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Supporting Online Material
www.sciencemag.org/cgi/content/full/314/5796/133/DC1
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Modulation of Cell Adhesion and Motility in the Immune System by Myo1f

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Although class I myosins are known to play a wide range of roles, the physiological function of long-tailed class I myosins in vertebrates remains elusive. We demonstrated that one of these proteins, Myo1f, is expressed predominantly in the mammalian immune system. Cells from Myo1f-deficient mice exhibited abnormally increased adhesion and reduced motility, resulting from augmented exocytosis of β2 integrin–containing granules. Also, the cortical actin that co-localizes with Myo1f was reduced in Myo1f-deficient cells. In vivo, Myo1f-deficient mice showed increased susceptibility to infection by *Listeria monocytogenes* and an impaired neutrophil response. Thus, Myo1f directs immune cell motility and innate host defense against infection.

In both mouse and human genomes, 16 genes encode conventional class II muscle and nonmuscle myosins, with 25 "unconventional" myosin genes encoding 11 other classes (*1*). Natural mutations of various myosin genes result in an array of genetic disorders, including cardiomyopathies, deafness, blindness, glomerular nephritis, and neuropathies (*2*, *3*). The class I myosins are the largest group of unconventional myosins and are evolutionarily ancient, existing in a wide range of species from yeast to vertebrates (*1*, *4*). Mice and humans have a total of eight class I myosin heavy-chain genes, six of which encode short-tailed forms (Myo1a, b, c, d, g, and h) and two of which encode long-tailed (amoeboid) forms (Myo1e and f) (*1*). All class I myosins consist of an N-terminal motor domain, light-chain–binding IQ motifs, and a basic tail homology 1 (TH1) domain thought to affect interactions with membranes (*2*). The long-tailed class I myosins have an additional proline-rich TH2 domain and a TH3 domain containing a single Src homology 3 (SH3) domain (*2*).

The class I myosins in *Dictyostelium* and yeast are involved in migration, phagocytosis, endocytosis, and actin remodeling (*5*, *6*). Short-tailed class I myosins in vertebrates are involved in more specialized functions, such as the adaptation of hair cells in the ear (*7*) and the transport of vesicles and organelles (*8*, *9*), as well as the structural maintenance of the enterocyte microvilli (*10*). However, the function of long-tailed class I myosins in vertebrates is poorly characterized (*11*–*14*).

Myo1f was first identified in our screen for differentially expressed genes in subsets of murine lymphocytes. In contrast to previous data suggesting the widespread expression of Myo1f in tissues (*15*), our results, which we obtained using specific probes, showed that Myo1f is selectively expressed in the spleen, mesenteric lymph nodes, thymus, and lung (Fig. 1A). By comparison, specific detection of Myo1e showed a predominant expression pattern in the spleen and mesenteric lymph nodes and moderate expression in the lung, small intestine, and large intestine (Fig. 1B). Within the lymphoid tissues, natural killer (NK) cells, macrophages, and dendritic cells were found to express considerable levels of both Myo1f and Myo1e; neutrophils and B cells showed selective expression of Myo1f and Myo1e, respectively (Fig. 1, C and D).

To determine the function of Myo1f in the vertebrate immune system, we generated Myo1f gene–deficient mice. We focused on neutrophils because Myo1f was detected exclusively in neutrophils (Fig. 1, C and D). Immunoglobulin G (IgG)–mediated phagocytosis was similar between wild-type and knockout (KO) neutrophils (Fig. 1E). To evaluate the degree of pathogen killing that follows phagocytosis, we measured the production of reactive oxygen species. Again, no considerable difference was detected between wild-type and KO neutrophils (Fig. 1F). Thus, Myo1f is dispensable for both the phagocytosis of bacteria and their destruction.

Integrin-mediated adhesion to the vascular endothelium is crucial in the process of neutrophil migration to infected tissue, and the dominant integrins involved in this process belong to the β2 integrin (CD18) family (*16*). Myo1f-deficient neutrophils exhibited strong adhesion to integrin ligands, including the intercellular adhesion molecule–1 (ICAM-1) (CD54) and fibronectin (Fig. 2, A and B). Activation of neutrophils by the proinflammatory cytokine tumor necrosis factor–α did not compensate for this difference, suggesting that increased adhesion did not result from changes in the activation status of Myo1f-deficient cells. Experiments with a blocking antibody showed that most of the adhesion was mediated by β2 integrin (Fig. 2, A and B). In addition, Myo1f affected only integrin-mediated adhesion, not integrin-independent adhesion to polylysine-coated substrate (Fig. 2C) (*17*). Spreading of Myo1f-deficient neutrophils on ICAM-1 was also increased as compared to that of wild-type neutrophils (Fig. 2D). Increased spreading was not due to a loss of cortical tension (fig. S2), which acts to maintain the round shape of the cells (Fig. 2E). In contrast, myosin I double mutants in *Dictyostelium* exhibit abnormalities

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研究報告の概要

○PRDT TSE親和性リガンドを用いた血中の内在性TSE感染性の除去及びMacoPharma P-Capt™ Filterへのリガンドの組み込み

背景:供血後にvCJDで死亡した供血者の血液を輸血されたことが判明し、疾患が検知可能となるまで生存した受血者19名のうち、vCJD輸血感染症例は3例で感染率は15%以上である。外科的に除去した組織の調査では、英国のvCJD潜伏症例は最低でも4000例と見積もられ、うち平均7%が受血者である。

目的:プリオンタンパク質およびTSE感染性に高親和性のリガンドを開発し、血液製剤によるTSE感染症伝播リスクを軽減するため、RBCやその他血液成分からTSE感染性を除去する器具に当該リガンドを組み入れること。

方法:Pathogen Removal and Diagnostics Technologies(PRDT)は、全血、RBCまたは血漿存在下の脳由来プリオンタンパク質およびTSE感染性と高親和性のリガンドを得るため数百万の化合物のスクリーニングを行った。血中の内在性TSE感染性の除去をテストするため、スクレイピー感染ハムスターから4時間以内に血液~500 mlsを採取し、商用フィルターで白血球除去を行い被験樹脂を通過させた。全血、白血球除去血液および樹脂通過血液を分注し、限界希釈法で力価測定を行った。ハムスター100匹に各サンプルの分注50μg(合計5mL)を接種した。ハムスターの自然死が起り始めた540日で実験を終了し、プリオンアミロイドの証拠がないか各動物の脳を調べた。

結果:もっとも優れた樹脂は、脳由来プリオンタンパク質でスパイクしたRBC 10^7ID/mlの力価を、4 log(10)ID(50)以上減少させ、血液やRBCに関して非常に低濃度の感染性に対して求められる性能を大きく上回った。脳由来物スパイクの妥当性が不確実であることから、血中に内在する感染源の除去能についても評価した。樹脂処理した血液を接種したハムスター100匹のうち、感染したハムスターはいなかった。当該樹脂は、TSE血液感染性(> 1.2 log(10)ID)を除去した。この樹脂は広範囲の血液適合性検査にパスした。これは、MacoPharmaにより白血球除去赤血球からTSE感染性を除去する独立型のP-Capt™ filterに取り入れられている。

結論:PRDTの樹脂は、高濃度のTSE感染性を吸着し、RBC存在下でも効果が高く、白血球除去血液中の低濃度内在性感染性を検出限界まで除去する。P-Capt™ filterとして実現した当該樹脂は、血液および血液製剤によるvCJD伝播のリスクを有意に減少させるはずである。

報告企業の意見	今後の対応
PRDT TSE親和性リガンドならびにリガンドを組み入れたMacoPharma P-Capt™ Filterを用いて血中に存在する内在性TSE感染性を除去できたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

to the whole blood and RCC filtrations, including losses in tubes and bags by transfer, of about 20 g Hb. The CompoSafe Pr filtration causes an additional loss compared to whole blood filtration of about 7 g Hb. The filtration over the CompoSafe Pr filter induced a slight increase of hemolysis (about 0.03% increase), but this did not result in a more rapid increase during subsequent storage: after 42 days 0.26% hemolysis for group I and 0.16% for group II were detected. For group I at day 42 1.3 % of the red cells were positive for AnnexinV (representing PS-exposure), whereas for group II this was 0.9 % (comparable to standard leukodepleted RCC in SAGM). The amount of ATP was predicted to be above 2.7µmol/g Hb at day 35, with about 15 mM glucose remaining at day 42.

Conclusion: Despite the double filtration step in the total procedure (one to leukoreduce the whole blood and one to remove prions from RCC), the remaining RCC met the European requirements for Hb content. The amount of Hb in the final RCC is similar to that in leukodepleted RCC prepared from whole blood after buffy coat depletion. The in vitro quality after 42 days was similar to those of standard leukodepleted RCC, prepared from whole blood after buffy coat depletion.

P-091
DETECTION OF INFECTIOUS PRION IN BLOOD BY PROTEIN MISFOLDING CYCLIC AMPLIFICATION TECHNOLOGY (PMCA)

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Background: Since the publication of three cases of probable transmission to British patients of the variant of Creutzfeldt-Jacob Disease (vCJD) by blood transfusion, it is likely that infectious prion protein (PrPsc) is present in human blood. Studies on rodent indicate that the estimated sensitivity level of assays needs to reach a minimum of 1 femtomolar (0.1pg/ml or 10IU/ml) in order to detect PrPsc in the blood of patients in the pre-clinical phase of the disease. No test actually available does reach this level of detection and the development of highly sensitive assay for detection of PrPsc in blood is essential to evaluate secondary transmission of the disease by blood transfusion.

Aim: We have chosen to amplify the PrPsc by saPMCA (serial automated Protein Misfolding Cyclic Amplification) prior to the detection of very low levels of infectious prion present in the blood.

Objective: to develop a screening test for PrPsc in human blood components. The first phase consists in the manual reproduction and optimization of the PMCA technology described by C. Soto on hamster brain, in order to adapt the method on an automated sonicator (Misonix S-3000), allowing the detection of PrPsc in blood.

Methods: PMCA allows accelerated production of infectious prions by successive incubation and sonication steps. During incubation, aggregates of PrPsc proteins are produced from low quantity of PrPsc. This template initiates transconformation of the normal prion protein (PrPc) into PrPsc. Sonication of the aggregates leads to numerous small amplification units, each one allowing new conversions. Amplified PrPsc is then detected by Western Blot.

Results: After optimizations, manual PMCA prior to detection by Western blot permits to amplify by 3log10 folds the initial input of PrPsc present in hamster brain or in leukocytes. The saPMCA has then been optimized for high-efficiency amplification of PrPsc. The performances of the Misonix S-3000 have been evaluated on hamster brain which has confirmed the high throughput of the assay. The saPMCA method is reproducible and specific. Adaptation of this technology for PrPsc detection in blood is in progress.

Conclusion: The saPMCA prior to detection by Western blot appears to be a powerful approach for early diagnosis of prion diseases. The development of this technology as a non invasive screening test for PrPsc in human blood donation will allow to evaluate the potential spread of the prion by blood transfusion.

P-092
REMOVAL OF THE ENDOGENOUS TSE INFECTIVITY PRESENT IN BLOOD USING PRDT TSE AFFINITY LIGANDS AND INTEGRATION OF THE LIGANDS INTO THE MACOPHARMA P-CAPT(TM) FILTER

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Background: To date, there have been three transfusion transmitted cases of vCJD among 19 recipients that are known to have received blood from persons that later died of vCJD and lived long enough themselves to have developed detectable disease. This is a transmission rate of ~15%. If any of the other 16 recipients were infected it will be even higher (www.cjd.ed.ac.uk/TMER). A survey of surgically removed tissues concluded that the number of incubating cases of vCJD in the UK was at least 4000 (Hilton et al. 2004, J. Pathol. 203:733-9). 7% of this group would on average be blood donors.

Aim: To develop ligands with strong affinity for the prion protein and TSE infectivity and incorporate them into devices to remove TSE infectivity from RBCs and other blood components to reduce the risk of transmission of TSE infections from blood products.

Methods: Pathogen Removal and Diagnostics Technologies, PRDT, screened millions of compounds to obtain high affinity ligands that strongly bind brain-derived prion protein and TSE infectivity in the presence of whole blood, RBC or plasma. To test for removal of the endogenous TSE infectivity that is present in blood, ~500 mls of blood was collected in < 4 hours from scrapie infected hamsters, leukoreduced by a commercial filter, and passed through the test resin. Aliquots of whole blood, leukoreduced blood and resin flow-throughs were titered by the limiting dilution method. A total of 5 mls of each sample was inoculated in 50 □l aliquots into 100 hamsters. The experiment was terminated at 540 days when hamsters begin to die of natural causes and the brain of every animal was checked for evidence of prion amyloid.

Results: The best resins reduced the titer of a brain-derived spike into RBC of 10(7) ID/ml by over 4 log(10)ID(50), far exceeding the capacity needed for the very low concentrations of infectivity associated with blood or RBC. To address the uncertainty about the relevance of brain-derived spikes, the lead resin was also assessed for its ability to remove the infectivity endogenously present in blood. There were no infections among the 100 hamsters inoculated with the resin treated blood. The resin removed > 1.2 log(10)ID of relevant TSE blood infectivity. This resin has also passed a broad range of hemocompatibility tests. It has now been incorporated by MacoPharma into the P-Capt™ filter, a stand alone device for removing TSE infectivity from leukoreduced red blood cells.

Summary: PRDT's lead resin adsorbs high concentrations (10(7) ID50/ml) of brain derived TSE infectivity with high efficiency (4 log(10)ID50) even in the presence of RBC, and removes the low concentrations (10 ID/ml) of endogenous infectivity that is present in leukoreduced blood to the limit of detection (>1.2 log(10)ID). Implemented as the Macopharma P-Capt(TM) filter it should significantly reduce the risk of transmission of vCJD by blood and blood products.

B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料Eにおいて集積報告を行っているため、添付していない）。