Conference report:

The 50th Annual Meeting of American Society of Hematology

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Place: San Francisco, CA, USA

Attendance: approximately 24,000 people

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Synopsis

This year, the American Society of Hematology (ASH) celebrated its 50th anniversary. The goal of ASH is to prevent and cure hematological diseases through the advancement and sponsorship of basic and clinical research. This year’s ASH meeting addressed important research topics including hematologic malignancies, bone marrow transplantation, sickle cell diseases, hematopoietic stem cells, thrombosis, hemostasis, and vascular biology. This report will focus on 1) the discovery of novel anti-coagulant agents, 2) the role of cell-surface molecules in vascular diseases such as sickle cell disease and inflammation, 3) the role of endothelial cells, platelets, and leukocytes in cancer, and 4) the platelet
signaling pathway regulated by integrins and other cell adhesion molecules. ([http://www.hematology.org](http://www.hematology.org)).

**Keywords:** vascular disease, thrombosis and hemostasis, inflammation, sickle cell disease, cancer

**I. Satellite Symposium:** There were several Satellite Symposiums preceding the annual meeting. One such symposium, “New Perspectives and Strategies in Venous Thromboembolic Disease” illustrated the weaknesses of currently available anticoagulants, described molecular mechanisms of factor Xa and thrombin inhibitors and their merits, and showed data from clinical trials of novel anticoagulants under development for effective prevention of venous thromboembolism. This has been a popular topic for ASH and other thrombosis conference including ISTH (International Society on Thrombosis and Haemostasis). New results regarding novel inhibitors of factor Xa and thrombin were also discussed. Dr. Charles T. Esmon (HHMI, Oklahoma Medical Research Foundation) explained the role of factor Xa and thrombin in the blood coagulation cascade. After vascular injury, tissue factor (a major initiator for blood coagulation) binds to factor VIIa. This complex activates factor X to factor Xa, thereby producing thrombin. Since factor Xa and thrombin are essential for fibrin formation, both molecules could be an attractive target for novel anti-coagulants. The major role of factor Xa is limited to thrombin generation, whereas thrombin regulates many responses including anticoagulation (through protein C activation and prostacyclin production), procoagulation (by feedback activation of coagulation factors), inflammation (by intravascular cell activation), and cellular proliferation (by PDGF and TGF production). Therefore, he mentioned that factor Xa inhibitors would inhibit blood coagulation specifically. In contrast, thrombin inhibitors might result in unwanted effects. More importantly, factor Xa activates blood clotting over a wider concentration range than thrombin. These results suggest that factor Xa inhibitors may have increased potency with lesser bleeding effects than thrombin inhibitors and could be safer.
The next speaker, Dr. Jeffery Weitz (McMaster University) has advocated the use of an orally-available direct thrombin inhibitor. He discussed RE-VOLUTION, a Phase II clinical trial of Dabigatran Etexilate, an orally-available direct thrombin inhibitor (Boehringer Ingelheim). This study has been performed in over 38,000 patients with primary, acute and secondary venous thromboembolism. Dabigatran at 110-220 mg (once a day, po) for 8, 13 and 33 days was comparable with Enoxaparin (low-molecular-weight heparin) at 40 mg (once a day, sc) for the same periods in terms of anti-coagulant efficacy and safety. Despite of its low oral bioavailability (6%), Dabigatran is currently marketed in Europe and Canada for thrombophylaxis after hip or knee replacement surgery. Dr. Weitz anticipated that potent thrombin inhibitors such as Dabigatran Etexilate could replace warfarin in the oral treatment of thrombotic diseases.

Dr. Alexander G.G. Turpie argued against Dr. Weitz’s talk. He claimed that direct factor Xa inhibitors have a safer therapeutic range than direct thrombin inhibitors. He showed new results from APPRAISE and ADVANCE (Phase III clinical trials of Apixaban, an orally-available direct factor Xa inhibitor, Bristol-Myers-Squibb). In the ADVANCE-1 (Efficacy of Apixaban for prevention of venous thromboembolism after knee replacement), Apixaban at 2.5 mg (twice a day, po) had an anti-coagulant activity similar to Enoxaparin at 30 mg (twice a day, sc). However, major bleeding of Apixaban was less severe than that of Enoxaparin. Another example is Rivaroxaban (an orally-available direct factor Xa inhibitor, Bayer HealthCare AG). The results of Phase II and III clinical trials of this drug (RECORD) were reported in the 49th Annual Meeting of ASH last year (in Atlanta, GA). This year, he reviewed the results from RECORD 1-4 in which the efficacy of Rivaroxaban at 10 mg (once a day, po) was compared to that of Enoxaparin at 30-40 mg (once a day or twice a day, sc) in the prevention of venous thromboembolism in arthroplasty and replacement of hip or knee. Rivaroxaban had similar or better antithrombotic effects and was safer with less bleeding than Enoxaparin. Further, Rivaroxaban is also in
Phase II and III (TIMI) trials for acute coronary syndrome, and the results are expected in mid 2009. In the Phase II study, Rivaroxaban slightly decreased the percentage of death, myocardial infarction, and stroke compared to placebo. The efficacy of Rivaroxaban in arterial thrombotic diseases is being tested in Phase III clinical trials. In conclusion, Dr. Turpie suggested that factor Xa inhibitors could be a safer anti-coagulant than thrombin inhibitors.

* The content of this Symposium was overlapped partly with the session of Thrombosis in Education Program.

**Ⅱ. Scientific Program**

**Ⅱ.1. Scientific Committee on Thrombosis and Vascular Biology and on Hemostasis:** All presentations in the Scientific Committee sessions were given twice to allow audiences to attend as many sessions as possible. I will articulate two sessions in detail: 1) Scientific Committee on Thrombosis and Vascular Biology (Crosstalk Between Vascular Inflammation and Thrombosis) and 2) Scientific Committee on Hemostasis (Hemostasis and Cancer).

The laboratory of Dr. Paul S. Frenette (Mount Sinai School of Medicine) has been working on the pathophysiology of sickle cell disease (SCD). Patients with SCD have classical “sickle-shaped” and various misshaped erythrocytes in blood. A single missense mutation substitutes glutamine to valine at the sixth residue of the beta-globin polypeptide. This mutation alters erythrocyte membrane and promotes its adherence, thereby causing the morbidity and mortality by painful vaso-occlusive episodes. In his talk, he explained the molecular mechanism of erythrocyte vaso-occlusion in SCD using multi-channel fluorescence intravital microscopy with pharmacologic and genetic approaches. Sickle
erythocytes interact specifically with adherent neutrophils. Most adherent neutrophils crawl along the inflamed venules, and endothelial cell E- and P-selectins are key molecules in vaso-occlusion in SCD. Recent work from his group (Hidalgo et al. Immunity 2007;26:477-89) has demonstrated that E-selectin-mediated signaling in adherent leukocytes plays a critical role in interaction of erythrocytes with adherent neutrophils. Further, they found that E-selectin ligand-1 mediates E-selectin-induced signaling and that alphaMbeta2 (Mac-1) mediates erythrocyte capture on adherent leukocytes. In sum, leukocytes adhere to E-selectin on the inflamed endothelial cells through leukocyte E-selectin ligand-1, which activates alphaMbeta2 regionally at the leading edge and allows erythrocyte adhesion to adherent leukocytes. These results give novel insight into how vaso-occlusion occurs in SCD.

Dr. Andy Weyrich (University of Utah) has been studying the machinery to produce new proteins in platelets. It was believed that platelets lack mRNA and translational factors and do not synthesize proteins. However, his work has demonstrated that platelets possess translational machinery consisting of ribosomes, mRNA, and translational factors and do synthesize proteins. In my opinion, these findings are still controversial because it is extremely hard to obtain pure platelets from blood without contamination from leukocytes. Therefore, some people still suspect that his observation is derived from contamination. This year, he showed that circulating platelets are divided into new platelets. Platelets are differentiated from megakaryocytes through proplatelets. Real-time microscopic images showed that platelets form cell bodies and generate more platelets in a platelet activation-independent manner. He also found that platelets cultured in serum-free media increase the mass and number. However, someone asked whether cultured platelets behave like circulating platelets in blood.

In the Scientific Committee on Hemostasis (Hemostasis and Cancer), Dr. Ajit Varki’s talk (University of California-San Diego) covered the role of cell-surface glycans (oligosaccharides and polysaccharides) in cancer cell metastasis. There are various classes of cell-surface glycans including N- and O-linked...
glycans, glycosphingolipids, and glycosaminoglycans. Specifically, he discussed one type of glycan consisting of sialic acid and fucose (sialyl-Lewis X). Sialic acids are a family of sugars with a shared nine-carbon backbone and are found at the terminal position of cell-surface molecules such as P-selectin glycoprotein ligand-1 (PSGL-1). The most prevalent sialic acid-binding proteins include the selectins (P-, L-, and E-selectins), which are important for cell-cell interactions in cancer, hemostasis, and inflammation. All three selectins may recognize sialo-fucosylated glycoconjugates, especially when the fucose residue near sialic acid contains alpha 1,3-glycosidic bond. Interaction of platelet and endothelial cell selectins with sialo-fucosylated moieties on PSGL-1 is believed to mediate an early step of cancer cell metastasis. Heparin contains a mixture of highly sulfated glycosaminoglycan chains. He explained that the effect of heparin on cancer cell metastasis results not from inhibition of blood coagulation but from inhibition of P- and L-selectin binding to sialo-fucosylated selectin ligands.

The next speaker, Dr. Jerry Ware (University of Arkansas) gave a presentation about how platelets contribute to malignancy. Platelets interact with certain tumor emboli, which prolongs tumor cell survival. He mentioned that this is because platelets protect tumor cells from natural killer cell-mediated death. Platelet glycoprotein Ibalpha (GPIbalpha) plays a critical role in platelet adhesion and accumulation by the interaction with von Willebrand factor (vWF) under high shear conditions. However, the role of GPIbalpha in tumorigenesis is unknown. His laboratory has generated congenic mice with dysfunctional GPIbalpha/IX receptors (a mouse model of Bernard-Soulier Syndrome). In a FeCl2-induced thrombosis model in mice, GPIbalpha-null mice prolong time to vessel occlusion significantly compared to wild-type mice, indicating a crucial role of GPIbalpha in thrombus formation. In addition, he reported that the absence of platelet GPIb/IX receptors results in a 15-fold reduction in the number of lung metastasis. Using mice lacking the extracellular domain of the alpha-subunit of GPIb, the extracellular region of GPIbalpha/IX complex is essential for the contribution of the receptor to
metastasis. These observations provide important evidence that circulating platelets regulate tumor metastasis through GPIbalpha.

Dr. Joseph S. Palumbo (Children’s Hospital research Foundation, University of Cincinnati) introduced his work on the role of tissue factor (TF) and factor XIII transglutaminase in tumor cell metastasis (His work was performed in collaboration with Dr. Jay L. Degen at the same institution). He generated mouse tumor cells expressing TF, TF lacking the cytoplasmic tail domain, or empty vector and injected into the dorsal subcutis of wild-type mice. He found that TF is crucial for tumor metastasis but not for tumor growth and angiogenesis. Tumor metastasis was not inhibited by injection of tumor cells expressing TF lacking the cytoplasmic domain, indicating that the cytoplasmic region of TF is not required for metastasis. Using mice lacking prothrombin, platelet function, fibrinogen, or natural killer (NK) cells, he demonstrated that metastasis of TF-expressing tumor cells depends on hemostatic system components. These results suggest that thrombin is a key molecule for coupling TF to metastasis. Consistent with these observations, mice lacking a platelet thrombin receptor, protease-activated receptor 4 (G protein-coupled receptor) did not support tumor metastasis, and Galphaq/- mice showed no platelet function and no tumor cell metastasis. Additionally, he showed that coagulation factor XIII transglutaminase, a thrombin substrate, stabilizes fibrin clots through covalent crosslinking. Mice lacking the catalytic factor XIII-A subunit exhibit significantly decreased tumor cell metastasis. He explained that factor XIII supports metastasis, presumably by impairing NK cell-mediated clearance of tumor cells. In sum, the platelet-fibrinogen axis supports spontaneous metastasis

### III. Oral Simultaneous Session

#### III.1. Platelet Activation: The laboratory of Dr. Ulhas P. Naik (University of Delaware) has been studying the role of platelet junction adhesion molecule-A (JAM-A) in integrin signaling. JAM-A-null
mice exhibit fast clot formation and enhanced platelet aggregation, suggesting that JAM-A plays a negative role in platelet function. He mentioned that in resting platelets, JAM-A is phosphorylated at a Tyr residue and is associated with platelet alphaIIbbeta3 integrin. However, interaction of JAM-A with alphaIIbbeta3 integrin is disrupted upon platelet activation, which results in activation of alphaIIbbeta3 integrin. In contrast, JAM-A is dephosphorylated at the Tyr residue but phosphorylated at a Ser residue in activated platelets. His work provides important evidence that the phosphorylation and dephosphorylation of JAM-A at Tyr and Ser residues regulate alphaIIbbeta3 integrin-mediated inside-out signaling.

The presence of platelet TF is controversial. As I summarized in Dr. Andy Weyrich’s talk, his work demonstrated the presence of mRNA in platelets, and in particular of mRNA encoding TF in activated platelets. However, his group used an unusual activation condition to detect platelet TF (Schwertz et al. J Exp Med 2006;203:2433-40). This year, Dr. Diego Mezzano (Pontificia Universidad Católica de Chile, Chile) presented that human platelets synthesize and express functional TF. Little TF mRNA was detected in resting platelets, whereas TF mRNA levels increase upon platelet activation. Using immunoprecipitation and immunofluorescence microscopy, he showed that activated platelets synthesize TF and platelet surface TF expression is increased in response to agonists. Puromycin treatment inhibited TF synthesis, suggesting that platelets synthesize TF by a de novo pathway. Interestingly, he found using confocal microscopy that TF colocalizes with GPIbalphalpha on the platelet surface. He mentioned that interaction of platelet GPIbalphalpha with vWF results in TF externalization through Src kinase activation. However, additional controls are needed to verify these results. My view is that the presence and regulation of platelet TF still remains an open question.

The laboratory of Dr. Barry Coller (Rockefeller University) has being working on the role of platelet alphaIIbbeta3 integrin in thrombotic diseases for a long time. This year, one of his graduate students
gave a presentation about a novel inhibitor of human platelet alphaIIb integrin subunit, RUC-1 (Merit Award Talk). This compound binds to the GENU domain of human alphaIIb subunit, thereby inhibiting aggregation of human platelets but not mouse platelets. To test this compound, his group generated mice expressing human alphaIIb/mouse beta3 integrin and mouse alphaIIb/human beta3 integrin. Using the mice, he confirmed that RUC-1 inhibits FeCl3-induced vessel occlusion in mice expressing human alphaIIb/mouse beta3 integrin completely. However, whether RUC-1 prolongs bleeding time remains to be determined. Also, this compound partially perturbed the alphav subunit due to the sequence conservation (approximately 50%) between alphav and alphaIIb subunits. From that point of view, I expect that this compound would act like ReoPro in vivo (Fab fragments of a chimeric monoclonal antibody against platelet alphaIIbbeta3 integrin).

IV. Special Symposium on the Basic Science of Hemostasis and Thrombosis: Since 2006, ASH has held the Special Symposium for scientists working in the field of hemostasis, thrombosis, and vascular biology. This year, Dr. Paul Kubes (University of Calgary, Canada) gave a very interesting presentation about how platelets trap bacteria in sepsis. He showed beautiful images using intravital microscopy ex vivo and in vivo that neutrophils adherent to endothelial cells interact with platelets in the presence of lipopolysaccharide (LPS). Platelets express Toll-like receptor 4 (TLR4), a receptor for LPS. His work demonstrated that LPS induces a platelet-neutrophil interaction which results in the formation of a web-like network. Such interaction was perturbed by TLR4 inhibitors, suggesting a critical role for TLR4 in the interaction between platelets and neutrophils. This web-like structure consists of sticky DNA, proteases, and other anti-microbial molecules. Platelets trap circulating bacteria into the network. His work gives new insight into how platelets help immune cells kill bacteria.
Dr. Scott L. Diamond (U. Penn) presented his work on the role of blood flow in the initiation of thrombus formation. As I mentioned earlier, interaction of platelet GPIbalpha with vWF is important for the initiation of platelet thrombus formation after vascular injury under which endothelial cells are denuded. GPIbalpha-vWF interaction occurs under high shear stress. His group developed a novel technology to measure the contribution of platelets and TF to thrombus formation. He explained that 1 dyne/cm² is required for platelets to adhere to A1 domain of vWF. Using this approach, he demonstrated how much TF is required for thrombin generation and subsequent fibrin formation under various shear conditions.

The next speaker, Dr. Leslie V. Parise (UNC-Chapel Hill) has been studying on the role of platelets in inflammation and in the immune response. Her talk covered the importance of CD40L, an inflammatory mediator, for SCD (sickle cell diseases). Platelets contain high levels of CD40L. CD40L is expressed on the platelet surface upon activation and further released as a soluble form. Soluble CD40L binds to platelets through CD40 and alphaIIbbeta3 integrin. She mentioned that such interaction results in platelet activation and thrombus formation. Further, CD40L activates vascular endothelial cells, B-cell proliferation, and TF expression. Using a mouse model of SCD (Berkeley mice) and SCD patients’ blood, her group found that biologically active CD40L is released from platelets of SCD patients, thereby increasing the level of soluble CD40L to 30-fold in patients’ blood. Treatment with integrilin (a specific antagonist for platelet alphaIIbbeta3 integrin, Millennium Pharmaceuticals) prevented release of soluble CD40L from patients’ platelets, suggesting that platelet alphaIIbbeta3 integrin plays an important role in CD40L release. She also focused on how multicellular aggregates form in the blood of SCD patients. She explained that platelets interact with monocytes through the interaction of P-selectin with PSGL-1. Moreover, plasma fibronectin links monocytes to red blood cells through recognition of alpha4beta1 integrin expressed on either cell. Therefore, she concluded that soluble factors such as...
CD40L and cell surface molecules including CD40 and alphaIIbbeta3 integrin could be a target for the treatment of SCD.

V. Concluding Remarks: Since its founding in 1958, ASH has grown to be one of the largest scientific associations in the U.S. ASH has maintained its status as a cutting-edge scientific organization through active participation of members, training young scientists, linking basic and clinical research, and garnering support from the U.S. government. As noted in the 50th anniversary logo, ASH has facilitated the tremendous progress in hematology that has occurred over the last half century by dedicating itself to the understanding and eradication of various blood diseases. The topics outlined in this report reflect only a small part of topics that are of interest to ASH and do not cover such hot fields as myeloma and hematopoietic stem cell research. Therefore, I apologize to scientists who study other such topics outside the field of thrombosis and vascular biology. To this end, I hope that the Korean Society of Hematology continues to support prestigious scientific meetings by networking with and forging close relationships with international scientists.