

**1995 MINUTES
ANIMAL MODELS SUBCOMMITTEE**

Saturday, 10 June, 1995, 13:00 - 15:00

Co-Chairs: Gerhard Johnson, USA; Thomas Griggs, USA

The subcommittee meeting was attended by 15 participants.

The group reviewed the project to establish an animal models registry and an electronic bulletin board using the Internet. The Registry of Animal Models of Thrombosis and Hemostasis will be available from "Sunsite" by file transfer protocol (FTP). The subcommittee has established and is operating a "listserv" bulletin board service on Sunsite. Access can be gained by sending a note or message to

Dr. Griggs by: Phone: (919) 966-5207
Fax: (919) 966-1743
E-mail: trg@med.unc.edu
or letter: CB #7075, Division of Cardiology
UNC School of Medicine
Chapel Hill, NC 27599-7075

Recommendation:

The subcommittee voted to make a formal recommendation that the ISTH support this effort by establishing an ISTH "homepage" on the World Wide Web (WWW).

Activities for the coming year:

1. Review and update current entries in the Registry of Animal Models of Thrombosis and Hemostasis.
2. Categorize current and future entries in the registry regarding their utility in assessments of hemostatic components (platelets, fibrin formation, inhibitors, etc.) and thrombotic variables (flow, platelet, endothelial cell function, fibrin formation, fibrinolysis, etc.).
3. To undertake a detailed review of the utility of animal models for assessing therapeutic modalities in the prevention of restenosis following arterial interventions with the goal of establishing guidelines for standardization of animal experimentation in preclinical assessment.

1996 MINUTES

ANIMAL MODELS OF THROMBOTIC AND HEMORRHAGIC DISORDERS SUBCOMMITTEE

Monday, June 24, 1996, 8:00 to 12:00

Fira Palace Hotel

Barcelona, Spain

Chair: Thomas Griggs, USA

Gerhard Johnson, USA; Lina Badimon, Spain

I. Review of the status of use of animal models for the study of restenosis following therapeutic interventions:

A. Dr. Griggs reviewed the clinical aspects of restenosis. Major points were:

1. Restenosis is an injury to atherosclerotic arteries.
2. Clinical restenosis is defined by angiographic criteria
3. Clinical restenosis involves geometric remodeling, thrombosis, proliferation, and matrix formation.

B. Dr. Badimon review work primarily using the atherosclerotic pig model of coronary artery injury. She emphasized the importance of thrombosis in the early stages, proliferation during the intermediate term and the late influence of matrix, a sequence similar to that seen in the human condition. She also showed data suggesting that the porcine model predicted the outcomes of clinical trials of several therapeutic agents better than did small animal models.

C. Dr. Johnson showed critical elements of small animal models. These included practical issues such as size and cost. Additionally, he highlighted key positive opportunities for use of small animals: These include:

1. Genetic control
2. Biochemical definition
3. Pharmacological correlation
4. Simulation of diseased vessels
5. Transgenic options
6. Genetic engineering

D. The subcommittee discussed the critical need for appropriate animal modeling of the stenosis problem both to study mechanisms and to define potential therapeutic benefit. There was extended discussion of the need to characterize the various models according to their best potential application. For instance, discussants agreed that small animal models are extremely helpful for understanding molecular mechanisms of proliferation but that large animal models have proven to be the best models for predicting clinical outcome of therapeutic approaches.

E. The group decided to publish the presentations of the subcommittee meeting as a review. Additionally, the subcommittee will consider using this review as a communication from the ISTH Subcommittee on Animal Models to key cardiology societies after discussion at the Florence meeting.

II. There was a demonstration of the Animal Models Registry in the internet format. A working party was appointed to develop a form for submission of new models and for establishing a mechanism for review and update of the Registry.

III. The agenda for the Florence meeting will include a program on the status, technology and science of local delivery of therapeutic agents to arteries and veins.

1997 MINUTES

Registry of Animal Models of Thrombotic and Hemorrhagic Disorders Subcommittee

Saturday, 7 June, 1997, 13:00-16:30

Giotto I, Fortezza da Basso

Florence, Italy

Chair: L. Badimon, Spain

Co-Chairs: T. Griggs, USA; G. Johnson, USA; D. Ginsburg, USA;

L. Drouet, France

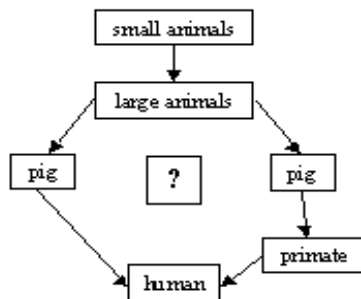
J.J. Badimon reviewed the present status of models to study local delivery devices. It is suggested that pig carotid artery is a necessary step before the application to the pig coronary to study local delivery.

L. Drouet presented four models in four different animal species to study thrombosis and surface passivation. It is suggested that a fiber of fibrin/fibrinogen plays a major role in passivation.

K. Mohlke (for D. Ginsburg) presented mouse genetic resources: genome project tools and transgenic mice. The indications and the cautions for the use of these genetically manipulated mice was discussed. References to Internet access to the mouse genome and transgenic animal databanks will be included on the Animal Model Subcommittee home page on the Internet (www.med.unc.edu/isth/).

G. Johnson presented the document of recommendation on "Restenosis: Which Animal Model is the Best?" by L. Badimon, T. Griggs, and himself on behalf of the Subcommittee. The discussion helped to solve some additional questions such as the following:

- Is there a best large animal model?
- What is the optimal sequence of pre-clinical studies?



- How close are we to a small animal model of atherosclerosis?

After an extensive discussion, the chairman proposed that the name of this subcommittee be changed to Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis. This was approved.

The next meeting will be held in [Ljubljana, Slovenia](#), with future subjects:

1. Gene transfer and regulation of gene expression in animal models
2. Review of models of venous thrombosis and pulmonary embolism which could give rise to a document of recommendation for models of venous thrombosis and pulmonary embolism.

1998 MINUTES
Animal, cellular, and molecular models of thrombosis
Monday, 22 June, 1998, 8:00-12:00
Cankarjev Dom
Ljubljana, Slovenia
Chair: L. Badimon, Spain
Co-Chairs: G. Johnson, USA; D. Ginsburg, USA;
L. Drouet, France

The Subcommittee meeting was chaired by G. Johnson. Approximately 50 persons attended the meeting. Active discussion of the presentations and resolution occurred.

PROGRAM

The meeting was devoted to presentations on the following three topics:

1. Animal models useful for the study of fibrinolytic mechanisms.

Dr. Jordi Felez described methods to study expression of plasminogen receptor binding sites on endothelial cells.

Dr. Peter Carmeliet, Leuven, Belgium, described the utility of gene knockout mice in the evaluation of the role of fibrinolytic parameters in the development of atherosclerotic vessels and myocardial response to injury.

Dr. Jose Paramo, Navarra, Spain, described studies of restenosis in atherosclerotic pig arteries and his observations of increased PAI-1 expression.

Dr. Paul Holvoet, Leuven, Belgium, described the important differences between the atherosclerotic lesions seen in rabbits, pigs, and humans, and he presented data on the increased expression of MDA modified LDL in acute coronary syndromes.

2. Animal models useful for the study of venous thrombosis.

Dr. Marcel Levi, Amsterdam, Netherlands, reported on an extensive review of the status of animal models of venous thrombosis. He emphasized the limitations of these models in predicting drug doses and efficacy in human venous thrombosis.

3. Animal models of restenosis.

Dr. Gerhard Johnson, Minneapolis, USA, presented a position paper on the use of animal models in the study of arterial restenosis.

ACTION The position paper on restenosis, "The Utility of Animal Models in the Preclinical Study of Interventions to Reduce Human Coronary Artery Restenosis: Analysis and

Recommendations," was approved by the Subcommittee for submission to the SSC and *Thrombosis and Haemostasis*.

FUTURE PLANS

1. To develop a position paper on animal models of venous thrombosis for presentation to the Subcommittee in 1999.
2. To explore the feasibility of having a program on *in vitro* models at the 1999 meeting.

1999 MINUTES

Animal, cellular, and molecular models of thrombosis AND Haemostasis

Saturday, 14 August 1999

8:00 to 12:00 PM

Room 10-12

Washington Convention Center

Washington, DC

Chair: L. Badimon, Spain

Co-Chairs: P. Carmeliet, Belgium; L. Drouet, France; G. Johnson, USA

The program and invited speakers addressed the issue of how representative are animal models of thrombosis and vascular disease of the respective human pathologies. Speakers were selected to discuss spontaneous atherosclerosis, restenosis, venous thrombosis and angiogenesis.

The first speaker was Dr. John MacGregor. He reported that the mechanism implicated in the initiation and perpetuation of vascular lesions and their subsequent rupture leading to thrombus formation and total lumen occlusion remains poorly understood. In an effort to investigate such mechanisms his laboratory selected the use of ApoE deficient mice (generated in the laboratory of Dr. Maeda in Chapel Hill, NC), and compared them to C57BL6 wild type, fed a fat or a chow diet. The work focused on the quantitative expression of major adhesion molecules (ICAM-1, VCAM-1, PECAM-1, P-selectin) on the aortic arch of ApoE deficient mice compared to controls (C57BL6). Immunohistochemistry and Northern blots were used to assay the levels of these adhesion molecules at the endothelial cell level or by the whole vessel. Results show modulation of the level of expression of these adhesion molecules at different stages of vascular lesions in ApoE deficient mice. A similar type of result has been observed for human tissue samples. However, no plaque rupture and/or thrombotic occlusion have been observed for these ApoE animals. Great caution has to be taken in interpreting the data observed in the mouse ApoE model. Further work needs to be performed to humanize the ApoE deficient mouse model.

Dr. J.J. Badimon reported on experimental studies of cell cycle inhibition as a therapeutic strategy to decrease coronary restenosis following stenting. Rapamycin was found to inhibit smooth muscle cell proliferation in pig coronary arteries by a direct effect on p27, a negative regulator of cell proliferation, rather than by an apoptosis-related mechanism.

Direct comparisons with data in ApoE KO mice in terms of markers of proliferation/apoptosis were discussed. More research is needed because intra-stent restinosis remains a significant problem in clinical revascularization.

Dr. M. Levi presented the results of a comprehensive review of the literature on animal models of venous thrombosis. In general, experimental venous thrombosis studies have been useful to determine if experimental agents are useful for the inhibition of thrombus formation. However, they have been of lesser utility in dose-finding and comparative pharmacology studies. Discussion followed on species differences regarding pharmacokinetics and coagulation/fibrinolytic system. Research is needed to develop new methods that can provide a clear insight in the etiology of venous thrombosis.

Dr. L. Drouet described the characteristics and utility of a large animal model of chronic venous thrombosis. Thrombi with the same morphologic characteristics as chronic human venous thrombi developed in Gortex grafts implanted in pig external jugular veins, although the distal normal segments did not thrombose. Studies of anticoagulant therapy in this model indicated that heparin was more effective than low molecular weight heparin and low-dose hirudin was ineffective.

Existing models of venous thrombosis, and namely the Wessler model (and derivatives) are:

- studying only the acute thrombotic reactivity for a few hours (while patients have clinical manifestations of thrombosis after days of evolution of the thrombotic process),.
- developed in small rodents (rabbit, rats, etc.) whose flow condition (due to vessel size) are different from human pathology.

A model of chronic venous thrombosis in the pig seems of relevance because the coagulation system of this animal is fairly well known and quite similar to the human system and this animal is accepted as a good model of arterial pathology. Ultrastructural examination shows, as in human thrombosis, an onion skin like structure of the thrombus. It is formed with several layers of dense platelet deposits alternating with layers of fibrin network imprisoning various amounts of red blood cells. The rate of occlusion was 100%.

Dr. P. Carmielet described studies on embryonic and neonatal angiogenesis in mice. He showed data on the role of VEGF in angiogenesis. VEGF knockout mice were found to have defective vascular sprouting. VEGF 121 mutant mice were observed to have a significant decrease in the density of coronary blood vessels. These mice had decreased intramyocardial blood flow and subendocardial ischemia, and they died prematurely of heart failure. Placental growth factor (PLGF) was also found to interact with VEGF in the differentiation of large blood vessels. PLGF seems to play a role in pathological angiogenesis while VEGF seems to play a role both in physiological and pathological angiogenesis. VEGF was found to increase myocardial capillary formation in wild type mice but not in UPA knockout mice.

Dr. G. J. Johnson reported that the Subcommittee report titled "The Utility of Animal Models in the Preclinical Study of Interventions to Prevent Human Coronary Artery Restenosis: Analysis and Recommendations" was recently published in *Thrombosis and Haemostasis*.

Dr Badimon reported that because the content of the document was of interest to other scientific communities, the editors of *Circulation* were contacted. They showed interest and asked for a document that possibly could be published as an editorial.

Finally, Dr. Badimon, as the Chair of the Subcommittee, proposed new tasks for the next year. Due to the lack of models specifically addressing the pathophysiology of venous thrombosis, a consensus document on this issue will be prepared by Drs. Levi, Drovot, Johnson, and Badimon. The plan is to have a preliminary draft circulated by January 2000 and a final draft ready for presentation at the next SSC meeting. It will be submitted for approval at a later date.

This year we had a large attendance at our SSC meeting (over 100 participants).

Next year we plan to focus on models of pulmonary embolism and on coagulation factors in animal models

ANIMAL, CELLULAR AND MOLECULAR MODELS OF THROMBOSIS AND HAEMOSTASIS

15 June 2000

08:00 to 12:00

Room 0.4

Maastricht Meeting and Convention Center

Chairman: P. Carmeliet--Belgium

Co-chairmen: L. Badimon--Spain; L. Drouet--France; P. Jagadeeswaran--USA;

G. Johnson--USA; N. Maeda--USA

The Subcommittee meeting was attended by 30-40 persons. The program was devoted to new animal and cellular models. G. Johnson, Co-Chair, presided, and Pudur Jagadeeswaran, Co-Chair, attended and presented.

PROGRAM

Luc Schoonjans (Leuven) presented new data on novel transgenic techniques using embryonic stem (ES) cells and cloning. The majority of gene targeting experiments have been performed using 129 ES cells. However, the 129 strain is not well characterized, and the knockout phenotype depends on the genetic background of the mice. Therefore, a program was developed to derive ES cells from the most commonly used mouse strains. This has resulted in the establishment of ES cells from 10 different mouse strains with germline transmission capability.

Virginie Mattot (Lille) presented the results of studies of transgenic mouse models expressing different VEGF isoforms. VEGF 120/120 animals manifested renal vascular abnormalities that resulted in sclerotic glomeruli and decreased tubule formation and impaired renal function. Thus, angiogenesis is normal in VEGF 120/120 animals, but vascular remodeling is abnormal.

Heike Beck (Erlangen) discussed rodent models of stroke. The advantages and disadvantages of single and extended thrombotic occlusion and embolic occlusion were described, and the roles of Ang-2, VEGF and apoptosis in post-stroke angiogenesis were discussed.

Vivian de Waard (Amsterdam) presented the results of studies of the role of PAI-1 and VN in an experimental arterial constriction model. Ligated carotid arteries demonstrated enhanced smooth muscle proliferation in PAI-1 $-/-$ and VN $-/-$ knock-out mice compared to wild-type controls. These studies indicate that PAI-1 and VN protect against smooth muscle-rich neointimal formation following arterial injury.

Pudur Jagadeeswaran (San Antonio) reviewed his studies of hemostatic parameters and thrombosis in zebrafish. Due to the ease of saturation mutagenesis and the rapidly progressing zebrafish genome project, this model has great promise for rapidly identifying specific genotypes. To evaluate hemostatic factors, micro-assays for coagulation, platelet function and bleeding time were developed, the effects of anticoagulants were assessed and thrombosis was

induced. These developments will enable further evaluation of the utility of this model in genetic research in hemostasis and thrombosis.

Anne Angellillo (Geneva) presented the results of studies of growth arrest-specific gene 6 (Gas6), a homolog of protein S, on platelet function. Contrary to expectations, Gas6^{-/-} mice were resistant to thrombosis. Protection from thrombosis was found to be attributable to impaired platelet aggregation in response to several agonists and to reduced endothelial tissue factor.

BUSINESS

The Subcommittee voted to approve submission of the manuscript, **USEFULNESS AND LIMITATION OF ANIMAL MODELS OF VENOUS THROMBOSIS**, written by M. Levi, J. Dorffler-Melly, G. Johnson, L. Drouet and L. Badimon on behalf of the Subcommittee, to the SSC for approval as an official SSC document.

The Subcommittee voted to authorize a writing committee to prepare a document on the assessment of bleeding risk in animal models. The committee will be appointed by the Chair.

The Subcommittee authorized the development of a program to be developed jointly with the Subcommittee on DIC for the 2001 meeting. Dr. M. Levi will coordinate organization of this symposium.

ANIMAL, CELLULAR AND MOLECULAR MODELS OF THROMBOSIS

**6 July 2001
08:00 to 12:00
Room 242
Palais des Congrès**

Chairman: P. Carmeliet--Belgium

Co-chairmen: L. Drouet--France; P. Jagadeeswaran--USA; G.J. Johnson--USA; N. Maeda--USA

The meeting was chaired by G. Johnson at the request of Chairman, P. Carmeliet

Attendance: ~100 persons

Scientific Presentations:

P. Jagadeeswaran, San Antonio, reported on innovative studies of hemostatic parameters and thrombosis in zebrafish. Using techniques developed in his laboratory, Dr. Jagadeeswaran has been able to perform clotting assays on extremely small volumes of blood, evaluate platelet aggregation and induce thrombosis. Anticoagulant effects on hemostasis and thrombosis and platelet inhibitory drugs have been evaluated. Genes for all the human coagulation factors, except Factors VIII and IX, have been identified in the Zebrafish. An additional Factor VII-like gene has also been identified. Combination of the functional assays with the enormous potential of the zebrafish for rapid genetic analysis make this model a very promising one for analysis of the complex interactions of multiple proteins in hemostasis and thrombosis.

G. Johnson, Minneapolis, presented an overview of the signaling defect that exists in thromboxane-insensitive dog platelets. This defect in activation of PLC-beta results in impaired secretion stimulated by thromboxane A₂ or thromboxane analogs. The cause of this signaling defect appears to be an elevated level of basal thromboxane receptor phosphorylation that is reversible by epinephrine. Thromboxane-insensitive dog platelets are a useful model for the study of the control of G protein-coupled receptors by phosphorylation.

L. Drouet, Paris, reported progress on the development of a pig model of unstable atherosclerotic plaque. Homozygous, LDL mutant, hypercholesterolemic pigs with spontaneously developing atherosclerosis had carotid stenosis lesions induced by application of an externally constricting collar combined with a ligature. These lesions develop flow reduction and down-stream thrombosis. The effects of angioplasty and antithrombotic agents on these lesions has been evaluated. Some spontaneous plaque rupture develops in this model when these animals are fed an atherogenic diet. Additional studies are in progress to further refine this very promising model.

Berend Isermann, Milwaukee, reported on a series of studies performed in mice with targeted disruption of genes for components of the thrombomodulin/protein C pathway. These studies have highlighted the contributions of components of this pathway to normal in-utero development. Thrombomodulin expression is required in the placenta during mid gestation,

before development of a functional cardiovascular system. During the second half of pregnancy and at birth, embryos with disrupted hemostatic systems often succumb to hemorrhage or thrombosis. Several successful strategies have been developed to circumvent the intrauterine lethality of mice with defects in the thrombomodulin\protein C pathway. Animal models with defects in the thrombomodulin\protein C pathway will be very useful in the identification of modifier genes for thrombosis, the study of vascular bed-specific hemostasis and the evaluation of thrombophilia-associated pregnancy complications.

Old Business: The manuscript, The Utility and Limitations of Animal Venous Thrombosis Models, approved for publication by the SSC at the 2000 meeting in Maastricht, has been revised to meet the editorial criteria of Thrombosis and Haemostasis. It has been reviewed and approved by the co-authors and the Chairman. It will be resubmitted to Thrombosis and Haemostasis as an official SSC publication.

New Business: Discussion of future activities included critical evaluation of the important characteristics of animal models that must be considered in the selection of appropriate models for the study of research topics, such as arterial thrombosis in large and small animals. Characteristics, such as levels of hemostatic parameters, availability of antisera, functional characteristics of platelets and endothelium, and other important parameters would be compiled and made available via a registry. A particular need exists for this type of information for mouse models. Subcommittee members will submit proposals for this type of analysis to G. Johnson who will coordinate this activity for the Subcommittee. A goal will be to have a draft developed for submission to the Subcommittee at the 2002 meeting.

Animal, Cellular & Molecular Models

July 18, 2002

13:00 to 17:00

Stanbro Room

Boston Park Plaza Hotel

Chairman: P. Carmeliet, Belgium

Co-chairs: L. Drouet, France; P. Jagadeeswaran, USA; G. Johnson, USA; N. Maeda, USA

Business: The meeting was chaired by Subcommittee CoChairs, G. Johnson and P. Jagadeeswaran. It was attended by approximately 100 persons.

Dr. G. Johnson proposed that the Subcommittee authorize a Working Party to develop a comprehensive data base on murine hemostatic parameters and models of thrombosis. The Working Party will tabulate published and volunteered unpublished data and prepare a manuscript for approval as an official SSC publication at the 2003 meeting. This proposal was approved by the Subcommittee. Dr. Johnson will Chair the Working Party.

Scientific Program:

Justin Hamilton, University of California at San Francisco, described studies of PAR 4 and PAR 3 knockout mice. Both knockouts manifested prolonged bleeding times, impaired thrombin-induced platelet aggregation and protection from experimental thrombosis. These studies demonstrate the important cofactor function of PAR 3 *in vivo*.

Mieke Dewerchin, Center for Transgenic Technology and Gene Therapy, Leuven, presented results of studies of antithrombin R47C mutant mice. This mutation resulted in a high rate of neonatal mortality. Survivors developed spontaneous thrombi in multiple organs. Thromboses were not prevented by the administration of pentasaccharide. These studies emphasize the critical functional role of the heparin binding site of antithrombin.

Brian Peterson, Scripps Research Institute, San Diego, spoke about the consequences of low tissue factor (~1% in brain) in mice. These animals had increased post-partum uterine hemorrhage, fatal hemorrhage in 20% and tissue fibrosis resulting in impaired cardiac function. These mice were resistant to experimental arterial thrombosis that was not corrected by transplantation of wild type bone marrow. Thus deficiency of vascular tissue factor appeared to be more responsible for hemorrhage and resistance to thrombosis than blood cell tissue factor.

Pudur Jagadeeswaran, University of Texas, San Antonio, presented an overview of his laboratory's extensive characterization of hemostatic parameters and thrombosis in zebrafish. The similarity of blood coagulation factors, platelets and thrombosis in zebrafish to those of mammals, combined with their utility as genetic models, make zebrafish a valuable model for the

study of mechanisms of hemostasis and thrombosis.

Laveena Sharma, Monash University, Australia, presented evidence from studies of mice with a targeted deletion of the cytoplasmic domain of tissue factor that the carboxyl terminus plays an important role in inflammation. Animals with the deletion challenged with LPS demonstrated improved survival, decreased pulmonary neutrophil accumulation and cytokine production, and they manifested impaired delayed hypersensitivity.

Ed Conway, Center for Transgenic Technology and Gene Therapy, Leuven, described studies that demonstrated the importance of the lectin domain of thrombomodulin in inflammation. Deletion of the lectin domain resulted in normal development and fertility, and normal interaction with protein C, but mice with this deletion had exaggerated responses to LPS and experimental arthritis, increased experimental myocardial infarction size and increased neutrophil adhesion to endothelial cells.

Anne Angellilo, University Medical Center, Geneva, spoke of studies of the role of Gas 6 in mouse platelet function. Gas 6 knockout mice had normal plasmatic coagulation, bleeding time and fibrinolysis, but they demonstrated impaired platelet aggregation and secretion. These mice were protected against experimental thromboembolic death. The impaired platelet aggregation was restored by addition of exogenous Gas 6. These mice also demonstrated impaired inflammatory responses.

Animal, Cellular & Molecular Models

July 13, 2003

08:00-12:00

Hall 5

The International Convention Center, Birmingham

Chairman: P. Jagadeeswaran, USA

Co-chairs: P. Carmeliet, Belgium; G. Johnson, USA; C. Kluft, The Netherlands;
T. Nichols, USA

Approximately 200 persons attended the meeting. Presentations were made by ten speakers describing a variety of animal models.

Scientific meeting:

B. Furie (Boston) described a technique to observe in vivo thrombus formation in real time using confocal widefield microscopy. He demonstrated sequential incorporation of platelets, fibrin and tissue factor, identified by fluorescent-labeled specific antibodies that allowed three color displays, into thrombi in mouse vessels. This is a powerful technique for studying the sequential events in thrombus formation.

G. Johnson (Minneapolis) presented results of studies of the potential serotonin contribution to the cardiopulmonary toxicity of fenfluramine. These studies were performed in canine model, which demonstrated decreased platelet serotonin, and increased plasma serotonin, resulting from decreased platelet uptake, and cardiac valve dysfunction. This model has potential utility for further study of cardiopulmonary toxicity induced by anorectic drugs that affect serotonin metabolism.

E. Rosen (South Bend) described in utero transplantation of fetal cells to rescue factor X null mice. Low levels of factor X expressed were sufficient to allow survival. The predominant site of localisation of the transplanted cells and their progeny was the liver although expression was demonstrated in other organs. This technique was projected to be useful for gene therapy.

S. Coughlin (San Francisco) presented the development of a PAR-4 knockout mouse that results in elimination of thrombin-induced platelet aggregation. Mice had prolonged tail bleeding times, but only a mild bleeding tendency. Using two different models (FeCl₃ thrombosis model and thromboplastin induced pulmonary embolism model) he demonstrated a significant protection from thromboembolism by PAR-4 deletion.

P. Jagadeeswaran (San Antonio) presented a comprehensive characterisation of zebrafish hemostatic pathways demonstrating all hemostatic pathways found in humans are present in

zebrafish. He developed methods to screen for hemostasis defects and isolated several mutants such as victoria. He also showed that young thrombocytes initiate while mature thrombocytes propagate the arterial thrombus. This model should be useful in identifying novel hemostatic mutations in future.

T. Nichols (Chapel Hill) described the effects of intravenous injection of *P. gingivalis* on inflammatory parameters and atherosclerosis in normocholesterolemic pigs. Increased CRP, IgG to *P.gingivalis* and atherosclerosis predominantly composed of smooth muscle cells were noted in *P. gingivalis* injected pigs. Additional studies in hypercholesterolemic pigs are in progress.

C. Kluft (Leiden) reviewed human data regarding the role of fibrinogen in atherogenic disease. Overexpression of fibrinogen in apoE3 mice did not result in accentuation of atherosclerosis. Since fibrin is a component of atheromatous lesions further development of animal models to study the role of fibrinogen in atherosclerosis was suggested.

S. Emeis (Leiden) summarised data regarding current understanding of fibrin participation in mouse models of atherosclerosis. In afibrinogenemic mice fatty streak lesions were less prominent although the number and the composition of lesions were unaffected. Further understanding of the contribution of fibrinogen to cardiovascular disease will require additional characterization of different forms of fibrinogen and strain specificity in mouse models.

E. Conway (Leuven) described studies of the role of endothelial survivin (an inhibitor of apoptosis) on angiogenesis. Fetuses lacking endothelial survivin showed extensive haemorrhage and had pericyte/endothelial cell abnormalities. These studies indicate survivin plays a role in vascular stability and integrity.

N. Mackman (La Jolla) described the generation of data on low tissue factor mice and illustrated blood borne tissue factor contributes to thrombus formation using bone marrow transplantation techniques. He analysed the role of TF in two different thrombosis models and observed a predominant contribution of vascular TF in one model and blood borne TF in the other model.

Business Meeting:

G. Johnson presented plans to review and analyze various methodologies useful in characterization of mouse hemostasis. Following the subcommittee decision made in Boston Dr. Johnson (USA) agreed to chair a working party composed of S. Lord (USA), A. Shet, (USA) M. Jirouskova (USA), S. Emeis (Netherlands), O. Matsuo (Japan), and E.M.Muchitsch (Austria) that will prepare a manuscript for the approval by Animal Model subcommittee to be submitted to the SSC for approval as an official communication at the Venice meeting 2004.

Animal, Cellular & Molecular Models

June 17, 2004

14:00 to 18:00

Barbantini Room

Fondazione Giorgio Cini

Chairman: P. Jagadeeswaran, USA

Co-chairs: G. Johnson, USA; C. Kluft, The Netherlands; T. Nichols, USA

The meeting was attended by 50 persons and the Drs. Johnson, Nichols, Kluft and Jagadeeswaran were present. The sessions were chaired by Drs. Jagadeeswaran and Johnson. There were a total of 9 presentations. The following are brief summaries of these presentations.

Barbara Furie (USA): Dr. Furie presented her work on the molecular basis for the ferric chloride and laser induced thrombosis in mice. The ferric chloride injury exposes collagen surface while in the laser induced thrombosis, the tissue factor was prominent at the site of the injury.

M. Dewerchin (The Netherlands): Dr. Dewerchin presented data on gene targeting studies on vascular abnormalities in neurological phenotypes. Low levels of VEGF in mice were associated with paralysis and axonal loss in sciatic nerve. The onset of paralysis was accelerated by SOD. She also presented data on haplotypes in VEGF gene promoter in ALS patients and found an association to a specific haplotype.

Tim Nichols (USA): Dr. Nichols presented sensitivity of assays to measure canine factor IX and factor VIII. He also presented historical perspectives of these assays and their applicability to the study of hemophiliac dogs.

Pudur Jagadeeswaran (USA): Dr. Jagadeeswaran presented an overview of zebrafish hemostasis and thrombosis and emphasized the significance of the zebrafish as a genetic model to study thrombosis. He presented data on isolation of zebrafish mutants such as Victoria, Leopold and Nicholas by using laser induced thrombosis assay. He also described the discovery of thrombocyte microparticles and their possible role as initiators of thrombus formation.

Bruce Furie (USA): Dr. Furie presented the data on microparticles, their measurement and role in thrombosis. An impressive series of videos presenting the accumulation of endothelial, leukocyte and platelet microparticles in the growing thrombus.

M. Jirouskova (USA): Dr. Jirouskova discussed methods for the measurement of platelet function in mice. She emphasized the importance of the method of obtaining blood. Samples obtained from retro-orbital vessels or the vena cava are most satisfactory. Platelet counts in mice must be performed with knowledge of their high counts, small size and strain variability. Platelet activation and aggregation studies have been performed using techniques suitable for human platelets, but a platelet-monocyte aggregation requires a better definition.

Susan Smyth (USA): Dr. Smyth presented comparison of human and mouse fibrinolytic components with respect to structures and function. She also reviewed the effects of gene knockout experiments in mice and insights gained in role of uPA, tPA, uPAR and tPAR.

E.M. Muchitsch (Austria): Dr. Muchitsch reviewed various coagulation assays in mice such as factor X assay using peptide substrates, factor IX, factor VIII one stage clotting assays. Standardization was performed using human deficient plasmas and verified by using factor deficient mouse plasma. She also presented vWF multimer assays. She discussed the limitations of tail bleeding time.

Gerhard Johnson (USA): Dr. Johnson presented the interesting results of the working party on Mouse Hemostatic and Thrombotic Parameters. Contributions to the work of the working party have been obtained in three major categories; platelets, plasmatic coagulation and fibrinolysis. Work on this project is nearing completion, a manuscript is being prepared.

New Business: The report of the working party on Mouse Hemostatic and Thrombotic Parameters was accepted by the Subcommittee for submission, on final completion, to the SSC for approval as an official SSC communication.

Animal, Cellular & Molecular Models

Chairman: H. Weiler, USA

Co-Chairs: S.R. Coughlin, USA; J.L. Degen, USA; P. Jagadeeswaran, USA; C. Kluft, The Netherlands;

N. Mackman, USA; T. Nichols, USA

The session had approximately 20 attendees.

The outgoing chairman of the subcommittee, **Dr. Jagadeeswaran**, reviewed SSC activities 2004 to 2006, including organization of the scientific program of the subcommittee during the ISTH bi-annual meeting in Sydney, Australia.

Dr. Johnson reported on the status of the Murine Parameter Project. The Project is a comprehensive, 3-part compendium of murine hemostasis, including methods to assess the status of coagulation, platelet function, fibrinolysis and thrombosis in normal mice, and a review of hemostatic function/phenotypes in genetically altered mice. The compendium has undergone successful peer review and is scheduled for eventual publication in the ISTH periodical, Journal of Thrombosis and Hemostasis.

Scientific program:

Dr. Jagadeeswaran (University of Northern Texas, TX, USA) reviewed progress in genetic approaches to hemostasis in zebrafish. Data suggest the existence of functionally diverse platelet subpopulations in zebrafish, and the existence of proteases secreted into the environment with potential hemostatic activity.

Dr. Taylor (OMRF, Oklahoma City, OK, USA) critically evaluated the interplay of hemostasis and inflammation in primate models of sepsis. A key conclusion drawn from the synthesis of many studies is that the relevant pathogenic mechanisms operating in diverse sepsis models depend on the severity of the inflammatory challenge (degree of ensuing lethality).

Dr. Nichols (UNC, Chapel Hill, NC, USA) reviewed the development of novel pig models of aortic and coronary atherosclerosis.

Dr. Stoll (Universitaet Muenster, Muenster, Germany) introduced novel genetic methods to detect genetic predictors for plaque development. Dr. Stoll reviewed the concept and approach of comparative genetics, and demonstrated the power of the approach by showing several novel candidate genes that have been identified.

SSC Agenda 2006-10:

The agenda was discussed in an open session by all attendees. Consensus was reached that the Animal Subcommittee will continue its activities according to previous schedules. The following items were identified as future projects:

1. Complete the Murine Parameter Project by achieving publication
2. Organize scientific program for the ISTH meeting in Geneva, 2007. Potential topics are comparative genomics of hemostasis. Efforts will be made to have a representative from the European Mouse Phenome Consortium, and corresponding efforts in other countries.
3. Establish a www.-based registry for genetically defined animal models with altered hemostatic function. The registry supports the identification, distribution and characterization of relevant animal models.
4. An effort will be made to integrate the subcommittees work with ongoing efforts by NIH to optimize the development of animal models for atherosclerosis/inflammation. One of the priorities will be to produce animal models and/or protocols with improved predictive power for efficacy of therapeutic intervention in clinical studies. The chairman will establish a working group that will include much needed expertise from pharmaceutical/biotech enterprise.

Animal Models

Chair: H. Weiler (USA)

Co-Chairs: Shaun Coughlin (USA), Jay L. Degen (USA), Cornelis Kluft (Netherlands), Nigel Mackman (USA), Tim Nichols (USA), Susan Smyth (USA)

The focus of the session was to give attendants a review of emerging technology and animal models relevant to thrombosis and hemostasis. The session addressed three themes:

Session 1. Genetic manipulation of hemostasis in mice

Dr. Degen reviewed available mouse strains with altered function of key hemostatic factors, thrombin and fibrinogen. In addition to loss-of-function models knockouts for prothrombin and fibrinogen, several mutations have been introduced into the fibrinogen gene cluster to abolish specific interactions of fibrinogen with integrins that are relevant in inflammation and infection. Novel models included mice with selective expression of Aalpha-chain isoforms (“long” and “short”), and a “non-clottable” form of fibrinogen that lacks the thrombin cleavage sites for fibrinopeptide removal. This model will be instrumental in dissociating functions of fibrinogen, as compared to fibrin. A novel approach was presented to delete the prothrombin gene in a temporally and spatially regulated manner. This model allows an investigator to temporally induce a state of almost complete prothrombin deficiency in adult mice.

Dr. Conway provided an excellent overview of approaches to alter gene functions in a cell type-restricted manner in endothelial cells. He introduced experimental concepts that will allow not only endothelial cell-specific manipulation of gene expression, but in addition target the endothelium of specific organs, in particular the brain. An important aspect of the presentation was to emphasize that several approaches to achieve endothelial cell-selective gene expression/inactivation also affect bone marrow-derived cells.

Dr. Coughlin focused on the role of protease activated receptors in platelet function, i.e. Par4, and gave a comprehensive summary of available data validating Par4 function in platelet-driven hemostasis and thrombosis in mice, as compared to humans.

Dr. Mackman reviewed existing data from the analysis of mice expressing lower or higher amounts of TF in different organs. Findings emphasize the concept of organ-selective functions of tissue factor, and of an organ-selective balance of hemostasis.

Dr. Isermann addressed experimental approaches and use of animal models to study the role of altered hemostasis in chronic, as opposed to acute models of disease. This concept was illustrated in the paradigms of atherosclerosis and of the role of the protein C pathway in diabetes. This presentation stressed the value of animal models that introduce –as compared knockout models– more subtle alterations in the hemostatic balance that more closely mimic the situation in human populations.

Session 2. Coagulation – inflammation axis

Dr. Ploplis reviewed published data from a set of transgenic mice expressing various levels of protein C. These animals, as opposed to complete knockouts for protein C, are viable but exhibit numerous derangements causing spontaneous thrombosis and inflammation, and cause pregnancy failure.

Dr. Lupu gave an overview over models of septic inflammation and DIC in Baboons. These models have been exploited to examine the function of the protein C pathway in inflammation. An important outcome of these studies is the recognition that inflammatory disorders such as sepsis with or without DIC comprise several different pathogenic mechanisms in different stages of disease. In contrast to mouse models, Baboons can be analyzed with reagents/methods developed for, and applicable to humans, because of evolutionary conservation of proteins and pathways.

Dr. Nichols gave an overview over the use of several pig breeds in research relevant to atherosclerosis, and an update on technological aspects of large vessel function analysis in these animal models.

Session 3. Analytical tools in rodents

Dr. Ruf presented data from experiments in mice using pharmacologic reagents to manipulate coagulation in settings of inflammation (sepsis/LPS); and dissect the role of coagulation and coagulation receptors in hemostasis as compared to cell signaling. An important outcome of these studies in mice was that coagulation activation appears to make only a minor contribution to the inflammatory derangements in mouse models of inflammation triggered by LPS.

Dr. Poncz reviewed strategies to manipulate and target gene expression in murine platelets. Using the paradigm of fVIII gene delivery to platelets and correct hemophilia, analytical methods were discussed to monitor functional outcomes of gene manipulation, such as in vivo imaging of platelet thrombus formation and measurement of bleeding times.

Dr. Smyth could not attend. The theme of her presentation was scheduled to be the use of genetic analysis to delineate novel traits relevant to thrombosis and hemostasis. This topic will be the lead theme for the coming sessions of the Animal model SSC.

The session was extremely well attended. The Chairs / Cochairs came to the consensus that similar sessions with an educational focus on technology will be valuable in future sessions during regular ISTH meeting years.

Animal, Cellular, and Molecular Models of Thrombosis and Haemostasis

**5 July 2008
Vienna, Austria**

Chair: *Hartmut Weiler, USA*

Co-Chairs: *Shaun Coughlin, USA; Jay L. Degen, USA; Cornelis Kluft, The Netherlands; Nigel Mackman, USA; Tim Nichols, USA; Susan Smyth, USA*

The Symposium "*Appropriate Use of Animal Models in Preclinical Development of Hemostasis and Thrombosis Therapeutics*" was held on July 5, 0800-1130.

Time Subject / Title; Speaker

Animal Models of Thrombosis and Hemostasis for Pharmaceutical Development:
Industry's Perspective

Eva-Maria Muchitsch; Baxter AG, Vienna

Dr. Muchitsch provided an overview of regulatory requirements and guidelines governing the development and preclinical testing of anticoagulants in pre-clinical animal models. Discussions highlighted interest to feature this topic in a potential collaborative review to be submitted as a publication of the working group.

Multiyear Safety and Efficacy of AAV-Mediated Gene Transfer For the Hemophilias
Timothy Nichols; Univ. North Carolina; Chapel Hill; U.S.A

Dr. Nichols summarized and up-dated outcomes of long-term studies addressing the efficacy and safety of viral gene delivery to correct the bleeding defects in hemophilic dogs.

Evaluation of a Novel Global Hemostatic Agent in Hemophilia A Dogs
David Lillicrap; Queens University; Kingston; Australia

Dr Lillicrap reviewed and extended on recently published findings on the efficacy of sulfated polysaccharides isolated from seaweed to ameliorate bleeding in hemophilic dogs.

Development of Transgenic Mice Through Lentiviral Transgenesis For Hemophilia A and B

Robert Montgomery; Blood Center of Wisconsin; Milwaukee, U.S.A.

Dr. Montgomery presented data on lentivirus-mediated gene therapy in mouse models of hemophilia A and B. The studies document the feasibility of cell-type selective expression of fVIII and IX with lentiviral vectors, discussed the potential

benefits of this approach with respect to the presence and development of inhibitory antibodies.

Blood flow devices in animal and man as models of hemostasis and thrombosis
Kjell Sakariassen; KellSa s.a.s.; Biella, Italy

Dr. K. Sakariassen (Biella, I) described the use of ex vivo flow models based on a shunt-device in translational medicine. The device resembles a stenotic flow chamber for whole blood assays. The chamber produces shear-rates conducive to rapid platelet thrombus formation, and is suitable for analysis of platelet function as well as coagulation.

Arterial Thrombosis and the Role of Thrombin in Atherosclerosis
Hugo ten Cate; University of Maastricht; Maastricht, Netherlands

Dr. TenCate presented data from the analysis of mouse models with defects in the protein C pathway and thrombin function, and how these defects modify the expression of atherosclerosis in mice. Discussions focused on the challenge of modeling in rodents the atherothrombotic phenotypes of plaque rupture/stability.

Imaging techniques for small animal in-vivo explorations: Current possibilities and evolutions
Antonio Servadio; ANIMASCOPE, Lyon, France

Dr. Servado gave a comprehensive overview of state-of-the-art non-invasive imaging in small laboratory animals. Approaches included MRI, two-photon-microscopy, and ultrasound in combination with selective molecular probes.

Discussion and Planning
Hartmut Weiler

The subcommittee will continue to provide a forum for selective topics on the use of animal models in hemostasis research. Drs. Muchitsch, Nichols, Lillicrap, and Weiler will draft a framework for a potential review of regulatory issues pertaining to the use of animal models in hemostasis research, that could serve as a reference for facilitating the establishment of animal protocols complying with regulatory requirements.