British Heart Foundation

Sticking Together

Impact of BHF support for haemostasis and thrombosis research

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Contents

Sticking together: Impact of BHF support for haemostasis and thrombosis research

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Introduction: BHF supported research in haemostasis and thrombosis: 1961 – 2021

His Royal Highness, Prince Philip, was the Patron of the British Heart Foundation since the charity was founded in 1961. In 2016, Prince Philip hosted a reception at St James's Palace to celebrate his 55-year patronage and, in a short speech, said that when he had first been approached about the role that he had asked his GP for advice. His GP was strongly in favour, as increasing his own awareness could help him to avoid a thrombosis. At the reception, the BHF presented the Prince with a framed image of a thrombus taken on an electron microscope (from the laboratory of Robert Ariëns, University of Leeds). His Royal Highness quipped that having now seen a thrombus, he certainly did not want one!

Over the last 60 years, there have been many remarkable advances in the prevention and treatment of thrombosis. Major changes over this period include: the recognition of smoking as a major cause of heart disease, the importance of a healthy lifestyle, advances in surgical procedures, and the introduction of drugs including statins, antiplatelets and anticoagulants.

Over this period, the BHF has awarded almost 1000 research grants to support research in haemostasis and thrombosis. In this document, we have set out to show how these have contributed to many of the key developments over the last 60 years. We present these as a series of short articles which summarises the research and the contribution of the BHF, and the names of researchers who have gained international recognition through this support.

The UK's contribution to the field of haemostasis and thrombosis in the 1950s and 1960s

A summary of the contribution of the BHF to the field of haemostasis and thrombosis over the last 60 years would not be complete without mention of the work of four researchers, who made fundamental contributions the 1950s and 1960s, that established the UK as a leader in the field of coagulation and platelet biology.

In the 1950s, Robert Macfarlane and Rosemary Biggs working in Oxford established the basis of the blood coagulation cascade, having identified several of the factors themselves (Biggs *et al.*, 1953). Together they wrote the first guidelines for the treatment of patients in the UK with haemophilia. The Medical Research Council established the Blood Coagulation Research Laboratory in Oxford in 1967, headed by Biggs. Whilst their work was not supported by the BHF, several grants were awarded to their colleague, Peter Esnouf, to support his work on the action of vitamin K antagonists on the coagulation cascade (see Chapter 10).

In the early 1960s, Gustav Born, at the Royal College of Surgeons in London, developed a measurement of platelet clumping based on the absorption of light, known as light transmission or Born aggregometry (Born 1962). The introduction of this simple technique had a dramatic effect on platelet research and on the clinical evaluation of patients. To this day it remains the gold standard for the evaluation of platelet function disorders in patients throughout the world. The introduction of the Born aggregometer provided a way to study the activation of platelets and the effect of drugs in the absence of other cell types. Today, a Born aggregometer can be found in all leading platelet research laboratories. Born also made several other key contributions to the field of haemostasis and thrombosis including the development of intravital microscopy. He received several grants from the BHF.

Sir John Vane, also in the Royal College of Surgeons discovered the mechanism of action of aspirin and the role of the cyclooxygenase pathway in platelet activation (Vane 1971). Vane received the Nobel Prize in 1982 for 'discoveries concerning prostaglandins and related biologically active substances'. In 1973 Vane moved to Industry, to take up the position of Director of Research in the Wellcome Foundation. The work of Vane was not supported by the BHF.

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Chapter 1: Discovery of GPIb and GPIIbIIIa

Overview

The clumping (or aggregation) of platelets is mediated through binding of von Willebrand factor (VWF) and fibrinogen in the blood to their two major platelet glycoprotein receptors, GPIb-IX-V and GPIIbIIIa, respectively. Research supported by the British Heart Foundation in the late 1960s and early 1970s was pivotal in the discovery of both receptors and started the career of Alan Nurden who has been a leader in the field for over 50 years. Drugs that block the binding of fibrinogen to GPIIbIIIa were introduced into the clinic over 20 years ago for patients with acute coronary syndromes and are still used today.

The research supported by the British Heart Foundation

John French, a skilled electron microscopist and Reader in the Sir William Dunn School of Pathology in Oxford, was awarded a project grant from the BHF to support his work on thrombus formation in 1968. This supported a research assistant, Alan Nurden, who, with his supervisor, Eric Adams, was asked to describe the elements of the platelet membrane surface. It was hypothesised that these would hold the key to unlocking the role of platelets in adhesion to the injured vessel wall and to platelet aggregation. The approach was to compare the sugar (oligosaccharide) structures obtained from the surface of cow and pig platelets, and endothelial cells (Adams et al., 1970). Because of the untimely death of French, this work was continued by Nurden through a further BHF Project Grant with the help of Gustav Born in the Nuffield Institute of Comparative Medicine, London. This work benefitted from access to the platelets from a large array of mammals residing in London Zoo, including non-human primates. A breakthrough in methodology used for protein separation, known as polyacrylamide gel electrophoresis (PAGE), enabled Nurden to identify a large, highly acidic glycopeptide on the platelet surface (Nurden, 1974). A further refinement in the method of separation of proteins, known as SDS-PAGE, led to the discovery of two further major glycoproteins (Nurden and Caen, 1975). The three proteins became known as GPIb, GPIIb and GPIIIa.

The importance of the discovery of these proteins to platelet biology was not known at the time. Nurden then began a collaboration with Jacques Caen in Paris, to study two groups of patients with inherited disorders that were associated with bleeding: Glanzmann thrombasthenia and Bernard-Soulier syndrome. Platelets of Glanzmann thrombasthenia patients are unable to aggregate in the presence of physiologic agonists but the reasons for this were not understood. Upon glycoprotein analysis, Nurden discovered that GPIb was present, but that GPIIb and GPIIIa were absent. Thus, a link between the two absent membrane glycoproteins, which are now known to form the complex GPIIb-IIIa, and platelet aggregation was formally established (Nurden and Caen, 1974).

Patients with Bernard-Soulier syndrome have a low platelet count and giant platelets, a combination known as macrothrombocytopenia. These large platelets show a decreased surface expression of sialic acid, and are unable to bind to bovine Factor VIII. Upon glycoprotein analysis, Nurden observed the opposite results as for the Glanzmann thrombasthenia patients. Here, no GPIb was present, but GPIIb and GPIIIa appeared normal (Nurden and Caen, 1975). Observations by others quickly unveiled that VWF was an important protein required for the adhesion of platelets to damaged vessel walls. Since this interaction had been shown to be abolished in patients with Bernard-Soulier syndrome, Nurden's findings helped to piece together the importance of the VWF-GPIb

interaction in platelet adhesion. It was also established around this time that GPIb complexed with GPIX and GPV on the platelet surface, to form the GPIb-IX-V receptor complex. It is now known that this complex also binds to key components in the coagulation cascade such as thrombin, Factor XI, Factor XII and P-selectin to support platelet aggregation.

Thus, studies funded by the BHF were at the origin of the discovery of the major platelet receptors for adhesion and aggregation, key players in thrombus and haemostasis. Nurden continued to work on the genetics of Glanzmann's thrombasthenia and Bernard-Soulier syndrome, and the function of GPIIb-IIIa and GPIb-IX-V, in Paris and Bordeaux until his retirement.

Perspective

The later work of Barry Coller, a clinical haematologist, in the USA built on the work of Nurden by showing that he could inhibit the aggregation of activated platelets with a blocking antibody. This discovery in 1983 led to the production of the first GPIIb-IIIa blocker. In 1994, the Fab fragment, abciximab, based on one of Coller's monoclonal antibodies, was introduced into the clinic. Abciximab is primarily used to prevent thrombus formation in patients undergoing procedures to widen narrowed or blocked blood vessels. During such procedures, damaged tissue is a prime target for platelets to bind to and undergo activation and aggregation. Without therapeutic intervention, this can cause the repaired vessel to re-occlude and ultimately result in myocardial infarction.

GPIb has also been an attractive target for drug development, but while anti-GPIb candidates have shown promise in animal models, they have yet to come to fruition in the clinic.

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Chapter 2: Large-scale genetic screens in patients with inherited bleeding disorders

Overview

The study of patients with inherited bleeding disorders is a very powerful way to understand the events that underlie haemostasis and to potentially identify new targets for the prevention of thrombosis. The study of Glanzmann's thrombasthenia and Bernard Soulier syndrome patients led to identification of the function of GPIIb-IIIa and GPIb-IX-V, respectively, with the bleeding disorders taking the name of the clinicians who first described the conditions (Chapter 1). This same approach has led to the discovery of many of the major proteins involved in coagulation and platelets, including Factor VIII, which causes haemophilia, and the collagen receptor, GPVI. The start of the genomic revolution in the 1980s led to the first blueprint of the human genome in 2002. Since then, continuing advances in the rapid sequencing of genomes have taken hold and have led to sequencing platforms playing a critical role in the diagnosis of patients with suspected bleeding disorders. The BHF has been a major supporter of this genetic revolution. Genetic testing of patients has led to the identification of many new genes involved in haemostasis, some of which are targets for the development of novel antithrombotic drugs.

The research supported by the British Heart Foundation

Patients with a severe bleeding disorder are at risk of excessive bleeding, which can be life-threatening. Such severe inherited disorders are extremely rare and are nearly always diagnosed in early life. So-called mild disorders are also rare but are more difficult to diagnose, notably in the case of platelet disorders, due to the absence of a gold standard test and an overlap in the responses of healthy controls. Prior to the sequencing of the human genome, there were relatively few examples of gene defects linked to mild bleeding, and some of these were found due to their association with other clinical symptoms. Functions disrupting mutations in the tyrosine kinase enzyme Btk give rise to an immunodeficiency syndrome, X-linked agammaglobulinemia (XLA). Based on the discovery of similarities in signalling by immune cells and platelets, a BHF student, Lynn Quek, observed a defect in activation by collagen in the platelets of XLA patients (Quek *et al.*, 1999). Inhibitors of Btk have since been developed for the treatment of patients with B cell malignancies and have been proposed as a potential novel anti-thrombotic that preserves haemostasis, by a BHF clinical fellow (Nicolson *et al.*, 2021).

The sequencing of the human genome fuelled two large genomic studies in the UK aimed at identifying causative gene defects that give rise to bleeding. The UK Genotyping and Platelet Phenotyping (GAPP) study tested blood from patients with suspected platelet disorders, identified by Haemophilia Care Centres throughout the UK, to a battery of functional tests and to whole exome sequencing. This approach led to the identification of a series of novel platelet-related genes and mutations, including patients with defects in genes encoding platelet receptors, such as ADP and thromboxane A_2; and the transcription factors *RUNX1* and *SLFN14* (Watson *et al.* 2013; Fletcher *et al.* 2013). In a much larger and wider-ranging study, that involved several international partners, Willem Ouwehand and colleagues in Cambridge used the power of whole exome sequencing of over 10,000 patients to identify novel causative genes associated with blood clotting and red blood cell formation (Truro *et al.* 2020). As part of this, they developed a

thrombogenomic high throughput screen for testing the known, most commonly disrupted genes, as the first step in diagnosing a bleeding disorder. They then tested this on almost 2,400 index cases (Downes *et al.* 2019). The result led to a molecular diagnosis in over one third of patients and the identification of over 700 unique variants. This work was supported by several major grants including from the BHF, NIHR and European Union. Together, these studies have revolutionised our understanding of bleeding disorders and the approach to diagnosis.

Perspective

While the development of the thrombogenomic high throughput screening platform has changed the way that we study patients with bleeding disorders, the definitive diagnosis of a mild bleeding disorder continues to remain challenging. Further, it is now recognised that bleeding in many of the patients is a complex trait, caused by defects in two or more genes. The development of more complex algorithms and improved functional tests are required before we can rely solely on genetic sequencing to identify the underlying cause of bleeding in disorders which are not caused by a single genetic mutation (see Chapter 18 for discussion of the future of genomics and platelet function testing).

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Chapter 3: Major clinical trials supported by the BHF

Overview

Clinical trials of antiplatelet, anticoagulant and fibrinolytic drugs have had a sound impact on the survival chances of patients presenting to hospital with thrombotic conditions such as heart attack, ischaemic stroke and pulmonary embolism. Patients presenting with these conditions can now expect radically better prognosis as a result of these antithrombotic drugs in addition to other evidence-based medications such as beta-blockers, statins and blood-pressure-lowering medications. Assimilating the evidence from numerous trials into meta-analyses has promoted the adoption of effective antithrombotic strategies into routine clinical practice. In terms of primary prevention of thrombotic events, there remain outstanding questions about which patients to target with which antithrombotic drugs and large clinical trials provide insights into more precise targeting of individual patients.

Some clinical studies supported by the British Heart Foundation

The ISIS-1 trial was an early large-scale study of 16,027 heart attack patients that demonstrated a protective effect of the beta-blocker atenolol and established the routine use of beta-blockers in these patients (ISIS-1 Collaborative Group 1986). This trial was initiated by Peter Sleight, the first BHF of Cardiovascular Medicine in Oxford. Subsequently, the characterisation of aspirin's effects on blood platelets as well as the recognition of the role of platelets in heart attacks underpinned the pivotal ISIS-2 study, which assessed the effect of aspirin versus placebo, as well as the thrombolytic drug streptokinase versus placebo, in patients presenting to hospital with major heart attack. The BHF provided support for this study alongside the manufacturer of streptokinase and the results were published in The Lancet in 1988 (ISIS-2 Collaborative Group 1988). The study demonstrated that aspirin saved a similar number of lives to streptokinase and that the two drugs together were the most effective strategy, reducing mortality from 13.2% with neither drug to 8.0% with both drugs. This phenomenal success led to the standard use of aspirin in the acute management of heart attack, a practice that persists to this day.

Many trials looked at the effects of aspirin and other antiplatelet drugs in a variety of patient groups, generating a huge amount of evidence. A lot of these studies were smaller and less conclusive than the ISIS-2 study so it was necessary to perform meta-analyses in order to synthesise evidence that could guide clinical practice. Supported by the BHF, the Antiplatelet Trialists' Collaboration, morphing subsequently into the Antithrombotic Trialists' Collaboration, performed a series of meta-analyses that were published between 1994 and 2009 in high-impact journals; these proved highly influential in international clinical guideline recommendations for the use of aspirin (Antiplatelet Trialists' Collaboration 1994-2009).

Much uncertainty has surrounded the use of aspirin for primary prevention in patients with diabetes. Consequently, the BHF supported the ASCEND trial of more than 15,000 diabetes patients, randomising them to aspirin or placebo (ASCEND Study Collaborative Group 2018). This trial clearly delineated the balance between benefits and risks of aspirin in this population and underpinned the latest guideline recommendations on the use of aspirin for primary prevention in diabetes.

Perspective

Over the last 40 years, the BHF has played a key role in supporting large-scale clinical trials and the synthesis of evidence that is the foundation for our current evidence-based practice in cardiovascular medicine and has informed many international guideline recommendations on the use of antithrombotic drugs, thus improving survival in heart attack and stroke patients and preventing future heart attacks, strokes and cardiovascular deaths.

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Chapter 4: The $P2Y_{12}$ ADP receptor: a target for antiplatelet drugs

Overview

Adenosine diphosphate (ADP) plays a critical role in reinforcing platelet activation and thrombus growth through the $P2Y_{12}$ receptor, which is the target for antiplatelet agents used to treat or reduce the risk of heart disease and stroke. The thienopyridine clopidogrel, an irreversible inhibitor of the $P2Y_{12}$ receptor, was approved for clinical use in 1997. Clopidogrel is a prodrug, which delays its onset of action, and its irreversible nature means that its effects cannot be stopped in patients with bleeding episodes. In the early 1980s, the BHF supported the work of Noel Cusack, who developed a series of ADP analogues as reversible inhibitors of platelet activation (Cusack and Hourani, 1982). The prototype of these led to the development of the reversible $P2Y_{12}$ receptor antagonist, cangrelor, which entered the clinic in 2015 for intravenous use, due to its rapid onset of action. This work also catalysed a discovery programme by Fisons (now AstraZeneca), that led to the development of the first oral reversible inhibitor of the $P2Y_{12}$ receptor, ticagrelor, which entered the clinic in 2010.

The research supported by the British Heart Foundation

ADP and thromboxane A_2 (TxA_2) are feedback agonists that reinforce platelet activation at sites of injury and are targets for the major two classes of antiplatelet agent. Aspirin inhibits the formation of TxA_2 and was recognised as an antiplatelet agent in the early 1980s, but it was not until the late 1990s that the $P2Y_{12}$ receptor antagonist, clopidogrel, was approved for clinical use. While clopidogrel is still one of the most commonly used drugs in the world, it is not ideal. This is due to its irreversible nature meaning its effects cannot be stopped, except through transfusing donor platelets. In addition, the antiplatelet effect of clopidogrel is unpredictable, as a result of wide inter-individual variation in plasma levels of its active form and interactions with other drugs (Judge et al., 2020). The introduction of two reversible antagonists (cangrelor and ticagrelor), initiated by the BHF-funded research of Cusack and Hourani (Cusack and Hourani, 1982), has addressed these two concerns. Significantly, ticagrelor was shown to reduce cardiovascular mortality compared to clopidogrel in the PLATO study (Wallentin *et al.* 2009).

Other than the support of Noel Cusack's research, there was relatively little support from the BHF on platelet activation by ADP until sequencing of purinoceptors in the late 1990s led to the discovery that platelets express three members of the family, $P2Y_1$, $P2Y_{12}$, and $P2X_1$. The effects of clopidogrel, cangrelor and ticagrelor are all mediated by blockade of the $P2Y_{12}$ receptor. The discovery of the three receptors stimulated a series of grants from BHF, which included an intermediate and senior fellowship award to Stuart Mundell in Bristol. This work has confirmed that $P2Y_{12}$ is the most important of the three receptors, and that it synergises with other platelet receptors in mediating activation. Further studies by Mundell on the nature of the inhibitory action of ticagrelor are anticipated to lead to the development of superior antagonists (Aungraheeta *et al.* 2016).

The BHF have also supported the research of Robert Storey in Sheffield on the role of the $P2Y_{12}$ receptor in pathways beyond arterial thrombosis, namely in inflammation and neointimal hyperplasia (Judge *et al.* 2005; Evans *et al.* 2009). This work has identified a

mechanism whereby $P2Y_{12}$ receptor antagonists might prevent restenosis of coronary arteries, which opens up their potential use in a range of cardiovascular diseases caused by inflammation (a field known as thrombo-inflammation). This potentially includes their use to ameliorate the vascular inflammatory effects of COVID infection.

Given the importance of the $P2Y_{12}$ receptor in platelet activation, it is unsurprising that patients with mutations which disrupt the function of the receptor have an increased risk of bleeding. Some of the first patients to be identified were in the UK and have provided new information on the multigenic nature of bleeding disorders and regulation of the $P2Y_{12}$ receptor. For example, Daly *et al.* (2009) showed that mutations in the $P2Y_{12}$ receptor were enriched in patients with the bleeding disorder von Willebrand disease, while Nisar *et al.* (2011) demonstrated the importance of internalisation in regulating $P2Y_{12}$ receptor function based on the identification of a patient with a novel mutation that prevented recycling to the platelet surface.

In work supported by the BHF, the group of Tim Warner at Queen Mary University of London have questioned whether the combination therapy of aspirin and $P2Y_{12}$ inhibition is more effective than $P2Y_{12}$ blockade alone (Leadbeater *et al.* 2011). This work prompted studies of the early cessation of aspirin after percutaneous coronary intervention in ticagrelor-treated patients, thus reducing the risk of bleeding. Further BHF-supported work provided some clarity on the contribution of aspirin to platelet inhibition in these patients (Hennigan *et al.* 2020).

Perspective

The BHF's support of the work of Noel Cusack in the 1980s eventually led to the introduction of reversible $P2Y_{12}$ receptor antagonists into the clinic. The long delay from discovery to clinical use reflects the challenges of introducing new medicines to treat those patients at risk of thrombosis. Fundamental research supported by the BHF will determine the future clinical use of $P2Y_{12}$ receptor antagonists in additional settings, including in thrombo-inflammation.

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Chapter 5: von Willebrand factor and GPIb-IX-V

Overview

Von Willebrand factor (VWF) is a large multimeric protein in the blood that captures platelets in the fast-flowing arterial circulation by binding to a unique receptor on the platelet surface, GPIb-IX-V, and bridging to exposed collagen fibres. VWF also binds to Factor VIII and prevents its degradation. GPIb-IX-V binds to several other proteins including Factors XI and XII, and integrin Mac-1 on leucocytes. Patients with low levels or mutant forms of VWF have a bleeding disorder which ranges from mild to severe, known as von Willebrand's disease.

Patients with mutations in GPIb-IX-V have large platelets and may also have a severe bleeding disorder. VWF is made in megakaryocytes and endothelial cells and released as large multimers which are broken down by a metalloproteinase in the blood, ADAMTS13. A small number of patients develop antibodies to ADAMTS13, usually in adulthood, and this gives rise to the life-threatening blood disorder thrombotic thrombocytopenic purpura (TTP), which is associated with blood clots and a low platelet count.

The research supported by the British Heart Foundation

GPIb was first identified by the BHF-funded studies of Nurden, as discussed in Chapter 1. The BHF supported early structural studies of Michael Barnes on the interaction of VWF and collagen in the 1980s (Fitzsimmons *et* al., 1988; Cockburn *et al.* 1989), and on the genetics of von Willebrand's disease by Martina Daly in Sheffield in 2000 (Allen *et al.*, 2000a and 2000b). From the start of the millennium, the BHF have funded a series of grants on the characterisation and structure of VWF, GPIb and ADAMTS13 from the groups of Crawley, Laffan and Lane at Imperial College London, and Emsley at the University of Nottingham.

The BHF has funded the research of David Lane and Jim Crawley (a former BHF Intermediate Fellow) since 2002 to investigate many aspects of ADAMTS13-VWF function, most notably how the structural domains of ADAMTS13 help it to recognise and cleave VWF (South *et* al., 2014; Xiang *et al.*, 2011), and how the level of ADAMTS13 in the body can contribute to stroke and heart attacks (Anderson *et al.*, 2012). ADAMTS13 has a large and complex structure and uniquely cleaves VWF at a single site, thereby breaking down large multimers of VWF in the blood which could otherwise cause thrombosis and platelet consumption. A small number of patients develop antibodies to ADAMTS13 which disrupt function and give rise to the life-threatening disorder thrombotic thrombocytopenic purpura (TTP).

The research of Mike Laffan and Tom McKinnon (also a former BHF Intermediate Fellow) has contributed to our understanding of the role glycans in determining the function of VWF. This includes the role of glycans in regulating the function of the A1 and A2 domains in VWF, which bind to GPIb and harbour the site of cleavage by ADAMTS13, respectively. VWF is one of the plasma proteins carrying the ABO blood group antigens. N-linked and O-linked glycosylation are the most common co/posttranslational modification of VWF, accounting for approximately 20% of its total molecular weight. Laffan and McKinnon produced the first site specific map of VWF glycans and characterised them in unprecedented detail (e.g. Canis *et al.* 2012). They have provided evidence of the critical

role that individual N-linked glycans have on both the synthesis and expression of VWF (McKinnon *et al.* 2010). They have shown that under shear stress O-linked glycosylation modulates VWF-platelet interactions, as well as influencing the ability of VWF to adhere to collagen and itself (Nowak *et al.* 2012), and reduce the susceptibility to cleavage of VWF by ADAMTS-13 (Nowak *et al.* 2013).

Jonas Emsley has studied the structural basis of GPIb both in regard to how mutations in Bernard Soulier syndrome prevent association with the GPIX subunit in the receptor complex (McEwan *et al.* 2011) and how this supports its interaction with the integrin Mac-1 on leukocytes (Morgan *et al.* 2019). The BHF have supported studies on GPIb signalling, notably from the group of Alastair Poole in Bristol (Falati *et al.* 1999). GPIb is a weak stimulus of platelets and this appears to be a minor role relative to its significance as an adhesive receptor and binding protein.

Perspective

The risk of severe bleeding has held back research into the therapeutic targeting of the VWF-GPIb interaction, despite its involvement in many blood disorders, including thrombosis. In 2018, a dimeric nanobody (antibody fragment) that blocks the binding of WVF to GPIb, caplacizumab, entered the clinic for the treatment of patients with TTP. Treatment with caplacizumab is associated with an increase in bleeding and this prevents its use as a routine anti-thrombotic. We predict that future work will reveal other ways to perturb the VWF-GPIb axis without causing excessive bleeding. For example, Lane has proposed the use of forms of ADAMTS13 with enhanced function in the treatment of stroke (South and Adams, 2018). The further understanding of site-specific variation of glycyosylation in influencing function will be important in designing further haemostatic variants. For example, the newly licensed recombinant VWF lacks ABO blood groups and has a significantly longer half-life than plasma derived VWF.

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Chapter 6: Collagens and collagen receptors

Overview

Collagen has been termed the most thrombogenic (i.e. platelet-activating) component in the blood vessel wall and plays a critical role in platelet adhesion and platelet activation. Collagen binds to von Willebrand factor (VWF) at sites of injury and bridges it to its receptor, GPIb. In addition, collagen directly supports adhesion and activation of platelets through two receptors, the integrin receptor GPIa-IIa (which is related to GPIIb-IIIa, Chapter 1) and glycoprotein GPVI, respectively. The BHF has played a critical role in dissecting the role of collagen in platelet adhesion and aggregation through the research of Michael Barnes and Richard Farndale in Cambridge, and Steve Watson initially in Oxford and then as a BHF Chair in Birmingham. Barnes and Farndale have identified the regions in collagen which bind to GPIb, GPIa-IIa and GPVI, and made collagen-based peptides that target these regions. Watson elucidated the mechanism of activation of platelet by collagen showing that it signals through a pathway that is shared with Fc and antigen receptors.

The research supported by the British Heart Foundation

The backbone of collagen is a three amino acid repeat, with every third amino acid being glycine. This sequence enables collagens to form a unique helical structure and makes the study of collagen highly specialist. The BHF first supported the work of Michael Barnes's laboratory in the 1980s, which was continued on his retirement by Richard Farndale in the 1990s. Their work led to the generation of a series of synthetic collagen-related peptides, which mediate binding to VWF, GPIa-IIa and GPVI. A GPIa-IIa specific peptide has been co-crystalised with its receptors (Emsley et al., 2000), a major advancement since knowledge of the binding site on the receptor is a prerequisite for the development of blocking agents. The collagen-related peptides have become critical tools to the community for studying the functional significance of collagen in platelet adhesion and aggregation.

Watson demonstrated that platelet activation by collagen was triggered by the binding of the tyrosine kinase, Syk, to the Fc receptor □-chain. Until then, this pathway was thought to be unique to Fc and antigen receptors in white cells. The discovery of this mechanism of platelet activation eventually led to the identification of GPVI as the major signalling receptor for collagen. Watson and others went on to further characterise this pathway, benefitting from the use of transgenic mouse models made by other laboratories. This was one of the first times that transgenic had been used to study the mechanism of platelet activation. This work revealed that the glycoprotein receptor signals through the sequential activation of Src, Syk and Btk families of tyrosine kinases, as well as through other proteins shared with myeloid cells and lymphocytes (Nieswandt and Watson, 2003).

Perspective

Collagen is enriched in atherosclerotic plaques. Plaque lesion mediates the powerful activation of platelets through GPVI which is therefore a target for a new class of antithrombotic agent. The inability to generate small molecule inhibitors of GPVI has held back this field. Recently a French academic, Martine Jandrot-Perrus, has taken a blocking

antibody to phase II trial for thrombotic stroke. In addition, inhibitors of Src, Syk and Btk kinases have entered the clinic in the treatment of blood cancers and immune thrombocytopenia and represent an alternative mechanism of GPVI (Harbi et al., 2020). Many of the inhibitors of Btk are irreversible which, by analogy to aspirin, would allow them to be used at a 'low dose' to selectively target platelets which cannot make new protein to overcome the inhibition.

The collagen-related peptides developed by Barnes and Farndale (Farndale et al., 2008) have been used by over one hundred research groups world-wide and are now available through a university spin-out company.

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Chapter 7: Thrombo-inflammatory disease: a new paradigm in thrombosis

Overview

There is increasing recognition of the role of the interaction between blood coagulation, endothelial cells, extracellular vesicles and leucocytes (white cells) in diseases of the heart and circulation. This has developed into a new branch of thrombosis research, termed thrombo-inflammation. In this miscellaneous group of disorders, uncontrolled inflammation can drive thrombosis and, in turn, be perpetuated by thrombosis. This vicious cycle of events can lead to chronic inflammation in the vessel wall from which a variety of thrombotic complications can arise such as deep vein thrombosis (DVT) The understanding of the mechanisms of thrombo-inflammation opens up new opportunities for intervention in diseases such as DVT, infection and ischaemia-reperfusion injury. BHF-funded researchers have played a pivotal role in recognising this as a distinct molecular pathway of vascular inflammation and thrombosis.

The research supported by the British Heart Foundation

Until the early 1970s, the cause of heart attacks and strokes was not known. Through the research of the BHF Chair, Michael Davies, and others, we now know that these are caused by blood clots in the coronary and carotid arteries, at sites of atherosclerotic plaque formation (Davies MJ 1996). Rupture of the plaque exposes a prothrombotic surface that triggers an occlusive thrombus. While, the mechanism of plaque formation is beyond the scope of this review, around the mid-1990s and early 2000s a new mechanism of vessel wall injury became recognised, in which inflammatory events triggered thrombosis and vice-versa, and, paradoxically, bleeding in patients with a low platelet count. This is now recognised as a new branch of thrombosis, termed thrombo-inflammation, and refers to conditions in which the interplay of inflammatory cells, blood coagulation, extracellular vesicles and endothelial cells triggers a vicious cycle of events that lead to thrombus formation.

BHF funded researchers, including Ed Rainger and Gerard Nash at the University of Birmingham, were among the first to recognise the interaction between platelets and leucocytes (neutrophils and monocytes) in the vasculature in the 1990s. In the early 2000s, this interaction was also shown to occur at the vessel wall and to be influenced by surrounding cells (e.g. smooth muscle, and fibroblasts), causing endothelial cell activation and the generation of a prothrombotic and proinflammatory environment (Tull *et al.*, 2006; Kuckleburg *et al.*, 2011). This work has since been extended, to show the importance of extracellular vesicles in modifying the endothelial surface (Kuravi *et al.*, 2019).

The discovery that low platelet counts are associated with bleeding at sites of inflammation was made by researchers in the USA and France. However, the BHF has funded research that shows the underlying mechanism varies between vascular beds (Rayes *et al.*, 2018), coining the term inflammatory haemostasis to describe this unique mechanism of blood clotting.

Perspective

The recognition of thrombo-inflammation as a distinct form of thrombosis has evolved over recent years. While the definition remains imprecise, it has opened up a new approach to thrombosis. This is illustrated by the work of a BHF Senior Researcher, Alex Brill at the University of Birmingham, through the discovery of a role for mast cells in triggering thrombosis in DVT suggesting that mast cell stabilisers may be a novel treatment for DVT without causing an increase in risk of bleeding (Ponomaryov *et al.*, 2017).

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Chapter 8: CLEC-2 – a new target in inflammation-driven thrombosis?

Overview

In 2006, the unexpected discovery of a new class of activating receptor on platelets, CLEC-2, was reported by the group of the BHF chair-holder, Steve Watson. We now know that CLEC-2 plays a critical role in the activation of platelets during development of the vasculature in the brain and of the lymphatics, and in supporting haemostasis at sites of inflammation in the vessel wall in adults. In addition, CLEC-2 has been shown using mouse models to drive thrombosis in the venous system in disorders such as deep vein thrombosis. CLEC-2 is also activated by haem released from damaged red blood cells, suggesting that it may play a role in driving thrombosis in vascular microangiopathies such as sickle cell disease. CLEC-2 has a minor role in haemostasis and is present at low level on a sub-population of other white cells therefore making it an attractive target for a new class of antiplatelet drug. The discovery of CLEC-2 also brought attention on the non-haemostatic role of platelets, notably in inflammation. The understanding of the non-haemostatic role of platelets is critical for predicting off-target effects of blocking CLEC-2 function.

The research supported by the British Heart Foundation

A variety of venomous toxins have been shown to mediate powerful activation of platelets. In 2003, the BHF awarded a grant to a PhD student, Gemma Fuller, to look for the receptor that mediated platelet activation by the snake venom toxin, rhodocytin. Working with a postdoctoral fellow, Katsue Suzuki-Inoue, Fuller was identified a novel activating receptor on platelets, CLEC-2, using rhodocytin as an affinity ligand (Suzuki-Inoue et al., 2006). This discovery was unexpected as the existence of a novel activating receptor was not predicted. The later identification of the membrane protein podoplanin as the endogenous ligand by Suzuki-Inoue after her return to Japan was also unexpected and raised the question as to the physiological significance of the interaction with CLEC-2. Podoplanin is expressed on many cell types including epithelial and stromal cells, and inflammatory macrophages, but is absent in the vasculature.

The first clues on the function of CLEC-2 in platelets came from studies on the embryos of mice deficient in the rhodocytin receptor, which revealed its role in the development of both the brain vasculature and the lymphatic system. The deletion of CLEC-2 post-development is well tolerated and mice are viable with a normal life-expectancy. However, the loss of CLEC-2 is associated with an increase in bleeding at sites of inflammation in the vessel wall, which is now recognised as a distinct from of haemostasis, termed inflammatory haemostasis (Rayes *et al.*, 2019). CLEC-2 has been shown to drive thrombosis in mouse models of thrombo-inflammation, such as deep vein thrombosis and in infection (Hitchcock *et* al. 2015; Payne *et al.* 2017). CLEC-2 has recently been identified as a receptor for haem and proposed to play a role in thrombosis in conditions associated with red blood cell lysis, such as sepsis (Bourne et al., 2021).

Perspective

The majority of the research on CLEC-2 has been performed on mouse models, as humans lacking functional CLEC-2 have not been identified, possibly because this is lethal *in utero*. Clues as to the function of CLEC-2 in humans have come from the observation that its ligand, podoplanin, is up-regulated at sites of inflammation such as in liver disease (Chauhan *et al.,* 2020). Btk inhibitors which are used to treat blood cancer block activation by CLEC-2 (Nicolson *et al.,* 2021) and are associated with a reduced incidence of deep vein thrombosis (Nicolson *et al.,* 2020) suggesting that they may be repurposed as novel antiplatelets for prevention of venous thrombosis. This work was driven by Pip Nicolson who held a BHF Clinical Studentship. Further Btk inhibitors can be used at 'low dose' as, like aspirin, they are irreversible and so this will minimise their off-target effects.

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Chapter 9: Fibrin and clot structure

Overview

Research into the structure of blood clots started some 20-30 years ago and has progressed considerably in the last decade, with UK-based efforts and BHF funding playing a leading role. Clots are mainly composed of platelets, red blood cells and fibrin, the latter produced from fibrinogen by thrombin-cleavage and provides a filamentous network to support the clot. Research into fibrin clot structure is of essential interest due to key considerations: 1) it results from the combined effects of coagulation factors and their inhibitors, thereby reflecting the overall potency or status of coagulation, and 2) it plays a critical role in functional characteristics such as clot mechanical properties, resistance to fibrinolysis (Chapter 11) and its interaction(s) with vascular cells (platelets, red and white blood cells, and endothelial cells). Recent efforts have started to disentangle the molecular and cellular mechanisms that regulate clot structure, other than coagulation activity status alone, thereby indicating potential future targets for the development of new treatments for the prevention and breakdown of deadly clots. The advent of endovascular thrombectomy devices (minimally invasive clot removal devices) has helped to provide novel ways of treating deadly clots in the heart or brain. Increasingly, however, the structure and mechanical properties of the clots to be removed are found to impact on the effectiveness of such procedures, further emphasising the importance of clot structure for patients with cardiovascular disease.

The research supported by the British Heart Foundation

Early BHF-funded research in this area by Robert Ariens in Leeds focussed on factor XIII crosslinking sites in fibrin, demonstrating key roles of crosslinking in the visco-elastic or mechanical properties of fibrin (Standeven *et al.* 2007). Further studies, by Peter Grant in Leeds, investigated the interactions between factor XIII on fibrinogen (the soluble precursor molecule to fibrin), showing a specific binding site for factor XIII on the fibrinogen □C-region (Smith *et al.* 2013). Together, these studies have started unpicking key molecular mechanisms that impact on clot mechanical properties through crosslinking by factor XIIIa. Additional mechanisms that regulate fibrin clot structure were elucidated by Nicola Mutch in Aberdeen (who held a BHF Intermediate Fellowship), showing that polyphosphate, which is released from the dense granules of activated platelets, changes the distribution of fibrin fibres and delays clot breakdown by the fibrinolytic system (Mutch *et al.* 2012).

Recent studies in this area have revealed how thrombin and a common fibrinogen splice variant determine protein packing in fibrin fibrils (Domingues *et al.* 2016). These Leeds-based studies showed, for the first time, how this fibrinogen variant can change the molecular make-up of fibrin fibrils, thereby impacting on clot stability and strength. Another key new discovery is the description of a new fibrin film that covers the borders of blood clots and protects the clot from infection (Macrae *et al.* 2018). This discovery resulted from a junior BHF-funded PhD student, Fraser Macrae, challenging dogma in the field that such films were an artefact of sample handling procedures. Macrae produced stunning data that convinced his supervisor, Robert Ariens, that the films were indeed a physiological entity, not an artefact, which required further detailed study. Subsequent studies showed that fibrin films on clots are formed as protein films and are important to stop microbial infection. These films have also been observed to support clot contraction

and are present on coronary thrombi, where their function and role remains to be fully described.

It has become increasingly apparent that changes in clot structure underpin the risk of thrombosis and cardiovascular disease in clinical studies. Recent BHF-funded studies have shown how the mechanical and elastic properties of clots are critical for venous thromboembolic disease (Baker *et al.* 2019). Crucially, new BHF-funded prospective studies, by Robert Storey in Sheffield and Ramzi Ajjan in Leeds have shown how abnormal clot structure and resistance to fibrinolysis are observed in a large cohort of patients with coronary arterial disease, and that they associate with poor outcome, disease recurrence and mortality (Sumaya *et al.* 2018). This was particularly notable in the patients with diabetes (Sumaya *et al.* 2020). These important results indicate for the first time that abnormal clot structure and resistance to fibrinolysis are not merely spectators in cardiovascular disease, but play a causal role in disease progression and evolution.

Perspective

With the help of BHF support, researchers in the UK have been able to characterise and establish clot structure as an important contributor, mechanism and risk factor for thrombosis in cardiovascular disease. These studies have provided key insights into the molecular regulation of clot structure, its mechanical properties and resistance to breakdown. Elegant clinical studies have shown the predictive nature of clot structure and resistance to lysis in cardiovascular disease. But many questions remain and the mechanisms by which the fibrin clot interacts with platelets, red blood cells, neutrophils and endothelial cells remain to be fully characterised. While we understand more about the mechanisms that regulate fibrin polymerisation and blood clot architecture, future efforts should focus on ways to change clot structure, making clots structurally more sensitive to breakdown when no longer needed, yet sufficiently strong to stop bleeding.

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Chapter 10: Thrombin generation and development of new anticoagulants

Overview

The blood clotting system (or coagulation cascade) serves to generate thrombin, which stimulates powerful activation of platelets and the formation of fibrin. Fibrin polymerises (gels) to form a rope-like structure which hold the clot together. Funding from the BHF to Peter Esnouf, in Oxford, throughout the 1970s played a key role in our early understanding of the coagulation cascade and the mechanism of action of vitamin K antagonists, such as warfarin. However, the major impact of the BHF to this field resulted from the support of protein structural work of Lane, Carrell and Huntington in the 1980s – 2000s in Cambridge and Imperial. The BHF supported the elucidation of the structures of major proteins involved in blood clotting, including antithrombin, factor X and thrombin. Resolving the structural aspects of these key molecules involved in clotting has helped to pave the way for the development of new anticoagulants, now known as direct oral anticoagulants (DOACs), by the pharmaceutical industry.

The research supported by the British Heart Foundation

The BHF was founded in 1961, by which time two British researchers, Rosemary Biggs and Robert Macfarlane, had mapped out the basis of the blood coagulation cascade. While Biggs and Macfarlane never benefited directly from BHF funding, their work paved the way for Esnouf to help establish the molecular mechanisms by which warfarin inhibits coagulation (Esnouf *et al.*, 1978). Warfarin is a key drug used in the prevention of thrombotic stroke in patients with atrial fibrillation, although it is now being increasingly replaced by the newly developed DOACs. Subsequent research funded by the BHF on the coagulation cascade focussed on powerful analytical tools to study protein structure, such as X-ray crystallography, in addition to nuclear magnetic resonance (NMR) and mutational analysis, and using such tools to unravel the structural molecular basis of thrombin generation and its inhibition. Studies using mutational analysis, by David Lane in London, investigated molecular structures and mechanisms involved in the inhibition of thrombin by antithrombin (Lane *et al.* 1987). Further studies elucidated how thrombin interacts with factor XIII (Philippou *et al.* 2003), and how a common mutation in factor XIII, Val34Leu, accelerates this interaction and consequently factor XIII activation by thrombin (Ariens *et al.* 2000). Robin Carrell, in Cambridge, focussed on the structural characterisation of thrombin, factor Xa and the inhibition of thrombin by antithrombin (Carrell and Owen. 1985; Carrell *et al.*, 1994; Chang *et al.*, 1996) These investigations provided detailed information on the structure and molecular mechanisms that underpin how thrombin interacts with heparin. They also elucidated an important serpin (serine protease inhibitor) based mechanism of protease inhibition, employed by antithrombin, applicable to serpins across the wider biochemistry field.

Further research by Jim Huntington in Cambridge built on this work, leading to the description of the detailed molecular structures and mechanisms involved in thrombin inactivation by antithrombin and heparin (Huntington *et al.* 2000). Novel insights were gained regarding the interaction of thrombin with its substrates, as well as the structures and mechanisms of thrombin-generating (prothrombinase) protein complexes. Serendipitously, serpin polymerising mechanisms and how this leads to serpin inactivation and thrombosis were described (Yamasaki *et al.* 2008). The detailed molecular and structural information on how thrombin and factor Xa interact with their substrates and

inhibitors has helped to pave the way for pharmaceutical companies to develop orally available thrombin and factor Xa inhibitors. This is because medicinal chemists could then use the structures to develop small molecules that fit their active site more snugly. Collectively called direct oral anticoagulants (DOACs), these 'designer' inhibitor molecules have taken treatment of different thrombotic diseases to a new level. DOACs have increasingly replaced vitamin K antagonists, in certain thrombotic diseases, due to their ease of administration and effective reduction in thrombosis. An additional advantage is that they do not require regular monitoring through blood sample analysis, as is needed for warfarin.

Perspective

While the main coagulation pathway was described over 60 years ago, new molecular and cellular mechanisms of regulation have since emerged, including the interactions with other biochemical and cellular systems, such as the innate immune and complement systems. More recent studies have focussed on the ultrastructure, architecture and functional characteristics of the final product of coagulation: the blood clot or thrombus (Chapter 9). Future studies may yield new opportunities for the targeting of coagulation in the treatment of thrombosis and bleeding. New oral anticoagulants that target coagulation factors other than thrombin and factor Xa may be developed in the near future and provide further advances in the treatment of thrombosis, by increasing safety and therapeutic windows.

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Chapter 11: Contact activation

Overview

The processes that lead to pathological thrombosis and physiological haemostasis have classically been considered opposite sides of the same coin. However, recent findings indicate that there are particular molecular and cellular mechanisms that may be more specific in driving thrombosis, while not being involved in haemostasis. One such mechanism involves the contact pathway of coagulation (also known as the intrinsic pathway), initiated by coagulation factors XII and XI. These factors have both been associated with thrombosis, but their deficiencies in patients are not associated with bleeding (factor XII), or with very mild bleeding (factor XI). This opens up exciting possibilities of targeting either of the two main contact pathway factors, to treat or prevent thrombosis, while at the same time not increasing the bleeding risk, as is associated with current anticoagulation treatment using vitamin K antagonists or DOACs (Chapter 10). The BHF has funded critical aspects of work in this area including (i) elucidation of the molecular structures of the proteins and protein complexes involved in the pathway, and (ii) development of small molecule inhibitors to target factor XII.

The research supported by the British Heart Foundation

Early work by Jonas Emsley, in Nottingham, focussed on the characterisation of the molecular structure of factor XI, one of the two key proteins involved in contact activation of coagulation, revealing a critical mechanism in its activation (Papagrigoriou *et al.* 2006). Further studies by the Emsley lab have since elucidated the molecular structure of the second protein involved in contact activation, factor XII (Pathak *et al.* 2015), enabling medicinal chemists to design molecules that fit the enzyme's active site precisely, for potent inhibition of its activity. Recent work, funded in part by the BHF, of Helen Philippou and Richard Foster, in Leeds, has identified potent and specific small-molecule inhibitors of activated factor XII(a). These inhibitors have the potential to block thrombosis without increasing bleeding risk and a spin-out company, LUNAC Therapeutics, has been created which aims to develop these molecules into clinical drug candidates targeting factor XIIa. In addition, BHF-funded studies by the Philippou lab recently identified a novel pathway by which kallikrein directly activates factor IX, bypassing factor XI (Kearney *et al.* 2021), contributing to a revision of the classical coagulation pathway. Earlier studies by Nicola Mutch, in Aberdeen, characterised and identified an important role for polyphosphates released from the dense granules of platelets in the activation of factor XII during thrombosis (Engel *et al.* 2014).

Perspective

The identification of molecular or cellular mechanisms that play a role in thrombosis but not haemostasis could provide the Holy Grail for the field of anti-thrombotic that does not cause bleeding. Disentangling the mechanisms involved in these two processes could facilitate the development of new treatments for thrombosis that do not incur the risk of bleeding associated with currently used anticoagulants. Recent BHF-funded research into the contact pathway of coagulation activation has brought this ideal closer to fruition and is expected to deliver improvements in patient treatment and care in the foreseeable future. Future endeavours in this area should focus on which pathological processes leading to thrombosis are driven by contact activation, as well as further developing the promising candidate molecules currently available, to progress their pharmacokinetic and

pharmacodynamic properties. As with all new mechanisms discovered by basic science, conclusive proof of their effectiveness will only be obtained when molecules with suitable properties are finally tested in patients with, or at high risk of, thrombosis.

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Chapter 12: Fibrinolysis

Overview

Excessive fibrin deposition during physiological or pathological coagulation needs to be controlled. In addition, once the fibrin clot is no longer required, it has to be eliminated. The body has developed an exquisitely controlled system to dissolve the fibrin clot on demand, called the fibrinolytic system. It is composed of serine protease enzymes, similar to those of the coagulation system, and also involves serpin-style inhibitors. The main enzyme responsible for cleaving and eliminating fibrin is called plasmin, which is generated from plasminogen by an enzyme called tissue plasminogen activator (tPA), released by endothelial cells. Platelets contain an inhibitor called plasminogen activator inhibitor (PAI-1), while blood plasma contains two inhibitors, □2-antiplasmin and thrombin-activatable-fibrinolysis-inhibitor (TAFI). Research funded by the BHF from the 1980s onwards, notably in Aberdeen, has helped to establish the molecular and cellular mechanisms involved in fibrinolysis, including inhibition of fibrinolysis and how the fibrinolytic system interacts with platelets. More recent BHF-funded research has helped to hijack the fibrinolytic system, and in particular the fibrinolytic factor tPA, in the treatment of deadly clots in the brain.

The research supported by the British Heart Foundation

Fibrinolysis, haemostasis and thrombosis are intimately linked through molecular cross-talk mechanisms, and the fibrinolytic pathway is the target of two widely-used classes of drugs that prevent or promote fibrinolysis. Tranexamic acid, which was first developed in 1962, inhibits the conversion of plasminogen to plasmin and is used to prevent excessive bleeding in a variety of conditions, such as trauma. The other class of drug is fibrinolytics and includes the recombinant tPA, alteplase, which is the current drug of choice for patients with ischaemic stroke, and Tenecteplase.

The BHF has funded basic research into the fibrinolytic pathway from the late 1980s onwards by Nuala Booth and in the 2000s by Nicola Mutch, a former BHF Intermediate Fellow, at the University of Aberdeen. Together, they established the UK as a leader in the field of fibrinolysis. The collaborative studies by Booth and Mutch evaluated the relative contributions of plasma and platelet PAI-1 (Booth *et al.* 2007) and the contribution of the different inhibitors PAI-1, TAFI and □2-antiplasmin in the inhibition of physiological and pathological fibrinolysis (Mutch *et al.* 2007). Mutch has also shown how the fibrinolytic system interacts with platelet compound polyphosphate on the platelet surface (Mitchell *et al.* 2016; Whyte *et al.* 2017) and found new cellular mechanisms of fibrinolysis by showing how fibrinolytic proteins such as PAI-1 and plasminogen interact with platelets, including via the surface receptor, Plg-RKT (Morrow *et al.* 2020 and Whyte *et al.* 2021).

Studies by Gordon Lowe, in Glasgow, focussed on measurements of fibrinolysis as biomarkers for stroke incidence in older men (Wannamethee *et al.* 2012). One of the measures studied by Lowe, D-dimer, is used clinically in the diagnosis of pulmonary embolism and as a more general biomarker of thrombosis. Research by the BHF Chair holder David Newby in Edinburgh established and characterised the effects of the bradykinin and angiotensin systems on tPA release by the vascular endothelium (Labinjoh *et al.* 2001). Further work described how inflammation regulates endothelial tPA release (Chia *et al.* 2003) and shows the usefulness of a clinical model in the assessment of endothelial function and endogenous fibrinolysis (Lucking *et al.* 2013). Studies by Ramzi

Ajjan, Robert Ariens and Peter Grant in Leeds have focussed on genetic polymorphisms and their effects on clot structure and fibrinolysis (Ajjan *et al.* 2008). Recent studies by Ajjan have developed novel proteins to regulate fibrinolysis (Kearney *et al.* 2019).

Drug treatments to promote fibrinolysis have the potential to save the lives of stroke patients. The drugs used in stroke are usually based on tPA, produced by recombinant DNA technologies and occasionally contain minor engineered modifications to improve efficacy. The BHF is supporting a programme of trial work led by Keith Muir, in Glasgow, on the evaluation of alteplase and low-dose Tenecteplase in stroke patients (ATTEST-2 and TEMPO-2 studies). Additionally, UK participation in another trial of Tenecteplase, in patients waking up with a stroke (TWIST), is being led by Thompson Robinson, in Leicester. Tenecteplase is an engineered recombinant variant of tPA that has higher specificity for its target, fibrin, and when used at a low dose may increase the benefit versus safety window. It should be noted that the treatment of stroke patients with tPA and its analogues is not always successful, and that administration of the drug within 3-4 hours from symptom onset is essential.

Perspective

Fibrinolytics still represent the only class of drug treatment available that has been shown to reduce and sometime resolve the ischaemic impact in stroke. The interaction of fibrinolysis with platelets is a new area of research that deserves further study. As an example, an early phase trial using fibrinolytics, in combination with a blocking antibody to GPVI, has been started by the Biotech Company, Acticor based on research supported by the BHF showing that fibrin activates GPVI, leading to a pathway that increases fibrin generation (Alshehri *et al.* 2015). Interactions of the fibrinolytic pathway with other vascular cells is also a key topic for further research, including in angiogenesis.

Future studies should address whether newly developed inhibitors of fibrinolysis can be exploited to treat bleeding, and whether fibrinolysis, for the treatment of thrombosis, can be achieved by targeting TAFI, PAI-1 or □2-antiplasmin. Such endeavours may lead to the future development of new agents to treat diseases associated with thrombosis and bleeding.

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Chapter 13: Signalling pathways regulating platelet activation

Overview

Towards the end of the 1970s and early 1980s, platelets became a widely used model to study signalling pathways, as they could be obtained from human volunteers in high yield and are enriched in signalling proteins. For example, studies on platelets played a critical role in the elucidation of the protein kinase C pathway by Yasutomi Nishizuka in Japan and the development of dyes for measuring Ca^{2+} elevation by the Nobel Laureate Roger Tsien in Cambridge (neither funded by the BHF).

The breakthrough in molecular biology in the 1980s heralded a new approach to the study of signalling pathways because it was now possible to alter the genetic makeup of a cell. Platelets however are not amenable to genetic techniques, as they lack DNA, and so investigation into the mechanisms of regulation of platelets lagged behind that of other cells. This changed in the late 1990s and early 2000s through the use of transgenic mice and techniques for rapid sequencing of proteins (proteomics) which enabled the composition of the platelet to be determined. The BHF was a major supporter of this new era in platelet research and established the careers of many platelet researchers in the UK. As a result, we now have a detailed understanding of the mechanisms that underlie platelet activation in health and disease.

The research supported by the British Heart Foundation

Poole, Gibbins and Watson, in Oxford, in the 1990s were among the first to use mouse models for the dissection of mechanisms of platelet activation (in this case for the collagen receptor GPVI, funded by the Wellcome Trust), establishing a new era in platelet research. Many platelet researchers around the globe have now used mouse models to dissect the events that underlie platelet activation and their contribution to haemostasis and thrombosis. The development of proteomics in the early 2000s further allowed researchers to study the protein composition of platelets leading to discovery of platelet-specific proteins, such as CLEC-2 (Suzuki-Inoue *et al.* 2006) and G6b-B (Senis *et al*, 2007) and semi-quantitative information on levels of expression. Thus, we now know the make-up of human platelets and through access to transgenic mice can study their function with the caveat of qualitative and quantitative differences between species.

Through the use of mouse models, specific inhibitors and the small number of patients with genetic defects that give rise to platelet dysfunction, we now have detailed understanding of the mechanism of platelet activation by G protein-coupled and tyrosine kinase-linked receptors, and the pathways that give rise to platelet aggregation, secretion and phosphatidylserine exposure (critical for regulation of the procoagulant, or clotting, activity of platelets). Specific examples include: mapping of proteins involved in secretion and aggregation by Alastair Poole and Ingeborg Hers in Bristol, receptor trafficking by Stuart Mundell in Bristol, movement of ions between platelets by Martin Mahaut-Smith in Leicester and Jon Gibbins in Reading, cyclic nucleotide regulation and function by Khalid Naseem in Leeds, tyrosine kinases and phosphatases by Steve Watson and Yotis Senis in Birmingham, and membrane organisers (known as tetraspanins) by Mike Tomlinson in Birmingham. These are a sample of the many researchers who have benefitted from funding from the BHF in the last 20 years in the study of platelet signalling pathways which has also given rise to a new generation of researchers studying signalling mechanisms in

platelets, including Matthew Harper in Cambridge, Alice Pollitt in Reading and Amanda Unsworth in Manchester.

Perspective

Research over the last 25 years into mechanisms of platelet activation can be described as a golden-era. This has underpinned the careers of many in the UK platelet field and led to the discovery of potential new targets for antiplatelet drugs. However, as yet, this not yet been translated into new therapeutics. In many cases, this is because of concerns over side effects (many proteins are expressed in other cell types) but also because of the challenge in targeting intracellular proteins with small molecule inhibitors that have to cross the membrane and the high cost of clinical trials. The recent interest in repurposing tyrosine kinase inhibitors as targets for a new class of antiplatelet agent however illustrates the potential of research in this area (e.g. see Chapter 8). The further understanding of signalling mechanisms is also likely to lie in precision medicine through the development of algorithms that improvement the estimation of risk of thrombosis and bleeding, and which will therefore guide treatment with current and future antiplatelet drugs.

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Chapter 14: Fluorescent- and light-based imaging in haemostasis and thrombosis

Overview

Technology is vital for scientific progress as illustrated by the fact that 60% of Nobel Prizes in Physiology or Medicine have been awarded for breakthroughs in methodology. The discovery of the platelet, in the 19th century was indeed the result of an advance in technology, namely the light microscopy. The first sighting of small particles in the microscope fuelled debate as to whether this was a cell or a cell fragment, or possibly bacteria or fragments of fibrin, which was only resolved through the demonstration of the clumping function of platelets by the Italian pathologist Giulo Bizzozero, in 1881.

The BHF is a strong supporter of advances in technology through its main funding schemes, as well as through the 'Infrastructure' and 'New Horizons' grant schemes that support the purchase of specialist equipment and research outside of the traditional domains of biology, respectively. Below, we describe how the application of fluorescence and other light transmission-based microscopy and related techniques have supported our understanding of haemostasis and thrombosis. Through advances in microscopy we can visualise thrombus formation in real time and in fixed samples to a remarkable level of detail.

The research supported by the British Heart Foundation

The development of light transmission aggregometry, by Born in 1962, revolutionised the platelet field and is still used today in both research and in the clinical evaluation of patients with suspected platelet disorders (see introduction). This simple assay has been refined over the years, including to a 96-well plate format (Optimul), by Tim Warner's group in London, which reduces the sample volume and increase throughput, and has the potential for automation such that it has the potential to become the standard assays (Lordkipanidze *et al.* 2014; Chan *et al.*, 2018). Optimul also be combined with fluorescence-based methods to provide further information on platelet activation, such as granule secretion. Nevertheless, Born aggregometry remains the method of choice by clinical testing laboratories for diagnosis of platelet disorders because of the experience that has been built up over the years. The advances in genetic sequencing may reduce the number of tests of platelet function that are performed but it seems likely that Born aggregometry will still many years from now.

A major advance in the last forty years for monitoring platelet activation has been in the application of fluorescent-based techniques and probe development. These can be used to monitor events in real time, such Ca^{2+} localisation and the location of proteins, using fluorescent dyes, antibodies and related agents. One of the major applications is in flow cytometry, which can visualise up to tens of fluorophores at a time on individual platelets and platelet-derived extracellular vesicles. Flow cytometry has been a major benefit in the diagnosis of patients with defects in surface glycoproteins, such as the GPIIbIIIa receptor, and is a cornerstone of research in all BHF-funded research laboratories.

Fluorescence-based techniques have had a profound impact on light microscopy, such that today we can visualise structures and proteins below the diffraction limit of light and study their localisation in three-dimensions with high accuracy. Fluorescence microscopy can also be used to monitor platelet activation under flow conditions *in vitro* and *in vivo*. Examples of the application of fluorescence microscopy include the research of Warner, who has used confocal microscopy to show that young and drug-treated platelets are differentially incorporated into a thrombus (Armstrong *et al.*, 2017), that of Jon Gibbins in Reading, to show that platelets are connected through a series of channels which support thrombus formation (Vaiyapuri *et al.* 2012; Sahli *et al.*, 2021) and Yotis Senis in Birmingham,

who demonstrated a critical role of tyrosine phosphatases in regulating thrombus formation *in vivo*, using a technique known as intravital microscopy (Senis *et al.* 2009). Poulter and Thomas, in Birmingham, have used the super-resolution techniques of direct stochastic optical reconstruction microscopy (dSTORM) and structured illumination microscopy (SIM), to map the movement of receptors on the surface of resting and activated platelets (Pollitt *et al.*, 2014; Poulter *et al.*, 2015; Pallini *et al.*, 2021). Fluorescent microscopy can also be combined with electron microscopy (called correlative electron light microscopy) to show the location of cells and clotting factors at high resolution, for example within a clot.

The BHF has funded several infrastructure and other grants to enable these methods to be established within haemostasis and thrombosis research groups in the UK to enable experiments to be performed on fresh tissue and in real time, as well as honing the necessary expertise. This contrasts with methods such as genome sequencing, proteomics and structural studies which use central resources, partly due to cost and their wider applicability, but also because samples can be analysed remote from the laboratory.

Perspective

Advances in technology will enable a deeper understanding of the mechanisms of haemostasis and thrombosis to be achieved and will eventually allow us to better understand why some patients bleed and some thrombose, and ultimately improve the treatment of both. Many of the current microscopy methods however are still in their infancy and may not bear fruit for many years. For example, the BHF have recently provided a 'New Horizons' grant to Steve Thomas in Birmingham to develop the method of expansion microscopy, which seeks to increase the size of a sample to enable a more detailed visualisation of the cytoskeleton. While such an approach could not have been envisioned many years ago, it is through advances like these that we will be able to more clearly describe the events that thrombus formation, and thereby reveal new targets for drug treatment and account for the differences in responses between patients.

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Chapter 15: Nitric oxide, cyclic nucleotides and lipid mediators

Overview

The release of lipid mediators, such as prostacyclin, and the gaseous transmitter, nitric oxide (NO), from endothelial cells helps to maintain circulating platelets in a quiescent (or inactive) state through formation of the cyclic nucleotides, cAMP and cGMP, respectively. These are the two most powerful ways of inhibiting platelets. Conversely, release of thromboxane A_2 from platelets, which like prostacyclin is generated through the combined actions of phospholipase A_2 and cyclooxygenase, has a positive feedback role in platelet activation at sites of vessel injury. Low-dose aspirin is proposed to inhibit platelet but not endothelial cell cyclooxygenase due to the inability of platelet to make new protein, selectively inhibiting formation of thromboxane A_2 in platelets. However, this is now recognised to be an oversimplification as the selectivity is not absolute and other cells are affected. Aspirin can also used in combination with $P2Y_{12}$ receptor antagonists (dual antiplatelet therapy) in the treatment of patients with a high risk of acute coronary syndromes but this also raises the degree of bleeding which in a minority of patients can be life-threatening. It is proposed that a greater understanding of the regulation and interplay of paracrine and autocrine mediators, such as prostacyclin and phospholipase A_2, and nitric oxide, will lead to improvements in drug regimens and treatment. Research supported by the BHF over the last 20 years has focussed on the interplay of lipid mediators and inhibitors of cyclic nucleotides to improve therapeutic intervention.

The research supported by the British Heart Foundation

The BHF has supported research on lipid mediators and NO function from the 1980s and since 2000 have given sustained funding to the groups of Valerie O'Donnell in Cardiff, Khalid Naseem in Leeds (previously in Bradford and Hull), and Tim Warner in Queen Mary's in London. The work of Naseem has focussed on the mechanisms of platelet inhibition by the cyclic nucleotides, cAMP and cGMP, and how this is altered by platelets receptors and proteins in the circulation, including oxidized low-density lipoproteins and thrombospondin-1. For example, they have shown using mouse models that the platelet surface receptor CD36 and thrombospondin-1 are critical regulators of platelet function *in vivo* (Aburima *et al.* 2021; Berger *et al.* 2020) despite relatively modest effects *in vitro*. O'Donnell is a leading expert in the UK in the identification of new lipid species and has conducted much of her work in platelets, showing that platelets express ~5,600 distinct lipid species. In work supported by the BHF, O'Donnell has identified a novel platelet-derived lipid regulator of neutrophils (Hinz *et al.* 2016) and a critical role for lipids in regulating NO consumption, which is inhibited by aspirin (Williams *et al.* 2005). The group of Warner has focussed on the relevance of lipid-mediated pathways in antiplatelet therapy, including the interaction with modulators of cyclic nucleotide metabolism. For example, they have recently shown how the eicosanoid lipid changes with the dose regime thereby explaining why increasing aspirin dosage or aspirin addition to other drugs may lessen antithrombotic protection (Crescente *et al.* 2020). Similarly, they have shown that low doses of guanylyl cyclase synergise with $P2Y_{12}$ inhibition, to produce powerful anti-platelet effects without altering blood flow (Armstrong *et al.* 2020). The BHF is also supporting the Storey group in Sheffield to explore the dose-dependent effects of aspirin on lipid mediators and inflammatory responses with a view to understanding whether very-low doses of aspirin, which show haemostatic advantage over standard-dose

aspirin, may be preferable for long-term treatment, including when used in combination with $P2Y_{12}$ inhibition (Parker et al., 2019).

Perspective

We have come a long way in understanding the interplay of lipids, NO and cyclic nucleotides in the regulation of platelet function and how this is altered in the presence of aspirin, $P2Y_{12}$ inhibitors and modulators of NO metabolism. The challenge is to now use this information to drive treatment in the clinic. Modulation of the dose regimens of aspirin in long-term preventive therapy, either alone or in combination with $P2Y_{12}$ inhibition, may provide increase the therapeutic window in patients at risk of thrombosis relative to bleeding.

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Chapter 16: Particulate matter and atherothrombosis

Overview

Establishing the link between smoking and cardiovascular disease was one of the major health care advances of the twentieth century. We now know through the work of the BHF and other organisations that smoking cigarettes causes the build-up of fatty material and plaque formation, leading to thrombosis and blockage of major blood vessels in the heart and brain. In the early 2000s, the BHF played a major role in anti-smoking campaigns and in driving government policy that led to an increased recognition of the dangers of smoking, changes in advertisement and to a ban on smoking in public places. In more recent years, attention has focussed on environmental factors, including particulate matter and the link to thrombosis and cardiovascular disease.

The research supported by the British Heart Foundation

The major research focus on smoking has been on the build-up of atheroma in the vessel wall and the molecular basis of increased state of activation of circulating platelets. An increase in platelet reactivity on exposure to diesel particles in humans has been reported by the group of the BHF Chair holder David Newby in Edinburgh using an *ex vivo* flow assay (Lucking *et al.* 2008). A critical question in this and related work (e.g. the use of nanoparticles in medicine) is whether the increase in platelet reactivity is the consequence of damage to the vasculature and vital organs or due to a direct effect of particulate matter on platelet activation, or both. In support of a possible direct effect, Newby has shown accumulation of gold nanoparticles in blood and organs in human volunteers and in mice for up to 30 days (Miller *et al.* 2017). Mike Emerson, at Imperial College London, and the BHF Chair Steve Watson, at the University of Birmingham, have investigated the mechanisms of platelet activation by diesel particles and nanoparticles (Smyth et al., 2013; Zia et al. 2019), showing that diesel particles activate platelets through the glycoprotein receptors GPVI and CLEC-2 (Alshehri *et al.*, 2015).

Newby and colleagues have written influential reviews in this area, including systematic reviews on the link between air pollution and stroke, and air pollution and heart failure (Shah *et al.*, 2013; Shah *et al.*, 2015), as well as an expert position paper of the European Society of Cardiology (Newby *et al.* 2015). These have helped to highlight air pollution as a critical health issue in cardiovascular disease and a target for global health policy.

Perspective

There is now widespread recognition of the link between particulate matter in the environment and cardiovascular disease, although it remains unclear to what extent this is mediated through a direct effect on the haemostatic system and specifically platelets as the particulate matter will bind to proteins in blood. The joining of cardiovascular and pulmonary science will be required to fully uncover the mechanisms involved.

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Chapter 17: Making platelets in vitro

Overview

The discovery of the cytokine thrombopoietin as a major regulator of megakaryocytes and platelet formation in the 1990s opened up the possibility making platelets in large amount *in vitro*. This provides the potential to overcome major issues in transfusion medicine due to the shortage of donors, short shelf life of platelets and risk of infection, as well as to make genetically altered platelets to treat patients with genetic disorders that give rise to a low platelet count (thrombocytopenia) or dysfunctional platelets. In the future, it may be possible to engineer the platelets to release drugs upon activation and in this way deliver them directly to the site of injury (e.g. antithrombotic agents).

The research supported by the British Heart Foundation

The BHF recognised the importance of understanding the mechanism of platelet formation as early as the 1960s through a research grant to Gustav Born and later with the appointment of John Martin to a Chair in King's College London in 1990. Martin had studied the link between platelet size and thrombosis in Sheffield in the 1980s which gave rise to the concept of hyperreactive platelets as a contributor to thrombosis. In addition, the BHF supported the research of Neville Crawford in London and Leeds on platelets as carriers of antithrombotic drugs in the 1980s and 1990s.

This early work was held back because of the challenge in making platelets from megakaryocytes *in* vitro and this only changed with the discovery of thrombopoietin (TPO), in the mid-1990s. This advance, which was not funded by the BHF, enabled researchers to show that platelets could be made *in vitro*, albeit in small numbers, and later to the introduction of TPO mimetics into the clinic for treatment of conditions associated with a low platelet count such as immune thrombocytopenia. Nevertheless, 25 years on, and we are still only able to make relatively small numbers of human platelets in the laboratory.

The BHF has supported the work of Cedric Ghevaert on megakaryocyte development and platelet formation, initially as an intermediate fellow in Birmingham and now in Cambridge. Ghevaert has published key findings on novel mouse models that have given insights into a condition known as thrombocytosis, where the body makes too many platelets (Hobbs *et al.*, 2013), and on the generation of platelets *in vitro* from induced pluripotent stem cells (iPSCs) through a technique, called 'Forward Programming' (Moreau et al., 2016). Ghevaert's group has also shown that under the right conditions forward programming can be used to produce immature red blood cells, known as erythroblasts (Dalby et al., 2019).

Perspective

The use of iPSCs in platelet and red blood production has many advantages. iPSCs can be cultured almost indefinitely, since they are capable of producing copies of themselves, whilst retaining the capacity to differentiate into almost any cell type. Since neither platelets nor red blood cells contain genetic material (DNA), they can be irradiated and transfused into patients without the risk of metastases. Due to their derivation from iPSCs they can also be modified. For example, to deliver drugs to sites of bleeding, or, to minimise the risk of alloimmunisation, whereby the body rejects transfused cells it recognises as foreign, an issue particularly pertinent to patients who require multiple transfusions. The potential to use platelets in cell therapy could revolutionise many of the current forms of treatment of cardiovascular disease.

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Chapter 18: Reflections and thoughts on future research

We would like to express thanks to our colleagues in their help in compiling this overview, including their thoughts on future developments in the field.

It is simply not possible in a short review to do justice to the enormous contribution that the BHF has made to research on haemostasis and thrombosis over the last sixty years. There are also practical challenges in that the early records are incomplete, and many of those who were funded over this period are no longer with us. The contribution of a national charity to a global field of research is difficult to capture, and this overview is inward-looking, with the critical work of non-UK researchers not included. Similarly, the contribution of other UK funding bodies such as the Wellcome Trust and MRC to the work described above is only briefly referred to.

The contribution of the BHF also goes far beyond research outputs, and this is captured in a reply that we received from Peter Grant in the University of Leeds in response to a series of questions:

'*I would say that the BHF reach within medicine is so large that massive influences occur that are not necessarily related to specific publications or, indeed, funding. I was privileged to chair the ESC/EASD guidelines both in 2013 and 2019, which would never have happened without BHF research funding support which was crucial in allowing me to develop my own interests in CV disease. Although the guidelines are not specifically related to BHF funding, the BHF is clearly part of the overall picture, with many of the publications cited influenced by the reach of the BHF. I believe the BHF has also had a massive influence on patient care, by constantly raising expectations, awareness and standards as well as by funding research.*'

We have focussed the chapters on areas where there is a link to translation due to space limitations. We have also focussed on research in the later years, in part because of the paucity of the early records. We have highlighted individuals who have benefitted from BHF funding, notably Fellows and Chairs. Funding from the BHF has enabled many of these to become international leaders and the UK as a global player in the field.

In compiling this overview, several points have emerged and are worthy of comment:

- Many of the research areas have been led by a small number of individuals and institutions. As examples, the work of Nuala Booth and Nicola Mutch have established Aberdeen as a leading centre in fibrinolysis, and that of David Lane and Mike Laffan, and more recently Jim Crawley, have established Imperial as a leading centre in von Willebrand factor.

- There can be a long delay from discovery research to the clinic. This is illustrated by the thirty years for the $P2Y_{12}$ blocker, cangrelor, to enter the clinic based on the BHF-funded work of Cusack and Hourani in the early 1980s, and the related development of ticagrelor (Chapter 4).

- Only a handful of new therapeutics has been developed in this field, despite the fantastic progress in our understanding of haemostasis and thrombosis. This is in part because of concern over bleeding and the cost of clinical trials. Even so, it

surprising that innovative ways of targeting receptors such as GPIb and GPVI have not led to the introduction of new therapeutics. This may be addressed in the future by focussing on rare diseases (as exemplified by the nanobody caplacizumab), or by focussing on single-dose studies, thereby obviating the need for a costly trial (e.g. the current phase IIa trial of the GPVI blocker, glenzocimab, in stroke is based on a single dose). Neither example is from BHF-funded research.

- The scope and breadth of the research and numbers of research projects and researchers funded by the BHF are hugely impressive.

Future developments

We asked our colleagues their thoughts on the future challenges and developments in this field. Below, we have pasted the reply from Alan Nurden, whose work is summarised in Chapter 1 as a further example of the help and support that we have received in compiling this overview:

'The recent advances in the field of inherited platelet disorders means that a large proportion of the more common genetic causes will have been discovered. The continued application of whole genome sequencing (WGS) and sophisticated gene platforms will both extend the range of causative genes and uncover unknown causes (e.g. deep intron changes; miRNA effects, epigenetic influences) within both new and already known genes. Such studies are to be encouraged. Also to be encouraged are efforts to identify the influence of single allele mutations that only manifest as disease-causing when in combination. This will require large patient cohorts and multicentre collaborations both on a national and international level with link up to high-capacity centres both for sequencing and for bioinformatics. But most important is the need now to distinguish between the requirements of gene sequencing for disease identification, an essential need for patient management (including assessing the risk of cancer and leukaemic states in certain inherited thrombocytopenias), optimal treatment and for prospective gene therapy; and the need for a genetic algorithm for assessing bleeding risk. For example in Glanzmann thrombasthenia it is now clear that while mutations within ITGA2B and ITGB3 define the disease, bleeding severity is determined by a panoply of plasma, blood cell and vascular factors controlled by genetic variants many of which remain to be identified. The real future challenge of WGS is to be able to predict both disease evolution and bleeding risk allowing personalized medicine. It should be noted that probably often the same variants and challenges would also apply to personalizing anti-thrombotic therapy. It seems to me that the BHF is appropriately placed to participate in these challenges that will require considerable ethical controls.

I put this at the top of my list which also includes the more straightforward and obvious challenges such as

(i) putting together a safe and efficient gene therapy strategy,
(ii) better understanding the role of platelets in inflammation, immunology and in bacterial and viral infections,
(iii) improving treatments for bleeding by eliminating additive thrombotic or immunological risk

(iv) *filling the gaps in our knowledge in the long process of platelet biogenesis and megakaryocytopoiesis,*

(v) *improving our understanding of the role of platelets in tumour growth and obtaining a better evaluation of the mechanisms behind (and thereby preventing) the onset of immune-related thrombocytopenia during chemotherapy,*

(vi) *evaluating new platelet targets for antithrombotic therapy including the rapid dissolution of platelet-rich thrombi and*

(vii) *greatly expanding our knowledge of how defects of endothelial cells and especially of how modification of gap junctions contribute to bleeding disorders.'*

To this we would add:

(viii) *The generation of platelets for transfusion through iPSC technology, including genetic engineering of iPSCs to produce platelets that are suited to certain patient groups, for example, generating 'universal' platelets.*

(ix) *Generation of platelets in cell therapy. As examples: to produce platelets that promote angiogenesis to benefit a patient undergoing cardiac surgery; to use platelets to deliver enhanced pro-repair proteins and drug molecules in ischaemic damage in the heart.*

(x) *Better understanding how platelets, coagulation, fibrinolysis and other vascular cells interact in the process of thrombosis and the generation of thrombi of differing composition in various thrombotic disorders.*

(xi) *Use of new molecular imaging methods such as high resolution cryo-electron microscopy to shed new light on clotting mechanisms and receptor activation, as it allows for high resolution molecular imaging of dynamic cellular and biochemical processes.*

(xii) *Exploring new targets in coagulation and clotting, such as platelet proteins that support thrombosis but not haemostasis, for the development of antithrombotic treatments that incur a much lower risk of bleeding.*

(xiii) *Discovering COVID19 and other infection-related mechanisms of thrombosis and bleeding for better treatment of pathology*

Fundamental to these areas will be a deeper understanding of the biology and interplay of platelets and the coagulation system in haemostasis and thrombosis, and in other pathways, including in inflammation and cancer.

Note:

The article was written in January 2021 which was just a few weeks before the first patients with vaccine-induced immune thrombocytopenia and thrombosis (VITT) were described. This condition is now known to be caused by high affinity antibodies to platelet factor 4 and activation of the platelet low affinity immune receptor, Fc□RIIA. Several BHF funded

researchers were involved in the reporting and elucidation of the underlying mechanism which is similar to a previously known condition, autoimmune heparin-induced thrombocytopenia.