ - and β_1 -containing integrins. *J. Biol. Chem.* **276**:11830–11837.
27. **Peretz, D., R. A. Williamson, K. Kaneko, J. Vergara, E. Leclerc, G. Schmitt-Ulms, I. R. Mehlhorn, G. Legname, M. R. Wormald, P. M. Rudd, R. A. Dwek, D. R. Burton, and S. B. Prusiner.** 2001. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* **412**:739–743.
28. **Priola, S. A.** 1996. Similar protein signatures for BSE and vCJD. *Nat. Med.* **2**:1303–1304.
29. **Prusiner, S. B.** 1994. Inherited prion diseases. *Proc. Natl. Acad. Sci. USA* **91**:4611–4614.
30. **Rescigno, M., M. Urbano, B. Valzasina, M. Francolini, G. Rotta, R. Bonasio, F. Granucci, J. P. Kraehenbuhl, and P. Ricciardi-Castagnoli.** 2001. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat. Immunol.* **2**:361–367.
31. **Rosenfeldt, H. M., J. P. Hobson, S. Milstien, and S. Spiegel.** 2001. The sphingosine-1-phosphate receptor EDG-1 is essential for platelet-derived growth factor-induced cell motility. *Biochem. Soc. Trans.* **29**:836–839.