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

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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 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).

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 protein and were inhibited by blockade of sphingosine kinase, suggesting transactivation of sphingosine-1-phosphate-dependent cell motility by prion protein.

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 (PrPSc).

Evidence suggests that circulating blood cells may have a role in enteral 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 PrPSc (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 (PrPc) is strongly expressed in myeloid DCs, which may act as carrier cells for the spread and circulation of the abnormal isoform PrPSc (3). In the absence of prion disease, high levels of expression of PrP in human spleen occur principally on myeloid DCs immediately adjacent to the white pulp, whereas follicular DCs do not strongly express PrPc; 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 (PrP 106-126). Signal transduction mechanisms that may be involved in directed migration of monocyte-derived DCs toward PrP 106-126 are described.

PrP 106-126, which is toxic to neurons, increases chemotaxis, oxygen free radical release, and intracellular calcium concentration in neutrophils and monocytes (5). To determine whether PrP 106-126 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 PrP 106-126 (Bachem, Bubendorf, Switzerland) in a concentration-dependent manner, whereas PrP 106-126 was not chemotactic for mature DCs (Fig. 2). Maximum chemotactic activity of PrP 106-126 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 PrP 106-126 and PrP 118-135 was monitored. Neither the scrambled form nor PrP 118-135 exerted chemotactic effects on immature DCs (Fig. 2). Checkerboard analysis revealed that the migration of immature DCs toward PrP 106-126 is true concentration gradient-dependent chemotaxis (Table 1). The influence of PrP 106-126 on 6Ckine-induced chemotaxis of mature DCs was tested. Combination of 6Ckine (1 μg/ml) with PrP 106-126 (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 PrP 106-126 was 78 ± 6.3 μm (P < 0.05). The effects of PrP 106-126 and 6Ckine alone are shown in Fig. 1.

Tyrophostin-23 (a tyrosine kinase inhibitor), bisindolylmale-
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 G₁/G₀ protein and Gₛ protein, respectively. Patterns of migration toward other DC attractants, including

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**FIG. 1.** Cytocfluorometric analysis of DC surface phenotype. A total of 5 × 10⁵ 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 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.).

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**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 ± 6.2 μm for immature and 49 ± 8.3 μm 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.
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 G1/Gi (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, PrPsc is particularly concentrated in follicular DCs, whereas high levels of PrP 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 PrPsc 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 PrPsc in vitro and transport intestinally administered PrPsc 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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PrP&lt;sub&gt;106-126&lt;/sub&gt; (10 nmol/liter)</th>
<th>Substance P (1 nmol/liter)</th>
<th>fMLP (10 nmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>1.477 ± 0.208</td>
<td>1.529 ± 0.221</td>
<td>1.820 ± 0.048</td>
</tr>
<tr>
<td>GFX (500 nmol/liter)</td>
<td>1.112 ± 0.031*</td>
<td>0.723 ± 0.092*</td>
<td>1.654 ± 0.135 (NS)</td>
</tr>
<tr>
<td>Rolipram (10 μmol/liter)</td>
<td>1.034 ± 0.180*</td>
<td>1.029 ± 0.106*</td>
<td>1.816 ± 0.064 (NS)</td>
</tr>
<tr>
<td>Tyr-23 (10 ng/ml)</td>
<td>0.889 ± 0.209*</td>
<td>1.058 ± 0.098*</td>
<td>1.685 ± 0.028*</td>
</tr>
<tr>
<td>WTN (10 nmol/liter)</td>
<td>1.111 ± 0.043*</td>
<td>1.049 ± 0.243*</td>
<td>1.859 ± 0.069 (NS)</td>
</tr>
<tr>
<td>CTX (1 nmol/liter)</td>
<td>1.636 ± 0.244 (NS)</td>
<td>1.729 ± 0.172 (NS)</td>
<td>1.918 ± 0.064 (NS)</td>
</tr>
<tr>
<td>PTX (1 nmol/liter)</td>
<td>1.074 ± 0.080*</td>
<td>1.227 ± 0.03*</td>
<td>1.268 ± 0.037*</td>
</tr>
</tbody>
</table>

* DCs were incubated with GFX, rolipram, tyrphostin-23 (Tyr-23), WTN, CTX, and PTX for 20 min. Chemotaxis toward PrP<sub>106-126</sub>, substance P, and fMLP was tested after a washing using modified multwell Boyden chambers.

* Ratio between directed and random migration. The mean random migration distance was 54 ± 0.05 μm. Statistical analysis was done by the Mann-Whitney U test after Kruskal-Wallis analysis. *, P < 0.05; NS, not significant.
result of pathogen uptake, maturation might occur and DCs might subsequently be arrested at the sites of prion uptake.

We further observed an inhibition of migration after pre-treatment of cells with DMS, which was used as specific inhibitor of sphingosine kinase. Previously it was suggested for monocytes that PrP_{106-126} might use the formyl peptide-like receptor 1, a pattern recognition seven transmembrane receptor also expressed in DCs (12). This receptor is coupled to G proteins and may be sufficient for migration induction (22). The observation that PrP_{106-126}-induced chemotaxis is sphingosine kinase dependent suggests that activation of chemotaxis toward sphingosine-1-phosphate, a motaxis is sphingosine kinase dependent suggests that transduction of immune system: focus on dendritic cells. Crit. Rev. Immunol. 2001. Sphingosine 1-phosphate receptor 1, a pattern recognition seven transmembrane receptor is expressed in DCs (12). This receptor is coupled to G proteins and may be sufficient for migration induction (22). The observation that PrP_{106-126}-induced chemotaxis is sphingosine kinase dependent suggests that activation of chemotaxis toward sphingosine-1-phosphate, a motaxis is sphingosine kinase dependent suggests that transduction of immune system: focus on dendritic cells. Crit. Rev. Immunol. 2001. Sphingosine 1-phosphate receptor 1, a pattern recognition seven transmembrane receptor is expressed in DCs (12). This receptor is coupled to G proteins and may be sufficient for migration induction (22). The observation that PrP_{106-126}-induced chemotaxis is sphingosine kinase dependent suggests that activation of chemotaxis toward sphingosine-1-phosphate, motaxis involves sphingosine kinase. Inward high concentrations of prion protein and their accumulation in lymphatic system of the gut, migration of circulating DCs to sites of pathogen uptake, maturation might occur and DCs might subsequently be arrested at the sites of prion uptake.

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 lysosphospholipid. 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
1-phosphate-induced endothelial cell migration requires the expression of EDG-1 and EDG-3 receptors and Rho-dependent activation of αvβ3 and αvβ6-containing integrins. J. Biol. Chem. 276:11830–11837.


