医薬品 研究報告 調査報告書

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				2006. 8. 22	該当なし	

一般的名称	洗浄人赤血球浮遊液	研究報告の公表状況	AABB Weekly Report. 2006 Jul 21.	公表国 アイルランド
販売名(企業名)	洗浄赤血球「日赤」(日本赤十字社) 照射洗浄赤血球「日赤」(日本赤十字社)			

研究報告の概要

○アイルランド輸血サービスで、プリオン除去フィルターの治験中止
アイルランド輸血サービスは、クロイツフェルト・ヤコブ病(CJD)の病因となるプリオンを供血血液から除去する新しいフィルターを1年間使用した後、治験の中止を決定した。7月11日付Irish Examinerの記事によると、輸血サービスは昨年フィルターシステムを購入したが、十分な効果が得られず、CJDは検出されずに供血血液に混入し続けている疑いがあるとして、使用を中止した。

報告企業の意見	今後の対応
アイルランド輸血サービスが、プリオン除去フィルターの試験の中止を決定したとの報告である。	今後も引き続き、プリオン除去フィルターなど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

洗浄赤血球「日赤」
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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

According to a July 14 *Modern Healthcare Today* article, the Bush administration's nominee for deputy treasury secretary for tax policy has also agreed to revisit a longstanding IRS rule on community benefits, known as Revenue Ruling 69-545.

Science and Medicine

The first observational study to assess the progress of patients in the U.K. undergoing a controversial treatment for variant Creutzfeldt-Jakob disease (vCJD) has indicated that the drug may be beneficial in extending the life of sufferers. According to a July 12 *Guardian* article, Pentosan, or PPS, injected into the skull around the brain and administered during neurosurgery each month has resulted in prolonging the lives of patients of all ages. The treatment works by slowing the loss of brain tissue, but it does not halt it. Although eight patients in the U.K. have been given PPS, health officials do not officially sanction the drug, and some have expressed concern that it could cause the brain to hemorrhage.

Ian Bone, the Glasgow neurologist who led the study, noted that "the patients treated with PPS appear to have survived for unusually long periods." Bone cautioned, however, that "we cannot conclude with certainty that the treatment has a beneficial effect because it is impossible to make direct comparison with similar but untreated patients."

International

Europe's largest homosexual news organization, *Pink News*, recently reported that Russia may soon lift its ban on blood donations from homosexual donors. According to a July 14 article, the country's general prosecutor has responded to domestic campaigning and has stated that there is no law preventing homosexuals from donating blood. As a result of this inquiry, the general prosecutor has called on the Ministry of Health to rescind its guidance from 2001 that disallows the homosexual community from donating because of the belief that it was a group of high-risk individuals.

An audit of more than 8,000 transfusion episodes that took place at 217 U.K. hospitals reveals that health care personnel do not check the identity of bedside transfusion patients in 6 percent of all cases. Although the practice of confirming patients' identities is the single most important way to make sure they are given the correct type of blood, the Royal College of Physicians and the National Blood Service found that the practice was not happening as often as necessary. Even when this practice is observed, identifying bracelets do not always contain the necessary information. The audit noted that in 9 percent of wristbands worn, key data were missing. Reportedly, the main reason for not reconfirming the transfusion recipient's identity was that the patient was "well known" by nurses and physicians. The audit also revealed that 34 percent of patients were not monitored during the first 30 minutes following the transfusion, when it is critical to observe vital signs for indications of adverse events.

The implications of the findings are that more staff training is needed, especially to review the hazards and correct procedures for blood transfusion. The National Blood Transfusion Committee and the National Patient Safety Agency have also considered initiatives to overcome these shortcomings, such as improved use of automated systems and barcode patient identification.

After a year of study utilizing a new machine designed to clean the prions causing Creutzfeldt-Jakob disease (CJD) from donated blood, the Irish Blood Transfusion Service has decided to discontinue the trials. According to a July 11 *Irish Examiner* article, the transfusion service purchased the filter system last year but has halted its use due to concerns that it was not working sufficiently and that CJD could still pass undetected through blood donations.

The Irish Blood Transfusion Service also recently released its annual report for 2005, in which it revealed that blood donations increased 1.4 percent last year in tandem with increased need.

People

The lieutenant governor of Arkansas died last week after two bone marrow transplants failed to cure his unclassified myeloproliferative disorder. According to a July 16 *Reuters* story, Win Rockefeller was in the middle of a gubernatorial campaign when he was diagnosed with the blood disorder last July. He received treatment in both Washington and Arkansas, but died July 16 at the age of 57. The public servant had hoped to follow in the footsteps of his late father, who served as the governor of Arkansas from 1966 to 1970. The former governor died of cancer at the age of 60.

Upcoming Events

July

26 **Benchmarking**
AABB Audioconference
Contact: AABB Education Department
Tel: +1.301.215.6842
Fax: +1.301.215.6895
Email: education@aabb.org
Online: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audio_conf.htm

31 – August 2 **ASQ's 2006 Quality Institute for Healthcare Conference**
Houston, TX
Contact: ASQ
Tel: +1.414.298.8789 x7263
Email: fspano@asq.org
Online: www.asq.org

August

9 **Transfusion When Nothing is Compatible: Evaluating and Managing Risk**
AABB Audioconference
Contact: AABB Education Department
Tel: +1.301.215.6842
Fax: +1.301.215.6895
Email: education@aabb.org
Online: www.aabb.org/Content/Meetings_and_Events/Audioconferences/audio_conf.htm

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販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社)			カナダ

研究報告の概要

○細胞プリオン蛋白は活性血小板のエキソソーム上に放出されている

細胞プリオン蛋白(PrP^C)は、未知の機能を持つGPIアンカー型蛋白である。血球成分を含め体内の多くの組織に見られ、このうちヒトでは血小板で最も多く検出される。ミスフォールドされたプロテアーゼ耐性型PrP^CであるPrP^{Sc}が、伝達性海綿状脳症(TSE)グループの致死性神経変性疾患の病因であると広く信じられてきた。TSEの病因については完全にはわかっていないものの、疾患の進行にはPrP^Cが必要であることが知られている。故にPrP^Cの生理学的機能を明らかにすることが重要である。血中のPrP^Cの位置を解明することは、PrP^Cの機能に関して有益な手がかりを提供することになるだろう。PrP^Cは、以前は静止血小板のα顆粒膜上に見られた。最近の研究から、血小板の活性化が血小板表面上のPrP^Cの一時的発現とそれに続く微小胞およびエキソソームへの放出につながることが明らかになった。血小板由来エキソソーム上のPrP^Cの存在は、血中のPrP^Cの運搬と細胞間伝播のメカニズムの可能性を示唆する。

使用上の注意記載状況・その他参考事項等

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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

報告企業の意見	今後の対応
PrP^Cは、静止血小板のα顆粒膜上に見られたが、血小板の活性化が血小板表面上のPrP^Cの一時的発現とそれに続く微小胞およびエキソソームへの放出につながることが明らかになったとの報告である。	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。

Cellular prion protein is released on exosomes from activated platelets

Catherine Robertson, Stephanie A. Booth, Daniel R. Beniac, Michael B. Coulthart, Timothy F. Booth, and Archibald McNicol

Cellular prion protein (PrP^C) is a glycophosphatidylinositol (GPI)–anchored protein, of unknown function, found in a number of tissues throughout the body, including several blood components of which platelets constitute the largest reservoir in humans. It is widely believed that a misfolded, protease-resistant form of PrP^C, PrP^{Sc}, is responsible for the transmissible spongiform encephalopathy (TSE) group of fatal neurodegenerative diseases. Although the pathogenesis of TSEs is poorly understood, it is known that PrP^C must be present in order for the disease to progress; thus, it is important to determine the physiologic function of PrP^C. Resolving the location of PrP^C in blood will provide valuable clues as to its function. PrP^C was previously shown to be on the alpha granule membrane of resting platelets. In the current study platelet activation led to the transient expression of PrP^C on the platelet surface and its subsequent release on both microvesicles and exosomes. The presence of PrP^C on platelet-derived exosomes suggests a possible mechanism for PrP^C transport in blood and for cell-to-cell transmission. (Blood. 2006;107: 3907-3911)

Introduction

Cellular prion protein (PrP^C) is a membrane-bound, glycophosphatidylinositol (GPI)–anchored protein[1] found primarily in lipid rafts on the cell membrane of neuronal and non-neuronal cells, including tonsils, spleen, and of the secretory granules of epithelial cells in the stomach, as well as in cultured cell lines.[2,3] Although PrP^C has been shown to be present on the surface of a number of peripheral blood cells,[4] the relative levels on individual cell types have been contentious. Individual studies have reported that the majority of PrP^C is associated with both platelets[5,6] and red blood cells.[7] In the former case the surface expression of PrP^C is increased following stimulation, suggesting an additional internal membrane source of the protein,[8] recently shown to be alpha granule membranes.[9] Furthermore, platelet activation is associated with the accumulation of PrP^C in releasates,[10] and in platelet concentrates, stored for up to 10 days, there is an increase in initially the microsomal, then plasma levels of PrP^C.[11]

Transmissible spongiform encephalopathies (TSEs) are a family of neurodegenerative disorders, including Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Schienker syndrome, and fatal familial insomnia in humans; scrapie in sheep; and bovine spongiform encephalopathy (BSE) in cattle.[12,13] They are all characterized by the accumulation of a protease-resistant isomer (PrP^{Sc}) of PrP^C in the brain of affected individuals. It is generally considered that PrP^{Sc} acts as a template inducing the same structural changes within other normally folded PrP^C molecules on contact, thus propagating the misfolded state of the protein.[14]

The CNS is the site at which TSE pathology is apparent in prion infections; however, the agent must first replicate and be transported to the CNS after peripheral infection. The spread of PrP^{Sc} has been tracked from the gastrointestinal tract and the spleen to the CNS.[15] The lymphoreticular system (LRS) is believed to be an important site of prion replication, and an accumulation of PrP^{Sc} is apparent in spleen and lymph nodes after peripheral infection. Indeed, neuroinvasion is delayed without a functional LRS.[16]

A new variant of CJD (vCJD) identified in humans in the United Kingdom is almost certainly the result of infection with the BSE agent.[17] Patients infected with vCJD, in contrast to those with classic CJD, have been shown to have widespread deposition of PrP^{Sc} in the LRS.[18,19] Immune cells, in particular B cells and follicular dendritic cells, have been identified as harboring infectious PrP^{Sc} in the LRS. However PrP^{Sc}, the standard biochemical marker used for diagnosis of TSEs, cannot be detected by current technology in circulating lymphocytes or whole blood. Bioassays are a more sensitive assay for infectivity, and a number of studies have demonstrated that the infectious agent is present in blood and blood components, buffy coats, plasma, and platelets in animal models.[20-23] The removal of all white cells by standard leukoreduction reduced infectivity by only 42%, suggesting that other blood components carry PrP^{Sc}.[24] There is therefore a significant concern that blood transfusions may represent a portal for the transmission of TSEs. Indeed since 2004, 3 apparent cases of transmission of vCJD by transfusion have been reported in the United Kingdom.[25,26]

To understand the transmission of the disease by PrP^{Sc}, it is important to determine the physiologic behavior and function of normal PrP^C in blood cells and plasma. In the current study the cellular localization, and stimulus-induced redistribution, of PrP^C in platelets has been examined.

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Materials and methods

The protocols of this study were approved by the Human Research Ethics Board of the University of Manitoba.

Antibodies and reagents

Monoclonal antibody (Ab) 308, raised against amino acids 106 to 126 of human PrP^C, was purchased from Cayman Chemical, Ann Arbor, MI, and the polyclonal Ab FL253, raised against the full-length PrP^C, was purchased from Santa Cruz Biotechnology, Santa Cruz, CA. Anti-CD62P Ab (D541) was a generous gift from Dr Sara Israels, Manitoba Institute of Cell Biology, Winnipeg, MB.

Polyclonal Ab to human fibrinogen was purchased from Calbiochem-Novabiochem, San Diego, CA. Horseradish peroxidase– and FITC-conjugated Abs were purchased from DakoCytomation, Mississauga, ON. Secondary Abs conjugated to gold, along with bovine thrombin, protease inhibitors, and all other reagents were purchased from Sigma-Aldrich Canada, Oakville, ON, and were of the highest grade available.

Preparation of washed platelets

Blood was collected following informed consent, by venipuncture of human volunteers who had denied taking medication known to interfere with platelet function within the previous 2 weeks. The blood was drawn into syringes containing acid citrate dextrose anticoagulant (ACD; 3.8 mM citric acid,.7.5 mM trisodium citrate, 125 mM dextrose; 1.8 mL anticoagulant/8.1 mL whole blood). Washed platelets were obtained as previously described.[27,28]

Flow cytometry

Plasma-free platelet suspensions were prepared and incubated with agonist, or saline control, for the times indicated. The samples were fixed by the addition of an equal amount of 4% paraformaldehyde, then incubated with anti-PrP^C Ab 308 diluted in PBS/0.1% BSA. Following washing 3 times in PBS/0.1% BSA, the samples were incubated in a FITC-conjugated secondary Ab. The samples were finally washed 3 times in PBS and resuspended in PBS. Flow cytometry was carried out on a Becton Dickinson (Mississauga, ON, Canada) FacsCalibur flow cytometer with forward and side scatter set to a logarithmic scale.

An area was drawn around the unstimulated platelet sample according to the forward and side scatter properties and labeled platelet region (P). A second region was drawn to gate on smaller particles and labeled microvesicle (MVS) region.[29] Fluorescence backgating was used to determine the number of PrP^C-positive events in each region.

Microvesicle and exosome preparation

Microvesicles and exosomes were prepared according to the method of Heijnen et al.[29] Briefly, plasma-free platelet suspensions were prepared and incubated with agonist or saline control for the times indicated, and the reaction was stopped by the addition of an equal amount of ice-cold ACD. Samples were centrifuged (Beckman GH-3 swing bucket rotor [Beckman Coulter Canada, Mississauga, ON, Canada]; 800*g*; 15 minutes) to obtain the platelet pellet fraction (P). The supernatant was further centrifuged (Beckman F3602 fixed angle rotor [Beckman Coulter Canada, Mississauga, ON, Canada]; 10 000*g*; 30 minutes) to obtain the microvesicle fraction (MV), and the supernatant from the MV fraction was centrifuged (Beckman MLS-50 swing bucket rotor; 100 000*g*; 60 minutes) to obtain the exosome pellet (EX). All pellets were resuspended in PBS.

To ensure exosome isolation, the supernatants from MV preparations were mixed with 2 M sucrose, and a 0.8 to 0.25 M sucrose gradient was layered on top. The gradient was centrifuged (Beckman MLS-50 swing bucket rotor; 65 000*g*; 16 hours) and 500-μL fractions were collected. The fractions were diluted in 4.5 mL PBS and centrifuged (100 000*g*; 60 minutes; 4°C), and the resultant pellets were resuspended in PBS.

In some studies, fractions from the sucrose gradient were vacuum-transferred onto a nitrocellulose membrane using a Schleicher and Schuell (Dassel, Germany) slot blot apparatus and immunoblotted with anti-PrP^C Ab. Briefly, following blocking in 5% nonfat milk, the membrane was incubated in Ab 308, washed in TBS with 0.01% Tween 20 (TBS-T) followed by incubation in a horseradish peroxidase–conjugated secondary Ab. After washing in TBS-T 6 times, the membrane was incubated in Super Signal West Pico chemiluminescence substrate (Pierce Biotechnology, Rockford, IL), and protein bands were visualized on a Bio-Rad Fluoro S Max imaging system. Densitometry was performed using Quantity One software (Bio-Rad, Mississauga, ON).

Electron microscopy and immunocytochemistry

Exosomes for double immunolabeling studies were prepared from the sucrose gradient fractions. Briefly, the exosomes were resuspended in PBS and adsorbed onto 300 mesh carbon-coated formvar nickel grids for 10 minutes. Excess PBS was blotted with filter paper, and the grids were fixed with 2% paraformaldehyde in PBS for 10 minutes. After fixation, the grids were incubated in PBS with 1% BSA for 15 minutes, incubated with the anti-PrP^C Ab for 30 minutes, washed 3 times in PBS with 0.1% BSA, and incubated in a secondary Ab conjugated to 5 nm gold for 30 minutes. The grids were rewashed 3 times in PBS, followed by 3 changes of PBS with 1% BSA, incubated in the anti-CD62 Ab for 30 minutes, washed (3 × 5 minute) with PBS with 0.1% BSA, and incubated in a secondary Ab conjugated to 10 nm gold for 30 minutes. Finally, the grids were washed (3 × 5 minutes) with PBS, followed by deionized water (3 × 5 minutes), embedded in uranyl acetate methyl cellulose. All specimens were examined in a Tecnai 20 transmission electron microscope (FEI Systems, Toronto, ON, Canada) operating at 200 kV at magnifications ranging from 25 500 × to 135 000 ×. Digital images (1024 × 1024-pixel) were acquired from the TEM via an AMT Advantage XR-12 TEM camera (AMT, Danvers, MA). Digital images were arranged in final figure format using the Canvas software package (ACD Systems of America, Miami, FL).

Frozen sections of platelets were prepared according to a method by Tokuyasu,[30] as previously described.[31] Cryo sections were cut with an Ultracut UCT ultramicrotome (Leica Microsystems Canada, Richmondhill, ON), equipped with an electron microscope (EM) FCS-sectioning chamber. Immunolabeling was carried out as described for exosome preparations with gold-conjugated protein G replacing the secondary Ab. Following gold labeling, the sections were stained and embedded in a uranyl acetate/methyl cellulose mixture.[30,31]

Results

PrP^C is present on platelet alpha granule, but not dense granule, membranes

Frozen sections of quiescent human platelets showed the characteristic platelet intracellular architecture (Figure 1A). Immunoelectron microscopy studies of these platelets using an anti-PrP^C Ab, FL253, followed by gold-conjugated Protein G, confirmed the association of PrP^C with intracellular granules (Figure 1B-C) and the membranes of the open canalicular system (Figure 1C). A polyclonal Ab to fibrinogen was used to identify alpha granules (Figure 1D), and double staining of these sections using Abs to PrP^C and fibrinogen was consistent with both proteins localizing to the same organelle (Figure 1E-F).

PrP^C translocates to and is released from the platelet surface following activation

Lysates from thrombin-stimulated platelets (1 U/mL; 0-120 seconds) were immunoblotted with anti-PrP^C Ab 308. There was a decrease in PrP^C levels associated with the platelet pellet over time (Figure 2A). Although there was significant donor variability in the time course of the loss of PrP^C from the pellet, densitometric analysis indicated that by 120 seconds of stimulation the intensity

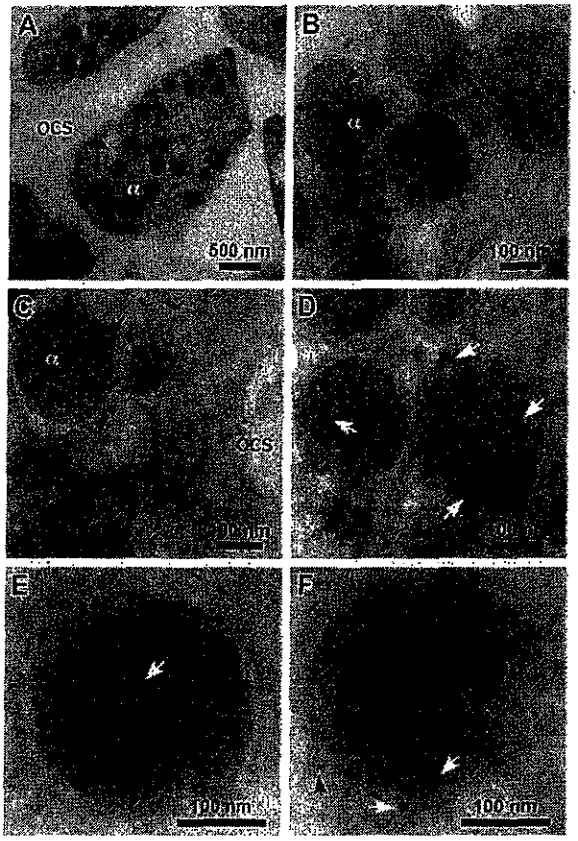

Figure 1. Immunoelectron microscopy of resting platelets. Frozen sections of resting platelets were incubated with Abs to PrP^C (FL253) and fibrinogen, followed by incubation in protein G conjugated to 5 or 10 nm gold. Normal resting platelets showing alpha granule (α) and open canalicular system (OCS) (A). PrP^C (10 nm gold, arrows) is seen in alpha granule and the open canalicular system (B-C). Alpha granules are identified by the presence of fibrinogen (5 nm gold, arrows; D). Double labeling with Abs to PrP^C (5 nm gold, black arrows) and fibrinogen (10 nm gold, white arrows) localized both proteins to the same granules (E-F).

of the PrP^C band from the platelet pellet had diminished to about 50% of control (Figure 2B). This decrease in platelet-associated PrP^C from activated platelets was accompanied by a corresponding accumulation of PrP^C in the releasate (Figure 2A-B).

PrP^C is present on platelet microvesicles

Flow cytometry, using anti-PrP^C Ab 308, demonstrated the presence of PrP^C on the surface of unstimulated platelets (Figure 3). Following thrombin stimulation (1 U/mL) there was a transient increase in the expression of PrP^C on the platelet surface, followed by its release into the supernatant. There was a corresponding shedding of microvesicles from the platelet surface in response to thrombin; however, the level of PrP^C on the surface of the microvesicles was low (< 10% of total released PrP^C) and remained constant with time (Figure 3). Platelet activation with A23187 (20 μM) was accompanied by increased levels of microvesicle production when compared with thrombin; however, the level of PrP^C associated with the microvesicles remained low (data not shown).

Immunogold labeling demonstrated the presence of PrP^C on the surface of unstimulated platelets (Figure 4A). Following activation with thrombin, PrP^C was observed around the periphery of the platelet and at the tips of pseudopods (Figure 4B-C). In addition, PrP^C was associated with small (< 100 nm) membranous vesicles released from the platelets (Figure 4D-E).

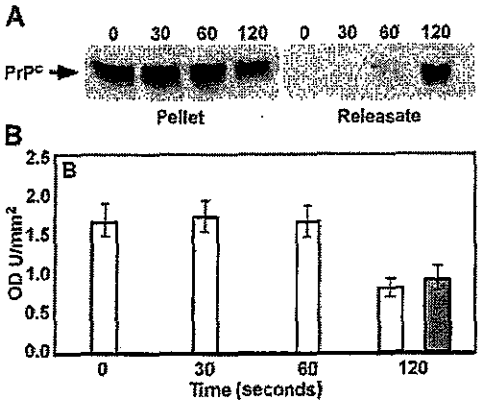

Figure 2. Immunoblotting of pellets and releasates in activated platelets. Platelets were incubated with 1 U/mL thrombin for 0, 30, 60, or 120 seconds. Following termination, platelet pellet and releasates were prepared, separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and immunoblotted with anti-PrP^C Ab 308 (A), and densitometry was performed (B) on pellet (□) and releasates (▨) (n = 3). Error bars indicate standard error of the mean.

PrP^C is present on platelet exosomes

Previous studies have shown that, in addition to microvesicles, thrombin stimulates the release of exosomes from platelets.[29] Given the relatively low levels of PrP^C on the surface of released microvesicles (Figure 3) and its presence on the surface of smaller membrane fractions (Figure 4D-E), the possible association of PrP^C with exosomes was examined.

Exosomes were prepared by differential centrifugation, and separation through a sucrose gradient, of the releasate of thrombin-stimulated platelets. Immunoblotting using anti-PrP^C Ab 308 was consistent with the presence of PrP^C in these exosome fractions (Figure 5A).

Immunoelectron microscopy of fractions 3 and 4 using anti-PrP^C Abs (308 or FL253) demonstrated the presence of PrP^C on vesicles ranging from 40 to 100 nm (Figure 5B), the size being consistent with previous reports of platelet-derived exosomes.[29] Double-labeling these fractions with anti-CD62 Ab D541 confirmed that these exosomes were derived from alpha granules (Figure 5C).

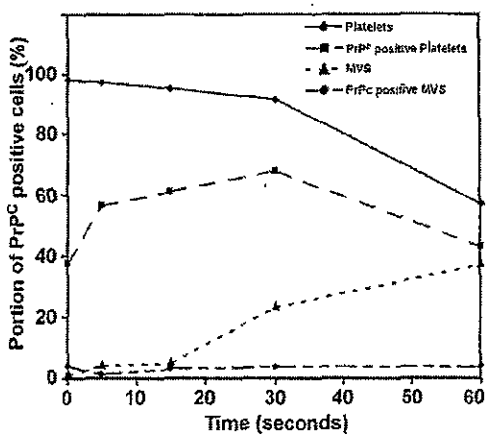

Figure 3. Flow cytometry of activated platelets. Platelets were incubated with 1 U/mL thrombin for 0, 5, 15, 30, or 60 seconds and labeled with anti-PrP^C Ab 308. Flow cytometry was used to distinguish platelets from microvesicles on the basis of their forward-scatter (FSC-H) and side-scatter (SSC-H) profiles (platelets, solid line ◆, microvesicles, broken line ▲). Fluorescence backgating determined the percentage of PrP^C-positive cells in each region. (Percentage of platelet region positive for PrP^C, ■; percentage of microvesicle region positive for PrP^C, ●).