Spotlight: David Newby, BA, BSc, PhD, BM, DM, DSc, FACC, FESC, FMedSci, FRSE

“We Found That People Who Smoke Cigarettes Release Markedly Less Tissue Plasminogen Activator Than Nonsmokers”

David Newby, British Heart Foundation John Wheatley Chair of Cardiology, director of research and development, NHS Lothian, Scotland, director of the Wellcome Trust Clinical Research Facility, director of the Clinical Research Imaging Centre, and consultant interventional cardiologist, Edinburgh Royal Infirmary, University of Edinburgh, Edinburgh, Scotland, talks to Monika Polak, PhD.

Early in his research career, in 1999 and 2001, David Newby, BA, BSc, PhD, BM, DM, DSc, FACC, FESC, FMedSci, FRSE, British Heart Foundation John Wheatley Chair of Cardiology, director of research and development, NHS Lothian, Scotland, director of the Wellcome Trust Clinical Research Facility, director of the Clinical Research Imaging Centre, and consultant interventional cardiologist, Edinburgh Royal Infirmary, University of Edinburgh, Edinburgh, Scotland, coauthored 2 influential and widely cited articles in Circulation. The first article investigated the release of a clot dissolving protein, tissue plasminogen activator (t-PA) in the forearm; the second, which resulted from his first research grant, showed similar findings but in the heart circulation.

He explains, “At the time people were looking at how the endothelium works and were focusing mainly on blood flow, but my innovation was to look at t-PA release. We found that people who smoke cigarettes release less of this clot-dissolving protein than nonsmokers.” He adds, “So that was the main finding that I was most proud of from my British Heart Foundation Junior Fellowship. I suddenly realised, having been reluctant to do research [he had previously turned down several clinical research opportunities], that I loved it, and I could not wait to do more.”

“This Was the First Ever Description of Worsening ST-Segment Depression When Exercising in a Polluted Environment Compared to Exercising in Filtered Air”

After his fellowship, Professor Newby secured a lecturer’s position at the University of Edinburgh and began cardiology training in parallel with developing his academic interests. His next “big thing” was securing a long-term programme grant from the British Heart Foundation to investigate the effects of air pollution on the heart. This project was conceived through a chance meeting on a train between his colleague, Nick Boon, MD, FRCP, a consultant cardiologist at Edinburgh Royal Infirmary, and a respiratory physician, Professor Bill MacNee, MD, FRCP, and particle toxicologist, Professor Ken Donaldson, PhD, FRCPath, of the Medical Research Council University of Edinburgh Centre for Inflammation Research. The theory was that air pollution affected the heart in the same way as cigarette smoke. Other key people involved were Professors Thomas Sandström, MD, PhD, and Anders Blomberg, MD, PhD, of Umeå University, Umeå, Sweden, who had spent ≈15 years researching the effects of diesel exhaust and chronic obstructive pulmonary disease, and Nick Mills, MD, PhD, who carried out the research for his PhD. Professor Newby says, “Nick exposed people to dilute diesel exhaust and showed exactly the same as

Spotlight: Alma Zernecke, MD

Professor Zernecke and her coworkers recently published an article in Circulation that conclusively demonstrated for the first time that plasmacytoid dendritic cells and their stimulation drive early atherosclerotic lesion development.
we saw in smokers—that t-PA release is impaired.”

The initial study involved healthy volunteers, so the research was repeated in patients with heart disease, who undertook mild exercise during the study and were monitored with electrocardiography. The results showed that ST-segment depression significantly worsened during diesel exhaust exposure. Professor Newby says, “This was the first ever description of worsening ST-segment depression when exercising in a polluted environment compared to exercising in filtered air, suggesting that patients with heart disease are particularly vulnerable to air pollution, and again they did not release as much clot-dissolving t-PA.”

A further series of studies investigated whether commercially available particle traps fitted onto exhausts could prevent these adverse effects. Professor Newby explains, “We were able to show that these blood vessel effects occur with dilute diesel exhaust, but are removed when a particle trap is used. We were also able to show that you produce less clot if you use a particle trap. This research demonstrates how interventions can make a real difference to cardiovascular health.”

Professor Newby was also involved in another intervention study in 2008 examining the impact of the smoking ban in Scotland that also generated positive results. The Study of Public Place Intervention on Tobacco Exposure (STOPIT), led by Professor Jill Pell, MD, FFPHM (see http://circ.ahajournals.org/content/120/6/f31), at the University of Glasgow, Glasgow, Scotland, found a 17% reduction in cases of myocardial infarction presenting to hospital after the smoking ban in public places came into force compared to before the ban. This article was voted top in the American Heart Association’s Top 10 Research Advances of 2008. Professor Newby says, “These really were, I think, 2 landmark articles, and they have been widely quoted to support initiatives to reduce air pollution including second-hand smoke in public places.”

The work on air pollution continues in China, with studies examining the effects of wearing a simple face mask incorporating an industrial filter (which reduces air pollution exposure by ≈98%) in terms of blood pressure and heart rate variability.

“It [Positron Emission Tomography Using Sodium Fluoride] Suggests that Aortic Stenosis Is Mostly About the Calcification Process and Not the Inflammation”

In 2009, Professor Newby became British Heart Foundation John Wheatley Chair of Cardiology and renewed his interest in imaging. Some 10 years earlier, he had worked on the Scottish Aortic Stenosis and Lipid Lowering Trial, Impact on Regression (SALTIRE) with Dr Boon, which had investigated lipid lowering in aortic stenosis, because researchers then believed that aortic stenosis could be atherosclerosis of the aortic valve. Jo Cowell, MD, Professor Newby’s wife, ran this trial in which a cohort of 155 patients received either atorvastatin or placebo and were followed with electrocardiography and computed tomography scans for 5 years.

Professor Newby says, “The end result was that it did not make a difference, which in a sense is disappointing. It did not show any change in progression, and we looked at it from every possible angle.” He adds that at the time of publication, the results were controversial because it was widely believed that statins must be the answer. However, 2 subsequent trials, Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) and Aortic Stenosis Progression Observation: Measuring Effects of Rosuvastatin (ASTRONOMER), each using a different statin, showed the same results.

Professor Newby continues, “So back to the drawing board; a bit of a heart sink, but I felt what we needed to do was imaging, to find out why it did not work and what will work.” With the help of James Rudd, MD, PhD, at the University of Cambridge, some of his most recent research has involved positron emission tomography scanning patients with aortic stenosis to determine the level of inflammation in the aortic valve using fluorodeoxyglucose as a marker. These studies showed a greater uptake of fluorodeoxyglucose in valves with more disease, but the results were not dramatic.

The team also used another tracer molecule, sodium fluoride, to indicate new calcification. Professor Newby says, “This was dramatically associated with how bad your valve was: the more severe the valve disease, the more sodium fluoride it took up. So it seems to suggest that aortic stenosis is mostly about the calcification process and not the inflammation. The inflammation might be a trigger—it might increase the calcium, but the thing that drives the disease seems to be calcium deposition and perhaps that is why statins did not work. Yes there is some low-level inflammation, but you really want to hit the calcium. We are looking at a potential new trial, SALTIRE2, to investigate drugs that might interfere with the calcium.”

In a follow-up article, which was awarded the American College of Cardiology Parmley prize, lead author Marc Dweck, MD, documented how sodium fluoride uptake in heart arteries appears to predict those at greatest risk of coronary heart disease. Professor Newby believes that sodium fluoride uptake, detected by positron emission tomography could be a novel and important marker of plaque vulnerability, and future work aims to investigate this further.
“I Lost a Stone in Weight, But I Learned So Much”

Professor Newby studied medicine more by accident than design. “No one in my family has ever been a doctor,” he says. “Initially, I applied to be a vet.” However, as a result of various factors he did not study veterinary science, but ended up studying medicine. He recalls, “At that time, the University of Southampton had a new course, which they pioneered, being a new medical school opened in 1966, the year I was born, and I liked the sound of it. Probably a theme of my career to date; a bit of serendipity I suppose.”

Professor Newby graduated in 1991 and then worked as a preregistration house officer in surgery and then in medicine for the next year to obtain full registration. “I did my surgery in Southampton. It was a 1 in 2 in those days [ie, providing 24-hour cover every other day in addition to a 40-hour week], so I worked 122 hours a week on average,” he recalls. A quiet week was 92 hours, a busy week 152 hours. Essentially you were locked in. I lost a stone in weight, but I learned so much. I did my medical job in Newcastle: a 1 in 5 job, and that was 72 hours a week, which was like heaven. I thought I was on holiday. I then did a 2-year general medical training rotation in the southeast of Scotland.”

Professor Newby has an interventional cardiologist since 2002 and now he spends half his time looking after patients. He says, “I do 100% equal with my full-time National Health Service colleagues in terms of on-call commitments. I do less Monday to Friday, but a lot of the out-of-hours. So I have quite a busy clinical commitment in terms of clinics, on-call, and doing interventions.”

In addition, Professor Newby has been National Health Service Health research and development director for 3 years, overseeing research for the southeast of Scotland, including the overall research strategy, funding, and resource allocation. For the past 10 years he has also codirected the Wellcome Trust Clinical Research Facility with hepatologist Professor Peter Hayes, MD, PhD. This facility has grown since its inception in 1997 and currently employs 100 people across 3 sites in Edinburgh, with 300 to 400 active research projects producing 100 to 200 articles per year covering 30 different specialties.

Professor Newby’s third additional role is as codirector of the Clinical Research Imaging Centre, together with radiologist Professor Edwin Van Beek, MD, PhD. He says, “In the Clinical Research Imaging Centre we have a 3 tesla magnetic resonance imaging scanner funded by the British Heart Foundation and the Medical Research Council, we have a 320-slice computed tomography scanner, which was funded by Royal Bank of Scotland, a time of flight computed tomography positron emission tomography scanner, and a cyclotron. I am chief investigator of a national multicentre study to look at the added value of computed tomography coronary angiography in evaluating patients with chest pain.” Professor Newby says, “I think my goals for the next 5 to 10 years are to move more into imaging. We are keen to explore aortic stenosis, look at atherosclerosis and myocardial infarction in terms of imaging, and move progressively into stem cell therapies.”

References

Contact details for Professor Newby:
British Heart Foundation/University of Edinburgh Centre for Cardiovascular Science, Chancellor’s Building, Royal Infirmary, 49 Little France Crescent, Edinburgh, EH16 4SA, United Kingdom

Monika Polak is a freelance medical journalist.
Spotlight: Alma Zernecke, MD

“Our Data Demonstrated for the First Time That Plasmacytoid Dendritic Cells and Their Stimulation Drive Early Atherosclerotic Lesion Development”

Alma Zernecke, MD, associate professor in vascular biology, Department of Vascular Surgery, Technical University of Munich, Munich, Germany, talks to Mark Nicholls.

A recent article in Circulation provides evidence for a hitherto unrecognised plasmacytoid dendritic cell-driven pathway of autoimmune activation in atherosclerosis that amplifies early atherosclerotic lesion formation.\(^1\) To investigate the role of plasmacytoid dendritic cells in atherosclerotic plaque, the final author of this article, Alma Zernecke, MD, now associate professor in vascular biology, Department of Vascular Surgery, Technical University Munich, Munich, Germany, and her group employed a plasmacytoid dendritic cell-depleting antibody administered to apolipoprotein E \(-/-\) mice fed a high-fat diet. They found that depleting plasmacytoid dendritic cells reduced the atherosclerotic plaque burden. Conversely, aggravated lesion formation was observed in high-fat diet-fed apolipoprotein E \(-/-\) mice injected with interferon-\(\alpha\) and in mice injected with CpG oligodeoxynucleotides to stimulate plasmacytoid dendritic cells, associated with enhanced anti-double-stranded DNA antibody titres.

Professor Zernecke comments, “These data conclusively demonstrated for the first time that plasmacytoid dendritic cells and their stimulation drive early atherosclerotic lesion development. Comparisons of early and advanced human carotid artery specimen revealed an increase in expression of plasmacytoid dendritic cell markers in advanced versus early lesions, suggesting that the presence of plasmacytoid dendritic cells correlates with plaque progression in human atherosclerosis. Notably, anti-double-stranded DNA antibodies were elevated in patients with symptomatic versus asymptomatic carotid artery stenosis.”

Professor Zernecke is intrigued by the immune mechanisms designed to protect organisms from infection that go astray and act against the organism to cause chronic inflammation. She says, “We are interested in the involvement of different leukocyte subpopulations and in particular the immune responses that participate in all phases of atherosclerosis. Given the remarkable role of immunity in atherosclerosis, the targeting of its cellular constituents appears to harbour the possibility for new therapeutic approaches to attenuate the disease process.

“We are particularly interested in vascular dendritic cells, which seem to contribute to plaque growth in different ways. These cells accumulate lipids in the vessel wall and control cholesterol metabolism by yet unknown processes. In addition, they promote antigen contact, instruct and recruit T cells, and may also egress from lesions. T cell responses affecting plaque growth seem to be primarily systemically modulated by dendritic cells within lymphoid organs, and a local direct interaction of T cells and dendritic cells may also play a role.”

“Further studies are now required to determine at which stage of lesion progression or degree of hyperlipidemia such mechanisms amount to proatherogenic or atheroprotective effects and how this may modulate proinflammatory versus tolerogenic dendritic cell functions.”

“Our Results Clearly and Directly Implicated Dendritic Cells and Their Effector Functions in Atherogenesis and Introduced CCL17 as an Attractive Therapeutic Target to Prevent Atheroprogression”

Recently, Professor Zernecke worked on the role of the cytokine chemokine (C-C motif) ligand 17 (CCL17) in atherosclerosis.\(^2\) It was “very long and enduring,” with most of the initial findings contrary to those her group had hypothesised. They had expected to see an increase in lesion size in CCL17-knockout mice but found a decrease. She explains, “Initially I was interested in the role of chemokines in leukocyte recruitment and atherosclerosis so I started to work on the role of CCL17, a chemokine that is exclusively expressed by dendritic cells in mice in atherosclerosis. Detection of an elevated expression of CCL17 in human atherosclerosis underscored a possible clinical relevance of this molecule. During the work on this dendritic cell chemokine, I became interested in the role of (auto)immunity in atherosclerosis, and we became familiar with immunological experimental approaches and questions, which really opened up and ignited my research interests in this area.

“In this study we investigated mice with a targeted replacement of the \(Ccl17\) gene by the enhanced green fluorescent protein gene (\(Egfp\)), i.e., \(Ccl17^{E/E}\) to visualise these cells in atherosclerosis and to investigate the pathogenic role of CCL17-expressing dendritic cells and their effector function in atherosclerosis. Using \(Ccl17^{E/E}\) or \(E^{+}\) reporter mice, we showed that mature dendritic cells expressing CCL17 accumulate within atherosclerotic lesions and can migrate from the vessel wall to lymphatic tissue. Genetic deletion of \(Ccl17\) as a dendritic cell-specific effector chemokine in apolipoprotein E \(-/-\) mice reduced development and progression of atherosclerosis in several disease models. Importantly, we provided the first evidence...
that CCL17+ dendritic cells restrain the homeostasis of Treg cells and thereby promote atherosclerosis. Besides peripheral lymphoid organs, this may also contribute to the suppressive effects exerted by lesional Treg cells in the aorta and atherosclerotic lesions. The withdrawal of suppressive effects by lesional Treg cells may correspondingly sustain inflammation and exacerbate plaque growth. Thus, our results for the first time directly implicated dendritic cells and their effector functions in atherogenesis and introduced CCL17 as an attractive therapeutic target to prevent atheroprogession.12

Other important research carried out by Professor Zernecke and her team is a study of endothelial apoptotic bodies. They revealed that apoptotic bodies convey paracrine alarm signals to vascular cells to induce the chemokine (C-X-C motif) ligand 12 (CXCL12) as a protective factor. This, she says, was mediated through transfer of microRNA-126 specifically enriched in apoptotic bodies, which unleashed an autoregulatory feedback loop by repressing regulator of G-protein signalling 16 (RGS16), a negative regulator of the CXCL12 receptor CXCR4. This protective mechanism limited and stabilised the growth of atherosclerotic plaques.3

Professor Zernecke comments that a major challenge for future work is the discovery of an increasing number of different immune cell subsets. Professor Zernecke says, “The plasticity and interplay of these cell subsets in disease development will be important but also challenging. New models of disease or more sophisticated animal models will have to be employed to single out functions of individual cells and to target these for therapeutic approaches.”

“During Autopsies, I Saw That Virtually All Patients Were Afflicted With Different Stages of Atherosclerosis” Professor Zernecke was born in Munich in 1976. During her final year at medical school in Munich, she was awarded a stipend from the Munich-Harvard Educational Alliance to study at Harvard Medical School, Boston, MA, for 4 months. She also spent some time at the Memorial Sloan-Kettering Cancer Center, New York, NY, and at the University of Toronto, Toronto, Canada.

During her studies in Munich, Professor Zernecke spent a semester carrying out research in the Department of Anatomy with Professor Ulrich Welsch, MD, PhD. She also joined the lab of Professor Christian Weber, MD, PhD (see http://circ.ahajournals.org/content/119/24/f139), who had just returned from his postdoc training in the United States. Here, she began investigating the role of adhesion molecules and chemokines in leukocyte recruitment. She says, “The supervisor of my medical thesis, Christian Weber, whom I followed from Munich to Aachen, shaped my career as he introduced me to the field of leukocyte recruitment and cardiovascular disease. In addition, he provided advice and guidance on acquiring funding and he encouraged me to develop my own ideas independently. Scientific discussions with Christian have always been constructive and sparked interesting ideas.”

Professor Zernecke trained in Internal Medicine, Cardiology at the Rheinisch-Westfaelische Technische Hochschule Aachen, which she describes as “a great inspirational environment, with many synergies and collaboration, but also some productive competition. However,” she adds, “it was clear to me that I wanted to pursue research, so I had planned from the start to halve my first 2 years of post-study training and spend 1 year in the clinic and 1 year in research.”

During this period of research, Professor Zernecke became familiar with animal models of cardiovascular disease to address the role of chemokines and different cell populations in vascular remodelling. She was also able to expand the focus of the lab and establish the techniques of intravital microscopy and workup of hearts and large arteries for studying primary atherosclerosis.

An award of third party funding from the German Research Foundation in 2006 led to Professor Zernecke’s promotion as a group leader in Professor Weber’s lab and allowed her to pursue her own research interest and focus on the immune aspects of atherosclerosis. While enjoying the research, she then felt she should follow medical specialisation training, so in 2007, she began training in pathology.
She recalls, “I learned a lot and became even more attracted to cardiovascular research. During autopsies, I saw that virtually all patients were afflicted with different stages of atherosclerosis; in addition, I was struck with the site specificity of some of these changes. For example, patients who had died from a heart attack had minimal disease in the aorta. The inescapable nature of atherosclerosis, regardless of age, gender, and lifestyle, shaped my way of thinking about this disease.” Professor Ruth Knüchel-Clarke, MD, head of pathology in Aachen, enabled Professor Zernecke to follow training in pathology while at the same time carrying out her research alongside the clinical routine. Professor Zernecke says, “Among the few women in medicine who chair a department in medicine, she was a role model for developing my career as a woman.”

A key event in 2009 then shaped Professor Zernecke’s career and its direction: She was granted a Heisenberg Stipendium by the German Research Foundation. At the same time, she was offered a junior research group at the Rudolf Virchow Center, German Research Foundation Research Center for Experimental Biomedicine, Würzburg. “Building up my own research group from scratch, I decided to abandon my aspirations to complete my training in pathology (at least for some years) and focus on research,” she says. In Würzburg, Professor Martin Lohse, MD, gave her the freedom to pursue areas she thought were interesting and recruited her to an environment where funding was generous and where she could independently build a research team free of much of the administrative burden.

Professor Zernecke comments, “The centre is unique in the sense that you are able to work in a free and open atmosphere alongside other junior research group leaders and established research workers in the same building, spanning a wide range of topics centred around target proteins, from structural biology to in vivo mouse models, with great technical expertise and equipment at hand.”

Professor Zernecke has recently taken up her current position as associate professor in vascular biology at the Technical University Munich. She says, “In the longer term, I will be heading the basic research in the department and I will be involved in clinical and translational research approaches, including expanding and developing a large biobank of biopsy and plasma samples for research.”

Professor Zernecke has already won a number of prizes and awards for her research. Looking to the future, she says, “Although I believe that atherosclerosis is a complex and multifactorial disease, and that autoimmunity certainly cannot explain everything, immune mechanisms are important, and in mouse models of disease, their targeting has been shown to be effective in slowing down disease progression, for example, dendritic cell-based vaccination strategies, and immunisation protocols for atherosclerosis with modified lipids or lipoprotein components as antigens aiming to induce specific and atheroprotective antibodies are under investigation. I think, and hope, that a vaccination approach could be feasible in the future.”

References

Contact details for Professor Zernecke:
Klinikum rechts der Isar, Technische Universität München, Klinik für Gefäßchirurgie, Ismaninger Str. 22, 81675 München, Germany. E-mail: zernecke@lrz.tum.de

Mark Nicholls is a freelance medical journalist.