

Prion Protein Expression and Release by Mast Cells After Activation

D. James Haddon,¹ Michael R. Hughes,¹ Frann Antignano,² David Westaway,⁵ Neil R. Cashman,^{3,4} and Kelly M. McNagny¹

¹The Biomedical Research Centre, ²Terry Fox Laboratory, ³Division of Neurology, Department of Medicine, and ⁴Brain Research Centre, University of British Columbia, Vancouver, and ⁵Centre for Prions and Protein Folding Diseases, University of Alberta, Edmonton, Canada

In this study, we compared hematopoietic stem cells and mast cells, using microarray expression analysis, and identified the cellular prion protein (PrP^C) as a potentially novel marker of mast cells. On further investigation, we found that PrP^C is expressed on the surface of human and mouse mast cells, is rapidly released by mast cells upon activation, and is released in response to mast cell–dependent allergic inflammation in vivo. Because mast cells are long lived and traffic to the brain and central nervous system, our observations could have important implications for the transmission and pathology of prion diseases.

Mast cells are highly granular immune effector cells that develop from hematopoietic stem cells (HSCs), circulate as committed precursors, and mature in their target tissues. Mast cells are located in many tissues, including the skin, gut, brain, and peritoneal cavity, and they tend to be in close proximity to nerves and blood vessels. When mast cells degranulate, they release an assortment of inflammatory mediators that can trigger anaphylaxis. Our group previously reported that mast cells share a number of surface markers with HSCs, including CD34, Sca-1, and c-kit [1].

The cellular prion protein (PrP^C) is a glycosylphosphatidylinositol (GPI)–anchored sialoglycoprotein and is the substrate

for the posttranslational, template-directed misfolding process implicated in transmissible spongiform encephalopathies (TSEs), including scrapie, bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease. TSEs are incurable, fatal, neurodegenerative diseases associated with accumulation and aggregation of the scrapie isoform of PrP^C (PrP^{Sc}) in the brain. The mechanism by which infectious prions reach the brain after peripheral inoculation, including the cell types involved, is incompletely understood. PrP^C is expressed by many cell types, including hematopoietic cells, but expression of PrP^C by mast cells has yet to be investigated [2, 3].

In this study, we compared Lin[−]Sca-1⁺c-kit⁺ (LSK) cells, which are highly enriched for HSCs, and mast cells, using microarray expression analysis, and identified PrP^C as a potentially novel marker of mast cells. On further investigation, we found that PrP^C is expressed on the surface of human and mouse mast cells, both in vitro and in vivo; is not required for mast cell differentiation or tissue homeostasis; is released by mast cells at steady state and rapidly on activation; and is released in response to mast cell–dependent allergic inflammation in vivo. Because mast cells are long lived and traffic to the central nervous system (CNS), our observations could have important implications for the transmission of PrP^{Sc} and the pathology of prion disease.

Materials and methods. C57Bl/6 (B6) and B6.Cg-Kit^{W-sh}/HNihrJaeBsmJ (*Kit*^{W-sh/W-sh}) mice were acquired from Jackson Laboratory (Bar Harbor, ME). *Zrch 1 Prnp*^{0/0} mice were maintained on a B6 background [4]. Mice were at least 4 weeks old at the time of experimentation, matched by age and sex, and kept in accordance with the Canadian Council on Animal Care guidelines.

Bone marrow mast cells (BMMCs) for microarray analysis were prepared as previously described [1]. Total RNA was isolated from 3 independent BMMC cultures at 14–16 days (for immature cells) or 8–15 weeks in culture (for mature cells), using a Qiagen RNeasy Mini RNA isolation kit. Total RNA samples were amplified by Affymetrix GeneChip eukaryotic small sample target labeling and hybridized to Affymetrix GeneChips (MOE430A and MOE430B). The resulting gene expression data, as well as data retrieved for LSK cells, are available from StemBase (available at: <http://www.stembase.ca>) and the National Center for Biotechnology Information's Gene Expression Omnibus (available at: <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3776> and [GSE3234](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE3234)) [5, 6]. Differentially expressed transcripts were those that were called “present” (Affymetrix MAS5.0 algorithm) and had a >2-fold, sta-

Received 3 January 2009; accepted 3 April 2009; electronically published 30 July 2009.
Potential conflicts of interest: none reported.

Financial support: Canadian Institutes for Health Research (CIHR); Stem Cell and AllerGen Network Centres of Excellence (to K.M.M.); Natural Sciences and Engineering Research Council of Canada (to D.J.H. and F.A.); Faculty of Medicine, University of British Columbia (UBC; to D.J.H. and F.A.); UBC Centre for Blood Research CIHR/Heart & Stroke Foundation of Canada Strategic Training Program in Transfusion Science (to M.R.H.); Michael Smith Foundation for Health Research (to F.A.).

Reprints or correspondence: Dr. Kelly M. McNagny, The Biomedical Research Centre, 2222 Health Sciences Mall, Vancouver, Canada V6T 1Z3 (kelly@brc.ubc.ca).

The Journal of Infectious Diseases 2009;200:000–000

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0022-1899/2009/20005-00XX\$15.00

DOI: 10.1093/infdis/jin102

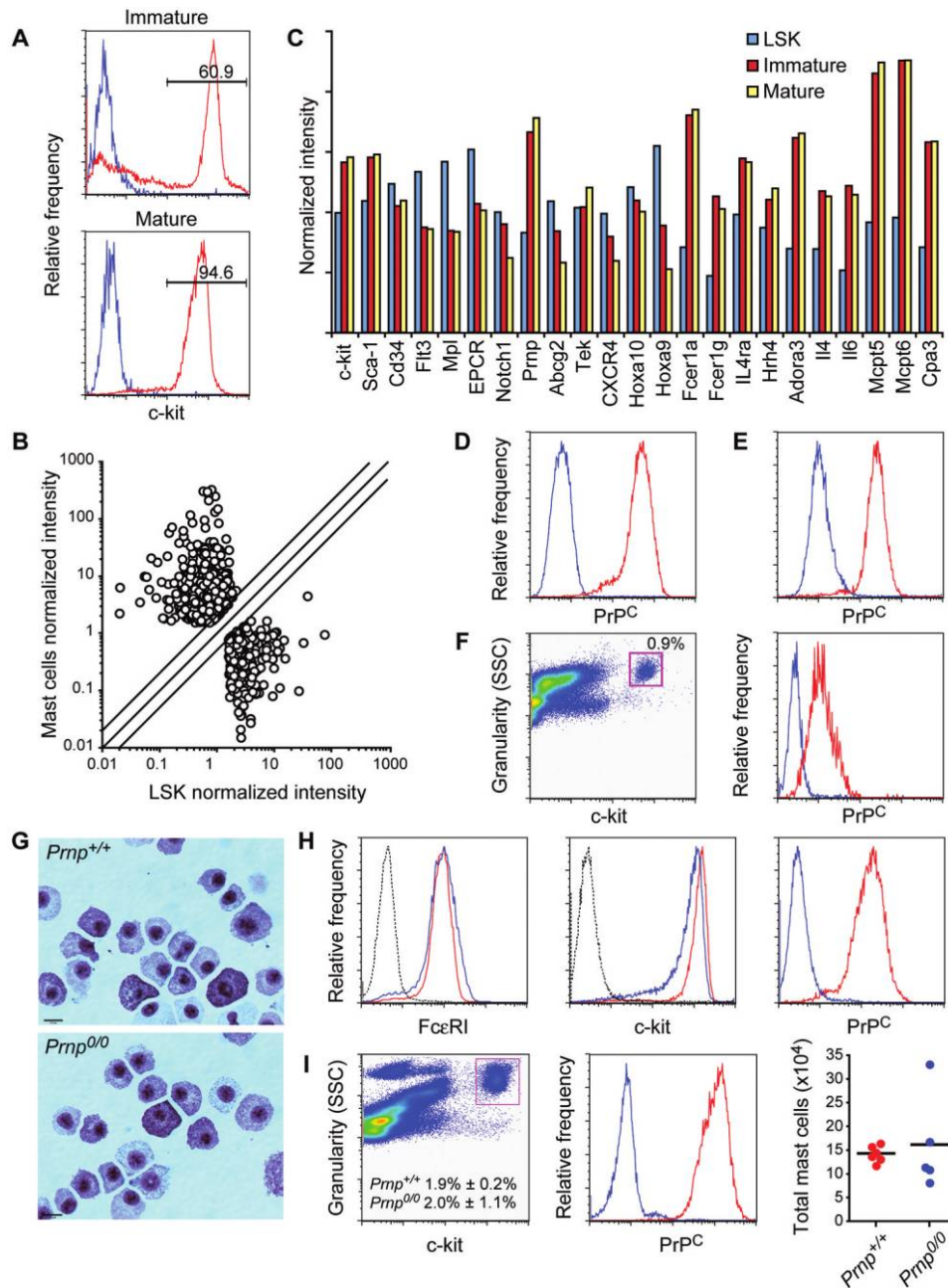


Figure 1. Gene expression analysis of mast cells and hematopoietic stem cells (HSCs) identifies the cellular prion protein (PrP^C) as a marker of human and mouse mast cells, in vitro and in vivo. *A*, Representative flow cytometry histograms of immature (14–16 days in culture) and mature (8–15 weeks in culture) mast cells from which total RNA was isolated for microarray analysis. C-kit expression is indicative of mast cell differentiation (blue, unstained/isotype; red, anti-c-kit-PE). *B*, Scatter plot of 1228 differentially expressed transcripts between mature mast cells and Lin[−]Sca-1⁺c-kit⁺ (LSK) cells (each circle represents a transcript; diagonal lines represent 1- and 2-fold cutoffs). *C*, Levels of selected HSC- and mast cell-associated transcripts in LSK cells, immature mast cells, and mature mast cells as determined by microarray. Flow cytometry analysis of bone marrow mast cells (BMMCs; *D*), HMC-1 (*E*), and c-kit^{hi}SSC^{hi} peritoneal wash mast cells (*F*) for surface expression of PrP^C (blue, isotype; red, anti-PrP^C 6H4). *G*, *Pmp*^{+/+} and *Pmp*^{0/0} BMMCs had similar morphologic characteristics, as shown by Wright-Giemsa stained cytopsins (bars = 10 μm). Images were acquired on a Zeiss Axioplan 2 microscope (Zeiss) with a 40×/0.75 dry objective and a Qimaging Retiga EX CCD camera, using Openlab 4.0.4 software (Improvision). *H*, Flow cytometry analysis revealed that *Pmp*^{0/0} BMMCs (blue line) had similar expression of FcεRI and c-kit to *Pmp*^{+/+} BMMCs (red line) but lacked surface PrP^C (dashed line, isotype). *I*, The total number (graph) and frequency (gate statistics) of c-kit^{hi}SSC^{hi} peritoneal wash mast cells were comparable between *Pmp*^{+/+} (red line) and *Pmp*^{0/0} (blue line) mice. Flow cytometry data are representative of 3 independent experiments.

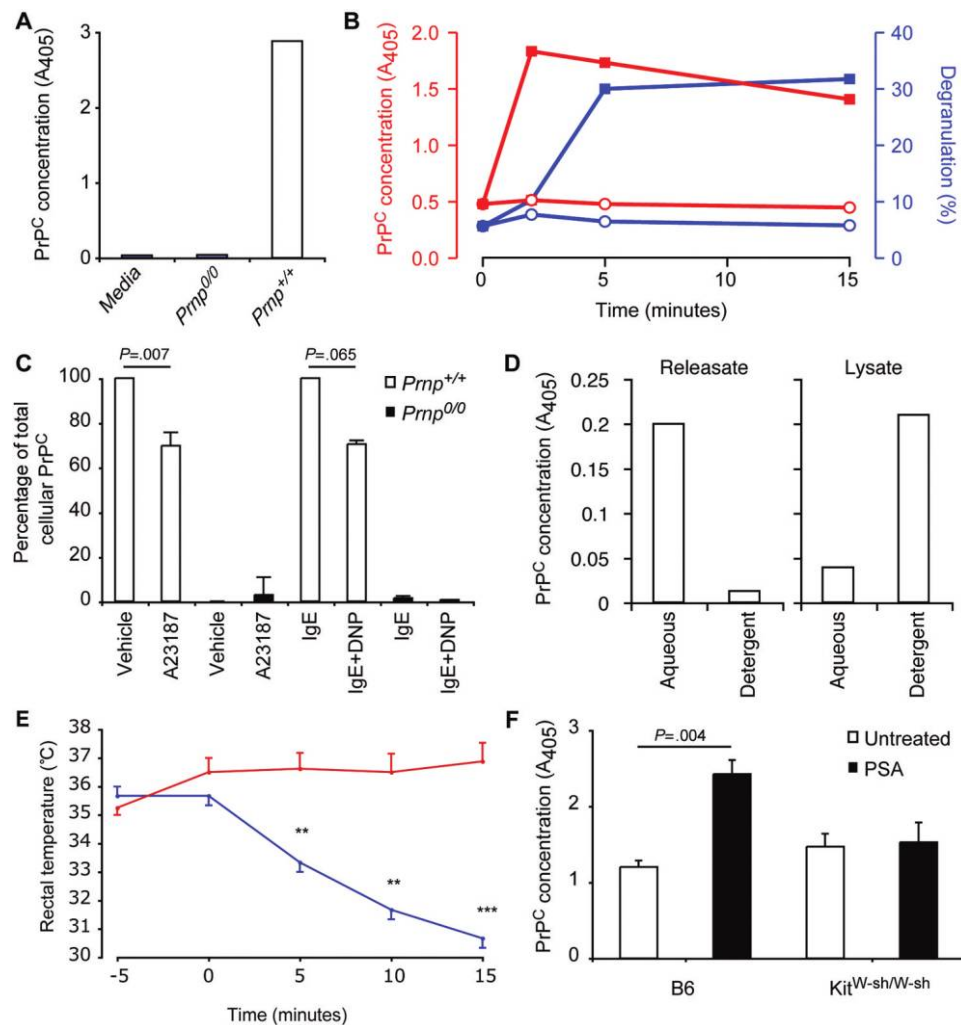


Figure 2. Mast cells release PrP^C at steady state and upon stimulation in vitro and in vivo. *A*, Prnp^{0/0} and Prnp^{+/+} bone marrow mast cells (BMMCs) were seeded at 1×10^5 cells/mL and cultured for 1 week, and high levels of the cellular prion protein (PrP^C) were found in the supernatants of Prnp^{+/+} BMMCs by PrP^C enzyme immunoassay (EIA). *B*, BMMCs were stimulated with A23187 (solid squares), and PrP^C was detected in the supernatant within 2 min (PrP^C EIA, red lines) and before maximal degranulation (β -hexosaminidase activity, blue lines), compared with vehicle controls (open circles). Data are representative of 3 experiments. *C*, EIA of whole cell lysates (1 volume of Tyrode's buffer with 0.1% bovine serum albumin (BSA), 0.5% NP-40, and tissue culture protease inhibitor cocktail) from Prnp^{+/+} BMMCs stimulated with A23187 or IgE plus dinitrophenol (DNP) for 15 min revealed a loss of 30% of total cellular PrP^C, compared with vehicle and IgE-alone controls. Data represent pooled results from 2 (IgE plus DNP) or 3 (A23187) independent experiments. *D*, BMMCs were stimulated with A23187 for 2 min, and a phase extraction was performed on the pellets (10^6 cells) and releasates (from 10^7 cells) in Tyrode's buffer with 0.1% BSA, 0.5% Triton X-114, and tissue culture protease inhibitor cocktail. PrP^C EIA of the aqueous and detergent phases revealed that, in contrast to the lysates, the majority of PrP^C in the releasate segregated to the aqueous phase. *E*, To induce passive systemic anaphylaxis, B6 ($n = 3$; blue line) and Kit^{W-sh/W-sh} ($n = 3$; red line) mice were sensitized (at $t = 0$) with anti-DNP IgE (20 μ g intravenously) and challenged the following day with DNP-HSA (1 mg intravenously). *F*, PrP^C EIA of presensitization and postanaphylaxis serum revealed that anaphylaxis induced a 2-fold increase in serum PrP^C in B6 but not Kit^{W-sh/W-sh} mice. Bars represent standard errors. * $P < .05$, ** $P < .01$, and *** $P < .001$, by 2-tailed unpaired t tests.

tistically significant difference in expression between conditions ($P < .05$ by the t test, with Benjamini and Hochberg false discovery rate correction performed with Genespring GX).

For all other experiments, BMMCs were prepared from bone marrow harvested from the femurs of B6 (Prnp^{+/+}) or Prnp^{0/0} mice and placed in Iscove's modified Dulbecco's medium (IMDM) supplemented with 15% fetal calf serum,

monothioglycerol (MTG; concentration, 150 μ mol/L), penicillin (100 U/mL), streptomycin (100 μ g/mL), and rmIL-3 (30 ng/mL). At 6 weeks, BMMCs were >90% pure, as determined by flow cytometry (Fc ϵ RI and c-kit). HMC-1 cells, maintained as described elsewhere [7], were a gift from Joseph H. Butterfield (Mayo Clinic [Rochester, MN]).

To induce degranulation, we incubated BMMCs overnight

in IMDM containing 0.1% bovine serum albumin (BSA) and MTG (concentration, 150 $\mu\text{mol/L}$) with or without mouse anti-dinitrophenol (DNP) IgE SPE-7 (1 $\mu\text{g/mL}$). We washed and resuspended the cells in Tyrode's buffer containing 0.1% BSA, and stimulated with 10 ng/ml DNP-HSA or 1 $\mu\text{mol/L}$ A23187 for the indicated times. β -hexosaminidase activity was determined as previously described [8]. PrP^C enzyme immunoassay (EIA; SPI-bio) was used to determine the relative levels of PrP^C release.

Flow cytometry samples were blocked with goat serum and anti-CD16/32, labeled with anti-c-kit-PE (Pharmingen), anti-PrP^C 6H4 (Prionics), or anti-Fc ϵ RI-biotin (eBioscience), followed by SA-FITC (Pharmingen). Anti-PrP^C was labeled with a Zenon AlexaFluor488 mouse IgG₁ labeling kit (Molecular Probes) or with goat anti-mouse IgG-FITC (Serotec). Acquisition was performed on a BD FACSCalibur and analyzed with FlowJo (TreeStar).

Results and discussion. To further compare mast cells and HSCs, we evaluated the gene expression of LSK cells, which are highly enriched for HSCs, to immature and mature mast cells, using microarray analysis (figure 1C). We identified 1228 differentially expressed transcripts between mature mast cells and LSK cells (figure 1A). Mast cells expressed higher levels of mast cell-associated genes (including *Fcer1a*, *Mcpt5*, and *Mcpt6*) (figure 1B). For the most part, LSK cells expressed higher levels of HSC-associated genes (such as *Cd34*, *EPCR*, and *Flt3*), whereas mast cells expressed higher levels of Sca-1, c-kit, and, surprisingly, *Prnp* [9].

We were intrigued to find that mast cells express high levels of *Prnp* transcript, because to our knowledge PrP^C expression by mast cells has not been reported. We found that BMMCs, PWMCs, and the HMC-1 human mast cell line all expressed high levels of surface PrP^C (figure 1D–F). To test whether PrP^C is required for the formation of mast cells, we attempted to derive BMMCs from *Prnp*^{+/+} and *Prnp*^{0/0} bone marrow. After 4 weeks of culture, both had formed Fc ϵ RI⁺c-kit⁺ mast cells (figure 1G and 1H). We also found that *Prnp*^{0/0} PWMCs were present at normal numbers and had normal levels of c-kit expression (figure 1I).

Some cell lines release PrP^C into the culture supernatant at steady state [10, 11]. We found that *Prnp*^{+/+} BMMCs also released high levels of PrP^C into the culture supernatant at steady state (figure 2A). Furthermore, we found that, on activation, BMMCs rapidly released 30% of their total cellular PrP^C (figure 2B and 2C). Activation-dependent release of PrP^C likely occurred through a previously reported proteolytic or lipolytic loss of its GPI anchor, because the majority of PrP^C in the releasate segregated to the aqueous phase when subjected to a Triton X-114 phase extraction, whereas PrP^C in BMMC lysates segregated to the detergent phase (figure 2D) [12]. Additionally, because the EIA relies on binding within PrP^C's octapeptide

repeats and to amino acids 144–153 (DYEDRYREN), it rules out intervening cleavages at amino acids 96 and 111 [13, 14]. To investigate whether mast cell-dependent allergic inflammation can provoke release of PrP^C in vivo, we induced passive systemic anaphylaxis in B6 and mast cell-deficient *Kit*^{W^{-sh}/W^{-sh} mice. Although *Kit*^{W^{-sh}/W^{-sh} mice were resistant to passive systemic anaphylaxis, B6 mice had severe hypothermia (figure 2E) [15]. Strikingly, anaphylaxis induced a 2-fold increase in serum PrP^C (figure 2F).}}

In summary, we report that PrP^C is expressed on the surface of human mast cells and mouse mast cells, both in vitro and in vivo; that PrP^C is not required for mast cell differentiation, or tissue homeostasis; that PrP^C is released by mast cells at steady state and rapidly on activation; and that PrP^C is released in response to mast cell-dependent allergic inflammation in vivo.

Via the oral route, prions are ingested, and they initially infect the gut-associated lymphoid tissue before propagation to the CNS. Because mast cells are migratory, located in gut, brain, CNS, and in close proximity to nerves and blood vessels, and express high levels of PrP^C, they are well suited to be infected with prions and may present a unique target to disrupt the propagation of infectivity to the CNS. Furthermore, mast cells may be a unique system to investigate the normal function of PrP^C.

Acknowledgments

We thank Dr. Michael Rudnicki and Pearl Campbell (Ontario Genomics Innovation Centre), for collecting the microarray data; and Dwayne Ashman, Alison Hirukawa, and Jing Yang, for technical assistance.

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