Could you describe the background to your collaboration?

AM: As an independent investigator at Stony Brook University, I was involved in the identification and characterisation of phospholipase D enzymes: intracellular enzymes that regulate cell signalling, cytoskeletal organisation and vesicular transport. Because these enzymes generate phosphatidic acid, we also identified other classes of enzymes involved in phosphatidic acid metabolism, including soluble and integral membrane phosphatidic acid phosphatase enzymes. Some localise to the surfaces of cells so we became interested in their possible role as regulators of bioactive lysophospholipid signalling. My collaboration with Susan Smyth leverages my interest and expertise in the basic biochemistry, cell biology and genetics of lysophospholipid signalling, allowing us to investigate the roles played by lysophospholipids in human disease processes.

SS: While at the University of North Carolina, I focused on integrin biology and the interplay between inflammation and thrombosis. We became interested in mediators produced and released by platelets in response to tissue ischaemia or injury, and identified lysophospholipids as potential platelet-derived mediators of vascular disease. This resulted in a longstanding collaboration with Andrew Morris and his group to understand the role of lysophospholipid signalling, and in particular the nexus of autotaxin, lysophosphatidic acid and the lipid phosphate phosphatase 3 (encoded by the PPAP2B gene), in cardiovascular disease (CVD).

Can you summarise the aims of your work?

SS: Our current work focuses on identifying mechanistic links between the dietary intake of lipids and CVD risk. Our investigations are exploring the possibility that choline-containing lipids and adipose-derived autotaxin serve as links between diet and CVD risk.

Lysophosphatidic acid can be hydrolysed and inactivated by lipid phosphate phosphatase (LPPP) enzymes present on cell membranes. A genome-wide association study identified the PPAP2B gene encoding LPP3 as a novel locus associated with coronary artery disease susceptibility. We have established that LPP3 expression in mice is critical to attenuate inflammation, reduce smooth muscle cell proliferation and maintain endothelial barrier function. Our studies are attempting to understand how heritable variation in the PPAP2B gene predisposes humans to developing atherosclerosis.

What links have you identified between lipid intake and CVD?

SS: The lysophospholipase D autotaxin catalyses the hydrolysis of circulating or cell-associated lysophosphatidylcholine (LPC) to generate lysophosphatidic acid, which has potent, receptor-mediated effects on blood and vascular cells. Lysophosphatidic acid is a proteotypic member of a family of bioactive lipid phosphonic acids that function as receptor-active mediators with roles in cell growth, differentiation, death and development.

Adipose tissue serves as an important source of autotaxin circulation, and autotaxin levels increase with obesity. LPC is the major lipid substrate in plasma for autotaxin, and can be produced during intestinal absorption of phosphatidylcholine or during esterification of cholesterol. Phosphatidylcholine is the most abundant phospholipid in the diet and is also the predominant phospholipid constituent of bile. It is secreted into the intestine in response to meals high in fat.

Recent studies link dietary phosphatidylcholine intake with atherosclerosis, demonstrating that trimethylamine N-oxide – which is derived from dietary phosphatidylcholine and other choline-containing foods by intestinal microbiota – is a powerful predictor of atherosclerosis in humans and mice. In addition, phosphatidylcholine may also be converted via LPC to lysophosphatidic acid.

AM: Building on these ideas, we have shown that circulating levels of lysophosphatidic acid are acutely sensitive to fasting and feeding in humans. Our mouse studies also indicate that higher plasma levels of lysophosphatidic acid, and its association with atherogenic lipoproteins, contributes to the development of heart disease.

To what extent might autotaxin prove a suitable therapeutic target for small-molecule therapeutic agents?

SS: The recent development of small-molecule inhibitors of autotaxin and lysophosphatidic acid receptor antagonists provides an impetus for exploring the possibility that pharmacological inhibition of lysophosphatidic acid synthesis and signalling could be a viable strategy to mitigate human CVD risk. Indeed, some experimental drugs are able to inhibit its synthesis and signalling mechanisms, and these can be tested in mouse models in preparation for studies on human subjects.

Can you provide an insight into your long-term research ambitions?

AM: A particular challenge of our research in the future will be to determine how other gene variants and environmental exposures contribute to the processes by which lysophosphatidic acid and PPAP2B determine human CVD risk.
A new link to cardiovascular disease risk

A collaborative study between physician scientists and lipid biochemistry researchers at the University of Kentucky aims to understand how genetic variation and environmental exposure interact to promote the development of atherosclerosis. The researchers intend to identify and harness a natural cardioprotective mechanism to protect individuals from coronary artery disease.

Atherosclerosis, the leading cause of death in Western societies, involves the narrowing and hardening of the arteries. Risk factors for its development include smoking and metabolic conditions such as diabetes, high blood pressure, high blood cholesterol and obesity. Genetic factors are also implicated, and individuals with a family history of early heart disease are at greater risk of developing the condition. Indeed, multiple interactions between genetics, and environmental and metabolic risk factors likely increase susceptibility to atherosclerosis and its complications, such as heart attacks and strokes.

Initially thought to be a metabolic disease with lipid deposition in blood vessels, atherosclerosis is now widely accepted to be a disease of inflammation and thrombosis as well. How diet interacts with genetic make-up to increase the risk of developing atherosclerotic vascular disease is the subject of a four-year study underway at the Gill Heart Institute in the University of Kentucky, USA. The Principal Investigators are Professors Susan Smyth and Andrew Morris. Smyth is also Chief of Cardiovascular Medicine and Medical Director of the Gill Heart Institute.

The central hypothesis of their study is that lysophospholipid signalling promotes the development of cardiovascular disease (CVD). “Our overarching hypothesis is that obesity and diet converge via elevated expression of autotaxin, and in combination with increased intestinal exposure to phosphatidylcholine from diet and bile, they promote the autotaxin-lysophosphatidic acid (LPA)-signalling nexus and accelerate atherosclerosis,” outlines Smyth. “Furthermore, we have evidence that the lipid phosphate phosphatase 3 (LPP3) enzyme – encoded by the PPAP2B gene – may attenuate the effects of autotaxin and its product LPA, and it may function to suppress the development of atherosclerosis”.

Seeds of collaboration

In 1998, the Morris laboratory identified the PPAP2B gene, enabling the researchers to conduct biochemical and genetic studies into it. They identified PPAP2B as a member of a family of enzymes that degrades lysophospholipids and determined that PPAP2B regulates cellular responses to bioactive lysophospholipids. Along the way, the team developed expertise in mass spectrometry, which it currently uses to define bioactive lipid signalling pathways and study them in the context of normal and pathologic states.

Using techniques developed by the Morris group, Smyth’s laboratory characterised the production of LPA in the circulatory system – as well as the metabolic pathways it utilises – providing the first evidence of a pathologic role for LPA receptors in response to arterial injury. Together, members of the laboratories have identified key structural and functional elements of autotaxin, and its mechanisms of functional regulation. “Andrew and I have highly complementary skills and expertise that we have applied to understanding lysolipid signalling in the vasculature from molecules to mice to men [and women],” reflects Smyth.
LYSOLIPID SIGNALLING

OBJECTIVES
To elucidate the molecular and cellular mechanisms involved in lysolipid signalling in the vasculature and translate the information into novel ways to prevent and treat human cardiovascular disease.

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SUSAN SMYTH is a physician scientist who combines clinical practice in cardiology with research focused on the interplay between inflammation and thrombosis in vascular biology. Her work centres on extracellular mediators and blood and vascular cell surface signalling receptors. Smyth’s team applies genetic and pharmacologic strategies in animal models of cardiovascular disease, in order to define cellular and molecular pathways, and then tests those pathways in clinical studies in humans.

ANDREW MORRIS studies the role of lipid metabolism and signalling in mechanisms that determine environmental and heritable cardiovascular disease risk. Current areas of interest include studies of integral membrane lipid phosphatases and bioactive lysophospholipid mediators and investigations into mechanisms linking diet induced obesity and exposure to environmental pollutants to human disease. He is also developing and applying mass spectrometry methods to structural analysis and quantitation of lipids and other biologically important molecules.

The central hypothesis of their study is that lysophospholipid signalling promotes the development of cardiovascular disease

SIGNALS THAT HEIGHTEN RISK
Strong evidence suggests that adipose tissue serves to produce some portion of the circulating autotaxin. In blood, the major lipid substrate for autotaxin is lysophosphatidylcholine (LPC), which is elevated in response to meals high in fat, in part by metabolism of phospholipids in the diet and bile, and also because it is a byproduct of esterification of cholesterol in the liver. Interestingly, circulating levels of trimethylamine N-oxide, another metabolite of dietary choline containing phospholipids that is generated by the intestinal microbiota, is also a strong predictor of CVD risk in humans. “Our observations suggest that elevated levels of autotaxin and LPC may converge to heighten LPA generation and thereby promote atherosclerosis,” Morris discloses.

How autotaxin and LPA affect blood and vascular cells is highly complex. LPA generated by autotaxin acts on cell-surface receptors to regulate many cellular processes, often through the activation of Ga12/13 and Rho signalling pathways. LPA promotes endothelial cell migration, disrupts endothelial barrier integrity and contributes to changes in the nature of vascular smooth muscle cells in response to injury. It also triggers neutral lipid uptake and accumulation of monocytes, and is implicated in the formation of blood clots through its effects on platelets.

The development of atherosclerosis can be modelled in mice by forcing the accumulation of ‘bad’ cholesterol – that is, low-density lipoprotein (LDL) cholesterol – in the blood, such as occurs in LDL receptor-knockout mice. As lipoproteins are deposited in the vessel wall, macrophages and other inflammatory cells are recruited to lesions. Administering LPA to hyperlipidemic mice further stimulates the accumulation of inflammatory cells; this accelerates the development of atherosclerotic plaque and can also result in intraplaque haemorrhage in the carotid arteries. “Mice lacking certain LPA receptors are protected in these models of CVD,” Morris notes. “However, the precise means of LPA generation in atherosclerotic lesions, relative to its accumulation from circulating sources, remains unclear.”

THE GENETIC LINK
Another element that Morris and Smyth have found in the pathway to high cardiovascular risk is an allele of the PPAP2B gene that appears to confer protection against coronary artery disease in humans. “In large scale genome-wide association studies, a common polymorphism in the PPAP2B gene was associated with lower risk for heart attacks,” Morris shares. Recent evidence suggests that the protective allele of PPAP2B dictates higher expression of LPP3.

How LPP3 may protect against the development or complications of atherosclerosis is not presently known, but Smyth and Morris have made substantial progress in addressing this in vascular disease, which they put down to the complementary nature of their collaboration, as well as the high levels of expertise in their respective laboratories. For example, their preliminary investigations show that deleting the PPAP2B gene, and thereby reducing LPP3 expression, accelerates the development of atherosclerosis in the mice, suggesting that LPP3 may normally function as an atherosclerosis suppressor. However, more research is needed to determine how genetic variation at the PPAP2B allele influences the development of disease in humans.

The team is also investigating whether the mechanisms that regulate LPP3 expression vary in the different cell types involved in CVD and what signalling pathway or pathways are controlled by LPP3. Undoubtedly, much remains to be discovered about to the nature of hereditary predisposition to atherosclerosis. “Our work on PPAP2B illustrates the power of human genetics for identifying the underlying causes of human disease,” Morris summarises. “It is likely that other genes and environmental exposures also contribute to CVD risk.”