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Cutting Edge: The Neurotoxic Prion Peptide Fragment $PrP_{106–126}$ Is a Chemotactic Agonist for the G Protein-Coupled Receptor Formyl Peptide Receptor-Like 1[1,2]

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Prion diseases are transmissible and fatal neurodegenerative disorders which involve infiltration and activation of mononuclear phagocytes at the brain lesions. A 20-aa acid fragment of the human cellular prion protein, $PrP_{106–126}$, was reported to mimic the biological activity of the pathologic isoform of prion and activates mononuclear phagocytes. The cell surface receptor(s) mediating the activity of $PrP_{106–126}$ is unknown. In this study, we show that $PrP_{106–126}$ is chemotactic for human monocytes through the use of a G protein-coupled receptor formyl peptide receptor-like 1 (FPRL1), which has been reported to interact with a diverse array of exogenous or endogenous ligands. Upon stimulation by $PrP_{106–126}$, FPRL1 underwent a rapid internalization and, furthermore, $PrP_{106–126}$ enhanced monocyte production of proinflammatory cytokines, which was inhibited by pertussis toxin. Thus, FPRL1 may act as a "pattern recognition" receptor that interacts with multiple pathologic agents and may be involved in the proinflammatory process of prion diseases. ***The Journal of Immunology*****, 2001, 166: 1448–1451.**

Creutzfeldt-Jakob disease in humans, scrapie in sheep, and spongiform encephalopathy in cattle (BSE, or "mad cow disease") are transmissible and fatal neurodegenerative diseases (1). The etiological agent of these diseases is proposed to be an aberrant isoform of the cell surface glycoprotein, the prion protein (PrPc) (1). The pathologic isoform of PrPc (PrPSc) is deposited in the extracellular space of diseased CNS at sites infiltrated by activated astrocytes and mononuclear phagocytes (microglia) (2, 3). Although a direct neurotoxic effect of prion or its peptide fragment is reported to account for the neurodegeneration in prion diseases (4), other evidence implicates an indirect pathway, mediated by neurotoxins and proinflammatory cytokines released by prion-stimulated microglial cells (5, 6). A 20-aa fragment of the human prion protein, $PrP_{106–126}$, has been shown to form fibrils in vitro and to elicit a diverse array of biological responses in mononuclear phagocytes, i.e., monocytes and microglia, including calcium mobilization, protein tyrosine phosphorylation, and production of cytokines (7–10). However, the identity of the cellular receptor(s) that mediate the activity of $PrP_{106–126}$ remains unresolved. Our data show that the $PrP_{106–126}$ induces directional migration of human monocytic phagocytes and further demonstrates that $PrP_{106–126}$ uses the G protein-coupled receptor formyl peptide receptor-like 1 (FPRL1)[4] to activate cells. To our knowledge, this is the first receptor described to mediate the biological activity of $PrP_{106–126}$.

Materials and Methods

Reagents and cells

$PrP_{106–126}$ was purchased from Bachem Bioscience (King of Prussia, PA) or synthesized and purified by the Department of Biochemistry, Colorado State University (Fort Collins, CO), according to the published sequence (4). The purity was >90% and the amino acid composition was verified by mass spectrometry. The endotoxin levels in the dissolved peptide were undetectable. $PrP_{106–126}$ was dissolved in DMSO at 10 mM as stock solution and was diluted in RPMI 1640 containing 1% BSA for experiments. The final concentration of DMSO in a solution of 50 μM $PrP_{106–126}$ was 0.5%. This concentration of DMSO was used in control medium and we found no effect on cell responsiveness. The synthetic *N*-formyl-methionyl-leucyl-phenylalanine (fMLF) was purchased from Sigma (St. Louis, MO).

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[4] Abbreviations used in this paper: FPRL1, formyl peptide receptor-like 1; fMLF, *N*-formyl-methionyl-leucyl-phenylalanine; DAPI, 4′,6′-diamidino-2-phenylindole; PT, pertussis toxin; FPR, formyl peptide receptor.

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Human peripheral blood monocytes were isolated from buffy coats (Transfusion Medicine Department, National Institutes of Health Clinical Center, Bethesda, MD) enriched for mononuclear cells by using an iso-osmotic Percoll gradient. The purity of the cell preparations was examined by morphology and was >90%. Rat basophilic leukemia cell line (RBL-2H3) transfected with epitope-tagged formyl peptide receptor (FPR) (designated ETFR) was a kind gift from R. Snyderman (Duke University, Durham, NC). cDNA cloning and establishment of FPRL1-transfected HEK/293 cells (FPRL1/293) have been described previously (11). All of the transfected cells were maintained in DMEM supplemented with 10% FBS (HyClone, Logan, UT), 1 mM glutamine (Life Technologies, Grand Island, NY), and 800 μg/ml geneticin (G418; Life Technologies).

Chemotaxis assays

Chemotaxis assays were performed using 48-well chemotaxis chambers (Neuroprobe, Cabin John, MD) as described previously (12). The chemotaxis index was used which represented the fold increase in the number of cells migrated in response to chemoattractants over the spontaneous cell migration (in response to control medium).

Calcium mobilization

Cells (2×10^7 cells/ml) were incubated with 5 μM fura-2-acetoxymethyl ester (Molecular Probes, Eugene, OR) in loading medium (DMEM, 10% FBS, 2 mM glutamine) for 30 min at room temperature. Ca^{2+} mobilization induced by stimulants was measured with a luminescence spectrometer (LS-50B; Perkin-Elmer, Beaconsfield, U.K.) as previously described (12).

FPRL1 internalization

FPRL1/293 cells cultured on chamber slides (Nalge Nunc International, Naperville, IL) were stimulated for 15 min at 37°C with peptides. Cells were fixed in 4% paraformaldehyde for 10 min at room temperature. After washing with PBS, cells were incubated with PBS containing 0.05% Tween 20 and 5% normal goat serum for 1 h to block nonspecific binding sites and to permeabilize the cells. The slides were incubated for 1 h at room temperature with a rabbit polyclonal Ab that recognizes the C-terminal 20 aa of FPRL1 (a kind gift from C.-C. Li, Science Applications International Corporation-Frederick, National Cancer Institute-Frederick; 1:50 dilution in PBS-Tween 20-normal goat serum). The slides were then washed three times with PBS and further incubated with a FITC-conjugated goat anti-rabbit IgG (Sigma; 1:150 dilution in TBS containing 3% BSA) for 30 min. The slides finally were mounted with an anti-fade water-based mounting medium with 4′,6′-diamidino-2-phenylindole (DAPI; Vector Laboratories, Burlingame, CA) and analyzed with a laser scanning confocal fluorescence microscope (Leica TCS-4D DMIRBE; Leica, Heidelberg, Germany). Excitation wavelengths of 365 nm (for DAPI) and 488 nm (for FITC) were used to generate fluorescence emission in blue and green, respectively.

Measurements of cytokine production

Monocytes were first preincubated with or without 50 ng/ml pertussis toxin (PT) at 37°C for 4 h and then incubated with stimulants for another 24 h in RPMI 1640 without FCS or BSA. Supernatants were collected and secreted cytokines were measured by ELISA using QuantiGlo ELISA kits (R&D Systems, Minneapolis, MN).

Statistical analysis

All experiments were performed at least three times and the results presented are from representative experiments. The significance of the difference between test and control groups was analyzed with Student's *t* test.

Results and Discussion

Microglia, the resting monocytes in the brain, and peripheral blood monocytes accumulate and are activated at sites of prion plaques (2, 3). We therefore investigated the capacity of $PrP_{106-126}$ to induce directional migration of monocytes, a crucial step in the accumulation of cells at sites of origin of chemotactic factors. Freshly dissolved $PrP_{106-126}$ induced a dose-dependent migration of human monocytes with a maximal cell response occurring at 50 μM of the peptide (Figs. 1 and 2*A*), a concentration comparable to or lower than needed to activate other functions of microglia or monocytes (5, 7–10). When incubated at 37°C (500 μM $PrP_{106-126}$ diluted in PBS) to promote the formation of fibrils, the aggregated peptide induced a comparable level of monocyte migration as freshly dissolved $PrP_{106-126}$ (Fig. 2*A*). The monocyte migration is dependent on the chemotactic rather than the chemokinetic activity of the $PrP_{106-126}$, as evaluated with checkerboard analyses (data not shown). A peptide with a scrambled amino acid sequence of $PrP_{106-126}$ was completely inactive (data not shown). Since many known leukocyte chemotactic factors use G protein-coupled receptors to induce directional cell migration, we investigated whether $PrP_{106-126}$ also activated such a receptor. Monocyte migration in response to soluble $PrP_{106-126}$ was completely inhibited by treatment of the cells with PT, an inhibitor of G_i and G_o proteins (Fig. 2*B*), suggesting that a G protein-coupled receptor was involved. This was supported by induction of transient mobilization of intracellular calcium (Ca^{2+}) in monocytes by $PrP_{106-126}$ (Fig. 2*C*), which was also completely inhibited by pretreatment of the cells with PT (data not shown).

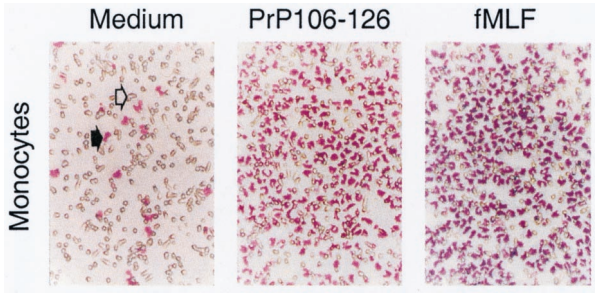

FIGURE 1. Monocyte chemotaxis induced by $PrP_{106-126}$. *Left,* Monocyte migration in the absence of stimuli. Open arrow indicates a micropore on the polycarbonate filter. Filled arrow denotes a monocyte migrated across the filter. *Center,* Monocyte migration in response to 50 μM freshly dissolved $PrP_{106-126}$. *Right,* Monocyte migration induced by 10 nM bacterial chemotactic peptide fMLF. Original magnification, ×400.

We then examined the capacity of a variety of other chemoattractants to cross-desensitize $PrP_{106-126}$-induced Ca^{2+} mobilization. This method has been effectively used previously to identify the sharing of receptors by chemotactic factors. The bacterial chemotactic peptide fMLF clearly attenuated $PrP_{106-126}$-induced Ca^{2+} flux in monocytes (Fig. 2*D*). Since high concentrations of fMLF were required to completely desensitize the cell response to $PrP_{106-126}$ (Fig. 2*D*), and such concentrations of fMLF have been found to additionally activate a G protein-coupled receptor FPRL1, which is also termed as the low-affinity fMLF receptor based on its homology to the high-affinity fMLF receptor FPR (13), we postulated that $PrP_{106-126}$ might share FPRL1 with fMLF. It was therefore determined that $PrP_{106-126}$ induced significant Ca^{2+} mobilization in FPRL1/293 cells (Fig. 3*A*), which was also completely inhibited by PT (data not shown). Untransfected parental cells (data not shown) or cells transfected with other chemoattractant receptors including FPR (Fig. 3*E*) did not respond to $PrP_{106-126}$. $PrP_{106-126}$ signaling in FPRL1/293 cells was desensitized by prior stimulation of the cells with high concentrations of fMLF (data not shown) and bidirectionally by another ligand for FPRL1, a synthetic peptide MMK-1 (14) (Fig. 3, *B–D*). Furthermore, FPRL1/293 cells, but not parental 293 cells or cells transfected with FPR, migrated in response to $PrP_{106-126}$ (Fig. 3*F*). The concentrations required for $PrP_{106-126}$ to activate FPRL1 were similar to those for monocytes, suggesting a major role for FPRL1 in monocyte activation by this prion protein fragment.

The possibility that FPRL1 contributed to the intracellular accumulation of prion fibrils was examined. Confocal microscopy showed that $PrP_{106-126}$ induced a rapid internalization of FPRL1 in FPRL1/293 cells (Fig. 3*G*). As a peptide control, it was shown that another synthetic peptide WKYMVm (W peptide), a ligand

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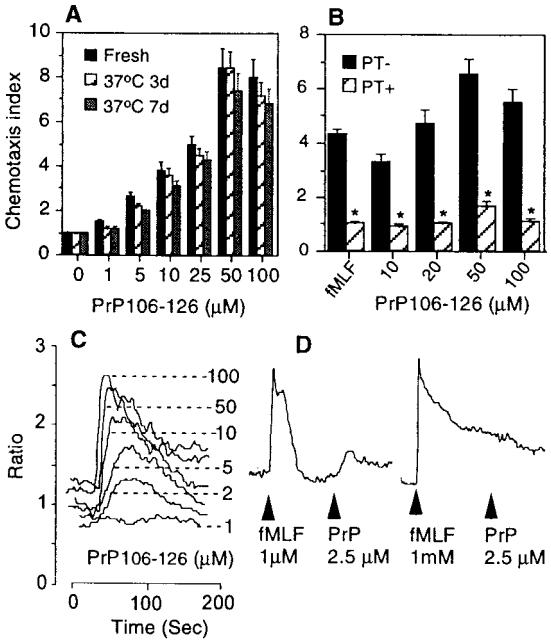

FIGURE 2. Activation of human monocytes by $PrP_{106-126}$. *A,* Migration of monocytes induced by freshly prepared $PrP_{106-126}$ and $PrP_{106-126}$ "aged" at 37°C for 3 or 7 days. *B,* Effect of preincubation with medium (PT−) or PT+ (100 ng/ml, 37°C, 30 min) on monocyte migration to $PrP_{106-126}$. fMLF at 100 nM was used as a control. *C,* Ca^{2+} mobilization induced by $PrP_{106-126}$ in monocytes. *D,* Attenuation of $PrP_{106-126}$-induced Ca^{2+} flux in monocytes by fMLF.

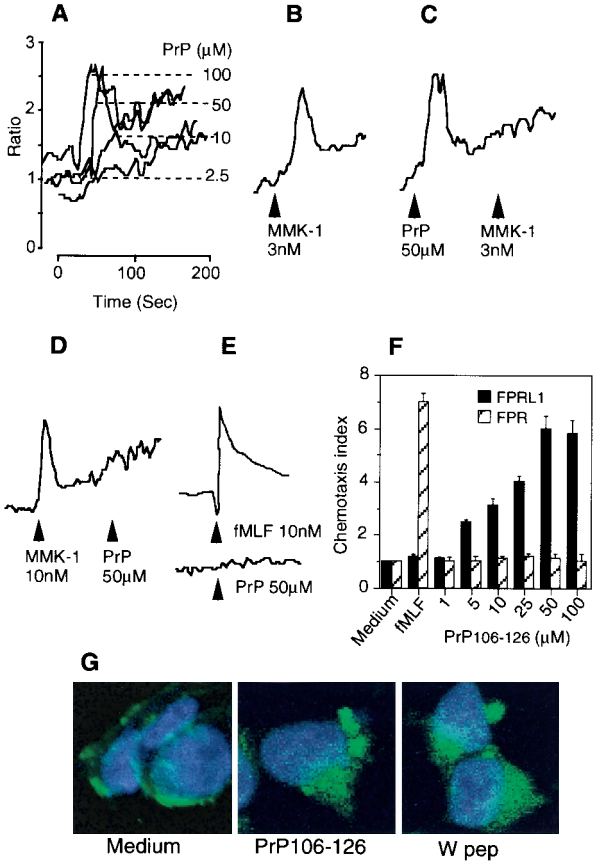

FIGURE 3. Activation of FPRL1/293 cells by $PrP_{106-126}$. *A,* Ca^{2+} mobilization induced by $PrP_{106-126}$ in FPRL1/293 cells. *B–D,* Cross-desensitization of calcium mobilization between $PrP_{106-126}$ and MMK-1 in FPRL1/293 cells. *E,* Ca^{2+} flux induced by fMLF and $PrP_{106-126}$ in FPR-expressing RBL cells (ETFR). *F,* Migration of FPRL1/293 and ETFR cells induced by $PrP_{106-126}$. fMLF at 100 nM was used as a control. *G,* Confocal micrographs of FPRL1/293 cells stained with anti-FPRL1 Ab (green) showing localization of FPRL1 in the cell membrane (*left*). FPRL1 internalization is induced by $PrP_{106-126}$ (50 μM; *center*) and WKYMVm (W peptide, 2 μM; *right*) in these cells. The nuclei appear blue (DAPI counterstain). Original magnification, ×600.

for FPRL1 (12), similarly induced internalization of FPRL1. These results suggest that FPRL1 may also play a role in the uptake of prion peptide by FPRL1-bearing cells.

We further investigated the capacity of ligand activation of FPRL1 to enhance the production of proinflammatory cytokines in monocytes. $PrP_{106-126}$ at concentrations that induced FPRL1-mediated cell migration stimulated a PT-sensitive production of proinflammatory cytokines in monocytes (Table I). The FPRL1 ligand MMK-1 had similar effects. In contrast, cytokine production in response to bacterial LPS, which does not use G protein-coupled receptor, was resistant to PT. Thus, in addition to mediating cell migration, FPRL1 activation by $PrP_{106-126}$ induced the release of proinflammatory cytokines that have been implicated in the neurotoxic effect of monocyte supernatants (10).

$PrP_{106-126}$ was derived from the amino acid sequence of human prion. In vitro, this peptide fragment has a high propensity to form amyloid-like aggregates and to induce apoptotic death of neuronal cells (4–6, 10). It was subsequently established that the neurotoxic effect of $PrP_{106-126}$ requires the presence of microglia or monocytes (4–6, 10), and $PrP_{106-126}$ induces signaling events in these cells that typically involved activation of cell surface receptor(s) (7–10). Our study identifies FPRL1 as a functional receptor used by $PrP_{106-126}$ to induce monocytic cell migration and activation.

FPRL1 is a G protein-coupled receptor that is capable of interacting with a number of agonists, including peptide domains derived from HIV-1 envelope proteins (13) and at least four host-derived molecules, serum amyloid A (15), lipid metabolite lipoxin A4 (16), β amyloid peptide,[5], and cathelicidin (17). FPRL1 mediates the chemotactic activity of peptide agonists and serum amyloid A, which is an acute phase protein and forms amyloid deposit during chronic inflammation (18). Consequently, FPRL1 behaves as a "pattern recognition" receptor that is activated to transduce signals by a wide variety of unrelated ligands. Stimulation of FPRL1 by its chemotactic agonists triggers a series of G protein-mediated signaling cascades leading to cell adhesion, migration, protein tyrosine phosphorylation, release of reactive oxygen intermediates, as well as gene activation and production of proinflammatory cytokines (13). These properties of FPRL1 account for various biological activities reported for $PrP_{106-126}$. FPRL1 is expressed in a number of cell types including cells of the nonhematopoietic origin such as epithelia (13). We found that human astrocytoma cells express FPRL1 and can be activated by FPRL1-specific agonists (19). We also detected FPRL1 gene expression in a neuroblastoma cell line and in a murine microglial cell line N9 (Y. Le, unpublished observation). Whether normal human neurons and astroglial cells express the functional FPRL1 and its role in glial activation and neuronal destruction are under further investigation.

A relatively high concentration (50 μM) of $PrP_{106-126}$ was required to induce an FPRL1-dependent maximal monocyte chemotactic response. Therefore, $PrP_{106-126}$ appears to interact with

[5] Y. Le, et al. Amyloid β_{42} activates a G-protein-coupled chemoattractant receptor FPR-like 1. *Submitted for publication.*

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Table I. *Induction of IL-1β and IL-6 by PrP106-126*[a]

Stimulant	IL-1β (pg/ml)		IL-6 (pg/ml)	
	−PT	+PT	−PT	+PT
Medium	19.3 ± 1.5	29.6 ± 3.5	12.8 ± 1.2	19.1 ± 1.1
MMK-1	46.9 ± 2.1*	32.2 ± 2.3†	62.3 ± 1.7*	19.9 ± 1.8†
$PrP_{106–126}$	84.8 ± 2.0*	46.2 ± 3.8†	75.7 ± 3.2*	23.6 ± 1.3†
LPS	3,615.3 ± 19.5*	3,634.2 ± 58.3	34,611.8 ± 365.6*	33,929.2 ± 357.3

[a] Human monocytes (10×10^6 cells/ml) were incubated with or without PT (50 ng/ml) at 37°C for 4 h and were further cultured in the presence of stimuli (MMK-1, 1 μM; $PrP_{106–126}$, 30 μM; and LPS, 0.5 μg/ml) for 24 h. The supernatants were measured for IL-1β and IL-6 with ELISA.
*, $p < 0.01$ compared with cells cultured with medium alone; †, $p < 0.01$ compared with cells not pretreated with PT.

FPRL1 with a relatively low affinity. However, many chemoattractants, including some chemokines, also show low-affinity interaction with their receptors and such interactions nevertheless contribute to the recruitment of leukocytes to the sites of inflammation (20). Although our results are based on in vitro models, they have repeatedly been shown to correlate directly with in vivo disease states (2, 3) in which infiltration of mononuclear phagocytes (monocytes and microglia) was found in and around prion disease lesions in association with proinflammatory reactions (2, 3). Thus, the low-affinity $PrP_{106–126}$ and FPRL1 interaction may help direct monocytes/microglia migrate to the vicinity of prion lesions which contain high concentrations of these amyloidogenic precursors and aggregated fragments including $PrP_{106–126}$ (4, 21–24). Interestingly, a recent study revealed that $PrP_{106–126}$ was detected in brain lesions of some Alzheimer's disease patients, suggesting the coexistence of prion disease pathology in Alzheimer's disease (25). Our observation of FPRL1 as a functional receptor, used by $PrP_{106–126}$ to chemoattract and activate mononuclear phagocytes, should promote further assessment of this receptor as a mediator of proinflammatory responses in neurodegenerative diseases and as a potential therapeutic target.

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