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Germany

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CSL Behring, a world leader in the development of plasma-derived and recombinant therapeutics, recently announced the winners of the “CSL Behring – Prof. Heimburger Award 2011” at an annual symposium held in Marburg, Germany. Five young investigators were each awarded a grant of €20,000 to assist with their independent research projects, which were selected from a large number of applications by a panel of global experts.

The Prof. Heimburger Award recognizes and supports the work of young medical doctors with an interest in coagulation research. Set up in 2008 to commemorate the enduring legacy of Prof. Norbert Heimburger – a long-term employee of CSL Behring who pioneered the use of pasteurisation to produce virus-safe plasma products – the annual award helps to encourage research excellence and support the professional development of the next generation of coagulation disorder treatment specialists.

The distinguished panel of experts who reviewed the 2011 award applications were:

- Thomas Abshire, School of the Medicine, Emory University, Atlanta, Georgia, USA
- David Lillicrap, Department of Pathology and Molecular Medicine, Queen's University, Kingston, Ontario, Canada
- Claude Négrier, Regional Centre for the Treatment of Haemophilia, Edouard Herriot Hospital, Lyon, France
- Johannes Oldenburg, Institute of Experimental Haematology and Transfusion Medicine, University Clinic Bonn, Bonn, Germany.

For 2012, David Lillicrap and Johannes Oldenburg will step down from their role as committee members, and Patricia Casais from the Institute of Epidemiological Research, National Academy of Medicine in Buenos Aires, Argentina, and Barbara Zieger from the Children's Hospital at the University of Freiburg, Freiburg, Germany, will commence as new members.

Over the 4 years since the Award's inception, the committee has reviewed around 200 applications and helped to assign over €400,000 to coagulation research projects around the world. “This is a wonderful opportunity to be involved in the growth of young people within coagulation,” said Dr. Thomas Abshire at the award symposium. “We consider it an honour and a privilege to do this.”

Award winners 2011

Anne Angelillo-Scherrer

From the Service and Central Laboratory of Hematology, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland, received an award for her project entitled *Role of Gas6 and protein S pathways in haemostasis, thrombosis and inflammation*.

The growth arrest-specific gene 6 (Gas6) and protein S (ProS) both belong to the vitamin K-dependent protein family and share many structural similarities; both bind to negatively charged phospholipid membranes via an N-terminal γ -carboxyglutamic acid domain (Gla domain) and to receptors via a C-terminal steroid hormone binding globulin-like (SHBG-like) domain. ProS is a cofactor for activated protein C, which inactivates activated factors V (FVa) and VIII (FVIIIa), and thus plays a central role in the coagulation cascade. A genetic deficiency of ProS in humans is one of the most severe inherited risk factors for thrombosis.

The role of Gas6 *in vivo* remains incompletely characterised. Unlike ProS, it has no anticoagulant activity and appears to be redundant for normal haemostasis. Previous studies conducted by Dr. Angelillo-Scherrer have shown that Gas6-deficient mice do not reveal any 'spontaneous phenotype' and they appear to be protected against thrombosis (1). Detailed analysis of these mice has revealed

that Gas6 plays an 'amplifier' role in haemostasis where it is critical for effective platelet aggregation upon vascular injury (2).

Dr. Angelillo-Scherrer is keen to establish more clearly the precise roles of Gas6 and ProS in haemostasis, thrombosis, and inflammation, and to explore the possibility that Gas6 agonists or antagonists may have value in the treatment of bleeding disorders, thrombosis, or sepsis. In order to do so, and as part of her award-winning study, she plans to generate a new range of viable animal models of ProS and Gas6 deficiencies – including conditional ProS knock-out mice – and to investigate the contribution of platelet and endothelial ProS to thrombosis and inflammation. Ultimately, she hopes to generate other tissue-specific ProS knock-out mice in order to investigate the ability of ProS to function as a ligand of tyrosine kinase receptors of the Tyro3 (TAM) family in various cell types and biological processes.

Dr. Angelillo-Scherrer will also study the role of Gas6/ProS pathways in inflammation and haemostasis. As she explained, previous studies have demonstrated that Gas6, when secreted by monocytic cells in response to endotoxins, dampens cytokine release from these cells (3). In contrast, Gas6-deficient endothelial cells respond to tumour necrosis factor- α without increases in adhesion molecules and tissue factor exposure, and leucocytes transmigrate less extensively through these cells than through wild-type cells. With this in mind, Dr. Angelillo-Scherrer will complete her studies by investigating the influence of Gas6/ProS pathways on coagulation parameters during endotoxaemia and infection using conditional tissue-specific mice lacking Gas6, ProS, or one or more of the TAM receptors, and by investigating the Gas6/ProS pathway in the Lausanne Sepsis Cohort.

Jan Emmerechts

From the Center for Molecular and Vascular Biology, University of Leuven, Belgium, received an award for his project entitled *Impact on haemostasis of acute and chronic air pollution by particulate matter*.

The project has been designed to build on previous work undertaken by Dr. Emmerechts and colleagues (4) in which short-term intratracheal exposure to particulate matter (PM) was found to induce pronounced pulmonary in-

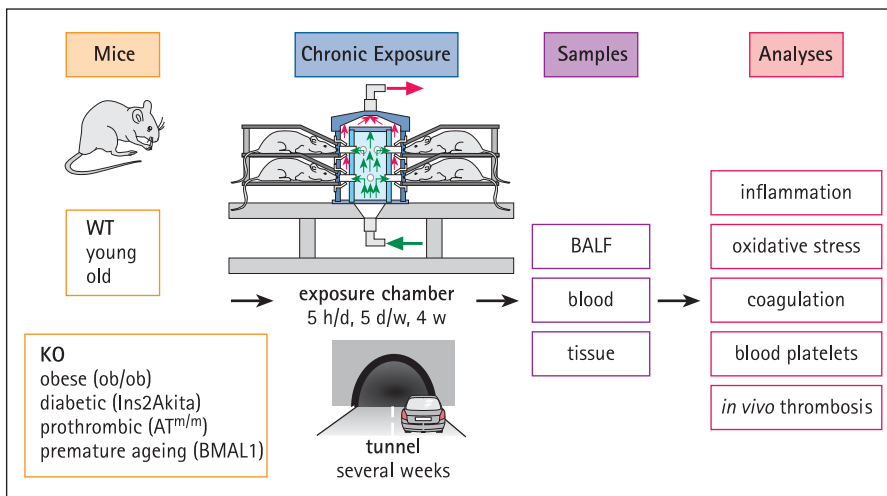


Figure 1: Development of a chronic mouse model of particulate matter (PM)-induced thrombogenicity. BALF, bronchoalveolar lavage fluid; d, days; h, hours; KO, knock-out; w, weeks; WT, wild type.

flammation, mild systemic increases in factor VII (FVII), FVIII and fibrinogen, and arterial (but not venous) thrombosis in healthy mice.

As Dr. Emmerechts explained, numerous epidemiological studies have reported consistent associations between exposure to urban air pollution and cardiorespiratory morbidity and mortality (5–8). He explained that there was now convincing evidence that chronic exposure to PM enhances atherosclerosis and that acute exposure induces blood platelet activation, triggering arterial thrombosis and myocardial infarction (9–13). Recent evidence also suggests that PM exposure is associated with an increased risk of venous thromboembolism (14–16).

The aim of Dr. Emmerechts' award-winning project is to unravel the pathophysiological pathways linking chronic PM exposure with coagulation activation and venous thromboembolism using *in vitro* cell culture systems, experimental animal models, and epidemiological research. For the first part of Dr. Emmerechts' study assessing the impact of ultra-fine particles on the pulmonary epithelial–endothelial barrier, he plans to expose human pulmonary microvascular endothelial cells to ultra-fine particles and measure the release of FVIII and von Willebrand factor (VWF) in the supernatant, to grow human bronchial epithelial cells and measure epithelial barrier function using trans-epithelial electrical resistance measurements, and to assess all the above parameters in a bi-culture system of endothelial and

epithelial cells. He also wants to investigate whether, *in vivo*, endothelial pulmonary cells are capable of FVIII production by transplanting a wild-type lung into a haemophilic mouse and assessing FVIII production by pulmonary endothelial cells before and after exposure to PM.

For the second part of his study, Dr. Emmerechts is hoping to develop a chronic mouse model of PM-induced thrombogenicity by exposing wild-type mice and knock-out mice with various pro-thrombotic phenotypes to PM using both an experimental exposure chamber and a confined roadside (tunnel) location (Fig. 1). Analyses will include markers of inflammation, oxidative stress, coagulation and *in vivo* thrombosis.

Finally, Dr. Emmerechts will be undertaking an epidemiological analysis of the association between air pollution exposure and increased risk of venous thromboembolism by monitoring air pollution exposure in 250 patients with diabetes. Classical markers of inflammation and coagulation will be assessed, as will several experimental parameters including an analysis of microvesicles by flow cytometry and thrombinoscopy.

Mindy L. Simpson

From the Rush University Medical Center, Chicago, Illinois, USA, received an award for her project entitled *Thrombin and plasmin gener-*

ation capacity as predictors of joint outcomes in children with severe haemophilia A.

The severity of haemophilia A is typically defined by blood levels of FVIII; however, there is significant phenotypic variability amongst patients with similar factor levels, suggesting that additional influences, such as co-inherited pro-thrombotic factors, may alter the phenotype. Joint disease is a major complication of severe haemophilia, affecting 90% of patients. However, predictors of joint outcome are lacking. This study will build on the framework of a completed, prospective, randomised controlled study in children with severe haemophilia A – the Joint Outcomes Study (17) – in an attempt to identify markers of bleeding and joint disease.

Dr. Simpson has previously been involved in the development of a 'global assay' assessing overall haemostatic balance that she hopes may have potential as a predictive tool for joint outcome in haemophilia patients (18). As she explained at the award symposium, the Simultaneous Thrombin and Plasmin generation (STP) assay uses fluorogenic thrombin and plasmin substrates, with coagulation initiated using dilute tissue factor, phospholipid, and calcium in platelet-poor plasma, and with fibrinolysis accelerated via tissue plasminogen activator. Unique thrombin and plasmin generation curves are produced (Fig. 2), enabling the measurement and calculation of several parameters including the maximum velocity of thrombin (V_{Tmax}) and plasmin generation (V_{Pmax}). Initial studies in FVIII-deficient plasmas suggest that thrombin generation is both delayed and decreased in the absence of FVIII (18).

Dr. Simpson believes the STP assay will discriminate children with severe haemophilia A from healthy age-matched controls and align with rates of clinical bleeding and magnetic resonance imaging (MRI) joint scores. The first aim of Dr. Simpson's award-winning study will therefore be to validate the STP in plasma samples from 25 children (<18 years) with FVIII:C ≤ 2 U/dl and to compare the results with those obtained from 90 healthy children.

The second aim of the study will be to evaluate the relationship between the STP V_{Tmax} and V_{Pmax} and joint outcomes using plasma samples and MRI scans from the Joint Outcomes Study, which evaluated prophylactic FVIII vs. intensive episodic treatment on joint outcomes in 65 children with severe haemophilia A (17). She

hopes to run the STP assay on available patient samples that are banked at the University of Colorado, compare the STP assay results of those on prophylaxis with those on episodic therapy, and to compare the final MRI scores with STP results from patients in the episodic arm who, she said, had a wider range of MRI scores than the patients in the prophylaxis arm.

Dr. Simpson believes that a global assessment of coagulation and fibrinolysis in the haemophilia population will help facilitate individualised treatment planning based on objective measures of the severity of the bleeding phenotype. In the future, she hopes to validate the prognostic value of the STP assay in a prospective, multicentre study of haemophilia patients.

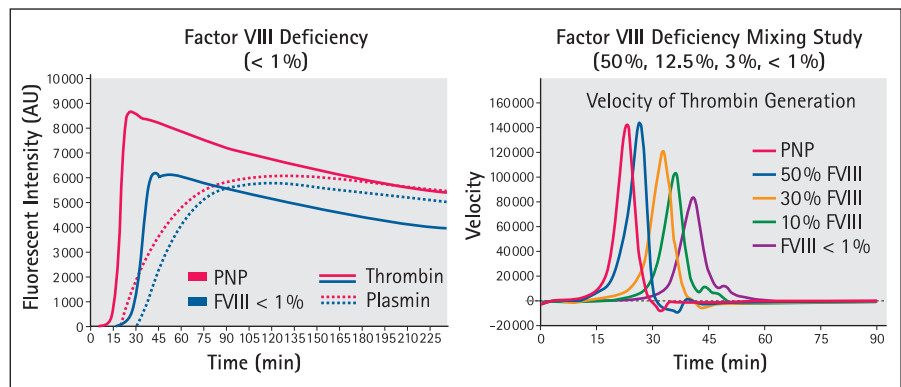


Figure 2: Simultaneous Thrombin and Plasmin generation (STP) curves (left panel) and velocity of thrombin generation (right panel) for factor VIII (FVIII) deficiencies relative to pooled normal adult plasma (PNP). AU, arbitrary units. Adapted from Simpson et al. (18).

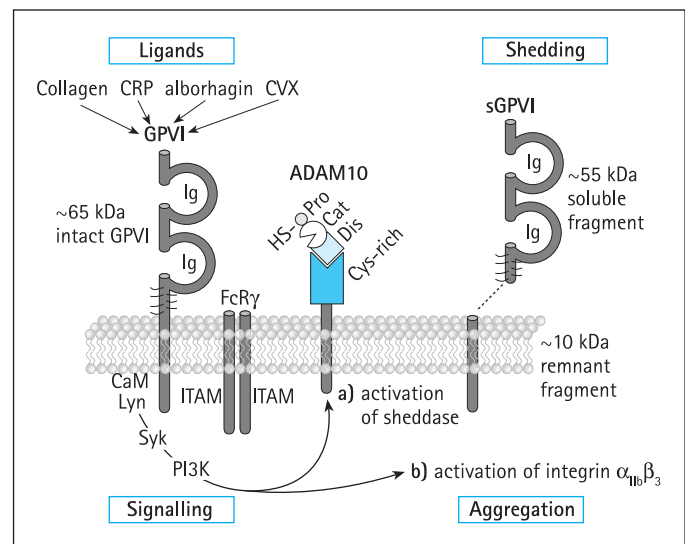
Chee Wee Tan

From the Northern Blood Research Centre, University of Sydney, Australia, received an award for his project entitled *Effect of factor Xa, thrombin and new anticoagulants on human platelet glycoprotein VI (GPVI) expression and GPVI-mediated coagulation*.

Human platelet glycoprotein VI (GPVI) is a 65-kDa membrane glycoprotein that plays an important role in collagen-induced activation and aggregation of platelets and, ultimately, in thrombus formation. After blood vessel injury, exposure of collagen fibres leads to platelet binding via both the GPVI receptor and the GPIb-IX-V complex which binds to sub-endothelial VWF, resulting in platelet adhesion to the vessel wall. GPVI forms a non-covalent complex with the FcR γ -chain, triggering a series of intracellular signalling pathways involving Src and Syk kinases that lead to activation of integrin $\alpha_{IIb}\beta_3$ – and platelet aggregation. Metalloproteinase-dependent ectodomain shedding (possibly via ADAM 10) can occur concurrently (Fig. 3). As Dr Tan explained, GPVI shedding can be induced *in vitro* by the GPVI ligands, collagen, collagen-related peptide and snake toxins, as well as by anti-GPVI and anti-platelet monoclonal antibodies.

In collaboration with the Australian Centre for Blood Diseases, it has been hypothesised that activation of coagulation/thrombin generation induces shedding of GPVI, which may serve to prevent thrombus growth, and this process may be differentially regulated by new-generation anticoagulants targeting activated factor X (FXa) (e.g. rivaroxaban) or thrombin

Figure 3: Structure, function, and shedding of glycoprotein VI (GPVI). CRP, collagen-related peptide; CVX, snake venom protein convulxin; Ig, immunoglobulin. Figure provided courtesy of Dr. Robert Andrews, Australian Centre for Blood Diseases, Monash University, Australia.



(FIIa) (e.g. dabigatran). *In vitro* experiments showed that GPVI shedding is induced in a time-dependent manner by coagulation – independent of added tissue factor/phospholipid – and by FX activation (addition of Russell viper venom). Shedding is inhibited by FXa inhibitors (enoxaparin, rivaroxaban) and metalloproteinase inhibitors (GM6001, GI2540230), but not by thrombin inhibitors (dabigatran). However, effects of coagulation on platelet GPVI expression *in vivo* have not been widely assessed.

Dr. Tan's proposed research now aims firstly to delineate the mechanisms by which FXa engages ADAM metalloproteinases in coagulation-induced GPVI shedding, and secondly, to determine if coagulation-induced GPVI shedding is occurring *in vivo*. He intends to achieve the second aim by measuring platelet GPVI in a

wide range of hypercoagulable states and to evaluate GPVI shedding in patients on anticoagulation therapy (i.e. FXa or FIIa inhibitors, warfarin, and low-molecular-weight heparin).

"We believe that coagulation-induced GPVI shedding involves FXa and this may be an important homeostatic mechanism against further thrombus growth," said Dr. Tan. "New generation anticoagulants that preferentially target FXa or thrombin may modulate this process, so any off-target effects of these drugs on platelet function may be important clinically and potentially impact on risk-benefit profiles of these drugs in individual patients."

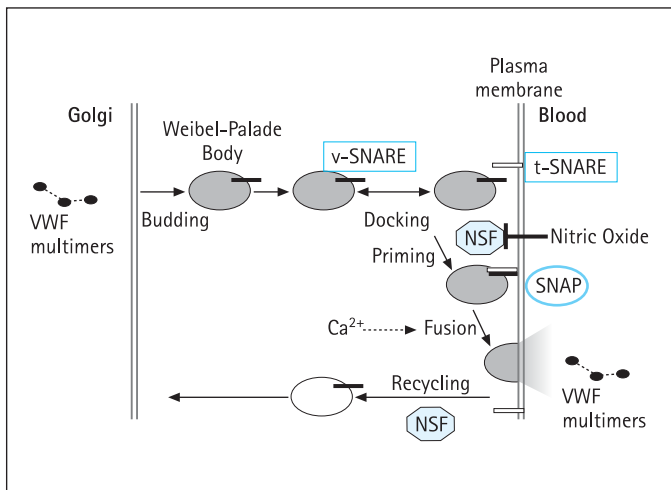


Figure 4: 'SNARE hypothesis' of vesicle exocytosis. NSF, N-ethylmaleimide-sensitive factor; SNAP, soluble NSF attachment protein; SNARE, soluble N-ethylmaleimide-sensitive factor activating protein receptor; t-SNARE, target SNARE; v-SNARE, vesicle SNARE; VWF, von Willebrand factor. Adapted from Lowenstein et al. (20).

with type I VWD. To do this, she plans to genotype single nucleotide polymorphisms in STXBP5 and STX2 in samples from approximately 150 patients with type I VWD enrolled in a nationwide cross-sectional study of moderate and severe VWD in the Netherlands (the WiN study) (21) using Custom TaqMan Genotyping Assays. She will correlate these findings with clinical and laboratory data already available.

“We hope that by investigating this relationship, we may clarify part of the aetiology and the variable clinical presentation of type I VWD,” she explained. “Our findings may form a basis for improving the diagnosis and treatment of patients with type I VWD in the future.”

Janine Eliza van Loon

From the Erasmus Medical Center, Rotterdam, the Netherlands, received an award for her project entitled *Role of snare protein genes in the regulation of von Willebrand Factor levels and bleeding phenotype in patients with type I von Willebrand disease*.

Type I von Willebrand Disease (VWD) is the most common inherited bleeding disorder and is characterised by a reduction of structurally normal VWF. Diagnosis of type I VWD is difficult because of high variability of VWF plasma levels within and between individuals and incomplete penetrance of the phenotype. Also, the clinical presentation of VWD tends to be highly variable; some patients bleed excessively, whereas others with similar VWF levels have no or only mild bleeding problems.

Although it has been anticipated for a long time that all type 1 VWD cases are caused by VWF mutations, there is now a growing expectation that mutations in other genes may be involved in the pathogenesis of type 1 VWD. A recent meta-analysis of the genome-wide association studies of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium identified two novel candidate genes highly associated with VWF levels: genes encoding syntaxin binding protein 5 (STXBP5) (q24 region of chromosome 6) and syntaxin 2 (STX2) (q24.3 region of chromosome 12), both of which are soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) proteins (19).

SNARE proteins are a superfamily of transmembrane proteins that are important in the

regulation of many secretory events and drive exocytosis by fusing granule and target membranes. According to the 'SNARE hypothesis' described by Dr. van Loon (Fig. 4), the release of VWF multimers from the Weibel-Palade storage bodies involves vesicle SNARE proteins on the granules that bud off from the Golgi apparatus interacting with target SNARE proteins on the plasma membrane prior to exocytosis and release of VWF into the bloodstream (20). Dr. van Loon hypothesises that genetic variations in the STXBP5 and STX2 SNARE proteins may affect VWF:Ag levels in patients with type I VWF and, in doing so, influence the penetration and clinical presentation of VWD.

Dr. van Loon's award-winning study will assess the relationship between genetic variation in SNARE protein genes, VWF plasma levels and the bleeding phenotype. She wants to establish if genetic variations in STXBP5 and STX2 are associated with VWF:Ag levels in patients with type I VWD, if the association between genetic variations in STXBP5 and STX2 VWF:Ag are dependent on blood group, and what the effect of genetic variations in STXBP5 and STX2 might have on the bleeding phenotype in patients

Applications for the 2012 award

Applications for the CSL Behring Prof. Heimburger Award 2012 are now being accepted and will close 7 October 2011. More information about the award and specific criteria for 2012 applications can be found at: www.cslbehring.com/ProfHeimburgerAward.

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