



ACBD

Australian Centre for Blood Diseases

Laboratory Descriptions and Projects

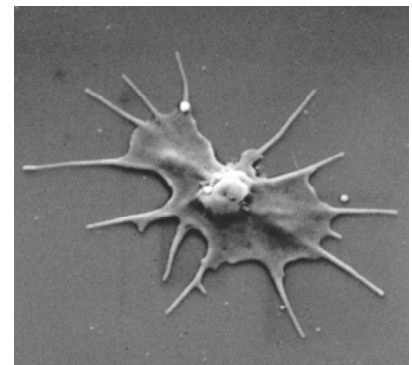
Thrombosis Research Unit

Platelets and Cardiovascular Disease

Platelets are a key ingredient in the development of blood clots (thrombosis) that lead to devastating diseases such as heart attacks and strokes. No other single cell type is responsible for as much death and disability as the platelet and, as a consequence, it represents a major target for therapeutic intervention. The growing awareness of the importance of platelets in cardiovascular diseases, as well as in the development and pathogenesis of atherosclerosis is reflected in the increasing number of patients receiving anti-platelet therapy (e.g. aspirin) to prevent cardiovascular disease. There are, however, significant drawbacks with existing therapies in many people: anti-platelet medicine doesn't provide protection against cardiovascular disease and in many patients, complications such as unwanted bleeding occur. As a result, it is difficult to predict a person's response to anti-platelet therapy: a low response doesn't afford cardiovascular protection; a high response compromises patient safety. Our laboratory's goal is to discover a 'magic bullet' that selectively targets pathological thrombus formation without causing bleeding complications. Such a discovery would help save millions of lives each year. Our recent achievements, as recognised in prestigious, high-impact journals such as *Nature*¹, include unravelling new molecular events regulating thrombosis, thereby providing promising new avenues to solving this long-standing problem.

Projects:

1. *Investigation of the effects of new anti-cancer agents on platelet function - A role for apoptosis in regulating platelet reactivity*
2. *Identification of a new family of proteins regulating blood clotting - Role of Dok2 in regulating platelet function*
3. *A new approach for the treatment of heart attack and stroke - Defining the roles of platelet protease-activated receptors in thrombosis*
4. *Investigating a new anti-clotting approach - Regulation of platelet adhesion and thrombus formation by the GPIIb/IIIa adhesion receptor*
5. *Investigating new therapeutic approaches to prevent heart attack and stroke - The role of Rho kinase inhibitors as clot lysing agents.*
6. *A New Class of Antithrombotic Agents - Investigating the role of PI 3-kinases in haemostasis and thrombosis*



Scanning electron micrograph of a spreading platelet.

¹ Jackson SP and Schoenwaelder (2003) Antiplatelet therapy: in search of the 'magic bullet'.

Nat Rev Drug Discov. (10):775-89. Review.

Jackson SP, Schoenwaelder SM, et al, (2005). PI 3-kinase p110beta: a new target for antithrombotic therapy. **Nature Medicine**, May; 11(5):507-14.

Jackson SP, and Schoenwaelder SM. (2006) Type I phosphoinositide 3-kinases: potential antithrombotic targets? **Cellular and Molecular Life Sciences (CMLS)**.



A C B D

Australian Centre for Blood Diseases

Fibrinolysis & Gene Regulation Laboratory

The Importance of Fibrinolysis in Preventing Organ Damage

While it is important to avoid blood clots in arteries leading to the heart and brain, once they are formed, it is vital that the blood clot is dismantled so blood flow can be restored thereby minimising the damage to the affected organ. The body has a natural clot dissolving enzyme system (the plasminogen activating cascade) that targets fibrin, the scaffolding of a blood clot. Clinically, proteases such as tissue type plasminogen activator (t-PA) have been used to treat patients with ischaemic strokes and heart attacks by restoring blood flow to oxygen depleted tissue. However, while blood flow is often successfully restored, t-PA can cause serious complications. For example, it is now known that t-PA has a direct action on neurons and can lead to neuronal death in ischaemic stroke—the cure can be worse than the cause.

Our laboratory studies the cell and molecular biology and regulation of the plasminogen activating cascade. We are particularly interested in understanding how t-PA and related proteases damage normal cells in the brain. We are also interested in understanding how gene expression of t-PA and other components of the plasminogen activating system are controlled and regulated in the central nervous system. Our recent work has shown that a vampire bat saliva derivative (desmoteplase) has the potential to be a clot busting drug like t-PA but without the side effects. This research has been featured on *CNN*, in *Scientific American* and *Stroke* and was considered by the American Heart Association as one of the top ten advances in 2003.

Projects:

7. *The role of mRNA stability in the regulation of tissue-type plasminogen activator gene expression*
8. *Is the expression of the plasminogen activator inhibitor type 2 gene subject to Post-transcriptional regulation?*
9. *Investigating the importance of tissue-type plasminogen activator in the formation of secondary injury following traumatic brain injury.*



Vampire Bat



ACBD

Australian Centre for Blood Diseases

Serpin Biology Laboratory

There's more than one way to skin a clot

The prevention and treatment of blood clots is a delicate act: too much anti-platelet or anti-coagulation therapy can lead to unwanted bleeding which can have catastrophic consequences (such as haemorrhagic stroke), while fibrinolysis therapy can cause brain damage. But is there another way of treating and preventing blood clots that can avoid these complications?

The Serpin Biology Laboratory focuses on the serpin (serine protease inhibitors) super family of proteins. We are developing ways of treating thrombosis patients by manipulating antiplasmin, the natural inhibitor of the clot dissolving enzyme, plasmin. In addition, our laboratory is examining the role serpins play in many diseases such as lymphoma and lung disease. Of great interest is our research into expression of centerin in lymphomas. Expression of this serpin may be useful as a diagnostic or prognostic marker in lymphoid malignancies.

Projects:

- 10. Manipulation of the plasmin-antiplasmin system to dissolve blood clots.*
- 11. Development of a clinically useful assay for centerin in B cell lymphoma*





ACBD

Australian Centre for Blood Diseases

Myeloma Research Group

Research Undertaken in the Myeloma Research Group

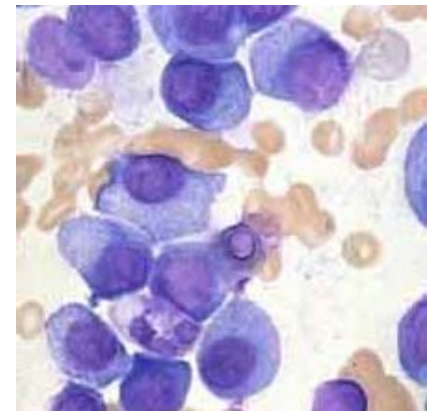
Multiple myeloma (MM) is a clonal malignancy of plasma cells characterised by the presence of a monoclonal protein in serum and/or urine, widespread osteolysis, renal failure and anaemia. Patients diagnosed with the disease have a median survival of only 2.5-3.0 years. The introduction of high-dose chemotherapy conditioned autologous stem cell transplantation (ASCT) has resulted in median survivals in the range of 5.0-6.0 years. MM remains incurable despite significant advances in treatment over the past decade with successive relapses manifesting increasing drug resistance, invariably culminating in uncontrollable and fatal disease. New treatments are constantly sought to overcome this disease.

The MRG has a long standing relationship with Novartis as exemplified by both the present translational collaboration and ongoing large-scale clinical trials of Novartis compounds being administered by the MRG under the auspices of the Australasian Leukaemia and Lymphoma Group (ALLG). Furthermore, the ongoing evaluation of additional novel compounds in a collaborative manner is also presently being carried out. The MRG has a long standing relationship with Pharmion Pty Ltd. Based on our preliminary data with azacitidine, Pharmion have agreed to support a Phase II 'proof-of-concept' trial examining azacitidine in relapsed/refractory MM.

The MRG's involvement in both clinical trials and laboratory research enables the accessibility of human bone marrow biopsy samples, facilitating our research in MM.

Projects:

- 12. Role of the bone marrow micro-environment on drug sensitivity, migration, adhesion and cytokines production by myeloma cells.*





ACBD

Australian Centre for Blood Diseases

Vascular Biology Laboratory

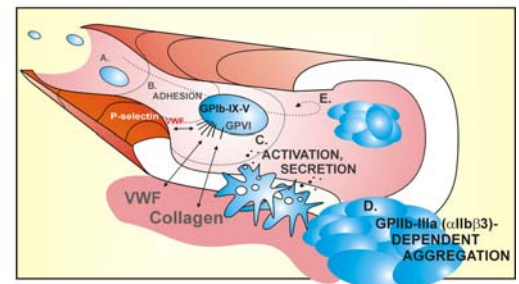
Department of Immunology, AMREP

Glycoprotein (GP) Ib-IX-V, which binds von Willebrand factor and other ligands, and GPVI that binds collagen, form a unique adhesion-signaling complex on the platelet surface, and initiate thrombus formation at arterial shear rates relevant to thrombotic diseases such as heart attack and stroke.

Research in the Vascular Biology laboratory focuses on the ligand binding, signaling and proteolytic regulation of GPIb-IX-V, GPVI and other platelet receptors. Recent research findings include the identification of dual proteolytic pathways regulating the platelet collagen receptor GPVI and the platelet Fc receptor, FcγRIIa, via metalloproteinase-mediated ectodomain shedding or intracellular calpain-mediated deactivation, respectively, relevant to thrombotic abnormalities associated with autoimmune disease or immunodysfunction.

Projects:

13. To identify binding sites for GPIb-IX-V ligands, and how these interactions regulate cell adhesion and coagulation.
14. To examine the physiological and pathological significance of receptor shedding from platelets, and assess plasma soluble GPVI as a biomarker of thrombotic abnormalities.
15. To dissect the molecular interactions and assembly of platelet receptor signalling complexes and initiation of thrombus formation.



- Shen Y, Cranmer SL, Aprico A, Whisstock JC, Jackson SP, Berndt MC, Andrews RK. Leucine-rich repeats 2-4 (Leu60-Glu128) of platelet glycoprotein Ib regulate shear-dependent cell adhesion to von Willebrand factor *J Biol Chem* 2006; 281:26419–26423
- Arthur JF, Shen Y, Kahn ML, Berndt MC, Andrews RK, Gardiner EE. Ligand binding rapidly induces disulfide-dependent dimerization of glycoprotein VI on the platelet plasma membrane *J Biol Chem* 2007; 282:30434-30441
- Gardiner EE, Karunakaran D, Arthur JF, Mu F-T, Powell MS, Baker RI, Hogarth M, Kahn ML, Andrews RK, Berndt MC. Dual ITAM-mediated proteolytic pathways for irreversible inactivation of platelet receptors: De-ITAM-ising FcγRIIa *Blood* 2008;111:165-174
- Mu F-T, Andrews RK, Arthur JF, Munday AM, Cranmer SL, Jackson SP, Stomski F, Lopez AF, Berndt MC. A functional 14-3-3ζ-independent association of PI3-kinase with glycoprotein Iba, the major ligand-binding subunit of the platelet glycoprotein Ib-IX-V complex *Blood* in press
- Andrews RK, Karunakaran D, Gardiner EE, Berndt MC. Platelet receptor shedding: a mechanism for downregulating platelet reactivity *Arterioscler Thromb Vasc Biol* 2007;27:1511-1520
- Arthur JF, Dunkley S, Andrews RK. Platelet glycoprotein VI-related clinical defects *Br J Haematol* 2007;139:363–372