Spring Meeting for Clinician Scientists in Training

“The UK hosts a remarkably diverse community of medical scientists, one that is truly second to none in the world today.”
**Comment**

1. Nurturing tomorrow’s clinician scientists
   J Tooke, J Mass
2. Science: a new generation
   R Horton
3. Anne Johnson and Patrick Vallance: same starting point, different outcomes
   G Watts
4. The researcher of the future...makes the most of social media
   S Scott
5. The researcher of the future...engages with industry
   J Fallowsfeld
6. The researcher of the future...takes advantage of international opportunities
   N Day
7. The researcher of the future...develops powerful networks
   R Lee

**Young Investigator Award Abstracts**

11. Assessment of calcification and inflammation with postmortem emission tomography in aortic stenosis and atherosclerosis
   M R Dweck and others

12. Highly potent human haemopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region
   A vanos and others

13. PTEN mutations as a cause of constitutive insulin sensitivity and obesity in human beings
   A Pol and others

14. Interleukin 23 is critical in the pathogenesis of spondyloarthropathy and acts on a novel population of interleukin 23R+ enthesal resident cells
   J P Sherlock and others

**Oral Plenary Abstracts**

15. Loss-of-function mutations in the immunoglobulin superfamily member 1 gene (IGSF1) cause a novel, X-linked syndrome of central hypothyroidism and testicular enlargement
   N Schoomakers and others

16. The BRC Allergene Project: heritability of nickel allergy and genetic determinants
   T Tsakok and others

17. microRNA-135b promotes cancer progression acting as a downstream effector of oncogenic pathways in colon cancer
   N Volen and others

**Poster Abstracts**

18. Effects of bariatric surgery on human small artery function: evidence for reduction in perivascular adipocyte inflammation, and restoration of normal anticontractile activity despite persistent obesity
   R Aghamohammadzadeh and others

19. Correlation between ultrasound imaging of major salivary glands and histopathological findings of glandal biopsy samples in Sjögren’s syndrome
   S Al and others

20. Safety and efficacy of liraglutide in patients with type 2 diabetes with elevated liver enzymes: individual patient data meta-analysis of the LEAD programme
   M J Armstrong and others

21. N-acetylcysteine and liberase improve success of hepatocyte isolation and viability of hepatocytes isolated from normal and diseased liver
   D C Bartlett and others

22. Neutrophil recruitment in response to intradermal endotoxin challenge in man
   A Rasam and others

23. Manipulation of liver regeneration with macroporphages to influence the hepatic progenitor cell niche
   T G Bird and others

24. Use of ENCODE and eQTL data to identify potential functional genetic variants at the 3q13 psoriasis arthritis susceptibility locus
   J Bluett and others

25. Lentiviral-vector-mediated gene therapy for X-linked lymphoproliferative disease restores humoral and cellular functions
   C Booth and others

26. Transcribed ultraconserved regions are aberrantly expressed and can be modulated by interleukin 6 in cholangiocarcinoma
   C Bricoti and others

27. Genetic and functional investigation of a Mendelian form of systemic lupus erythematosus
   T A Briggs and others

   D J Carr and others

29. Outer retinal transduction can be achieved after intravitreal delivery of adeno-associated virus serotype 2 vector with glycosidic enzymes
   J Chege-Kapotanovic and others

30. Influence of acute matrix metalloproteinase activity on myocardial dysfunction associated with urgent cardiac surgery: cardioprotective effects of inhibition
   E S Teh, J Chambers

31. Invasive pneumococcal disease among HIV-1 seropositive individuals in the era of highly active antiretroviral therapy: does HIV-1 impair the macrophage host response to pneumococci?
   P J Collin, D J Dockrell

For the Spring Meeting for Clinician Scientists in Training Abstracts see
http://www.thelancet.com/journals/lancet/specialissue

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The Spring Meeting was established by the Medical Research Society in 2002. The Medical Research Society merged with the Academy of Medical Sciences in October, 2011.
32 Neurodegeneration caused by intronic expansions of C9ORF72 is a clinically heterogeneous but pathologically distinct disease
J Cooper-Knock and others
33 Urinary transferrinase 2 as a potential biomarker of chronic kidney disease detection and progression
M da Silva Lodge and others
34 The epigenetic phenotypic switch of vascular smooth muscle cells involved in atherosclerosis
M Davies and others
35 The anti-citrullinated antibody repertoire in periodontitis: a role in the induction of autoimmunity in rheumatoid arthritis?
P de Pablo and others
36 Effect of perhexiline on myocardial protection during coronary artery surgery: a two-centre randomised double-blind placebo-controlled trial
N E Dreyer and others
37 Reassessing long-term risk of suicide after a first episode of psychosis
R Dutta and others
38 Imaging abnormal skin sensations: a novel functional MRI study
J A Eccles and others
39 Plasma biomarkers of abdominal aortic aneurysm
S Ehan and others
40 Microalbuminurina could improve risk prediction of stroke in patients with transient ischaemic attacks and minor strokes
S Elyas and others
41 Viscoelastic characterisation of chordae tendineae of the mitral valve: requirements for future replacement materials
A G Wilcox, D M Espino
42 Characterisation and immunogenicity of a decellularised skeletal muscle scaffold for laryngeal tissue engineering
J M Fishman and others
43 Outcomes of kidney transplantation in HIV-positive patients: the UK experience
E Gathagho and others
44 Antigen-sensitive CD8 T-cell clones with tough HIV-1 suppression
J M Glanville and others
45 Abdominal aortic aneurysm repair outcomes in aortic stiffening
Y J Gokani and others
46 Next generation sequencing of RNA from muscle biopsies shows marked differences between inflammatory myositis, inclusion body myositis, and controls
P Hamann and others
47 Tumour necrosis factor-related apoptosis-inducing ligand is a novel therapeutic target in pulmonary arterial hypertension
A G Hameed and others
48 High bone mass is associated with an increased prevalence of joint replacement
S A Hardcastle and others
49 Plasma membrane proteomics identifies Notch1 as a potential regulator of ras-induced senescence
M Hoare and others
50 Is there a correlation between lung function values and cardiopulmonary exercise outcome?
M Homsy and others
51 Urinary C-peptide creatinine ratio to detect absolute insulin deficiency in type 2 diabetes
S V Hope and others
52 Patients with early inflammatory arthritis who fulfil the 2010 American College of Rheumatology–European League Against Rheumatism classification criteria for rheumatoid arthritis have increased mortality compared with those who do not: results from the Norfolk Arthritis Register
J N Humphreys and others
53 Effects of phosphodiesterase type 5A inhibition on intracellular calcium handling and its implications for cardioprotection and antiarrhythmogenesis
D C Hutchings and others
54 Mechanical unloading reverses transverse tubule remodelling and normalises local calcium-induced calcium release in a rodent model of heart failure
M Ibrahim and others
55 A chemical-genetics approach to study the molecular pathology of central serotonin abnormalities in fetal valproate syndrome
J Jacob and others
56 Investigation of idiopathic inflammatory myopathy for shared genetic risk factors with other autoimmune diseases: results from the European Myositis Network
M Janis and others
57 Early rheumatoid arthritis and resolving fibroblasts segregate according to Dickkopf related protein 1 expression
M Juarez and others
58 Congenital melanocytic naevus syndrome is caused by a post-zygotic mutation in codon 61 of NRAS, predisposing to melanoma development in affected tissues
V A Kinsler and others
59 Differential effect of amisulpride on cognition in schizotypy: validation of models for the early identification of cognitive enhancing agents
I Krychev and others
60 Best clinical care versus the common good of research: a solution for challenging surgical trials
Y I Kulikov and others
61 Isocitrate dehydrogenase mutation analysis in gliomas as a diagnostic and prognostic biomarker
K M Kuriyan and others
62 First-in-man evidence of the mechanistic effects of biventricular pacing on coronary physiology
A Kyriacou and others
63 Expression of inhibitory Fc receptor (FcyRIIB) is a marker of poor response to rituximab monotherapy in follicular lymphoma
C S Lee and others
64 A novel subset of functional interleukin-10 secreting CD8 regulatory T cells infiltrate human hepatocellular carcinoma
K K Li and others
65 Sequential expression of CD39 regulates late developmental T helper type 17 plasticity imparting a regulatory cell phenotype
M S Longhi and others
66 Association between anti-tumour necrosis factor therapy and risk of ischaemic stroke in patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Registers–Rheumatoid Arthritis (BSRBR-RA)
A S L Low and others
Molecular regulators of cardiovascular valves in development and disease

Seralogical status: a predictor of response to intensive therapy in rheumatoid arthritis

Prediction of treatment response in psoriasis with measurement of serum levels of adalimumab, etanercept, and antiderug antibodies: a pilot study

Diabetes and cardiovascular events in women with polycystic ovary syndrome: a 20-year retrospective cohort study

Depot-specific and sex-specific secretion of leptin and interleukin 6: higher leptin release in women and lower interleukin-6 release from femoral adipose tissue in vivo

Sensitivity to cuteness in baby faces is not influenced by pregnancy

Characterisation and therapeutic potential of endothelial progenitor cells

Signatures of CD4 T-cell help and CD8 exhaustion predict clinical outcome in autoimmune, infection, and vaccination

Incidence of eating disorders in the UK: findings from the UK General Practice Research Database

Novel in-vitro model to study first responses of airway epithelial cells to allergen and pro-inflammatory stimuli at birth

Endocrine disruption in the human fetal testis: use of a xenograft system to assess effects of exposure to environmental agents and pharmaceutical drugs

TIIE2-expressing monocytes regulate revascularisation of the ischaemic limb

Hepatic inflammation and fibrosis biomarkers are associated with cardiovascular risk factors but not cardiovascular disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study

Preventing dedifferentiation of human exocrine enriched pancreatic cells in culture

Adverse impact of heart failure on the electrophysiological response to ischaemia-reperfusion in human myocardium

Does muscle inflammation influence recovery of muscle strength and function in patients undergoing total hip replacement?

CXCR6 and CXCL16 in liver disease

Endothelial progenitor cells in smokers are dysfunctional because of increased DNA damage and senescence
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>The Rheumatoid Arthritis and Falls (RAF) study: a prospective study of fall risk factors in adults with rheumatoid arthritis</td>
<td>E K Stanmore and others</td>
</tr>
<tr>
<td>104</td>
<td>Fn14 is expressed on neoplastic cholangiocytes in intrahepatic cholangiocarcinoma and promotes necrosis after interaction with TWEAK</td>
<td>B Stephenson and others</td>
</tr>
<tr>
<td>105</td>
<td>Investigation of the pathogenesis of uromodulin-related genetic disease</td>
<td>A P Stewart and others</td>
</tr>
<tr>
<td>106</td>
<td>A strong correlation between expression of Wntless and of human epidermal growth factor receptor 2 in gastric, ovarian, and breast cancers suggests a novel-signalling pathway involving NFκB and STAT3</td>
<td>J Stewart and others</td>
</tr>
<tr>
<td>107</td>
<td>Molecular effects of UVA1 in human skin in vivo</td>
<td>A Tewari and others</td>
</tr>
<tr>
<td>108</td>
<td>Analyses of blood outgrowth endothelial cells reveal an endothelial HOX gene signature in human beings</td>
<td>M Toshner and others</td>
</tr>
<tr>
<td>109</td>
<td>Investigation of the role of B lymphocytes and tertiary lymphoid tissue in a murine model of renal chronic allograft damage</td>
<td>G Tse and others</td>
</tr>
<tr>
<td>110</td>
<td>The first inborn error of manganese metabolism caused by mutations in SLC30A10, a newly identified manganese transporter</td>
<td>K Tuschl and others</td>
</tr>
<tr>
<td>111</td>
<td>Parallel pathways of glutamate and ATP-mediated excitotoxicity cause significant neural cell death during ischaemia: potential for novel neuroprotective strategies</td>
<td>P Vermehren, R Fern</td>
</tr>
<tr>
<td>112</td>
<td>Hypoxia modulates the expression and secretion of inflammation-related adipokines in differentiated human adipocytes</td>
<td>B Wang, P Trayhurn</td>
</tr>
<tr>
<td>113</td>
<td>Selective recruitment and retainment of regulatory T cells in human colorectal cancer</td>
<td>S T Ward and others</td>
</tr>
<tr>
<td>114</td>
<td>Mutations of TCF12, encoding a basic-helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis</td>
<td>V P Sharma and others</td>
</tr>
<tr>
<td>115</td>
<td>Defining the functional role of laminin isoforms in the adult hepatic progenitor cell response</td>
<td>M J Williams and others</td>
</tr>
<tr>
<td>116</td>
<td>Trimodal pattern of C9ORF72 GGGGCC normal allele repeat number in sporadic amyotrophic lateral sclerosis and lack of association with disease risk and age at onset</td>
<td>I Woollacott and others</td>
</tr>
<tr>
<td>117</td>
<td>Mitochondrial DNA damage promotes atherosclerosis and is associated with vulnerable plaque</td>
<td>E Yu and others</td>
</tr>
<tr>
<td>118</td>
<td>The contact electrogram and its architectural determinants in atrial fibrillation</td>
<td>J A B Zaman and others</td>
</tr>
<tr>
<td>119</td>
<td>Loss of CaMKKβ attenuates endotoxin induced hypotension</td>
<td>J Zhao and others</td>
</tr>
</tbody>
</table>

**Index of Poster Abstracts**

| 120  | Index table                                                                 |
Nurturing tomorrow’s clinician scientists

Clinician scientists are a small but critically important sector of the medical profession. We must nurture them if society is to reap the rewards of medical science. Clinical science has been an historic strength in the UK, yet surveys conducted by the Medical Schools Council in 2001, revealed a decline in clinical academic numbers. There was evidence not only of a decline in the number of those seeking a career in clinical academia, but also of a reduction in the number of trainee specialists intent on a career devoted to clinical practice who had undertaken any form of research training. This “binary divide” in the medical workforce, where a minority pursue research as an integral part of their professional career and a majority have little or no research exposure, denies the reality that all doctors must be research aware if they are to critically appraise new developments that could impinge on their practice, or facilitate research led by others.

The Medical Schools Council data precipitated important moves by the National Institute for Health Research, the Wellcome Trust, the Higher Education Funding Council for England, and others in England, and similar responses in the devolved administrations, to address the deficit, and in particular to attend to the critical early development stages of doctoral training and postdoctoral lecturer positions. More recent surveys show encouraging trends, with lecturer posts having increased by 34% since 2006, although several disciplines still remain vulnerable.

The importance of ensuring a robust pipeline of talent cannot be overestimated. It is increasingly well established that the criteria for impactful biomedical research include close linkages between fundamental and clinical science and industry, and research questions being closely informed by clinical need. Furthermore, increasingly sophisticated phenotyping to which the clinician scientist is particularly well equipped to contribute, will be an essential part of the journey towards “precision medicine”—more effective therapy based on a deeper understanding of the molecular pathology of disease.

The way new drugs are developed is already changing. Conventional industrial models of drug discovery are failing, with the time and cost associated with the successful introduction of new therapy spiralling. New models are being sought that involve closer alliance between academia and industry such that the discovery capacity of universities can be harnessed and earlier proof of concept verified. Tomorrow’s clinician scientist will need to be able to work effectively in such partnerships if the new models are to succeed. Cultural barriers will need to be overcome, not least through increased permeability between the two sectors leading to a greater appreciation of one another’s roles.

The path of the clinician scientist is a challenging one, combining as it does clinical service commitments with the demands of a research career to which are often added teaching and managerial responsibilities. Those challenges may be especially profound for women in medicine with surveys revealing marked under-representation in senior clinician scientist positions, although the proportion of women in lecturer grade posts is slowly improving (an increase of 43% since 2004). But the career of a clinician scientist is also a deeply fulfilling one, if trainees are appropriately prepared to rise to those challenges and if positive measures are taken to address gender imbalance. The recent requirement for institutions in receipt of

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Comment

National Institute for Health Research funding to show commitment to gender equality has provided a timely and effective stimulus in that regard.

Many influences spur aspiring clinician scientists on their way: a receptive health service environment that values research is crucial. The binary divide referred to above can mitigate against this, but the renewed commitment of the National Health Service to its research function and the creation of Academic Health Science Networks in England to promote the adoption and diffusion of medical innovation should help. As in other branches of medicine, role models (including increasingly those who spend some or all of their time in industry) have a key role in enabling a trainee to envisage the successful career they would wish to emulate. Mentoring by more senior figures, who have trodden the path before yet are not directly associated with the trainees’ employment or role, can be hugely beneficial. The success of the Academy of Medical Sciences mentoring scheme has been externally validated. A booklet and suite of short introductory films provide useful guidance for those wishing to establish such schemes. Professional bodies such as the Royal Colleges in the UK, which help define professional training standards, must embrace the needs of those who have embarked on academic careers and also take a leading role in creating this receptive environment for research. A number of Royal Colleges are introducing research competencies into their curricula for all trainees, including the Royal College of Physicians (RCP). The RCP is also developing an initiative to deliver this culture change by targeting support towards those clinicians who do not have formal academic roles but who facilitate the delivery of research.

Crucial too is the role of peer support and the opportunity to meet fellow trainees at a similar stage of development, particularly in those disciplines where numbers in any one centre may be extremely limited. A real value of the Spring Meeting for Clinician Scientists in Training, jointly hosted by the Academy of Medical Sciences and the RCP and building on the legacy of the Medical Research Society, is that it combines many of these positive influences to good effect. Primacy is given to trainee presentations, interspersed with talks from established senior figures reflecting on their own work and experience. Adequate opportunities to network socially complement the more formal part of the programme. The formula is by no means unique, but is a winning one as judged by the quality of the debate and the formal and informal feedback. Successful it must be if society is to benefit from promised and achievable advances in medical practice.

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what most scientists could imagine. In the UK, we try to be more sanguine. Those leading the Research Excellence Framework—a reductive Court of Horrors that holds a sword over the neck of individuals and institutions alike—say they reject impact factors when judging the quality of work they receive. Quite right. But for journals that hope to be global—Nature, Science, New England Journal of Medicine, and The Lancet, for example—a fall in impact factor would send a shock wave of uncertainty across many national science communities. This ridiculous situation has been created by those very same national science communities. There are, as yet, few signs that it will end soon.

In the 1990s, we decided we wanted to open up our pages to talented young scientists. We understood that it was unlikely these young researchers would have successfully completed a randomised trial among 50 000 participants or a piece of basic research of Lasker or Nobel standards. The only way to get young scientists into the journal was to create a new opportunity, which we called “research letters”. There, short descriptions of interesting new findings could be reported, and so the journal would not be seen as an exclusive club for small elite. What a terrible idea that was. Over the coming years, as we published as many as ten research letters each week, our impact factor collapsed. Who cares? Surely, I reasoned, sensible people are past the point where they took the impact factor seriously. How wrong I was. Wherever I went people asked if I was unwell. Didn’t we care any longer about quality? How soon did I think it would be before I was fired? I laughed these questions off, but secretly knew we had made a gloriously stupid mistake. Though many very intelligent people know and say that impact factors represent a corrupt game, they continue to pay attention to these strange, even dangerous figures. We couldn’t seem to escape their tyranny. At least, we couldn’t escape unless every serious journal decided at the same time to renounce the number that ruled our lives. And they weren’t about to do so.

We killed research letters. We had to. And we threw talented young scientists on the funeral pyre of bad ideas. Until recently. At most scientific meetings today there will be thousands of oral presentations and posters. Once, a long time ago, you carried around an enormous, back-breaking abstract book to guide you through the event. Now, you will find those same abstracts on CDs, sticks, or just online. The problem with this technology is that you can take the science or leave it, and many people simply leave it. Which is a pity. For in these abstracts you will find hidden gems of exciting new work completed by brilliant young researchers. So what about an alternative model—a small meeting, perhaps with no more than a hundred or so abstracts, printed and widely distributed freely online. You can read a hundred abstracts quite easily. You can dig deep into the meeting and get a good sense of how a field is evolving and who its leaders are.

We began with a global health metrics conference held in Seattle in 2011. Last year, we published abstracts from young investigators at the World Health Summit, held in Berlin. And we co-sponsored a London meeting of public health science, which revealed a new era of multidisciplinary ingenuity in public health research. This year, we will continue with each of these three relatively small (by gargantuan global standards) conference abstract collaborations. And now we are delighted to add a fourth—a meeting of young clinician scientists organised by the Academy of Medical Sciences and the Royal College of Physicians. As this abstract book shows, the UK hosts a remarkably diverse community of medical scientists, one that is truly second to none in the world today.

Journals aren’t liked by everybody. Some see us as having too much influence on scientific research. They would prefer it if we died and the scientific community moved to massive online databases where there is a bias to publish everything that passes a minimum quality threshold. I can see the practical attractions of that idea. But be careful what you wish for. Journals have other roles too. We can advocate, encourage, praise, celebrate, applaud, question, monitor, reflect, incentivise, improve, amplify, and spotlight work that might otherwise drown in a sea of well-intentioned mediocrity. There is nothing mediocre in what you will see here. Please read each abstract. You will find something of remarkable interest on every page.

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Anne Johnson and Patrick Vallance: same starting point, different outcomes

A glance at the career histories of this year’s keynote lecturers reveals little in common. True, they both opted for academic medicine and have both worked (one still does) at University College London (UCL). But while Patrick Vallance trained in clinical pharmacology and is now a senior research director in the pharmaceutical industry, Anne Johnson opted for public health with a focus on the epidemiology of infectious disease. Not much similarity there. But get them talking about their original motives and they turn out to have one thing in common; both were initially drawn to medicine by something more than the straightforward urge to be a doctor.

Prof Vallance, now president of research and development at GlaxoSmithKline (GSK), has a love of science that dates back to his school days. But he didn’t want to be what he calls “a pure scientist”. He wanted to do something that involved its application. Since he was also attracted by the idea of human interaction, medicine became the natural choice.

Anne Johnson, professor of infectious disease epidemiology at UCL, says she was always interested in the association between socioeconomic conditions and health. “A lot of the things that determine whether or not people live or die are not to do with doctors”, she points out. So while she studied medicine at Cambridge, she also chose to incorporate courses in social and political science: a choice that foreshadowed her subsequent career in public health.

First to Patrick Vallance. Following his graduation in 1984 he spent the late 1980s and early 1990s in the Department of Clinical Pharmacology at St George’s Hospital Medical School. His field of interest during this time was mainly but not exclusively cardiovascular. He then moved to UCL as professor of clinical pharmacology, becoming head of the Division of Medicine in 2002.

It was 4 years later that he was invited to join GSK. No one was more surprised than Vallance himself when he accepted the offer. He’d had no intention of forsaking academic life, he says. In fact he was already anticipating becoming UCL’s head of biomedicine. His only previous relationship with the drug industry had been as a member for 2 years of GSK’s research advisory board. When the company offered him a full time job his immediate response was thanks…but no thanks. By the following morning he’d changed his mind. “I thought I can either spend the rest of my career being somewhat critical of industry, or I can go into it and see if I can make a difference. The switch flicked overnight. The next morning I thought, yes, I’m going to do it.” So, far from being the fulfilment of a long held ambition, taking the new job was a sudden change of course.

The move, he says, has proved a happy one. “I think more about the breadth and future of medicine and what needs to be done now than at any time in my career. You have to keep thinking about how health care is going to evolve and where the need will be across a whole range of diseases. It’s been a mind-broadening change.”

GSK offered him the job, he thinks, because it wanted someone in charge of discovery who had a science background, current clinical knowledge and experience, and the capacity to concentrate that experience on the search for clinically important medicines. The focus of his first few years was in reorganising the company’s research effort. The aim was to create interdisciplinary groups to provide leaders across a range of areas, and so avoid having all decisions made by just one or two people. “We wanted these individuals and their teams to apply judgment, intuition and scientific nous to the decisions they made, rather than viewing what they did as a conveyor belt process.” The intention was to make the company’s efforts in research and development more outward looking, more part of the global scientific community.

By contrast, one of Anne Johnson’s long standing concerns has been with the polar opposite of looking outwards. Although her current interests include preparations for global warming and the impact of seasonal and pandemic influenza, the work for which she is best known are surveys of peoples’ sexual behaviour—surveys prompted by the advent of HIV and dependent for their success on honest introspection by the many thousands who, over the years, have agreed to take part in them.
Her first involvement with HIV and other sexually transmitted diseases was in 1985 when she became a lecturer in genitourinary medicine at the Middlesex Hospital Medical School, London. She soon realised that predictions about the likely scale of the HIV epidemic were meaningless without reliable data on peoples’ sex lives. So she and some colleagues designed and carried out a pilot study. It demonstrated that such surveys were feasible.

Together with a like-minded group at the London School of Hygiene and Tropical Medicine she ran a second pilot study, and then applied to the Economic and Social Research Council for a grant. After some delay this was refused, allegedly on account of the disapproval of the then Prime Minister Margaret Thatcher. In little more than ten days the Wellcome Trust, persuaded of the importance of the work, came to the rescue. The first National Survey of Sexual Attitudes and Lifestyles (NATSAL 1) was saved. “It gave us an estimate of the proportion of the male population who had same sex contact”, says Johnson. It also yielded other data necessary to make the first reliable projections of the spread of HIV. Its evident value promoted the creation of a second survey, NATSAL 2, tracking changes in behaviour over time, and much else. A third is still in progress.

Anne Johnson’s view of the current state of HIV is mixed. Treatment, she points out, has undergone enormous developments. But she is less sanguine about prevention. “On the one hand people can have an almost normal life. On the other, around the world there is still a large number of new infections each year. Risk behaviours have increased because people are less concerned or less educated about HIV.”

Although surveying the sexual appetites of one’s fellow human beings and restructuring an international research enterprise are both essential tasks, they have little in common—a reminder that the same starting point, a degree in medicine, can have vastly different outcomes.

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The researcher of the future...makes the most of social media

Social media refers to a wide variety of online platforms within which people can interact, from providing online reviews on Amazon to updating information on Wikipedia pages. At the heart of social media lies the idea of user-generated content, with the implication that we as users don’t simply absorb online information, but actively contribute to it. As a researcher, I have found social media to be extremely useful in a number of different ways, and it has enabled me to both engage people in my research and to get involved in more public discussions. For me, Twitter has been the best medium for these activities. Twitter is a micro-blogging site, where users post short bursts of information (i.e., under 140 characters) known as tweets. There are over 500 million registered Twitter users sending approaching 350 million tweets daily. Twitter can be accessed via the website, apps on smart phones, or SMS (text) messages on mobile phones. Anyone can read tweets that have been posted, but you need to be registered to post tweets.

For me, the beauty of Twitter is that tweets can be comments photos or links to webpages and videos. Furthermore, unless tweets are kept private, they are public and searchable—for example, they can be found by search engines like Google. Users can help focus searches by including hashtags, phrases starting with a # sign (e.g., #neuroscience), in their tweets: people clicking on the hashtag can immediately find all the tweets referring to this topic, reply to them, or retweet them (i.e., repost them). Twitter uses the tag “join the conversation”, and like conversations, the precise content of people’s tweets is as wide and as variable as any human interest: many users discuss live television programmes as they go out (e.g., Newsnight) and the programme makers often encourage this by suggesting such hashtags; others use Twitter as a way of telling jokes or sharing news.

As a scientist, I find that Twitter provides an excellent and flexible forum for many different facets of my work. Twitter is an outstanding forum for...
promoting public engagement work. The Institute of Cognitive Neuroscience runs an annual Brains on Film competition, as part of Brain Awareness week, and I tweet links to the films on YouTube. These films have now been viewed thousands of times and picked up by other media (eg, national newspapers). I also use Twitter to promote new papers from my lab, and blogs that I and members of my lab have written for our website. Another example would be Science Showoff, a science communication event that I am involved with, at which anyone with an interest in science can present short demonstrations, songs, films and talks about science. Science Showoff, which recruits performers and promotes events solely via Twitter and Facebook, has been highly successful, with a wide variety of demonstrations and sell-out gigs.

Promotion of events and research is a key feature of Twitter, but it is not the only one. I find the more interactive aspects of Twitter to be extremely valuable, and enable me to get involved in more outward facing public engagement activities. Earlier in the year, UK Channel 4 showed a programme over two nights, Drugs Live, in which different aspects of the drug ecstasy were discussed and demonstrated with functional MRI (fMRI). This programme was enthusiastically discussed on Twitter and I became engaged in some specific conversations about the extent to which the fMRI component was adding to the debate, and the potential limitations of this technique. In this way, Twitter allows scientists to get involved in public discussions of science-relevant matters in a way that is both easily managed (in terms of time and amount of involvement) and immediate.

Finally, I have found Twitter to be a very useful scientific resource. In addition to being an effective and novel way of encountering other people’s research (via people tweeting about new papers or science in the news), Twitter can function as a forum for online journal clubs, where the discussion just happens to be public. I frequently live-tweet seminars that I attend, and I encourage my students to do so as well: not only does this mean that people can follow the speaker’s talk in real time, but it also is an excellent training in summarising points in a clear and concise fashion. I have also had opportunities to make scientific collaborations via social media. I’m collaborating on a project involving both medical and basic scientists as well as humanities researchers to look at the phenomenon of voice hearing: my role in this research came about entirely through connections made on Twitter.

More and more universities and funding bodies are realising the importance of public engagement activities, and social media allow a flexible and relatively non-time-intensive way of both engaging in debates and promoting events and research. Furthermore, it does so in a way that facilitates evaluation—for example, one can count Twitter followers and numbers of retweets to measure the ways one’s presence is having an impact on Twitter. As a scientist, I believe that public engagement is an essential activity, both for promoting and explaining our work as scientists, and for shining a light onto the nature of scientific investigations. Social media tools like Twitter permit us to provide a degree of public engagement that we can manage ourselves, and with which we can engage in as often or as intensively as suits us. I’d encourage you to join the conversation.

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The Academy of Medical Sciences tweets from @acmedsci, @amsmentoring, and @inspireresearch; a list of tweeting Academy Fellows is available online http://bit.ly/YHtzr9.
The researcher of the future...engages with industry

Innovation and collaboration are now regarded as crucial for the future success of industry, efficiency of the National Health Service, improved outcomes for patients, and catalysing economic growth in the UK. The scientific landscape has changed dramatically in recent years. Big pharma is increasingly outsourcing research and development to boost pipelines as drug development budgets contract, and universities are transforming from ivory towers to engines of economic growth. Academia needs industry. Industry needs academia. Consequently, there are great and varied opportunities for the more proactive, entrepreneurial researcher to exploit.

My personal experience of engaging with industry has had both highs and lows and reflects the complex nature of academic researchers’ interactions with biotechnology and pharmaceutical organisations. Like most academics, research-related factors (rather than commercialisation) have been my primary motivators for engagement.

Industry engagement often generates considerable benefits for academic research, including funding, in-kind resources (eg, high throughput facilities, small molecule libraries), commercial exploitation of technology or intellectual property, and cross-fertilisation of knowledge and skills. Accordingly, interactions between academics and industry can take multiple forms, such as collaborative (precompetitive) research often subsidised by public funding, contract research directly commissioned by companies, and consultancy services provided by individual academic researchers to industry.

It is important to recognise that the different objectives and ethos of academia and industry can potentially cause conflict. Whereas researchers may be concerned with restrictions to academic freedom and the right to publish, industry may be more focused on protection of confidential information and intellectual property, market penetration, and shareholder value. Likewise, the entrepreneurial university will also seek commercially relevant outputs such as patents and spin-offs. The Lambert review of business-university collaboration made a series of recommendations aimed at smoothing out the path between the UK’s strong science base and the business community. Responses to the report suggest that it should be possible to reconcile the often divergent interests of both parties through intelligent drafting of collaboration agreements, dealing upfront with publication rights, intellectual property, and confidentiality.

I am currently involved in a collaborative study with a major pharmaceutical company (an exploratory clinical trial). I first approached the company 3 years ago with what I considered to be persuasive laboratory data. Initially, I found it difficult to make any headway within the organisation. However, once I connected with the right person the collaboration rapidly took off. It was a physician within the company who knew my specialty who eventually catalysed discussions, highlighting the benefits to both academia and industry of individuals who move between sectors.

Exchange of knowledge and ideas is now free-flowing, facilitated by a robust confidentially agreement and effective channels of communication. We will benefit from an industry sponsored research fellow for the duration of the trial and, hopefully, the opportunity to engage fully in the developmental path to market. Expectations are high and the company will be looking for targets and deliverables within a tight timeframe.

By contrast, I have also experienced the rough end of engagement with industry. Along with colleagues, I had been working with a company for around 4 years on a diagnostic project. We suggested drawing up a UK Medical Research Council (MRC) industry collaborative agreement (MICA) and a heads of terms (HoT) agreement. The MICA form outlines the nature of the collaboration and...
the industrial partners’ contribution to the project, and the HoT form asks applicants to outline arrangements around intellectual property and project management. Negotiations were protracted and difficult and ultimately the project was cut without warning and the industry partners withdrew. Several years of trust and effective collaboration were blown away.

I also have experience of consultancy projects, which are usually commissioned directly by the industry partner; payments are either received by individuals or channelled back through university accounts to support research. Perhaps naively, I have undertaken this work primarily as a loss-leader to build personal relationships with industry contacts and to learn about industry problems and applications. Time will tell how useful this has been.

Academic-industrial partnership now lies at the heart of the strategic plans of both the MRC and Wellcome Trust, with numerous initiatives to support collaborative research and training and career development opportunities—for example, the MRC industrial CASE scheme for PhD students and the Wellcome Trust Interdisciplinary Training Programmes for Clinicians in Translational Medicine and Therapeutics (I currently supervise a PhD student under the Scottish wing of the Wellcome scheme). There is also increasing evidence of industry actively reaching out to academia (eg, GlaxoSmithKline’s R&D Esprit programme and Discovery Partnerships with Academia). Perhaps the exemplar model for how academia and industry can interact productively and sustain a durable partnership is the Division of Signal Transduction Therapy (DSTT)—a collaboration between the University of Dundee, MRC, and six of the world’s leading pharmaceutical companies to accelerate the development of improved drugs to treat global diseases such as cancer. Founded in 1998 and awarded a Queen’s Anniversary Prize for Higher and Further Education in 2006, DSTT has just secured renewed funding until 2016.

Engaging with industry opens up avenues for research that would not otherwise be possible, and although there are potential pitfalls, if we are serious about translational research, we cannot ignore the opportunities.

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The researcher of the future...takes advantage of international opportunities

For today’s clinician an international perspective on health has never been more important. An understanding of the global epidemiology and burden of disease is essential in a world where both international travel and migration are commonplace, and infectious diseases in particular are no longer neatly geographically constrained. And for today’s aspiring academic clinician the research landscape has never been more globalised. The internet, the proliferation of international conferences, and easy access to journals from around the world has meant that in all fields international collaborations are easy to pursue as well as essential to staying at the forefront of your chosen research area.

The past quarter of a century has seen a dramatic change in the way in which clinicians are trained in the UK. When I began my postgraduate career there was little concept of organised training for doctors once they passed their finals. For physicians there was the considerable hurdle of the MRCP(UK) Diploma, but other than that you just toiled through a series of career grades until you reached a point where an appointments committee would employ you as a consultant. If you aspired to an academic career, or just wanted to improve your chances against the competition, you would take several years out to pursue a PhD or MD, and that was about it. This system provided a great deal of freedom, and it was very much up to you...
Comment

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...
The researcher of the future...develops powerful networks

The rules of engagement for health researchers are starting to change. Traditional concepts of academic individualism and elite institutions are being challenged by a range of influences that are pulling researchers in a collegiate direction. Our obligation is to ensure that the fruits of our innovations are widely disseminated for the benefit of all.

We are increasingly incentivised—by the National Institute for Health Research (NIHR) and other bodies—to interact with each other. Far-reaching networks have been established, both to promote national engagement in clinical trials and to achieve high impact through the practical application of advances generated through health research across the National Health Service (NHS). These strategies are becoming embedded in usual clinical practice and the culture shift towards collective empowerment is now also trickling from applied research in common diseases through to early phase human research in tertiary care disciplines.

My experience in the NIHR Moorfields Biomedical Research Centre (BRC) reflects this, since the scientific theme in which I work uniquely enshrines a cross NHS partnership between London and Bristol to harness the strengths in UK ophthalmology and align these under a single strategy for experimental medicine. This pioneering and outward facing approach is achieving economies of scale in shared technologies and expertise.

Our projects have already extended beyond our initial goal of joint preclinical and early phase studies in inflammatory eye diseases to encompass later phase clinical trials of novel interventions for all major retinal disorders. Together, Bristol and Moorfields have access to a referral base of up to 20 million people across the south of England, which represents a network for clinical research in ophthalmology unmatched anywhere in the world.

Our most important such partnership to date was established in May, 2012, with the US National Institutes of Health (NIH) for the study of human ocular inflammation. As only the third NIH-UK consortium of its kind to have been signed, it promises to deliver advances that would have been impossible to achieve independently. The first studies to run across all three sites (Bristol, Moorfields, and Bethesda) are currently underway. The challenges of implementing an integrated programme of work across such large distances are daunting but not insurmountable, even though we must communicate across the Atlantic.

For me, the key to overcoming these geographical barriers lies in the strength of relationships between the collaborations’ leaders. This contact needs to be cemented with shared personnel who straddle institutional boundaries and visibly embody the collective ambition all parties have chosen to pursue. The otherwise separated teams at each site can then be brought together using widely available technologies for video conferencing.

Two or three times a month, the retinal investigators in Bristol and Moorfields now meet virtually to review their studies and agree strategy. Transatlantic conversations with the NIH are equally amenable to this approach, and although we have been clocking up our air miles to get the consortium off the ground we have also now started joint remote research meetings.

It is the regular and reliable availability of remote colleagues that make the bond real. Our post-doctoral researchers and PhD students now speak to each other with Skype or Google+ to troubleshoot and discuss day-to-day issues, at the click of button, much as you would do with a colleague down the hall.

Our example demonstrates that BRCs can provide subspecialist hubs around which other units with niche expertise can cluster, creating a focal point for wider collaboration. Being in the midst of an international collaboration is exciting and makes my everyday work interesting and dynamic. I hope that other researchers will be encouraged to boldly break down the silos which inhibit our collective talent.

The tools to foster these collaborations are already there at your fingertips. Why not give it a go?

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Dr Richard Lee is the Lead for the experimental medicine inflammation and immunotherapy theme for the NIHR Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, University Hospitals Bristol NHS Foundation Trust and the University of Bristol.
Assessment of calcification and inflammation with positron emission tomography in aortic stenosis and atherosclerosis

M R Dweck, N V Joshi, W Jenkins, C Jones, M W L Chow, Alison Fletcher, E J R van Beek, N A Boon, J H F Rudd, D E Newby

Abstract

Background Calcification and inflammation are key pathological processes in aortic stenosis and atherosclerosis. Using combined positron emission tomography and computed tomography (PET/CT), we sought to investigate their contribution to disease progression in aortic stenosis and to help identify vulnerable atherosclerotic plaque.

Methods In the first part of the study patients with calcific aortic valve disease stenosis were prospectively compared with age-matched and sex-matched controls with normal valves. Aortic valve severity was determined at baseline and 1 year by echocardiography and CT calcium scoring. Calcification and inflammation in the valve were assessed by sodium 18-fluoride (NaF) and 18-fluorodeoxyglucose (FDG) uptake with PET. In the second part of the study NaF and FDG activity was assessed in the coronary arteries both in patients with stable coronary disease and in patients after myocardial infarction.

Findings 101 patients with aortic stenosis were compared with 20 controls. Tracer activity (target to background ratio [TBR]) was higher in patients with aortic stenosis than in controls (mean NaF 2·87 [SD 0·82] vs 1·55 [0·17], FDG 1·58 [0·21] vs 1·30 [0·13]; both p<0·01). NaF uptake displayed a progressive rise with valve severity (r²=0·540) with a more modest increase observed for FDG (r²=0·218). Baseline NaF correlated closely with alkaline phosphatase staining on immunohistochemistry (r²=0·79) and was a better predictor of disease progression at 1 year (r²=0·44, n=20) than was FDG (r²=0·02) or baseline calcium score (r²=0·36, current best predictor). Increased NaF activity was observed in 45 (42%) of 106 patients with stable coronary atherosclerosis and was localised to individual coronary plaques. These patients had higher rates of previous major adverse cardiovascular events (p=0·016) and higher Framingham risk scores (p=0·011) than did patients without increased uptake. In patients after myocardial infarction (n=15) intense NaF activity was observed at the site of the culprit lesion, with increased uptake compared with the maximum uptake elsewhere in the coronary arteries (TBR median 1·56 [IQR 1·49–1·82] vs 1·23 [1·15–1·48], p=0·02).

Interpretation In the valve, NaF holds promise in predicting aortic stenosis progression. In the coronary arteries it identifies culprit plaque post myocardial infarction and stable patients at elevated cardiac risk.

Funding British Heart Foundation.
Highly potent human haemopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region

Andrejs Ivanovs, Stanislav Rybtsov, Lindsey Welch, Richard A Anderson, Marc L Turner, Alexander Medvinsky

Abstract
Background Haemopoietic stem cells (HSCs) are used in the clinic to treat various haematological disorders. These cells emerge during early embryogenesis and maintain haemopoiesis in the adult organism. In the vertebrate embryo, HSCs develop in multiple locations. Little is known about the embryonic development of human HSCs.

Methods Human embryonic and fetal tissues were obtained after elective termination of pregnancy. Preconditioned immunodeficient mice were used as recipients for human HSCs. Transplanted mice were bled every 1–2 months to assess human HSC contribution.

Findings We have found that human HSCs emerge first in the aorta-gonad-mesonephros (AGM) region and only later appear in the yolk sac, liver, and placenta. Transplantation of human AGM region cells into immunodeficient mice provides long-term high-level multilineage haemopoietic repopulation. We have shown that, despite the low number of HSCs in the human AGM region, their self-renewal potential is enormous. A single HSC derived from the AGM region generates around 600 daughter HSCs in primary recipients, which disseminate throughout the entire recipient bone marrow and are retransplantable.

Interpretation We provide a systematic spatiotemporal analysis of HSC emergence in the early human embryo and identify the AGM region as the primary source of powerful HSCs with enormous self-renewal capacity. This high potency of the first HSCs sets a new standard for in-vitro generation of HSCs from pluripotent stem cells for the purpose of regenerative medicine.

Funding UK Medical Research Council.
PTEN mutations as a cause of constitutive insulin sensitivity and obesity in human beings

Aparna Pal, Thomas M Barber, Martijn Van de Bunt, Simon A Rudge, Qifeng Zhang, Katherine L Lachlan, Nicola S Cooper, Helen Linden, Jonathan C Levy, Michael J O Wakelam, Lisa Walker, Fredrik Karpe, Anna L Gloyn

Abstract

Background Epidemiological evidence and genetic evidence link type 2 diabetes, obesity, and cancer. The tumour suppressor phosphatase and tensin homolog (PTEN) has roles in both cellular growth and metabolic signalling. Germline PTEN mutations cause Cowden syndrome, a cancer predisposition syndrome, providing an opportunity to study the metabolic effects of PTEN haploinsufficiency in human beings. We investigated insulin sensitivity and beta-cell function in patients with Cowden syndrome.

Methods Insulin sensitivity and beta-cell function were assessed in 15 carriers of the PTEN mutation and 15 matched controls by oral glucose tolerance tests and hyperinsulinaemic euglycaemic clamps. Insulin signalling was measured in biopsies of muscle and adipose tissue with immunoblot. The effect of PTEN haploinsufficiency on obesity was assessed with anthropometric indices, dual-emission x-ray absorptiometry, and skinfold thickness in patients and controls.

Findings All measures of insulin resistance were lower in mutation carriers, including fasting plasma insulin (mean 29 pmol/L [range 9–99] vs 74 [22–185], p=0.001). This was confirmed by hyperinsulinaemic euglycaemic clamps, which showed glucose infusion rates twice as high in patients as in controls (p=0.009). This higher insulin sensitivity was explained by enhanced insulin signalling through the PI3K-AKT pathway, evidenced by increased AKT phosphorylation in both muscle and adipose tissue. PTEN mutation carriers were obese compared with population-based controls (mean BMI 32 kg/m² [range 23–42] vs 26 [15–48], p<0.001). Increased body mass in patients with Cowden syndrome was due to augmented adiposity without corresponding changes in fat distribution.

Interpretation PTEN haploinsufficiency is the first description of a monogenic cause of profound insulin sensitivity. The constitutive insulin sensitisation is apparently obesogenic. This study demonstrates a potential divergent effect on diabetes and cancer risk, with PTEN mutations increasing risk of obesity and cancer but decreasing risk of type 2 diabetes via enhanced insulin sensitivity.

Funding UK Medical Research Council and Wellcome Trust.
Interleukin 23 is critical in the pathogenesis of spondyloarthropathy and acts on a novel population of interleukin 23R+ enthesal resident cells

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Abstract

Spondyloarthropathy is characterised by inflammation, bone erosion, and new bone formation at the enthesal insertions of tendons and ligaments to bone. Lack of understanding of the underlying mechanisms that drive enthesal disease has seriously inhibited design of therapeutics. Although anti-tumour necrosis factor (TNF) therapy reduces signs and symptoms of inflammation, residual inflammation continues and bone growth is not inhibited. This suggests that TNF is not the optimum target to modify enthesal disease.

We previously demonstrated that interleukin (IL) 23 is pivotal in autoimmune inflammation. Recently, genetic variants in the IL-23 receptor (IL23R) have been associated with development of spondyloarthropathy. Moreover, HLA-B27 (present in 90% of patients with ankylosing spondylitis) as well as associated bowel inflammation have been shown to induce IL-23 expression. However, the site and mechanism of action of IL23 are unknown and the reason why such dysregulation of IL23 is associated with inflammation specifically at the enthesis has been inexplicable. We now demonstrate that the entheses and aortic root contain a novel population of resident IL23R+ T cells, which allow the tissue to rapidly respond to IL23. Intravital microscopy reveals that these cells display an extremely restricted enthesal localisation and are absent from synovium and tendon proper. The cells express the molecule PLZF, which allows them to respond to cytokines extremely rapidly, and indeed entheses respond within hours to IL23 in vitro. Moreover, IL-23 expression in vivo in mice is sufficient by itself to rapidly induce the hallmark features of spondyloarthropathy with development of exceedingly specific enthesitis, aortitis, and typical bone erosion and new bone formation.

The highly restricted distribution of IL23R+ cells provides the fundamental basis for the anatomical localisation of inflammation observed in spondyloarthropathy, as well as allowing a unified understanding of the genetic associations. These findings suggest that neutralisation of IL23 may be a truly disease modifying therapeutic approach.

Funding Merck.
Loss-of-function mutations in the immunoglobulin superfamily member 1 gene (IGSF1) cause a novel, X-linked syndrome of central hypothyroidism and testicular enlargement


Abstract

Background Congenital central hypothyroidism occurs either as isolated thyroid-stimulating hormone (TSH) deficiency or in conjunction with other pituitary hormone deficits. Undetected central hypothyroidism is associated with developmental delay in children and adverse cardiometabolic sequelae in adults. Hitherto, mutations in the thyrotropin-releasing hormone receptor gene (TRHR) or the TSHb subunit gene (TSHB) are the only known causes of isolated TSH deficiency.

Methods Using whole exome and candidate gene sequencing, we have studied 11 unrelated families with males exhibiting isolated TSH deficiency, testicular enlargement, and variably low serum prolactin levels.

Findings We have identified eight distinct mutations and two whole gene deletions disrupting the X-linked immunoglobulin superfamily member 1 gene (IGSF1) in affected males. IGSF1 encodes a pituitary-enriched plasma membrane glycoprotein; disease-associated mutations block trafficking of IGSF1 from the endoplasmic reticulum to the membrane, consistent with loss-of-protein function. Adult male IGSF1 null mice exhibit central hypothyroidism with decreased pituitary TSH content and circulating T3 levels; TSH secretion in response to TRH is blunted and pituitary TRHR mRNA levels are decreased, suggesting that impaired TRH signalling may provide the basis for hypothyroidism.

Interpretation Our observations delineate a novel X-linked syndrome in which loss-of-function mutations in IGSF1 cause central hypothyroidism, testicular enlargement, and variable prolactin deficiency, and identify a previously unsuspected role for IGSF1 in hypothalamic-pituitary control of thyroid and testicular function. Variable biochemical penetrance in these families highlights the importance of genetic ascertainment in this syndrome, so that asymptomatic affected individuals can benefit from early initiation of thyroxine treatment.

Funding Wellcome Trust and National Institute for Health Research Biomedical Research Centre.
The BRC Allergene Project: heritability of nickel allergy and genetic determinants

Teresa Tsakok, Lydia Quaye, Lisa Bevan, Cristina Menni, Alireza Moayyeri, Idil Erte, Timothy Spector, Chris Hammond, Frank Nestle

Abstract

Background Allergies affect up to 30% of adults and are increasing in prevalence. However, little is known about the genetic aetiology of immediate (type I) and delayed (type IV) immunity underpinning allergic reactions. Delayed hypersensitivity to nickel is the commonest form of contact dermatitis, affecting 17% of women and resulting in considerable occupational disability. The importance of environmental factors in nickel allergy is well established, but the question of this trait’s heritability remains unknown.

Methods 780 women, comprising 120 monozygotic (MZ) and 270 dizygotic (DZ) twin pairs, were assessed for a 48-h response to nickel patch testing. Associations between nickel sensitivity and the abundance of 401 metabolites were assessed to identify a potential biomarker for this allergic response. A genome-wide association study (GWAS) of the abundance of the top-ranking metabolite was done to ascertain correlations with common genetic variants.

Findings A positive patch test to nickel was observed in at least one individual of 34 MZ and 102 DZ twin pairs. The case-wise concordance was approximately 50% for MZs and 30% for DZs, giving an estimated heritability of 54%. Metabolomic profiling showed that the strongest association was with isovalerate (p=1.97 × 10⁻⁴), a constituent of fatty acid metabolism. The GWAS of the abundance of isovalerate showed that the strongest association was with a single nucleotide polymorphism (SNP) of ANO6 (12q12), rs11183014 (p=1.8 × 10⁻⁶). There were also suggestive associations with four additional SNPs from ANO6 and a variant of CAMK1D (p<9.5 × 10⁻⁶).

Interpretation Nickel allergy seems to be moderately heritable, and preliminary genetic analyses may suggest functional roles for ANO6 and CAMK1D in this delayed hypersensitivity response. Combined use of metabolomic and genome-wide genotyping data offers great promise in the discovery of novel genetic variants for nickel allergy.

Funding King’s College London Biomedical Research Centre.
microRNA-135b promotes cancer progression acting as a downstream effector of oncogenic pathways in colon cancer

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Abstract

Background microRNAs (miRs) are small non-coding RNAs often deregulated in colorectal cancer. We aimed to identify miRs with a driver role in carcinogenesis altered by similar mechanisms in both human and mouse colorectal cancer. The goal was to use colorectal cancer mouse models for the preclinical development of anti-miRs as therapeutics.

Methods Azoximetane/dextran-sulphate (AOM/DSS) treated mice were used to study inflammation-associated colorectal cancer and CDX2P-NLS Cre;Apc<loxP> (CPC;Apc) mice were used to study sporadic APC-related colorectal cancer. Human inflammatory bowel disease associated (n=15) colorectal cancer and sporadic (n=62) colorectal cancer with their matched normal tissues were used for miR expression analysis. miR and gene expression profiling was assessed by nCounter technology. Anti-miR-135b probes for in-vivo studies were synthesised by Girindus.

Findings miR profiling from AOM/DSS and CPC;Apc colorectal cancer showed that miR-135b is one of the most upregulated miR in both models. miR-135b was overexpressed in human inflammatory bowel disease and sporadic colorectal cancer and was associated with poor survival. Molecular studies in mouse embryo fibroblast and human colorectal cancer cell lines defined APC/β-catenin and SRC-PI3K as key pathways in miR-135b activation. miR-135b up-regulation resulted in reduced apoptosis and increased cell growth due to the downregulation of TGFRB2, DAPK1, APC, and FIH. miR-135b silencing in vivo reduced tumour multiplicity and tumour load in the AOM/DSS colorectal cancer model. Mice treated with anti-miR-135b showed well-differentiated tumours with an acinar pattern whereas tumours in the control groups showed poor differentiation and adenomatous pattern. Tumours in mice treated with anti-miR-135b showed reduced proliferation and increased apoptosis.

Interpretation Our data suggest that miR-135b is a key molecule whose activation is downstream of driver genes frequently mutated in colorectal cancer. The in-vivo silencing of miR-135b shows preclinical efficacy with low toxicity and represents the first in-vivo study for use of anti-miRs in treatment of colorectal cancer.

Funding University of Glasgow.
Effects of bariatric surgery on human small artery function: evidence for reduction in perivascular adipocyte inflammation, and restoration of normal anticontractile activity despite persistent obesity

R Aghamohammadzadeh, Adam Greenstein, R Yadav, Maria Jeziorska, S Hama, P Pemberton, H Soran, B Ammori, A M Heagerty

Abstract

Background In lean healthy human beings, perivascular adipose tissue (PVAT) exerts an anticontractile effect on adjacent small arteries, but this is lost in obesity where there is evidence of adipocyte inflammation and increased oxidative stress. The aim of this study was to investigate the effects of bariatric surgery on small artery function and the underlying mechanisms.

Methods Small arteries were isolated from subcutaneous adipose tissue samples obtained by gluteal biopsies from morbidly obese patients (n=15). Vessels were prepared as segments with and without intact PVAT. Response of vessel segments to cumulative doses of norepinephrine was recorded with wire myography. Biopsy samples were re-taken 6 months after bariatric surgery and effect of PVAT on vessel contractility was re-assessed. Vessel segments with intact PVAT were also incubated with blocking peptide for adiponectin receptor 1 (1·6 × 10^{-4} mol/L, n=5). PVAT adiponectin levels were assessed by proteomic analysis of samples from obese (n=13) and control (n=10) individuals. Inflammatory profile of PVAT was assessed by staining for macrophages and tumour necrosis factor α.

Findings Obese patients had a mean body-mass index of 52 kg/m²; 6 months following surgery and significant weight loss (p<0·05), patients remained clinically obese. There were significant improvements in serum biomarkers including adiponectin (p=0·009), as well as inflammatory markers and measures of insulin sensitivity. Proteomic analysis of PVAT showed that adiponectin levels were low in the obese group compared with controls (fold change 2·1, p<0·05). Presence of PVAT made no difference in contractility of vessels to noradrenaline was re-assessed. Vessel segments with intact PVAT were also incubated with blocking peptide for adiponectin receptor 1 (1·6 × 10^{-4} mol/L, n=5). PVAT adiponectin levels were assessed by proteomic analysis of samples from obese (n=13) and control (n=10) individuals. Inflammatory profile of PVAT was assessed by staining for macrophages and tumour necrosis factor α.

Interpretation Damage to the anticontractile property of PVAT in obesity is in part due to a reduction in adiponectin levels within the PVAT and is rescued by weight loss following bariatric surgery.

Funding British Heart Foundation.
Correlation between ultrasound imaging of major salivary glands and histopathological findings of labial gland biopsy samples in Sjogren’s syndrome

Shabnum Ali, Jackie Brown, Rose Ngu, Troy Daniels, John Greenspan, Edward Odell, Penelope J Shirlaw, Kuldipsinh G Gohil, Bruce Kirkham, Genevieve Larkin, Stephen J Challacombe

Abstract

Background Ultrasound imaging has been recognised as a non-invasive, reproducible method for assessing the major salivary glands. The aim of this study was to investigate the relation between ultrasound imaging of major glands and focal lymphocytic score (FS) of minor salivary glands in Sjogren’s syndrome.

Methods Biopsies of labial glands and ultrasound imaging of major glands were done on 196 patients (mean age 55 years, 19 male, 177 female) attending the Sjogren’s Clinic at Guy’s Hospital. FS of labial glands was judged by two histopathologists whose assessment was standardised. Ultrasonography was done with a single Philips-iU22 Ultrasound machine, and a disease severity score (US) was determined. Patients were categorised into three groups: those diagnosed with Sjogren’s syndrome according to the American-European classification criteria (Sjogren’s syndrome group), those with non-specific sialadenitis (sialadenitis group), and those with normal biopsy results (non-salivary gland disease group).

Findings There was a highly significant correlation between FS and US scores (mean score 1·8 [SD 2·4, range 0–11·3] vs 2·7 [2·9, 0–9], r=0·665, n=196; p<0·0001). Mean FS and US scores for the Sjogren’s syndrome group were significantly greater than for the sialadenitis group (p=0·0001). For the Sjogren’s syndrome group, there was a significant correlation between FS and US scores (mean score 3·6 [SD 2·6, range 0–11·3] vs 5·0 [2·4, 0–9], r=0·395, n=87; p<0·0001). For the sialadenitis group, there was a significant correlation between FS and US scores (mean score 0·4 [SD 0·6, range 0–2·3] vs 1·1 [1·7, 0–7], r=0·321, n=66; p<0·005). No obvious correlation between FS and US scores existed for the non-salivary gland disease group (mean score 0·2 [SD 0·5, range 0–2·6] vs 0·5 [SD 1·2, 0–5], n=43).

Interpretation This is the first large-scale study to show the strong relation between US scores of major salivary glands, and FS scores of minor salivary glands, suggesting a uniform disease process. Ultrasound analysis proves to be an important methodology in management and studies of Sjogren’s syndrome.

Funding National Institute for Health Research.
Safety and efficacy of liraglutide in patients with type 2 diabetes with elevated liver enzymes: individual patient data meta-analysis of the LEAD programme

M J Armstrong, D D Houlihan, I A Rowe, W H O Clausen, B Elbrønd, S C L Gough, J W Tomlinson, P N Newsome

Abstract

Background Fatty liver disease has reached epidemic proportions in type 2 diabetes. Glucagon-like peptide-1 (GLP-1) analogues are licensed for treatment of type 2 diabetes, yet little data exist on efficacy and safety in liver injury. We aimed to assess the safety and efficacy of 26 weeks’ liraglutide on liver function compared with an active placebo.

Methods Individual patient data meta-analysis was done with patient level data combined from six 26-week, phase 3, double-blind randomised controlled trials on type 2 diabetes, which comprise the Liraglutide Effect and Action in Diabetes (LEAD) programme. In addition, the LEAD-2 sub-study was analysed to assess the effect on CT-measured hepatic steatosis.

Findings Of 4442 patients analysed, 2241 (50·8%) had an abnormal alanine aminotransferase (ALT) at baseline (mean 33·8 IU/L [SD 14·9] in female participants; 47·3 [18·3] in male participants). Liraglutide 1·8 mg reduced ALT in these patients compared with placebo (–8·20 vs –5·01 IU/L, p=0·003), and was dose dependent (no significant differences vs placebo with liraglutide 0·6 or 1·2 mg). This effect was lost after adjustment for liraglutide’s effect on reduction of weight (corrected mean ALT difference vs placebo –1·41 IU/L, p=0·21) and HbA1c (corrected mean ALT difference vs placebo 0·57 IU/L, p=0·63). Adverse effects with 1·8 mg liraglutide were similar between patients with and without baseline abnormal ALT. In the LEAD-2 sub-study, liraglutide 1·8 mg (26 weeks) improved hepatic steatosis (CT-measured liver:spleen attenuation ratio) from baseline (0·10, p=0·001) and showed a trend towards improvement compared with placebo (0·10 vs 0·00, p=0·07).

Interpretation 26 weeks of liraglutide (1·8 mg) is safe, well tolerated, and improves liver enzymes compared with placebo in patients with type 2 diabetes.

Funding Wellcome Trust.
N-acetylcysteine and liberase improve success of hepatocyte isolation and viability of hepatocytes isolated from normal and diseased liver

D C Bartlett, J Hodson, R H Bhogal, S C Afford, D H Adams, P N Newsome

Abstract
Background Successful hepatocyte isolation is crucial for development of cellular transplantation and biochemical research. Most researchers isolate hepatocytes from surplus donor tissue or normal tissue removed during resection of liver tumours. However, most tissue available for research is from explanted diseased liver and donor tissue rejected for transplant. We previously described our experience of hepatocyte isolation using liberase from such livers with a success rate of 51% and median viability of 40%. Liberase is a highly purified collagenase that has been shown to improve the viability of isolated porcine hepatocytes. N-acetylcysteine (NAC) has been shown to improve the viability of human hepatocytes isolated from steatotic donor tissue. The aim of this study was to determine the effect of both reagents in combination on the outcome of hepatocyte isolation from normal and diseased liver.

Methods Hepatocytes were isolated from 30 consecutive liver specimens as previously described (old protocol). A further 30 consecutive liver specimens were perfused with buffer containing NAC and standard collagenase substituted by liberase (new protocol). Success was defined as maintenance of cell adhesion and morphology for 48 h and/or their successful use in laboratory studies. Mann-Whitney tests were used to compare results. Fisher’s exact test was used for categorical data.

Findings Baseline factors were similar for both groups. The delay to tissue processing was slightly less in the new protocol group (median 2 h vs 4 h, p=0·007). The success rate improved from 40% (12/30) with the old protocol to 70% (21/30) with the new protocol (p=0·037), and the median viable cell yield increased from $7.3 \times 10^4$ to $28.3 \times 10^4$ cells per g tissue (p=0·003). After multivariable analysis adjusting for the difference in time delay, the success rate (p=0·014) and viable cell yield per g tissue (p=0·001) remained significantly improved.

Interpretation NAC and liberase greatly improve the success of hepatocyte isolation and result in a significantly higher viable cell yield. Use of these agents may improve the availability of hepatocytes for transplantation as well as laboratory research.

Funding UK Medical Research Council.
Neutrophil recruitment in response to intradermal endotoxin challenge in man

A Basran, L Bingle, D Dockrell, H L Wilson, M Whyte, R Sabroe, I Sabroe

Abstract

Background Neutrophil recruitment is a key component of the innate immune response, but it is difficult to study in vivo in man, where multi-time-point samples from disease or challenge models are hard to obtain. In-vitro data from our group suggest that neutrophilic inflammation in response to endotoxin will be initiated by a cytokine cascade in which interleukin (IL) 1 has an early and important role. To study this in human beings, we have established a model of intradermal challenge.

Methods Endotoxin and control solution (0·9% NaCl) were injected intradermally in the volar surface of the forearm of healthy volunteers. Serum was taken for blood count and C-reactive protein (CRP) measurements at 0, 1, 6, and 24 h. Temperature, blood pressure, and pulse were monitored. Sites were sampled by 4 mm punch biopsies at varying time points and after varying doses. Biopsy samples were analysed by immunohistochemistry and qPCR.

Findings Intradermal erythema and mild oedema were seen over a current dose range of 0·01–15 ng endotoxin per site. Total dose per individual was 1 to 45 ng. No volunteer had significant systemic reactions. After 5 h, a peripheral blood neutrophilia was seen in some individuals. Erythema area increased up to 6 h, and there was a trend to larger area with larger dose. Above 1 ng endotoxin per site, neutrophilic infiltration of the skin was visible microscopically, but this was much greater at doses of 5 ng or greater. A significant difference was seen between neutrophil elastase stained biopsy area at 2 h and 6 h post administration of endotoxin for the higher doses. There were no changes in serum cytokine levels post injection. By contrast, qPCR revealed a rapid local generation of IL-8 mRNA, and more sustained expression of IL1α/β.

Interpretation Intradermal administration of endotoxin within the limits described seems to be safe. It is an effective method of inducing significant local neutrophilic inflammation at an accessible site. Neutrophil infiltration of tissues starts before 2 h and continues beyond 6 h in response to a single dose of endotoxin. Neutrophilia (without CRP rise or symptoms), appears to be associated with a higher total endotoxin dose. IL-8 production at the inflamed site peaks early, whereas IL1α/β mRNA levels remain high at 6 h.

Funding National Institute for Health Research.
Manipulation of liver regeneration with macrophages to influence the hepatic progenitor cell niche


Abstract

Insufficient regeneration of the adult liver is believed to cause failure to recover from severe liver disease. An undifferentiated cell population with stem-cell-like qualities known as hepatic progenitor cells (HPCs) is hypothesised to have a central role in regeneration of the adult liver during massive or chronic liver disease. Stem cells in other organ systems are believed to reside in a specialised microenvironment or niche that supports their maintenance and function. The existence of a hepatic stem cell niche might provide a means of therapeutically manipulating endogenous HPCs in vivo as a regenerative therapy.

To investigate the physiological potential of HPCs to regenerate the mammalian liver, we have established a novel model of hepatocellular injury and HPC activation using genetic manipulation of hepatocytes. After hepatocyte senescence and death in this model (AhCre Mdm2fl), HPCs expand and bring about the complete regeneration of the liver parenchyma.

We demonstrate that a stereotypical niche, consisting partly of macrophages, exists in both animal models and correlating human disease. Using cell tracking, we show active recruitment of extrahepatic macrophages into this niche during injury. In health, intravenous injection of macrophages results in macrophage engraftment to the liver niche, with subsequent HPC activation and changes to liver structure and function.

Within the niche, macrophages use paracrine signalling to control both HPC proliferation and cell fate via TWEAK (tumour-necrosis-factor-like weak inducer of apoptosis) and the Wnt signalling pathway, respectively. After hepatocellular injury, macrophages ingest hepatocyte debris, and release Wnt which promotes HPC differentiation into hepatocytes. TWEAK is vital for HPC proliferation in the AhCre Mdm2fl model of regeneration. Here, the absence of TWEAK signalling results in liver failure and mortality.

This work has demonstrated for the first time the ability of a solid organ to fully regenerate in the adult mammal from progenitor cells, and additionally highlights mechanisms by which this process can be modulated by either small molecule or cell therapy.

Funding University of Edinburgh.
Use of ENCODE and eQTL data to identify potential functional genetic variants at the 5q31 psoriatic arthritis susceptibility locus

James Bluett, John Bowes, Pauline Ho, Neil McHugh, Anne Barton

Abstract

Background Psoriatic arthritis (PsA) is a chronic inflammatory arthritis associated with psoriasis. Results from family studies have shown a strong genetic component to risk for developing PsA with heritability estimates of 80–100%. There is evidence from subgroup analysis of a genome-wide association study (GWAS) that a locus on chromosome 5q31 is specifically associated with PsA and not psoriasis. This result has been replicated in two independent studies involving UK and Canadian individuals. A recent GWAS done in a UK population (unpublished data) has refined and strengthened this association to a region approximately 500 kb upstream of the original GWAS. However, substantial linkage disequilibrium within this region means that functional data are needed to determine the causal variant.

Methods Genotype data for a total of 179 602 single-nucleotide polymorphisms (SNPs) including the 5q31 region were available for 929 individuals with PsA and 4537 controls (www.wtccc.org.uk). Genotyping was performed with Illumina Immuochip, a custom genotype chip designed for fine mapping of established GWAS loci in autoimmune diseases. The lead SNP from the 5q31 region was selected, and SNPs highly correlated ($r > 0.8$) to the lead SNP were identified by interrogating 1000 genomes project (May, 2011, release). Data from ENCODE (Encyclopedia of DNA elements) and eQTL (expression quantitative trait loci) were searched with the Regulome database and other publicly available databases to explore evidence for transcription factor binding, DNase hypersensitivity sites, and eQTL.

Findings The lead SNP rs715285 ($p = 8.92 	imes 10^{-5}$, odds ratio 1.22) lies within an intergenic region and does not overlie a known transcription factor binding site or DNase1 hypersensitivity site but was associated with a significant change in gene expression of SLC22A5 ($p = 8.58 	imes 10^{-4}$) in monocytes. rs27437 is highly correlated with the lead SNP ($r = 0.88$) and lies within a region that has been identified as a transcription factor binding site, DNase peak and is associated with a significant change in gene expression of SLC22A5 ($p = 8.58 	imes 10^{-4}$) in monocytes.

Interpretation In conclusion, there is a wealth of experimental evidence demonstrating the functional impact of genetic variation in this region associated with PsA.

Funding University of Manchester.
Lentiviral-vector-mediated gene therapy for X-linked lymphoproliferative disease restores humoral and cellular functions

Claire Booth, Christine Rivat, Maria Alonso-Ferrero, Michael Blundell, Neil J Sebire, Adrian J Thrasher, H Bobby Gaspar

Abstract

Background X-linked lymphoproliferative disease (XLP) arises from mutations in the gene encoding SAP, an intracellular adaptor protein expressed in T cells, natural killer (NK) cells, and natural killer T (NKT) cells. SAP deficiency causes abnormalities of NK cell cytotoxicity, NKT cell development, and T-cell-dependent humoral function. Clinical manifestations are characterised by haemophagocytic lymphohistiocytosis (HLH), lymphoma, and dysgammaglobulinaemia. Curative treatment is limited to allogeneic haemopoietic stem-cell transplantation (HSCT). Somatic gene therapy offers a potential cure in XLP. We have developed a lentiviral mediated gene therapy strategy to correct immune defects in XLP.

Methods We designed a lentiviral vector incorporating codon-optimised human SAP cDNA and green fluorescent protein (GFP) driven by the elongation factor-1 short (EFS) promoter. Haemopoietic stem-cell progenitors from SAP-deficient mice were transduced with SAP-GFP (n=10) or GFP only (n=9) vectors before transplantation into irradiated SAP-deficient recipients. Animals were sacrificed 3 months later, 10 days post vaccination with NP-CGG to assess functional humoral reconstitution.

Findings Both SAP and GFP expression was evident in bone marrow, thymus, spleen, and peripheral blood cells. NK cell cytotoxicity was restored to wild type levels in mice receiving the SAP-GFP vector, and NKT cell development was seen in SAP-GFP transduced mice at levels significantly higher than those seen in SAP-deficient or control mice. Baseline IgG, IgM, IgG1, and IgG3 levels were significantly increased and T-cell-dependent humoral responses were also restored, with SAP-GFP transduced mice having levels of NP-specific immunoglobulin that were significantly higher than SAP-deficient mice or controls.

Interpretation We demonstrate correction of cellular and humoral defects in SAP-deficient mice through lentiviral-vector-mediated gene transfer into haemopoietic progenitor cells, providing proof of concept for gene therapy in XLP.

Funding Wellcome Trust and Leukaemia Lymphoma Research.
Transcribed ultraconserved regions are aberrantly expressed and can be modulated by interleukin 6 in cholangiocarcinoma

Chiara Braconi, Nicola Valeri, Pierluigi Gasparini, Jinmai Jiang, Takayuki Kogure, Carlo Croce, Thomas Schmittgen, Tushar Patel

Abstract

Background The inflammation-associated cytokine interleukin (IL) 6 has been implicated in cholangiocarcinogenesis. Transcripts encoded by a group of highly conserved regions (transcribed-ultraconserved regions [T-UCRs]) are novel promising non-coding RNAs that proved to have a role in human carcinogenesis and liver cancer progression. We aimed to study expression of T-UCRs in cholangiocarcinoma.

Methods MzChA-1, TFK, CC-LP-1, and KMCH human cholangiocarcinoma cells, and normal human intrahepatic biliary epithelial cells (HIBEC) were used. T-UCR expression profiling was done with real-time PCR. IL-6 dependent T-UCR expression was assessed in Mz-ChA-1 cells incubated with 10 ng/L IL6 for 24 h or stably transfected with full-length IL6 (Mz-IL6).

Findings The expression of 65 T-UCRs was altered in Mz-ChA-1 compared with normal HIBEC cells. Of these, 12 T-UCRs were upregulated by more than two fold and 48 T-UCRs were downregulated including ultraconserved RNA uc.169, which was dramatically reduced in other human cholangiocarcinoma cell lines and tissues. uc.169 was further reduced in Mz-ChA-1 cells overexpressing IL6. Incubation of MzChA-1 cells with IL6 reduced expression of uc.169, and pretreatment with the inhibitor of transcription actinomycin D inhibited this effect. Enforced expression of IL6 caused an increase in the microRNAs miR-21 and miR-26-a1, which are involved in promotion of cholangiocarcinoma. The uc.169 sequence includes target seed regions for miR-21 and miR-26-a1 and they may be responsible for the downregulation of uc.169. Overexpression of uc.169 in Mz-ChA-1 cells reduced cell proliferation and caused arrest in phase G0/1 of the cell cycle, with increased apoptosis.

Interpretation We report that T-UCRs are differentially expressed in malignant human cholangiocytes, and that IL6 can modulate the expression of selected T-UCRs, probably through a miRNA-mediated modulation. These studies highlight for the first time a role of ultraconserved non-coding RNAs as novel effectors of IL-6 signalling, and the involvement of these RNA genes in the pathogenesis of cholangiocarcinoma. They lay the bases to further investigation of the clinical implications of T-UCR in human cholangiocarcinoma.

Funding University of Glasgow.
Genetic and functional investigation of a Mendelian form of systemic lupus erythematosus

Tracy A Briggs, Jie An, Nalini Agrawal, Alice Wiedeman, Gillian I Rice, Keith B Elkon, Yanick J Crow

Abstract
We have shown that bi-allelic mutations in ACP5 result in a deficiency of the encoded protein, tartrate-resistant acid phosphatase (TRAP), which causes the immuno-osseous disease spondyloenchondrodysplasia. In addition to having a bone and neurological phenotype, patients with spondyloenchondrodysplasia exhibit a wide spectrum of autoimmune manifestations, including autoantibody production, systemic lupus erythematosus, increased interferon α (IFNα), and an interferon signature. In bone, TRAP produced by osteoclasts regulates cell migration by dephosphorylation of osteopontin. However, TRAP is also produced by immune cells, particularly macrophages and dendritic cells (DCs). In murine plasmacytoid dendritic cells (pDC), phosphorylated osteopontin is integral to IFN-α production. Whereas osteopontin has been shown to be a TRAP substrate in murine bone cells, nothing is known about whether TRAP regulates osteopontin in immune cells. We hypothesise that TRAP dephosphorylates osteopontin in human pDCs and negatively regulates IFN-α production.

We studied the interaction between TRAP and osteopontin. By confocal microscopy, we showed that TRAP co-localised with osteopontin in the Golgi in primary human macrophages, DCs, and pDCs. In a TRAP and osteopontin overexpression system in human embryonic kidney 293 cells, we co-immunoprecipitated TRAP and osteopontin, suggesting that they interact with each other. We also showed in an in-vitro assay that recombinant human TRAP could dephosphorylate osteopontin, demonstrating that osteopontin is a substrate for TRAP. These findings suggest that osteopontin is a target for TRAP in immune cells. To test whether TRAP deficiency leads to hyperphosphorylation of osteopontin and increased IFNα, we are generating TRAP knockdowns in a pDC cell line.

Understanding of the mechanism by which TRAP regulates IFNα, potentially via osteopontin, may eventually allow for directed therapeutic approaches in spondyloenchondrodysplasia and other autoimmune diseases associated with an interferon signature, particularly systemic lupus erythematosus.

Funding Wellcome Trust.
Prenatal adenovirus vascular endothelial growth factor gene therapy: a promising new treatment for fetal growth restriction

David J Carr, Raymond P Aitken, John S Milne, Vedanta Mehta, Donald M Peebles, John F Martin, Ian C Zachary, Jacqueline M Wallace, Anna L David

Abstract
Background Fetal growth restriction (FGR) is an important obstetric condition that is commonly associated with reduced uterine blood flow. In normal sheep pregnancies, adenovirus (Ad) mediated overexpression of vascular endothelial growth factor (VEGF) in the uterine arteries increases uterine blood flow. We hypothesised that enhancing uterine blood flow would improve fetal substrate delivery in a sheep model of FGR that is characterised by reduced uterine blood flow from mid-gestation.

Methods Singleton pregnancies were established with embryo transfer in 90 adolescent ewes subsequently overnourished to generate FGR (n=78) or control fed (n=12). In study 1, at a mean gestational age of 89 days (SE 0·2), 45 overnourished ewes were randomised to receive Ad-VEGF (n=18), Ad-LacZ (control vector, n=14), or saline (n=13) injected into each uterine artery. Control-fed ewes (n=12) received saline. Uterine blood flow was monitored with indwelling flowprobes until necropsy at 131 days’ gestation (0·6). Placental mRNA expression of VEGF and its receptors, Flt1/KDR, was assessed in the maternal and fetal placental compartments by quantitative reverse transcription PCR. In study 2, at 88 days’ gestation (0·7), 33 overnourished ewes received Ad-VEGF (n=17) or saline (n=16) and were allowed to deliver spontaneously at 141 days’ gestation (0·4). In both studies fetal growth and wellbeing were evaluated (blind) using serial ultrasound.

Findings In both studies ultrasound measurements of abdominal circumference (AC) were greater in Ad-VEGF-treated FGR fetuses than in control-treated FGR fetuses at 21 days (0·3) and 28 days (0·5) post injection (p<0·001–0·047). In study 1, fewer fetuses were markedly growth-restricted (weight >2 SD below control mean) in Ad-VEGF compared with Ad-LacZ plus saline groups (5/18 vs 18/27, p=0·038). Indices of head sparing (ultrasound biparietal diameter:AC ratios, relative brain weight, and brain:liver weight ratios) were lower (p=0·001–0·046) and maternal placental Flt1/KDR mRNA expression higher (p=0·028/0·034) in Ad-VEGF than in Ad-LacZ plus saline groups, but no measurable differences in uterine blood flow were detected. In study 2, Ad-VEGF-treated lambs tended to be heavier (p=0·081) with increased placental efficiency (p=0·074). There were no maternal or fetal complications or significant differences in the level of neonatal care required to ensure lamb survival.

Interpretation Ad-VEGF safely increases fetal growth in this ovine model of FGR.

Funding Wellbeing of Women.
Outer retinal transduction can be achieved after intravitreal delivery of adeno-associated virus serotype 2 vector with glycosidic enzymes

J Cehajic-Kapetanovic, A Allen, R J Lucas, P N Bishop

Abstract

Background Gene therapies for retinal disorders, including in current clinical trials, so far have relied on subretinal delivery of adeno-associated virus (AAV) vectors carrying therapeutic DNA into outer retinal cells. Subretinal injection has many limitations over the less-invasive route of administration into the vitreal cavity. However, at present only limited retinal transduction can be achieved after intravitreal delivery of AAV vectors. We hypothesise that the inner limiting membrane and extracellular matrix proteoglycans act as a barrier to AAV vector entry into and movement across the retina. Therefore, glycosidic enzymes, which degrade these extracellular barriers, can improve retinal gene therapy. In this study we investigated the effects of enzymatic digestion of extracellular matrices on the depth of vector penetration into the retina.

Methods The green fluorescent protein (GFP)-expressing AAV serotype 2 (AAV2) vector was co-injected intravitreally with glycosidic enzymes at their optimum concentration. Efficacy of virus transduction was assessed by visualising fluorescence in histological cross-sections with fluorescence microscopy. We also analysed safety of these treatments and retinal function using electroretinography.

Findings Glycosidic enzymes led to a significant improvement in retinal transduction after intravitreal delivery of AAV2. These enzymes markedly improved transduction of the outer retina, including photoreceptor cells. Electroretinograms were unchanged (compared with controls) even at much higher doses of enzymes than were needed for optimum retinal transduction.

Interpretation AAV2-mediated retinal transduction is improved by co-injection of glycosidic enzymes into the vitreous. Improved transduction efficiency may allow intravitreal injection to become the preferred route for delivering gene therapy to the inner and outer retina in both preclinical and clinical settings.

Funding UK Medical Research Council.
Influence of acute matrix metalloproteinase activity on myocardial dysfunction associated with urgent cardiac surgery: cardioprotective effects of inhibition

Elaine S Teh, David J Chambers

Abstract

Background Matrix metalloproteinase-2 (MMP-2), a gelatinase involved in cell structure degradation, is emerging as having an important role in acute ischaemia-reperfusion injury in the heart. We investigated whether MMP-2 plays a part in cardiac dysfunction when previously infarcted hearts were subjected to additional elective global ischaemia.

Methods Left coronary artery occlusion was surgically induced in male Wistar rats. After 7 days, the hearts were removed and subjected to isolated Langendorff perfusion (20 min), global ischaemia (30 min), and reperfusion (60 min) with or without s-phenanthroline (an MMP inhibitor). Mechanical function (left ventricular developed pressure [LVDP]) of the heart was monitored continuously by intraventricular balloon. MMP-2 activity was measured (with zymography) in the initial 6 mL of coronary reperfusion effluent and in the heart tissue (with an MMP-2 activity assay) at varying times of reperfusion.

Findings The final recovery in the infarcted hearts was significantly lower than in non-infarcted or in sham hearts (mean preischaemic LVDP 22% [SE 2] vs 39% [2] vs 39% [3], p<0.05). MMP-2 activity in infarcted hearts peaked at 5 min of reperfusion and was significantly higher than in normal hearts (1.03 ng/mL/g of protein vs 0.576ng/mL/g, p<0.05). MMP-2 release into the coronary effluent was also higher. Inhibition of MMP-2 activity improved recovery of LVDP in infarcted hearts to 47% [4] (p<0.05).

Interpretation In infarcted hearts, additional elective global ischaemia, as occurs during cardiac surgery, reduced mechanical function with increased MMP-2 activity. MMP-2 inhibition ameliorated cardiac dysfunction, suggesting a role for MMP-2. Clinically, inhibition of MMP-2 activity may improve cardioprotection of patients undergoing cardiac surgery after an acute coronary event.

Funding British Heart Foundation.
Invasive pneumococcal disease among HIV-1 seropositive individuals in the era of highly active antiretroviral therapy: does HIV-1 impair the macrophage host response to pneumococci?

Paul J Collini, David H Dockrell

Abstract

Background In the era of highly active antiretroviral therapy, incidence of bacterial pneumonia and invasive pneumococcal disease among HIV-1 seropositive individuals is still more than 30 times higher than for seronegative individuals. Risk remains elevated in those with CD4 cell counts in the normal range. A programme of host-mediated macrophage (Mφ) apoptosis ensures killing of *Streptococcus pneumoniae* when canonical phagolysosomal killing capacity is exhausted. HIV-1 infection is associated with resistance of Mφ to apoptosis. We hypothesised that HIV-1-mediated Mφ apoptotic resistance impairs *S pneumoniae* killing.

Methods The following Mφ models were used: healthy donor monocyte derived Mφ (MDM) infected with HIV-1<sub>inw</sub> (HMDM) or sham infected (SMDM), with infection confirmed with anti-p24 immunohistochemistry; and MDM treated with gp120 10–100 ng/mL (gpMDM) or vehicle (cMDM). Mφ were challenged with opsonised type 2 *S pneumoniae* multiplicity of infection=10 or mock-infected and apoptosis up to 20 h by observing morphological changes (counting condensed or fragmented nuclei), counting rates of positivity with the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labelling) assay, measuring cells with hypodiploid DNA, and measuring caspase-3/7 activity. Bacterial internalisation and killing were measured by gentamicin protection assay. Data are mean and SD, and analysed by paired *t* test, or are median and IQR with Wilcoxon matched pairs signed rank test or Kruskal-Wallis test if non-parametric.

Findings After *S pneumoniae* challenge, rates of initial *S pneumoniae* internalisation were similar for all MDM conditions. HMDM showed fewer apoptotic nuclei than did SMDM (19% [IQR 18–21] vs 33% [26–43], *n*=7, *p*<0·05), and smaller increases in caspase-3/7 activity and higher Mcl-1 expression on western blot after *S pneumoniae* challenge. Compared with cMDM, fewer gpMDM developed apoptotic nuclei after *S pneumoniae* challenge, which was dose dependent (cMDM 36% [IQR 30–48] vs gpMDM 20% [12–28] at 10 ng/mL and 18% [14–22] at 100 ng/mL, *n*=12, *p*<0·01, confirmed with TUNEL), and gpMDM exhibited significantly less caspase 3/7 activity (*p*<0·05). In gpMDM, reduced apoptosis was associated with bacterial survival.

Interpretation HIV-1 infection reduces Mφ apoptosis in response to *S pneumoniae*, and gp120 may be sufficient to mediate this. Apoptosis resistance may impair killing of *S pneumoniae* after phagocytosis and increase susceptibility to invasive pneumococcal disease in HIV-1 seropositive individuals.

Funding UK Medical Research Council.
Neurodegeneration caused by intronic expansions of C9ORF72 is a clinically heterogeneous but pathologically distinct disease

Johnathan Cooper-Knock, J Robin Highley, Judith Hartley, Antonio Milano, Stephen Sawcer, Alistair Compston, Antonina Frolov, Gavin Charlesworth, Nicholas Wood, Oliver Bandmann, Christopher J McDermott, Janine Kirby, Paul Ince, Pamela Shaw

Abstract

Background Crossover between neurodegenerative diseases is described but poorly understood. Hexanucleotide repeat expansions of C9ORF72 are a major cause of motorneuron disease (MND)/frontotemporal dementia but we and others have observed clinical heterogeneity within C9ORF72-positive probands and their families, including a high incidence of parkinsonism. We aimed to determine whether C9ORF72 expansions are an upstream cause of clinically and pathologically distinct neurodegenerative diseases. This would have significant implications for neurodegeneration research.

Methods In cohorts of patients with clinical parkinsonism (n=518), multiple sclerosis (MS) (n=215), and MND (n=563) we screened DNA for the C9ORF72 expansion, reviewed clinical histories, and undertook pathological evaluation of brain tissue where available.

Findings We identified the C9ORF72 expansion in one patient with clinical parkinsonism (0.2%), 23 patients with MND (13.7%), and none of the patients with MS. The C9ORF72 positive parkinsonian patient had a family history of MND and displayed pathology consistent with MND with C9ORF72 expansion in addition to α-synucleinopathy. Two further patients with MND were identified with α-synucleinopathy: one with the C9ORF72 expansion, the other without. Of five MND patients who initially presented with MS, four (80%) were positive for the C9ORF72 expansion. C9ORF72-MND is more rapidly progressive in the presence of preceding MS. Pathological examination of MND patients with C9ORF72 expansions revealed p62 positive, TDP-43 negative neuronal cytoplasmic inclusions in frontal cortex, hippocampus, and substantia nigra, which were relatively absent in MND patients without C9ORF72 expansions.

Interpretation C9ORF72 expansions are not a major cause of either classic Parkinson’s disease with α-synucleinopathy or MS. MS appears to increase the penetrance of the C9ORF72 expansion and exaggerate the severity. We suggest that p62 positive, TDP-43 negative neuronal cytoplasmic inclusions within the substantia nigra account for the association between C9ORF72 expansions and parkinsonism. Moreover we suggest that the distribution of these inclusions determines the clinical heterogeneity of C9ORF72 disease.

Funding UK Medical Research Council.
Urinary transglutaminase 2 as a potential biomarker of chronic kidney disease detection and progression

M da Silva Lodge, M El Nahas, T S Johnson

Abstract

Background This study aimed to determine whether urinary transglutaminase 2 (TG2) has the potential to act as an early biomarker of chronic kidney disease (CKD) and whether it can predict the rate of progression better than can albuminuria.

Methods Urine samples were collected prospectively from 292 patients with CKD at the Sheffield Kidney Institute and 33 controls and followed for a minimum of 3 years. The cohort consisted of 61·7% men of whom 89% were white. Major causes of CKD were: diabetic nephropathy (29·05%), chronic glomerulonephritis (23·01%), hypertensive nephrosclerosis (16·6%), atherosclerotic renovascular disease (8·3%), chronic interstitial nephritis (6·41%), and autosomal dominant polycystic kidney disease (4·52%). Urinary TG2 was measured by an in-house sandwich ELISA. One-way ANOVA with Bonferroni correction was applied to estimate differences between groups. Area under the curve for receiver operating characteristic (ROC) analysis was used to determine prediction accuracy. A p value of less than 0·05 was considered statistically significant.

Findings Urine TG2 was 41 times higher in CKD patients than in healthy individuals (mean 3381 ng/mL [SD 135·36] vs 81·6 [4·18], p<0·001). Levels were elevated 17 fold by CKD stage 2. The largest increase in urine TG2 was observed in patients with diabetic nephropathy (7005 ng/mL [376]) with a 85-fold rise in CKD compared with healthy individuals, followed by hypertensive nephrosclerosis (3899 ng/mL [277]). TG2:creatinine ratio was 9·72 ng/mmol. [1·29] in controls versus 605·77 ng/mmol [81·07] in patients with CKD (p<0·001). TG2 excretion was elevated in those patients with progressive (estimated glomerular filtration rate decline 2–5 mL/min/1·73m² per year, 8997·91 ng/mL) or rapidly progressive CKD (>5 mL/min/year, 17650·195 ng/mL) compared with non-progressors (<2 mL/min/1·73m² per year, 2764 ng/mL; p>0·05). There was no correlation between TG2 and total proteinuria (r=0–02808, 95% CI –0·09814 to 0·1534; p=0·6632). ROC curve analysis determined an 81·1% accurate prediction of progression for the TG2:creatinine ratio compared with just 71·4% for the albumin:creatinine ratio.

Interpretation Urinary TG2 was significantly increased in all causes of CKD. ROC curve analysis demonstrated that TG2:creatinine ratio is a better predictor of patients with progressive CKD than albumin:creatinine ratio indicating its potential as a non-invasive biomarker of progressive kidney scarring.

Funding University of Sheffield and Pfizer.
The epigenetic phenotypic switch of vascular smooth muscle cells involved in atherosclerosis

Mark Davies, Jennifer Harman, Haixiang Yu, Martin Bennett, Helle Jørgensen

Abstract
Atherosclerosis is a progressive and chronic inflammatory response to pathological fatty deposits in the arterial wall. The resulting disease spectrum ranges from angina pectoris, myocardial infarction, and sudden cardiac death to peripheral vascular disease and stroke. Vascular smooth muscle cells (VSMCs) are one of the major cell types contributing to neointimal formation of the affected arteries leading to atherosclerosis and in-stent restenosis. The severity of this disease has been linked to the cellular plasticity of VSMCs. At least two phenotypic states have been described: a contractile state, in which cells have increased cytoplasmic myofilaments and are involved in maintaining vascular tone, and a synthetic state, in which cells have relatively few contractile elements but in contrast upregulate the machinery required for protein synthesis and extracellular matrix secretion. In healthy vessels, VSMC can switch between these states, but regulation of this switch is disrupted in atherosclerosis and thought to contribute to the progression of disease.

Our aim was to characterise the epigenetic signature of these VSMC phenotypes to better understand the pathophysiology of atherosclerosis. We used primary mouse VSMC from aorta and characterised the phenotypic changes occurring in response to transforming growth factor β, which promote the contractile phenotype, and platelet-derived growth factor that is involved in VSMC proliferation and migration. First we showed that VSMC develop into a contractile or synthetic phenotype in response to growth factor stimulation. We then characterised the gene expression profile and pattern of epigenetic histone modifications of VSMC selective genes. This identified novel histone modification proteins which could be involved in promoting the atherosclerotic phenotype. We have also shown that key VSMC selective genes are regulated through different epigenetic gene regulation mechanisms. This cell model will be used to investigate the molecular pathways involved in VSMC phenotypic regulation and identify novel candidates for future therapeutic intervention.

Funding UK National Health Service and British Heart Foundation.
The anti-citrullinated antibody repertoire in periodontitis: a role in the induction of autoimmunity in rheumatoid arthritis?

Paola de Pablo, Thomas Dietrich, Iain Chapple, Michael Milward, Muslma Chowdhury, Peter J Charles, Christopher D Buckley, Patrick J Venables

Abstract

Background Data suggest that periodontal disease (periodontitis) may be a risk factor for rheumatoid arthritis. Anti-citrullinated peptide/protein antibodies (ACPA) are highly specific for rheumatoid arthritis, and epitope spreading arises years before clinical onset of the disease. The purpose of this study was to determine the immune reactivity to ACPA and their uncitrullinated control peptides in patients with periodontitis.

Methods ACPA serology was determined in patients with and without periodontitis, including anti-cyclic citrullinated peptide antibodies (anti-CCP), anti-mutated citrullinated vimentin antibodies (anti-MCV), and antibodies with specificity for different citrullinated peptides, including enolase (CEP-1), vimentin (cit-vim), fibrinogen (cit-fib), and their uncitrullinated forms (REP-1, vim, and fib, respectively).

Findings We included 96 patients with and 98 without periodontitis, none of whom had rheumatoid arthritis at inclusion. Compared with patients without periodontitis, patients with the disease had a higher frequency of antibodies against CEP-1 (3% [3/98] vs 12% [12/96], p=0·02) and REP-1 (2% [2/98] vs 6% [6/96], p<0·001). Antibodies against fib (negative control peptide for cit-fib) and cparg (negative control peptide for CCP) were also more common among those with periodontitis than in those without (26% [25/96] vs 3% [3/98], p<0·001; and 9% [9/96] vs 3% [3/98], p=0·06, respectively). 79 (49%) of 149 non-smokers with periodontitis had significantly higher titres of antibodies against CEP-1 (103% difference in titres, p<0·001), REP-1 (91%, p=0·001), vimentin (87%, p=0·002), and fib (124%, p=0·001), than did patients without periodontitis, independent of age and sex.

Interpretation In patients without rheumatoid arthritis, periodontitis is associated with higher titres of antibodies to both citrullinated and uncitrullinated peptides in non-smokers suggesting that periodontitis may contribute to the development of autoimmunity related to rheumatoid arthritis.

Funding Gums & Joints Collaborative EU Framework Programme 7 project and Birmingham and Black Country Comprehensive Local Research Network.
Effect of perhexiline on myocardial protection during coronary artery surgery: a two-centre randomised double-blind placebo-controlled trial


Abstract

Background Perhexiline is thought to modulate metabolism through the inhibition of mitochondrial carnitine palmitoyltransferase, reducing fatty acid uptake and increasing carbohydrate utilisation. Our group has shown that a glucose-insulin-potassium infusion enhances myocardial protection during coronary artery bypass graft (CABG) surgery through metabolic manipulation. This study assessed whether perhexiline improves clinical or biochemical markers of myocardial protection and analysed its effect on the myocardial metabolome.

Methods In a prospective randomised double-blind placebo-controlled trial, patients undergoing CABG at two centres were randomised to receive oral perhexiline or placebo for at least 5 days before surgery. The primary outcome was a low cardiac output episode in the first 6 h after removal of the aortic cross-clamp. A low cardiac output episode was defined as a cardiac index of less than 2·2 L/min/m² in the presence of adequate preload, afterload, and heart rate. All analyses were conducted according to the intention-to-treat principle with a 90% power to detect a relative risk of 0·5 with a one-sided α of 0·025. Left ventricular biopsy samples were taken before ischaemia, snap-frozen, and analysed with mass spectrometry-based (MS) metabolomics. This trial is registered with ClinicalTrials.gov, number NCT00845364.

Findings Over a 3-year period, 286 patients were randomised, received the intervention, and included in the analysis. There were no important baseline differences between groups. The incidence of a low cardiac output episode in the perhexiline arm was 36·7% (51/139) versus 34·7% (51/147) in the control arm (odds ratio 0·92 [95% CI 0·56–1·50]; p=0·74). There were no significant differences in inotrope usage, myocardial injury with troponin-T or electrocardiogram, reoperation, renal dysfunction, or length of hospital stay. No difference in pre-ischaemia left ventricular metabolism was identified between groups.

Interpretation Preoperative perhexiline does not improve myocardial protection in patients undergoing CABG. That perhexiline has no significant effect on the MS-visible polar myocardial metabolome in vivo in human beings supports the suggestion that it acts via a pathway that is independent of myocardial carnitine palmitoyltransferase inhibition.

Funding British Heart Foundation and Sussex Heart Charity.
Reassessing long-term risk of suicide after a first episode of psychosis

Rina Dutta, Robin M Murray, Matthew Hotopf, Judith Allardyce, Peter B Jones, Jane Boydell

Abstract

Background The long-term risk of suicide after a first episode of psychosis is unknown, because previous studies have often been based on prevalence cohorts, have been biased to more severely ill, hospitalised patients, have extrapolated from a short follow-up time, and have made a distinction between schizophrenia and other psychoses. The aim of this study was to determine the epidemiology of suicide in a clinically representative, retrospective inception cohort of patients with a first episode of psychosis.

Methods All 2723 patients who presented for the first time to secondary care services with psychosis in three defined geographical catchment areas in London (1965–2004, n=2056), Nottingham (1997–1999, n=203), and Dumfries and Galloway (1979–1998, n=464) were traced after a mean follow-up of 11·5 years. The main outcome measure was number of deaths by suicide and open verdicts according to International Classification of Diseases, editions 7–10.

Findings Case fatality from suicide was considerably lower than expected from previous studies: 1·9% (53/2723); proportionate mortality was 11·9% (53/444). Although the rate of suicide was highest in the first year after presentation, risk persisted late into follow-up, with median time to suicide being 5·6 years. Suicide occurred nearly 12 times more than expected from the general population of England and Wales (standardised mortality ratio 11·65, 95%CI 8·73–15·24), and 49 of the 53 suicides were excess deaths. Even a decade after first presentation, suicide risk remained almost four times higher than in the general population (3·92, 95%CI 2·22–6·89), a time when there may be less intense clinical monitoring of risk.

Interpretation The highest risk of suicide after a psychotic episode occurs soon after presentation, yet clinicians should still be vigilant in assessing risk a decade or more after first contact. The widely held view that 10–15% die from suicide is misleading because it refers to proportionate mortality rather than lifetime risk. Nonetheless, after a first episode of psychosis, risk of suicide is substantially increased compared with that in the general population.

Funding UK Medical Research Council.
Imaging abnormal skin sensations: a novel functional MRI study

Jessica A Eccles, Sarah N Garfinkel, Ruth E Taylor, Anthony P Bewley, Hugo D Critchley

Abstract

Background A subgroup of patients present to dermatological services with unexplained skin sensations, usually ascribing them to infestation; however, no medical cause can be found. This condition is referred to as delusional infestation, a rare and difficult to treat disorder with considerable impact on psychosocial functioning. The neurobiological mechanisms underlying this condition are unclear. We undertook the first functional MRI (fMRI) study in this group of patients.

Methods Five patients presenting with medically unexplained skin sensations were recruited from the specialist psychodermatology service at The Royal London Hospital, UK (mean age 52·8 years, four women, one man). Five healthy controls were matched for age and gender. Whole brain fMRI data were acquired with a 1·5 T scanner. In an event-related design, participants were randomly shown six classes of images: insects on skin, insects on leaf, other objects on skin, other objects on leaf, neutral images, and disgusting and fearful images. Functional images were analysed with statistical parametric mapping, version 8. A full factorial model was used to analyse the results with two factors—group and stimulus type.

Findings Results are reported at the significance threshold p<0·001 (whole brain analysis xyz co-ordinates, z score, k voxel number). Across the two groups the main effect of insect versus non-insect images was to activate occipital lobe (−52, −70, 6, z 4·04, k 92). In this contrast, patients showed greater activity in the right parahippocampus than did controls (22, −32, −4, z 3·35, k 2). The main effect of presentation of skin rather than leaf was to activate inferior parietal lobule (44, −40, 30, z 4·07, k 21), with patients showing increased activity in this area. Across all conditions patients showed greater activity in the right parahippocampus (26, −4, −32, z 3·77, k 21). Patients showed greater activity in bilateral temporal lobes when viewing disgusting or fearful images than when viewing neutral images.

Interpretation We have shown for the first time that brain activity differs between patients with abnormal skin sensations and controls when viewing pictures. This activity is in regions of brain supporting emotional awareness.

Funding UK Medical Research Council.
Plasma biomarkers of abdominal aortic aneurysm
S Ehsan, S E Slade, D Boocock, K E Herbert, R D Sayers, M J Bown

Abstract
Background Hypothesis-free global proteomics can provide the functional information required to select biologically plausible candidate biomarkers of abdominal aortic aneurysm (AAA). The aim of this study was to validate findings from mass spectrometry (MS) discovery studies and investigate the biological plausibility of candidates using in-silico network analysis.

Methods We compared data from three independent prospective plasma proteomics case-control discovery studies of AAA. Each study used hypothesis-free designs and different MS techniques (surface-enhanced laser desorption-ionisation time-of-flight MS, matrix-assisted laser desorption ionisation time-of-flight MS, and liquid chromatography quadropole time-of-flight MS). Selected upregulated proteins from the joint analysis of the discovery studies were tested in an independent case-control study (20 AAA, 16 control), using ELISAs to verify MS data. Web based networks analysis tools (the Database for Annotation, Visualization and Integrated Discovery and the Integrative biomolecular analysis for complex 'omics data; Ingenuity Systems, Inc), were used to further explore the MS data and identify network pathways associated with AAA.

Findings The selected upregulated proteins from the joint analysis of MS data from patients with AAA, apolipoprotein A-1 and kininogen-1, were verified with ELISAs in the independent case-control study (apolipoprotein A-1, p=0.03, area under the curve [AUC]=0.69) and kininogen-1 (p=0.003, AUC=0.86). Network analysis of the combined MS data showed that the inflammatory and defence response, blood coagulation, wound healing, and enzyme inhibition were the biological processes over-represented in the plasma of patients with AAA.

Interpretation Using MS techniques we have identified and verified plausible plasma biomarkers for AAA.

Funding National Institute for Health Research.
Microalbuminuria could improve risk prediction of stroke in patients with transient ischaemic attacks and minor strokes

S Elyas, A C Shore, H Eastwood, S Keenan, J Stewart, W D Strain

Abstract

Background Transient ischaemic attacks (TIA) and minor strokes are important risk factors for recurrent strokes; they precede 23% of strokes within 90 days. Identification of patients at high risk of developing further strokes is essential to allow early intervention and avoid the catastrophic outcome of strokes. Elevated urinary albumin excretion rate (AER) is a risk factor and predicts cardiovascular disease, stroke, and mortality. Elevated AER can be detected with a point-of-care bedside test.

Methods Patient demographics and the ABCD2 score were obtained for 150 consecutive patients with TIA who presented to the daily stroke clinic and the stroke unit. The ABCD2 score composite for age, blood pressure, clinical features, duration, and diabetes is the risk score presently used for stratifying patients with TIA. All patients had their albumin:creatinine ratio (ACR) measured from a urine sample obtained during their visit to the clinic or the stroke unit at Royal Devon & Exeter Hospital. Patients were followed up for any events, cardiovascular events, stroke, or death at day 7, 30, and 90.

Findings Nine patients had recurrent strokes or TIAs by day 7 and 13 by day 9. Patients who had a recurrent stroke or TIA at day 7 and day 90 had a significantly higher ACR than those who did not have an event (4·00 mg/mmol [95% CI 1·89–8·40] vs 1·89 [95% CI 1·58–2·25]; p=0·03 and 3·73 [95% CI 2·12–6·56] vs 1·85 [95% CI 1·55–2·22]; p=0·02, respectively). After adjustment for sex and ABCD2 score, the 90-day predictive role of ACR persisted for those with versus those without subsequent events (adjusted ACR 3·48 mg/mmol [95% CI 1·96–6·19] vs 1·87 [95% CI 1·56–2·24], p=0·04). Stratification of the population at an ACR of 3.0 mg/mmol identified 39 patients at higher risk. Cox proportional hazards of progressing to stroke by day 90 if ACR was more than 3·0 mg/mmol was 3·2 (95% CI 1·07–9·45, p<0·04).

Interpretation Increased urinary albumin excretion, as detected by urinary ACR, is significantly elevated in patients who present with TIA or minor strokes and go on to have further strokes. The use of clinic urinary ACR test could improve the risk prediction of currently available stroke risk scores such as the ABCD2 score.

Funding National Institute for Health Research and Stroke Research Network.
Viscoelastic characterisation of chordae tendineae of the mitral valve: requirements for future replacement materials

Ashleigh G Wilcox, Daniel M Espino

Abstract

Background Chordal rupture can cause mitral valve regurgitation, which can be corrected by chordal replacement. Development of novel replacement chordae requires measurement of chordal properties under loads as they occur in vivo. However, frequency-dependent viscoelastic properties of chordae—ie, the storage $E'$ and loss $E''$ moduli which measure elastic recoil and energy dissipation, respectively—are unknown. This study aimed to characterise viscoelastic properties of chordae tendineae over physiological frequencies.

Methods Dynamic mechanical analysis (DMA) was performed with a materials testing machine (Bose EnduraTEC 3200). 52 chordae were dissected from six porcine hearts and categorised as basal, marginal, strut, or commissural. After precycling, chordae were sinusoidally loaded at chordal-specific loads mimicking physiological loading (determined from literature). For example, different loading conditions were applied to marginal (0·6 N [± amplitude 0·5]) and basal (0·8 N [0·7]) chordae. Chordae were loaded at a range of frequencies between 0·5 and 5 Hz (hearts typically beat at around 1·2 Hz)—ie, physiological and pathophysiological heart rates.

Findings $E'$ (p<0·05), but not $E''$ (p>0·05), was frequency dependent for all chordae. $E'$ was greater than $E''$ for all chordae (p<0·05). $E'$ and $E''$ varied with chordal categories, with marginal chordae ($E'$ 816·9 MPa, $E''$ 80·9 MPa) being stiffest and strut chordae ($E'$ 137·1 MPa, $E''$ 12·3 MPa) the most compliant. This finding was consistent with $E'$ being inversely proportional to chordal thickness (p<0·05).

Interpretation Viscoelastic properties of chordae are dependent on both frequency and chordal type. The hierarchy of chordal stiffness (based on $E'$ values, highest to lowest) is marginal, commissural, basal, and strut. Differences in viscoelastic properties probably reflect chordal roles in the mitral valve: thinner marginal chordae are stiffer to ensure valve closure without regurgitation, and thicker basal and strut chordae transfer greater loads but are not responsible for valve competence and can, thus, be more compliant under dynamic loading. Future/novel replacement chordal materials must account for frequency dependent viscoelastic properties of chordae tendineae.

Funding Society of Biology, EU Seventh Framework Programme, and Arthritis Research UK.
Characterisation and immunogenicity of a decellularised skeletal muscle scaffold for laryngeal tissue engineering

J M Fishman, M Lowdell, T Ansari, P Sibbons, P De Coppi, M A Birchall

Abstract

Background Successful replacement of airways in patients has been done with tissue engineered constructs. However, replacing the larynx where active movement is crucial, requires functional muscle tissue.

Methods Decellularised scaffolds were characterised with histological, immunohistochemical, and molecular techniques and xenogenically implanted to determine the effect of implantation on scaffold biodegradation time and immunogenicity in vivo. The cellular host immune response to the scaffold was quantified by stereology and by fluorescence activated cell sorting in vitro.

Findings Decellularisation results in total DNA clearance and downregulation of MHC classes I/II and myosin heavy chain expression, with relative preservation of the scaffold’s structural integrity (collagen, elastin, s-glycosaminoglycans content) and biomechanical properties. Decellularisation altered the host response to the scaffold in vivo, resulting in a prolonged degradation time and increased neoangiogenic potential relative to fresh tissue. In addition, we proved for the first time that decellularised scaffolds trigger a lower cellular mediated immune response, resulting in a reduced T-cell proliferative response in vitro, and alter the cellular immune profile towards the scaffolds in vivo, with a reduction in CD3+ cells and a shift towards the M2-macrophage phenotype.

Interpretation Decellularised laryngeal muscles are non-immunogenic and may provide the optimum scaffold source for a tissue-engineered larynx.

Funding UK Medical Research Council, Sparks Children’s Charity, and Royal College of Surgeons of England.
Outcomes of kidney transplantation in HIV-positive patients: the UK experience

Esther Gathogo, Lisa Hamzah, Rachel Hilton, Neal Marshall, Mark Harber, Jeremy B Levy, Rachael Jones, Marta Boffito, Saye H Khoo, Martin Drage, Sanjay Bhagani, Frank A Post

Abstract

Background HIV infection is an independent risk factor for end-stage kidney disease in HIV-positive patients of black ethnicity. Highly effective antiretroviral therapy has allowed these patients to be considered for kidney transplantation (KT). We report the outcomes of KT in a national observational cohort study.

Methods We retrospectively identified HIV-positive patients who had undergone KT up to December, 2010, through all 25 UK KT centres and major HIV clinics, and included follow-up until December, 2011. Patient characteristics, treatments, and complications were described. Patient and graft survival rates and cumulative incidence of acute rejection were estimated with Kaplan-Meier and Nelson-Aalen analyses.

Findings 35 HIV-positive KT recipients (median age 40 years, 66% male, 74% black ethnicity) were identified. At the time of KT, all patients were stable on antiretroviral therapy with undetectable HIV RNA and median CD4 cell count of 366 cells per mL. Patient survival at both 1 and 3 years was 91·3%, and graft survival was 91·3% and 84·7%, respectively. In the first year after KT, blood concentrations of calcineurin inhibitors (CNI) were frequently outside the therapeutic reference range. At 1 year after KT, the cumulative incidence of acute allograft rejection was 48%, and the median estimated glomerular filtration rate 61 mL/min/1·73 m² (IQR 46–78). Although HIV viraemia and HIV disease progression were uncommon, renal complications were relatively frequent.

Interpretation Our study corroborates the feasibility of KT in HIV-positive patients. Co-administration of antiretroviral therapy and CNI is challenging, and sub-therapeutic CNI concentrations may contribute to the high rate of acute allograft rejection. The optimum immune suppression strategy in this population remains to be refined.

Funding King’s College London.
Antigen-sensitive CD8 T-cell clones with tough HIV-1 suppression

Julie M Glanville, Steve Taylor, Dilair Baban, Geraldine Gillespie, Philippa Easterbrook, Guillaume Stewart-Jones, Alison Simmons, Sarah Rowland-Jones, Tao Dong, Andrew McMichael

Abstract
CD8 T-cell sensitivity is crucial for optimum control of persistent viral infections, including HIV-1. The devastating HIV-1 pandemic may be countered by development of a cytotoxic T cell (CTL) based vaccine if qualities associated with protection can be defined at the molecular level. However, the heterogeneity of the total viral-specific CTL response confounds identification of protective correlates, and T-cell sensitivity is no exception and remains controversial. To address this we reduced the heterogeneity of the HIV-1 CTL response to single units, generating 19 CTL clones that recognise the same HIV-1 derived epitope restricted by HLA B*08. Correlation of functional assays directly with the ability of each clone to suppress HIV-1 replication in vitro, the so-called viral suppression assay, identified high functional avidity as a key quality for suppressive activity. Remarkably, four clones from this panel, isolated from one individual, a long-term non-progressor, all used an identical T-cell receptor (TCR) yet had distinct T-cell sensitivity and suppressive activity. Two of these clones were characterised in detail, and had distinct cytokine profiles, regulated by epigenetic mechanisms, and differential expression of a group of cell surface receptors with the potential to modulate the signalling threshold to antigen. This is the first evidence that avidity maturation in CD8 T cells with the same TCR affinity occurs in viral infections in man as reported in the mouse. Modulation of sensitivity in CD8 T cells is crucial for viral suppression.

Funding
UK Medical Research Council.
Abdominal aortic aneurysm repair results in aortic stiffening

V J Gokani, E Choke, M J Bown, B Williams, R D Sayers

Abstract
Long-term follow-up studies show that all-cause mortality remains unchanged after repair of abdominal aortic aneurysm (AAA), possibly because of an increased cardiovascular risk in this high risk group. Repair of the AAA introduces a semirigid conduit into the circulation with unknown effects on the central aortic haemodynamics, such as pulse-wave velocity (PWV). One recent study revealed that a 1 m/s increase in PWV confers a 15% increased risk of cardiovascular events. We investigated whether central aortic haemodynamic changes resulting from AAA repair could be contributing to this excess cardiovascular risk.

In nine patients undergoing endovascular aneurysm repair of infrarenal AAA who were assessed for changes in carotid-femoral PWV (cfPWV), mean cfPWV (n=9) was 10.3 m/s (SD 1.0) preoperatively. 1 week and 6 weeks postoperatively, mean cfPWV was 10.2 m/s and 11.2 m/s, respectively (mean difference at 6 weeks 0.9 m/s [95% CI 0.1–1.8], p=0.03).

AAA repair appears to result in a functional stiffening of the aorta. A larger powered study is in progress to confirm this finding and also investigate whether this phenomenon is sustained in the long term. Intensive cardiovascular risk monitoring and pharmacomodulation may be indicated in this high-risk population.

Funding British Heart Foundation.
Next generation sequencing of RNA from muscle biopsies shows marked differences between inflammatory myositis, inclusion body myositis, and controls

Philip Hamann, Mark Lindsay, Neil McHugh, James Heward

Abstract

Inflammatory myositis (IM) is a multi-system inflammatory disorder characterised by a variable triad of muscular weakness, interstitial lung disease, and cutaneous manifestations. A serological profile is now recognised in over 80% of cases and correlates closely with the phenotypic manifestation of the disease. The largest serological subgroup of IM is anti-Jo-1 (an anti-tRNA synthetase antibody). Present standard treatment is with systemic immunosuppression.

Inclusion body myositis (IBM) differs from IM by affecting peripheral and proximal muscles and tends to have little in the way of multi-organ involvement. IBM tends to respond poorly to immunosuppression, and currently does not have an as well recognised serological profile as IM.

Recent research suggests that non-coding RNA plays a crucial role in orchestrating protein transcription, and aberrant non-coding RNA expression may play a key part in IM and IBM. Advances in sequencing techniques now enable detailed analysis of RNA transcription and may help unravel the pathogenesis of IM and IBM.

In this pilot study we investigate the differences in expression of coding and non-coding RNA in IM (anti-Jo-1 antibody positive) and IBM. RNA was extracted from each muscle biopsy and pooled into three samples (IM, IBM, and control) for sequencing. RNA was extracted from five muscle biopsy samples for each of the IM and control group, and two for the IBM group. The muscle transcriptome was determined with next generation sequencing (Illumina Hi-Seq 2000, Poly A+ extraction). Data were mapped onto the human genome and changes in expression were analysed.

Preliminary data have shown significant differential changes in transcription of cell structure and inflammatory response proteins, as well as marked variation in skeletal-muscle-specific long non-coding RNAs, between the three groups.

Funding National Institute for Health Research.
Tumour necrosis factor-related apoptosis-inducing ligand is a novel therapeutic target in pulmonary arterial hypertension


Abstract

Background Pulmonary arterial hypertension is a fatal disease characterised by progressive narrowing of pulmonary arterioles, driven by aberrant cellular proliferation. Identification of key pathways in disease pathogenesis is required for the development of new-targeted therapies. We have previously reported tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) immunoreactivity within pulmonary vascular lesions from patients with idiopathic pulmonary arterial hypertension and animal models. Since TRAIL induces endothelial cell apoptosis and smooth muscle cell proliferation, we hypothesised that TRAIL is an important driver of disease in pulmonary arterial hypertension.

Methods We characterised the expression of TRAIL in human and rodent pulmonary arterial hypertension and determined the effects of TRAIL on pulmonary artery smooth muscle cells (PASMCs) in vitro. Using genetic deletion, pharmacological overexpression, antibody blockade, and bone marrow transplant (BMT) chimera experiments we determined the direct pathogenic role of TRAIL in three independent rodent models of pulmonary arterial hypertension. We then tested the efficacy of inhibiting TRAIL in halting or regressing established disease in two preclinical models. Terminal phenotyping included cardiac catheterisation, echocardiography, and pulmonary vascular immunohistochemistry.

Findings TRAIL mRNA and protein expression was upregulated in PASMCs from patients with pulmonary arterial hypertension. In vitro, TRAIL was a mitogen for PASMCs. TRAIL-deficient mice were protected from both hypoxia-induced and diet-induced pulmonary arterial hypertension. Antibody blockade prevented rats from developing toxin-induced disease. In BMT chimeras, only mice with expression of TRAIL restricted to tissue developed pulmonary arterial hypertension. In rodents with established pulmonary arterial hypertension, an anti-TRAIL antibody reversed pulmonary vascular remodelling, through reducing proliferation and inducing apoptosis, improved pulmonary haemodynamics, and significantly improved survival.

Interpretation Our studies are the first to determine the importance of TRAIL in the pathogenesis of pulmonary arterial hypertension and demonstrate its potential for translation into a novel therapeutic target.

Funding British Heart Foundation.
High bone mass is associated with an increased prevalence of joint replacement

Sarah Ann Hardcastle, Celia L Gregson, Kevin Deere, George Davey Smith, Paul Dieppe, Jon H Tobias

Abstract

Background Numerous epidemiological studies have reported an association between osteoarthritis and increased bone mineral density (BMD). To explore the nature of this association we examined whether osteoarthritis risk is increased in individuals with high bone mass (HBM), in whom bone mineral density is assumed to be elevated due to a primary genetic cause.

Methods 335 115 dual x-ray absorptiometry (DXA) scans were screened to identify HBM index cases (defined by DXA scan as an L1 Z-score of ≥+3·2 and total hip Z-score ≥+1·2 or total hip Z-score ≥+3·2 and L1 Z-score ≥+1·2). In relatives, the definition of HBM was L1 Z-score plus total hip Z-score ≥+3·2. Controls comprised unaffected relatives and spouses. Clinical indicators of osteoarthritis were determined by structured assessment. Analyses used logistic regression adjusting for age, gender, and body-mass index.

Findings 353 individuals with HBM (mean age 61·7 years, 77% female) and 197 controls (mean age 54·1 years, 47% female) were included. The prevalence of prior joint replacement surgery was higher in HBM cases (13·0%) than in controls (4·1%) (adjusted odds ratio 2·42 [95% CI 1·06–5·56], p=0·04). Adjusted use of non-steroidal anti-inflammatory drugs (NSAIDs) was also more prevalent in HBM cases than in controls (odds ratio 2·17 [1·10–4·28], p=0·03). Adjusted prevalence of joint pain and knee crepitus did not differ between cases and controls. Further comparison of unadjusted joint replacement prevalence in HBM cases aged 65 years or older with equivalent population data from the Health Survey for England 2005 also suggested an increase in joint replacement in HBM.

Interpretation Individuals with HBM have a higher prevalence of joint replacement and NSAID use compared with controls, suggesting an association between HBM and osteoarthritis.

Funding Arthritis Research UK, Wellcome Trust, and National Institute for Health Research.
Plasma membrane proteomics identifies Notch1 as a potential regulator of ras-induced senescence

Matthew Hoare, Michael P Weekes, Nicholas J Matheson, Suraj Menon, Robin Antrobus, Paul J Lehner, Masashi Narita

Abstract

Background Oncogene-induced senescence (OIS) is an intrinsic tumour suppressor mechanism leading to stable cell-cycle arrest in response to oncogene activation. OIS is a heterogeneous phenotype of multiple effector mechanisms; understanding of in-vivo OIS is lacking because of the difficulty in identifying senescence. We wished to establish a cell-surface phenotype of OIS.

Methods We used ER:Ras IMR90 human diploid fibroblasts, which undergo OIS after 6 days with 4-hydroxytamoxifen (4-OHT). We used SILAC (stable isotope labelling with amino acids in cell culture)-based proteomics and three labelling conditions (light, IMR90 plus 4-OHT; medium [lysine, K+4Da, arginine, R+6Da] ER:ras IMR90; heavy [K+8Da, R+10Da] ER:Ras IMR90 plus 4-OHT), combined with cell surface aminooxybiotinylation before streptavidin pulldown. Tryptic peptides were fractionated by high pH reversed-phase high performance liquid chromatography and then subjected to liquid chromatograph mass spectrometry. Data were processed by the analytical packages MaxQuant and MASCOT. Hits were validated by fluorescence-activated cell sorting (FACS), quantitative PCR, and immunoblotting. shRNA-mediated knockdown of protein expression used the murine stem cell virus-miR30 system.

Findings 899 proteins were identified, of which 73% were present at the cell surface by Gene Ontology annotation. Notch1 was significantly upregulated in OIS compared with both control conditions (3·1–3·4 fold). Upregulation was confirmed by both FACS and immunoblotting. Downstream Notch target-genes were also upregulated in OIS. Treatment with the γ-secretase inhibitor DAPT increased both senescence-associated heterochromatin foci (SAHF) and senescence-associated β galactosidase (SA-β-gal) activity, features of OIS. However, Notch1 knockdown reduced both SAHF and SA-β-gal. The effect of DAPT was not mediated through canonical Notch1 signalling since RBP-j knockdown had no effect upon DAPT-mediated SAHF and SA β-gal upregulation. Numb, a canonical Notch pathway inhibitor, is upregulated in OIS. Numb may underpin a transition switch from canonical to non-canonical Notch signaling in OIS.

Interpretation Plasma membrane proteomics has identified a cell-surface phenotype of OIS. Notch 1 cell surface expression and downstream target-genes are upregulated in OIS. The transition to OIS is correlated with a transition from canonical to non-canonical Notch1 signalling that may be driven by Numb expression.

Funding Cancer Research UK.
Is there a correlation between lung function values and cardiopulmonary exercise outcome?

M Homsy, M Gillion, B Lams, E Suh, M Dasaolu

Abstract

Background Cardiopulmonary exercise testing (CPET) has become an important tool for perioperative assessment because it may identify patients at risk of postoperative cardiopulmonary complications. An anaerobic threshold (AT) less than 11 mL/min/kg has been recommended as a way to stratify postoperative treatment in colorectal surgery patients. The British Thoracic Society guidelines recommend that a peak VO₂ (pVO₂) less than 15 mL/min/kg confers high risk in thoracic surgical patients. Because CPET can be challenging to carry out, this study aimed to determine whether lung function values correlated with CPET outcome and therefore could be used as an alternative measure.

Methods 500 pre-operative colorectal (388) and oesophageal (112) patients were analysed. Gas transfer and spirometry were performed to assess lung function. CPET was performed on a cycle ergometer to calculate pVO₂ and AT. The predictive capacity of forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), transfer factor of the lung for carbon monoxide (TLco), and carbon monoxide transfer coefficient (Kco) values compared with pVO₂ and AT was assessed using receiver operating characteristic (ROC) curves.

Findings The area under the curve (AUC) for pVO₂ and AT for FEV₁ was 0.56 and 0.55, respectively; for FVC 0.56 and 0.57; for TLco 0.72 and 0.64, and for Kco 0.63 and 0.56. There was a significant correlation between AT and pVO₂ (AUC 0.89); an AT greater than 12 mL/min/kg predicted pVO₂ greater than 15 mL/min/kg (sensitivity 77.3%, 1–specificity 13.7%).

Interpretation Lung function variables cannot reliably predict pVO₂ or AT outcome. However, of the variables recorded, TLco was the best marker for predicting a pVO₂ greater than 15 mL/min/kg. In preoperative assessment of patients undergoing thoracic surgery, an AT of more than 12 mL/min/kg could be used as an alternative to CPET if the patient is unable to achieve a pVO₂ greater than 15 mL/min/kg.

Funding King’s College London.
Urinary C-peptide creatinine ratio to detect absolute insulin deficiency in type 2 diabetes

S V Hope, A G Jones, M Shepherd, B Shields, D Strain, T McDonald, B A Knight, A T Hattersley

Abstract

Background National Institute for Health and Clinical Excellence guidelines (CG87) recommend neutral protamine hagedorn (NPH) insulin for the provision of basal insulin in type 2 diabetes, but use of analogue insulin is as much as 40%. Where residual endogenous insulin secretory capacity is present there is no evidence that analogue insulins provide any additional benefit over human insulins, and they come at an expensive premium. Anecdotally, however, there is a reluctance to switch people back to NPH insulin, partly because of a perceived risk of pancreatic failure and potential ketosis. Urinary C-peptide creatinine ratio (UCPCR) has been validated as a method for evaluating residual endogenous insulin secretion in type 1 and type 2 diabetes, with a UCPCR of no more than 0·2 nmol/mmol suggestive of absolute insulin deficiency. We aimed to evaluate the prevalence of true insulin deficiency among patients with type 2 diabetes with UCPCR, and confirm findings with the gold standard mixed meal tolerance test (MMTT).

Methods 191 insulin-treated patients with a clinical diagnosis of type 2 diabetes (diagnosed at or after age 45 years and who did not start insulin within the first year of diagnosis) collected a 2-h post-prandial urine sample for UCPCR measurement. Nine patients from two subgroups (UCPCR ≤0·2 nmol/mmol and UCPCR >0·2) completed a standard MMTT.

Findings 11 (5·8%) of 191 patients had two consistent UCPCRs of less than or equal to 0·2 nmol/mmol. Nine were able to do the MMTT, of whom five were confirmed to have absolute insulin deficiency (stimulated serum c-peptide <0·2 nmol/L). Three of these five patients were glutamic acid decarboxylase antibody-negative. Nine of nine patients with UCPCR of more than 0·2 nmol/L had confirmed endogenous insulin secretion in their MMTT. Those with insulin deficiency had a shorter time to starting insulin (median 2·5 years [IQR 1·5–3·0] vs 6·0 [3·0–10·75], p=0·005) and lower body-mass index (25 kg/m² vs 29, p=0·04) but no other significant differences in clinical characteristics.

Interpretation We have demonstrated a very low prevalence of true pancreatic failure in this population of insulin-treated patients with type 2 diabetes. This requires further exploration by comparison of a population being treated with NPH insulin with one on analogue insulin, and then determining whether UCPCR could act as a clinical decision support tool to safely switch from analogue insulin to NPH insulin.

Funding National Institute for Health Research.
Patients with early inflammatory arthritis who fulfil the 2010 American College of Rheumatology–European League Against Rheumatism classification criteria for rheumatoid arthritis have increased mortality compared with those who do not: results from the Norfolk Arthritis Register

J H Humphreys, S M M Verstappen, M Lunt, J Chipping, K L Hyrich, T Marshall, D P M Symmons

Abstract

Background  Mortality is increased in rheumatoid arthritis compared with the general population. Most studies have used the 1987 American College Rheumatology criteria to define rheumatoid arthritis when investigating mortality. The aims of this study were to examine whether, in a cohort of patients with early inflammatory polyarthritis, the 2010 American College of Rheumatology–European League Against Rheumatism classification criteria for rheumatoid arthritis identify those with decreased survival; and if so to identify which components of the criteria are the strongest predictors of mortality.

Methods  Adults with two or more swollen joints for 4 or more weeks were recruited to the Norfolk Arthritis Register (NOAR) between 1990 and 2009. Patients included in this analysis had symptom duration of at least 2 years and had not received any disease modifying therapy at initial assessment. Data on the components of the 2010 criteria, along with demographic details, were obtained at baseline-visit through a nurse-administered questionnaire and joint examination. Bloods samples were taken for C-reactive protein (CRP), rheumatoid factor (RF), and anti-citrullinated protein antibody (ACPA) estimation. All patients registered with NOAR are flagged with the UK Office for National Statistics (ONS) which provides mortality data. Survival analyses were performed with Cox proportional hazards models in univariate analyses, then adjusted for age and sex. A multivariable model was then developed including all components of the 2010 criteria as well as baseline smoking status, age, and gender.

Findings  1671 patients had complete data for analysis, with 20 488 person-years of follow-up. 1092 (65%) patients were female and there were 471 deaths reported by the ONS by Dec 31, 2011. 905 (54%) patients fulfilled the 2010 criteria at baseline, and they had a significantly increased risk of death compared with patients in NOAR who did not fulfil the 2010 criteria, both in univariate analyses and in the age-adjusted and sex-adjusted model (adjusted hazard ratio (HR) 1·39 [95% CI 1·15–1·68]). The multivariable model identified high titre RF, ACPA (or both) positivity (more than three times the upper limit of normal) and raised CRP as significant predictors of mortality (HR 1·64 [95% CI 1·31–1·97] and 1·25 [95% CI 1·02–1·52], respectively).

Interpretation  Patients presenting with early inflammatory polyarthritis who fulfil the 2010 criteria have significantly increased mortality compared with those who do not. The components of the 2010 criteria that seem to be important predictors of mortality are high titre RF or ACPA positivity, and abnormal CRP at baseline.

Funding  Arthritis Research UK.
Effects of phosphodiesterase type 5A inhibition on intracellular calcium handling and its implications for cardioprotection and antiarrhythmogenesis

D C Hutchings, M Lawless, D A Eisner, A W Trafford

Abstract
Phosphodiesterase type 5A inhibition with sildenafil improves cardiac function in heart failure. In addition, sildenafil in animal models of myocardial infarction has direct cardioprotective and antiarrhythmic effects. Sildenafil reduces L-type calcium current (\(I_{ca-L}\)) and attenuates adrenergically driven inotropism, but effects on calcium handling are largely undetermined.

Isolated adult rat ventricular myocytes were voltage clamped and calcium fluorescence measured with the indicator fura-2. Cells were paced at 0·5 Hz with depolarisations from –60 mV to +10 mV. Sarcoplasmic reticulum (SR) content was determined by application of caffeine (10–20 mmol/L) and integration of inward sodium-calcium exchanger current. Rate constants for calcium extrusion from the cell (\(K_{caff}\)) and calcium uptake into the SR (\(K_{SERCA}\)) were determined by fitting first order exponentials to decay phases of the respective calcium transients. Following the initial control protocol, a therapeutically relevant dose of sildenafil (1 µM) was applied. Differences were determined with student’s paired \(t\) tests.

Sildenafil reduced SR content by 26·5% (n=9, \(p<0.01\)). To a lesser extent, sildenafil also reduced calcium transient amplitude (by –13·6%, n=9, \(p<0.05\)); this was not accompanied by a reduction in \(K_{SERCA}\) (–2.3% with sildenafil, \(p=0.97\), n=5). Peak and integrated \(I_{ca-L}\) were also reduced with sildenafil (–9·1% and –6·0%, respectively, \(n=9\), \(p<0.05\)). The effect on \(I_{ca-L}\) was also seen in adult dog ventricular myocytes (reducing peak and integrated \(I_{ca-L}\) by 15·9% and 26·4%, respectively, \(p<0.05\) and \(p<0.01\), n=6, 23°C). These effects cannot be attributed to run-down effects.

Sildenafil substantially reduced SR content with no reduction in \(K_{SERCA}\), and thus may be mediated through ryanodine receptor modulation. Such reduction in SR load may reduce proarrhythmic SR calcium release, indicating a novel mechanism through which sildenafil exerts an antiarrhythmic effect. Acute reductions in calcium transient amplitude and \(I_{ca-L}\) with sildenafil indicate acute negative inotropic effects and may contribute to our understanding of its cardioprotective effects in the setting of hyperadrenergic drive in heart failure.

Funding British Heart Foundation.
Mechanical unloading reverses transverse tubule remodelling and normalises local calcium-induced calcium release in a rodent model of heart failure

Michael Ibrahim, Manoraj Navaratnarajah, Urszula Siedlecka, Christopher Rao, Priyanthi Dias, Alexey Moshkov, Julia Gorelik, Magdi Yacoub, Cesare Terracciano

Abstract
Calcium-induced calcium release (CICR) is crucial for contraction in cardiomyocytes. The transverse (t)-tubule system guarantees the proximity of the triggers for calcium release (L-type calcium channel, dihydropyridine receptors) and the sarcoplasmic reticulum calcium-release channels (ryanodine receptors). Transverse tubule disruption occurs early in heart failure. Clinical studies of left ventricular assist devices in heart failure indicate that mechanical unloading induces reverse remodelling. We hypothesise that unloading of failing hearts normalises t-tubule structure and improves CICR.

Heart failure was induced in Lewis rats by left coronary artery ligation for 12 weeks; sham-operated animals were used as controls. Failing hearts were mechanically unloaded for 4 weeks by heterotopic abdominal heart transplantation (HF-UN). Heart failure reduced the t-tubule density as measured by di-8-ANEPPS staining in isolated left ventricular myocytes, and this was reversed by unloading. The deterioration in the regularity of the t-tubule system in heart failure was also reversed in HF-UN. Scanning ion conductance microscopy showed the reappearance of normal surface striations in HF-UN. Electron microscopy revealed recovery of normal t-tubule microarchitecture in HF-UN. L-type calcium current density, measured with whole-cell patch clamping, was reduced in heart failure but unaffected by unloading. The variance of the time-to-peak of the calcium transient, an index of CICR dyssynchrony, was increased in heart failure and normalised by unloading. The increased calcium spark frequency observed in heart failure was reduced in HF-UN. These results could be explained by the recoupling of orphaned ryanodine receptors in heart failure, as indicated by immunofluorescence.

Our data show that mechanical unloading of the failing heart reverses the pathological remodelling of the t-tubule system and improves CICR.

Funding British Heart Foundation.
A chemical-genetics approach to study the molecular pathology of central serotonin abnormalities in fetal valproate syndrome

John Jacob, Vanessa Ribes, Steve Moore, Sean Constable, David Wilkinson, James Briscoe

Abstract
Valproate (VPA) is a commonly used drug worldwide despite increased awareness of its adverse effects, especially when used during pregnancy. Fetal valproate syndrome, characterised by birth defects, learning difficulties, and autism, is observed in up to 50% of children exposed in utero. The teratogenic properties of VPA are thought to result from altered gene expression through its inhibition of the chromatin modifying enzyme family of histone deacetylases (Hdac). Brainstem serotonergic abnormalities are believed to be important in mediating the behavioural manifestations of fetal valproate syndrome. Abnormal differentiation of these neurons has been reported in rodent models of autism and this syndrome. However, the role of Hdac and the identity of the salient genetic targets in VPA-associated serotonergic dysgenesis has not been determined; this has hampered the development of therapies that can ameliorate fetal valproate syndrome. Here we show that VPA specifically blocks serotonergic differentiation in a zebrafish model by downregulating the expression of the proneural gene, Ascl1b, which encodes a critical determinant of serotonergic fate. Using a chemical-genetics approach we show that VPA exposes cryptic transcriptional repression by tonic Notch signalling, which prevents the activation of Ascl1b. Chemical blockade of Notch signalling results in Ascl1b activation and the rescue of serotonergic differentiation in the presence of VPA. We find that the ability of VPA to silence Ascl1b and block serotonergic neurogenesis is specifically dependent on inhibition of Hdac1. Consistent with this finding, Hdac1 mutants fail to activate Ascl1b and lack 5-HT neurons. Moroever, treatment of Hdac1 mutants with a Notch inhibitor drug rescues 5-HT neurons, which confirms that Hdac1 mediates the effects of VPA. The current model of Hdac function proposes that cyclical Hdac activity is needed to prime active genes, such as Ascl1b, for further rounds of transcription. Our discovery of the interplay between chromatin modifiers and Notch in gene regulation adds a new layer of complexity to the epigenetic molecular model. Fetal valproate syndrome thus highlights the crucial role of the epigenetic interface in genome-environment interactions in disease.

Funding UK Medical Research Council
Investigation of idiopathic inflammatory myopathy for shared genetic risk factors with other autoimmune diseases: results from the European Myositis Network


Abstract

Background  Idiopathic inflammatory myopathies (IIM) are a group of autoimmune disorders characterised by inflammation of muscles. They may present as a primary disorder or may overlap with other diseases such as rheumatoid arthritis, systemic lupus erythematosus, or systemic sclerosis. The cause of IIM is largely unknown, but is thought to include a combination of both genetic and environmental factors. Recent genome-wide association studies (GWAS) have identified many genetic variants associated with autoimmune disorders, several of which are common to multiple disorders. We tested the hypothesis that genetic risk factors associated with other autoimmune disorders also predispose to IIM.

Methods  Single-nucleotide polymorphisms (SNPs) significantly associated with systemic lupus erythematosus, rheumatoid arthritis, juvenile idiopathic arthritis, coeliac disease, inflammatory bowel disease, psoriasis, type 1 diabetes, multiple sclerosis, and systemic sclerosis were identified from published GWAS in white populations. 233 unique SNPs were identified (\(p < 5 \times 10^{-8}\)), of which 99 had not been captured through our international myositis genetic collaboration GWAS. These SNPs were genotyped in a sample of 1043 European white individuals with adult or juvenile dermatomyositis or polymyositis who were compared with race-matched controls. Analysis was carried out with PLINK, followed by fixed-effects meta-analysis.

Findings  1001 individuals and 83 SNPs passed quality control filtering criteria and were genotyped. Two SNPs within the HLA region were significantly associated with IIM (rs2040406 near \(H LA-DQA1\), \(p = 2.23 \times 10^{-8}\) and rs615672 near \(H LA-DRB1\), \(p = 6.07 \times 10^{-10}\)). Two SNPs outside the HLA region achieved Bonferroni corrected significance levels (type 1 diabetes rs11171739, \(p = 6.07 \times 10^{-8}\) and multiple sclerosis rs3135388, \(p = 9.71 \times 10^{-9}\)). SNPs associated with the genes \(FAM107A\), \(TNFAIP3\), and \(BLK\), previously linked with rheumatoid arthritis and systemic lupus erythematosus, were also associated with IIM.

Interpretation  The association of HLA SNPs confirms the autoimmune nature of IIM. The association of SNPs previously associated with type 1 diabetes, multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis suggests that IIM may share genetic risk factors with other autoimmune disorders.

Funding  UK Medical Research Council and UK Myositis Support Group.
Early rheumatoid arthritis and resolving fibroblasts segregate according to Dickkopf related protein 1 expression

Maria Juarez, Dagmar Scheel Toellner, Emmanuel Karouzakis, Rowan Hardy, Lorraine Yeo, Rachel Bayley, Banesa de Paz, Karim Raza, Mark Cooper, Steffen Gay, Christopher Buckley, Andrew Filer

Abstract

Background Dickkopf related protein 1 (DKK1) has been proposed as the master regulator of joint remodelling. This Wnt signalling pathway inhibitor is involved in osteoblast growth and differentiation. In rheumatoid arthritis, increased DKK1 plasma levels correlate with inflammation and bone erosions. Furthermore, patients with rheumatoid arthritis who carry genetic variants in the DKK1 gene have higher serum DKK1 levels and more progressive joint destruction, suggesting a fundamental role for DKK1 in rheumatoid arthritis. In the diseased joint, synovial fibroblasts are key mediators of bone and cartilage destruction via secretion of matrix metalloproteinases and regulation of monocyte to osteoclast differentiation. In this study we analysed whether DKK1 secretion might contribute to this effect. We hypothesised that synovial fibroblasts from patients with early rheumatoid arthritis would be characterised by high DKK1 expression compared with those from patients with resolving arthritis.

Methods Synovial tissue samples were obtained by ultrasound-guided biopsy from patients with early synovitis within 12 weeks of symptom onset. 12 patients developed rheumatoid arthritis (according to 1987 American College of Rheumatology criteria) and eight had self-limiting disease within 18 months' follow-up. Synovial fibroblasts were cultured and expanded to passage three with established methods. mRNA expression was quantified with real-time quantitative PCR. DKK1 levels were measured in the supernatants of cultured cells with a commercial ELISA kit (R&D systems). The methylation status of the DKK1 gene promoter was assessed with methylated DNA immunoprecipitation assays.

Findings Expression of DKK1 mRNA was significantly higher in synovial fibroblasts from patients with early rheumatoid arthritis than in those with resolving arthritis (2^{-\Delta{Ct}} relative to glyceraldehyde 3-phosphate dehydrogenase 0·024 vs 0·009, p<0·005). Differential expression was confirmed at the protein level (median 23·2 ng/mL [IQR 11·1–48·5] vs 6·6 ng/mL [4·2–23·6]; p=0·07). Expression levels of DKK1 mRNA or protein did not correlate with disease duration, or with clinical indices. Analysis of DKK1 promoter methylation showed a trend towards promoter hypomethylation in samples from early rheumatoid arthritis compared with resolving samples (1·04-fold enrichment vs 1·52-fold enrichment, not significant). Further samples are being analysed to increase the power of the study.

Interpretation DKK1 is an inhibitor of the Wnt signalling pathway that promotes cell invasion and a pro-destruction imbalance of osteoblast and osteoclast activity. Our data suggest that expression of DKK1 by fibroblasts cultured from treatment-naive patients discriminates between persistent and resolving disease, and occurs early in the disease process in rheumatoid arthritis. This difference in gene expression might be under epigenetic control at the level of DNA methylation. Current studies are focusing on validating these findings.

Funding Wellcome Trust.
**Abstract**

Congenital melanocytic naevus (CMN) syndrome is the association of large or multiple CMN with neurological abnormalities, characteristic facial features, and an increased risk of melanoma that is not restricted to the skin. The genetic basis of the syndrome has previously been unknown, although various somatic mutations have been described in individual skin lesions. We hypothesised that one of these mutations might occur early in embryogenesis, leading to somatic mosaicism. 55 cutaneous, neurological, and blood samples were obtained from 15 accurately phenotyped patients with multiple CMN, and blood samples were taken from a further 44 patients with CMN. Site-directed mutagenesis generated restriction enzyme sites unique to the normal DNA sequence of codon 61 of NRAS (MIM 164790), allowing selective amplification of mutant alleles in mosaic tissue. Oncogenic missense mutations in codon 61 of NRAS were found in the affected neurological and cutaneous tissues of 12 of 15 patients, in a somatic mosaic pattern. In ten patients the heterozygous mutation was c.181C>A, p.Q61K, and in two c.182A>G, p.Q61R. No mutations were found in unaffected tissues or blood. Investigation of the primary melanoma samples in two patients revealed loss of heterozygosity at NRAS in one patient, and deletion of a region of chromosome 9p including CDKN2A (MIM 600160), a known melanoma gene, in both patients. CMN syndrome is therefore a mosaic RASopathy. This mosaicism underpins the associated risk of malignant melanoma and non-melanocytic central nervous system tumours by acting as a first hit in a multi-hit model of tumorigenesis.

**Funding**

Wellcome Trust.
Differential effect of amisulpride on cognition in schizotypy: validation of models for the early identification of cognitive enhancing agents

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Abstract

Background The cognitive impairment associated with schizophrenia is a major target for drug development but none of the drugs designed to address this problem has shown consistent efficacy. The most common causes for failure in the registration trials could be preempted by testing novel agents in milder forms of the disorder, such as schizotypy and assessing the effect with validated biomarkers. In this study we aimed to test this approach by comparing the effects of risperidone, amisulpride, and nicotine in a double-blind placebo-controlled three-centre study on the cognitive performance in schizotypy.

Methods We recruited healthy volunteers who scored high and average on the Schizotypal Personality Questionnaire (122 in each group). 244 participants were randomised to an acute dose of risperidone (n=62, capsule plus placebo patch), amisulpride (n=62, capsule plus placebo patch), nicotine (n=61, placebo capsule plus nicotine patch), or placebo (n=59, placebo capsule plus placebo patch) and proceeded to complete cognitive tests (n-back, verbal fluency, and spatial working memory tasks). Primary outcome measures were percentage correct, errors of commission, and response latencies (n-back), number of correct words and category transitions (verbal fluency), and between-search and within-search errors (spatial working memory tasks). This trial has not been registered.

Findings We found evidence for worse performance in the high schizotypy group on the n-back and verbal fluency tasks (p<0·01, η²=0·059 and p<0·01, η²=0·064, respectively). Amisulpride had a differential effect on the two groups in respect to these two tasks (p=0·02 and p=0·04, respectively): it improved performance in the high schizotypy group but impaired the controls. By contrast, risperidone impaired both groups while nicotine had a beneficial effect for the low baseline performers. There was a statistically significant increase in the reaction time latency in the n-back task in the risperidone arm compared with all other arms.

Interpretation We attribute the effects of amisulpride to its presynaptic dopamine enhancing action at low doses. The deterioration of performance with risperidone on the other hand was probably due to sedation while nicotine showed signs of general vigilance improvement. Our findings confirm that cognitive abnormalities in schizotypy are replicable in multisite studies and responsive to drug challenges. Importantly, these results underline the importance of dopamine agonism in the pathophysiology of the cognitive deficits in the schizophrenia spectrum disorders.

Funding P1vital Ltd.
Best clinical care versus the common good of research: a solution for challenging surgical trials

Yuri I Kulikov, Nicholas R Parsons, Damian Griffin

Abstract
Randomised controlled trials (RCTs) in surgery are expensive and labour intensive, yet considerable concern remains about applicability of results in clinical practice. Apart from quality issues, this lack of applicability is often due to strict eligibility criteria leading to selection bias. In addition, only a small proportion of eligible patients are randomised, since both clinicians and patients are rarely in equipoise and do not feel comfortable being excluded from the decision making process, especially when substantially different procedures (such as open versus minimally invasive or operative versus non-operative interventions) are compared or surgical innovations are involved.

The Patient Eligibility Assessment through Clinical Equipoise (PEACE) methodological framework is introduced. In this framework, every provisionally eligible patient is regarded as a complex case which is assessed on line by an expert panel. Collected opinions including that of the submitting surgeon are processed to calculate the level of clinical equipoise. A sufficient level of equipoise for random treatment allocation is confirmed or a panel consensus in preference for a certain treatment is advised.

The proposed framework was developed and panel assessment tested in collaboration with a national multicentre trauma randomised controlled trial comparing operative and non-operative interventions. 12 surgeons from nine hospitals acting as principal investigators in the trial formed the expert panel and assessed 77 real clinical cases eligible according to standard inclusion and exclusion criteria during the 3 year course of the trial.

Because every case is assessed for eligibility, initial entrance criteria can be pragmatic and less specific than with the standard approach. A treating surgeon is involved in the decision process through submitting an opinion and has the backing of an expert panel when treatment options and trial participation is discussed with a patient. Cases where there is treatment consensus can also be researched, but should not be randomised on ethical grounds.

Funding Warwick Medical School.
Isocitrate dehydrogenase mutation analysis in gliomas as a diagnostic and prognostic biomarker

Kathreena M Kurian, Harry R Haynes, Charlene Crosby, Kirsten Hopkins, Maggie Williams

Abstract

Background There is a high rate of isocitrate dehydrogenase (IDH) 1 and 2 mutations in low grade gliomas and in high grade gliomas derived from them. IDH analysis of gliomas is a novel adjunct to traditional classification and an independent prognostic marker. We compared antibody and sequencing methods for the detection of IDH mutations.

Methods 88 samples from 74 patients were identified: 16 patients had WHO grade II gliomas, 30 had WHO grade III gliomas, and 28 had WHO grade IV glioblastoma multiforme (GBM). 31 samples had insufficient material available for DNA extraction. For immunohistochemistry, sections were stained with anti-IDH1R132H antibody. For sequencing, DNA was extracted from fresh, frozen tissue.

Findings 20 (28%) of 72 patients were positive for the R132H IDH1 mutation by antibody. An IDH1 mutation was detected by molecular genetics in 21 (37%) of 57 patients, and no IDH2 mutations were detected (6% expected from previous studies). 5 (24%) of 21 patients had rare IDH1 mutations not detected by immunohistochemistry (7% expected). Three of these patients displayed the p.Arg132Cys mutation (two anaplastic astrocytomas, one fibrillary astrocytoma) and two displayed p.Arg132Gly (one anaplastic astrocytoma, one anaplastic oligoastrocytoma). Where sufficient tissue was available, immunohistochemistry and DNA analysis were fully concordant for the p.Arg132His mutation. We found a high rate of IDH1 mutations in lower grade lesions (WHO grade II and III) (54% [25/46]) and a low rate in GBMs (7% [2/28]). Both grade II gliomas and anaplastic astrocytomas showed a statistically different distribution of IDH1 mutation load compared with GBMs (p<0.0001 and p=0.0021, respectively).

Interpretation A rationalised combined approach involving R132H antibody testing and sequencing of negative cases would be ideal for the detection of IDH1 mutations. Antibody testing is cheaper than sequencing but sequencing demonstrates rare IDH1 mutations not detected by immunohistochemistry.

Funding Brain Tumour Bank Frenchay, British Neuropathological Society, and Brain Tumour Action.
First-in-man evidence of the mechanistic effects of biventricular pacing on coronary physiology

Andreas Kyriacou, Zachary I Whinnett, Justin E Davies, Punam A Pabari, Nicholas S Peters, Prapa Kanagaratnam, Jamil Mayet, Alun D Hughes, Darrel P Francis

Abstract

Background Normal coronary blood flow is principally determined by a diastolic backward travelling decompression (suction) wave. Dyssynchronous heart failure may attenuate suction. We hypothesised that biventricular pacing, by restoring left ventricular (LV) synchronisation and improving LV relaxation, might increase this suction wave and coronary flow.

Methods Ten patients with congestive heart failure (nine men; mean age 65 years [SD 12]; mean ejection fraction 26% [SD 7] with left bundle branch block (LBBB, mean QRS duration 174 ms [SD 18]) underwent atriobiventricular pacing at 100 beats per min. LV pressure was measured and wave intensity calculated from invasive coronary flow velocity and pressure, with native conduction (LBBB) and during biventricular pacing at atrioventricular (AV) delays of 40 ms (BiV-40), 120 ms (BiV-120), and separately pre-identified haemodynamically optimal AV delay (BiV-Opt). Data are given as median (IQR).

Findings Compared with LBBB, BiV-Opt enhanced coronary flow velocity time integral (VTI) by 15% (7–25, p=0.007), LV dP/dt_{max} by 17% (9–22, p=0.005), and _neg dP/dt_{max} by 17% (9–22, p=0.005). The cumulative intensity of the diastolic backward decompression (suction) wave increased by 26% (18–54, p=0.005). Much of the increase in coronary flow VTI occurred in diastole (69% [41–84], p=0.047). The systolic compression waves also increased: forward by 36% (6–49; p=0.022) and backward by 38% (20–55, p=0.022). BiV-120 generated a smaller LV dP/dt_{max} (by 12% [5–23], p=0.013) and _neg dP/dt_{max} (by 15% [8–40], p=0.009) increase than did BiV-Opt, with LBBB as reference; BiV-Opt and BiV-120 were not significantly different in coronary flow VTI or waves. BiV-40 was no different from LBBB.

Interpretation When biventricular pacing improves left ventricular contraction and relaxation, it increases coronary blood flow velocity, predominantly by increasing the dominant diastolic backward decompression wave.

Funding British Heart Foundation.
Expression of inhibitory Fc receptor (FcγRIIB) is a marker of poor response to rituximab monotherapy in follicular lymphoma

Chern Siang Lee, Margaret Ashton-Key, Sergio Cogliatti, Susanne Crowe, Mark Cragg, Hsu-Fang Schmitz, Michele Ghielmini, Peter Johnson

Abstract

Background FcγRIIB promotes rituximab internalisation on various B-cell targets, including in follicular lymphoma, which may lead to reduced efficacy. We analysed diagnostic tumour samples from the SAKK 35/98 trial, which has follow-up data of nearly 10 years to determine the relation of FcγRIIB expression with responses and clinical outcomes after rituximab monotherapy in follicular lymphoma.

Methods Available archived tissue samples were stained with an anti-human FcγRIIB antibody. Positive samples were graded into negative/low intensity staining (n=116) or medium/high staining (n=13) by a histopathologist masked to clinical outcomes. Failure-free survival (FFS) was defined as time from first rituximab infusion until failure to achieve complete/partial response at week 12, progression, relapse, a second cancer, or death from any cause. Objective response rate (ORR) was associated with intensity staining levels with Fisher’s exact test. All time-to-event endpoints were evaluated with the Kaplan-Meier method; groups were compared with the log-rank test. Hazard ratio (HR) was assessed with Cox proportional hazards models.

Findings Patients expressing medium/high levels of FcγRIIB were less likely to respond to rituximab than were those with negative/low levels (ORR 23·1% [95% CI 7·5–50·9] vs 58·6% [49·5–67·2], p=0·02). FFS was higher in the negative/low staining group than in the medium/high staining group (median 8·3 months [95% CI 2·8–13·4, IQR 2·76–28·5] vs 2·8 [not calculable, 2·76–2·76], p=0·002; HR 0·43 [95% CI 0·23–0·78]). There was a non-significant trend towards better overall survival in the low/negative group compared with the medium/high group (median 140·0 months vs 50·0, p=0·13; HR 0·56 [95% CI 0·26–1·20]).

Interpretation Elevated FcγRIIB expression level is associated with poor response to rituximab in patients with follicular lymphoma. This group may show better results with non-internalising type II antibodies, a hypothesis for validation in future prospective clinical trials.

Funding Cancer Research UK.
A novel subset of functional interleukin-10 secreting CD8 regulatory T cells infiltrate human hepatocellular carcinoma

Ka-Kit Li, Steve T Ward, Stuart M Curbishley, Henning W Zimmermann, Tony Bruns, David H Adams

Abstract

Background Tumour specific effector T cells can be detected in the blood and tumours of patients with hepatocellular carcinoma but they fail to mount effective immune responses. Attempts to amplify anti-tumour immune responses using immunotherapy show promise, but are hampered by the presence of suppressive regulatory T cells (Tregs) that inhibit anti-tumour immune responses. Tregs are crucial in the maintenance of immune homeostasis and in the prevention of autoreactive immune response but in the context of cancer they can suppress beneficial anti-tumour immunity leading to tumour progression. A novel subset of CD8 expressing Tregs has recently been described and we now report the presence of such cells in human hepatocellular carcinoma and define their functional and homing properties.

Methods Fresh tissue from hepatocellular carcinoma and matched distal non-involved tissue were obtained from patients undergoing liver resection or transplantation at the Queen Elizabeth Hospital, Birmingham, after informed consent. Liver-derived T cells were isolated and phenotyped with multicolour flow cytometry including intracellular cytokine staining. CD8$^{+}$Tregs were isolated with a Mo-Flow cell sorter for functional assays. Distribution of CD8$^{+}$Tregs was investigated by immunohistochemistry and immunofluorescence.

Findings The percentage of CD8$^{+}$Tregs (defined as CD8$^{+}$CD25$^{int}$CD127$^{low}$FoxP3$^{+}$) infiltrating hepatocellular carcinoma tumours was significantly greater than matched non-involved liver. Tumour-derived CD8$^{+}$Treg isolated by Mo-Flo sorting suppressed allogeneic effectors cells in vitro and secreted interleukin (IL) 10. By contrast, T-cell interferon-γ production was decreased within the tumour compared with matched non-involved liver. The chemokine receptor CXCR3 which is involved in T-cell recruitment to the inflamed liver was highly expressed on tumour-derived CD8$^{+}$Tregs.

Interpretation A novel subset of functional IL-10 secreting CD8$^{+}$Tregs may suppress anti-tumour immunity in hepatocellular carcinoma. Their expression of CXCR3 provides a potential mechanism for recruitment into the tumour environment.

Funding Wellcome Trust.
Sequential expression of CD39 regulates late developmental T helper type 17 plasticity imparting a regulatory cell phenotype

Maria Serena Longhi, Aiping Bai, Yan Wu, Terry B Strom, Simon C Robson

Abstract

Background CD39 is an immune cell phenotype marker that exhibits ectonucleotidase activity, converting extracellular nucleotides to nucleosides. Although CD39 has been associated with regulatory T cells (Treg), the ectoenzyme is also expressed by a subpopulation of memory T cells (CD4\textsuperscript{mem}) with effector functions. We postulate that CD39 imparts plasticity to effector T helper type 17 (Th17) as well as co-ordinating Treg cellular programmes of differentiation.

Methods Experiments were performed with peripheral blood mononuclear cells obtained from healthy blood donors. Sorted CD4\textsuperscript{+}CD45RO\textsuperscript{+} memory cells (CD4\textsuperscript{mem}) were exposed to interleukin (IL) 6 plus IL1\textbeta plus recombinant transforming growth factor β1 (rTGF) or IL6 plus IL1\textbeta plus IL23, or IL6 plus IL1\textbeta plus rTGF β1 plus IL23 to induce Th17 polarisation. Cells at Th17 stage were then treated with high-dose IL2 plus anti-CD3/anti-CD28 to favour Treg differentiation and then re-exposed to Th17 differentiating conditions to induce putative reverse or suppressive Th17 (rev/regTh17) cell. Impacts of purinergic mediators on cell effector phenotype and functions were assessed.

Findings CD4\textsuperscript{mem} could be differentiated sequentially to Th17, Treg, and rev/regTh17. In contrast to the inflammatory properties associated with prototypic Th17 cells, rev/regTh17 exhibited a suppressive phenotype (ie, CD39\textsuperscript{high}, CD73\textsuperscript{high}, FOXP3\textsuperscript{+}) and were able to control CD4\textsuperscript{+}CD25\textsuperscript{−} cell proliferation and pro-inflammatory cytokine (IFNγ, IL17) production. rev/regTh17 did not upregulate CD39, CD73, and FOXP3 and did not undergo increase in their suppressive function after culture with adenosine.

Interpretation Differential levels of expression of CD39 designate early Th17 cells from later Treg/revTh17 cell plasticity. The potential for Treg to revert to the inflammatory Th17 phenotype is mitigated by expression of CD39, as indicated by enhancements of suppressive function in vitro.

Funding UK Medical Research Council.
Association between anti-tumour necrosis factor therapy and risk of ischaemic stroke in patients with rheumatoid arthritis: results from the British Society for Rheumatology Biologics Registers-Rheumatoid Arthritis (BSRBR-RA)

Audrey S L Low, Mark Lunt, Louise K Mercer, James B Galloway, Rebecca Davies, Kath D Watson, BSRBR Control Centre Consortium, Deborah P Symmons, William G Dixon, Kimme L Hyrich, on behalf of the British Society for Rheumatology Biologics Registers (BSRBR)

Abstract

Background People with rheumatoid arthritis are at increased risk of cardiovascular morbidity and mortality, including stroke (cerebrovascular accident [CVA]). Anti-tumour necrosis factor (anti-TNF) therapy may influence the risk of CVA by reducing inflammation. The aim of the analysis was to study the association of anti-TNF therapy with risk of ischaemic CVA in rheumatoid arthritis.

Methods The British Society for Rheumatology Biologics Registers-Rheumatoid Arthritis (BSRBR-RA) is an ongoing national prospective observational cohort study. Patients with rheumatoid arthritis recently started on anti-TNF therapy and a biologic-naive comparator group treated only with non-biologic disease modifying anti-rheumatic drugs (nbDMARDs) were recruited to the BSRBR-RA from 2001 to 2008. Patients were followed by physician and patient questionnaires and also linked to the national death register. Incident CVAs were identified from all three sources of follow-up. CVAs were validated against WHO criteria for CVA and further classified as ischaemic CVA using CT brain reports or if ischaemic CVA was reported as the underlying cause of death from death certificates according to International Classification of Diseases 10 (ICD-10) code I63. Patients with a previous CVA were excluded. Risk of ischaemic CVA was compared between the nbDMARD cohort and people ever exposed to anti-TNF using a Cox regression model. Missing baseline data were replaced by multiple imputation. Adjustment was made for confounders using propensity scores stratified by deciles.

Findings To Oct 31, 2010, 130 verified incident ischaemic CVAs (21 in 3271 nbDMARD patients, 109 in 11 642 anti-TNF patients) had occurred during 11 973 and 61 226 person-years of observation, respectively (incidence rate 175 vs 178 per 100 000 person-years). After adjustment for confounders, there was no association between ever exposure to anti-TNF and ischaemic CVA risk (hazard ratio 0·88 [95% CI 0·46–1·71]).

Interpretation Exposure to anti-TNF therapy does not appear to be associated with risk of ischaemic CVA when compared with nbDMARD therapy. Further follow-up is needed to assess time-varying risk.

Funding British Society for Rheumatology.
Molecular regulators of cardiovascular valves in development and disease

O T A Lyons, A Sabine, S Grover, E Bazigou, N A Brown, T Petrova, T Makinen, A Smith

Abstract

Background Cardiac disease, lymphoedema, and venous reflux are associated in rare single-gene disorders but the overall molecular regulators of venous valve (VV) development and maintenance are poorly understood. Recently we compared the expression profile of murine and human VV, characterised normal VV formation in mice, and used knockout lines to show that genes required for regulating lymphatic valve development are required for VV development and maintenance. More recent developments will be presented and the genetic patterning of venous valves with respect to the genetics of human venous disease will be discussed.

Methods Murine valves were examined by light microscopy, whole-mount confocal immunofluorescence, and scanning electron microscopy in wild-type mice and genetic reporter lines. Human valves were examined by immunohistochemistry, scanning electron microscopy, and transmission electron microscopy. Tissue-specific conditional knockout lines were used to identify roles of genes in valve formation/maintenance.

Findings Murine and human venous valves exhibit a similar structural and protein expression pattern. Several novel regulatory genes were found to be required for valve formation/maintenance.

Interpretation We have established the use of murine knockout lines in the study of venous valve disease. Venous and lymphatic valves share a common gene-expression profile and some developmental pathways, which explains the shared phenotype of lymphoedema and venous reflux seen in the clinic. Further work should be aimed at defining other genetic and environmental factors required for the development and maintenance of these complex structures, and their role in disease.

Funding UK Medical Research Council.
Serological status: a predictor of response to intensive therapy in rheumatoid arthritis

M H Y Ma, C Dahanayake, I C Scott, G H Kingsley, A P Cope, D L Scott

Abstract

Background Rheumatoid arthritis is the most common chronic inflammatory disease in the UK. Serological status such as rheumatoid factor (RF) and anti-citrullinated peptide antibody (ACPA) positivity predict poor outcomes. Early intensive treatment regimens targeting remission reduce disease activity, structural damage, and long-term disability. However, we do not know whether all patients with active disease should have such intensive treatment regimens. Can serological status be used to predict the need for intensive therapy?

Methods We analysed samples from a published randomised controlled trial which compared four treatment regimens in patients with early active rheumatoid arthritis (disease duration <2 years): methotrexate monotherapy, double therapy (methotrexate plus either ciclosporin or prednisolone), and triple therapy (methotrexate plus ciclosporin plus prednisolone). The trial randomised 467 patients (68% female, median age 54 years [IQR 46–63]). Disease activity was assessed with the disease activity score of 28 joints (DAS28). Remission was defined as DAS28 less than 2.6 at 24 months. RF isotypes (IgM and IgA) and ACPA levels were measured with commercial ELISA kits. Statistical analysis used Pearson’s chi-squared test.

Findings 402 (86%) patients were positive for IgM RF, 346 (74%) for IgA RF, and 346 (74%) for ACPA. 98 (21%) patients achieved remission at 24 months. In RF IgM negative cases (n=65) the proportion of patients achieving remission at 24 months was similar in all treatment groups (25%, 22%, and 30% for monotherapy, double therapy, and triple therapy, respectively). In RF IgM positive cases, significantly fewer patients achieved remission with monotherapy (13/65, 17%) and double therapy (24/157, 15%) than with triple therapy (27/80, 34%) (p=0.001). There were similar, consistent findings with IgA RF and ACPA, with significantly more seropositive patients achieving remission with triple therapy than with monotherapy.

Interpretation Contemporary treatment of rheumatoid arthritis emphasises the use of intensive therapy to achieve remission. However, we have shown that not all patients require such an aggressive approach to therapy. Given the heterogeneity of the disease, treatment should be personalised to the individual, which would minimise costs of treatment as well as potentially toxic side-effects. Our study shows that only seropositive patients with rheumatoid arthritis should be given more intensive therapies.

Funding National Institute for Health Research.
Prediction of treatment response in psoriasis with measurement of serum levels of adalimumab, etanercept, and antidrug antibodies: a pilot study

S K Mahil, Z Arkir, G Richards, J N Barker, C H Smith

Abstract

Background A substantial proportion of patients with psoriasis do not respond or lose initial response to tumour necrosis factor antagonists. This may partly be attributable to development of an immunogenic antibody response which causes subtherapeutic drug levels because of the clearance of drug-antidrug complexes. The aim of this study was to investigate the association between serum drug (adalimumab and etanercept) levels, antidrug antibodies, and clinical response in a cohort of psoriasis patients.

Methods In a single-centre cohort of 56 adults with psoriasis initiated on adalimumab or etanercept between 2009 and 2011, drug and antidrug antibody levels were measured with a commercially available ELISA at the patients’ routine clinic reviews (4, 12, and 24 weeks of treatment and the last available observation). Responders were defined as having a 75% reduction in psoriasis area and severity index from baseline (PASI 75) within 6 months of treatment, or physician’s global score of clear or nearly clear. Non-responders were defined as not achieving a 50% reduction in PASI from baseline (PASI 50) within 6 months or having a loss of PASI 50 treatment response.

Findings After 4 weeks of therapy, adalimumab levels were significantly higher in responders than in non-responders (median 5.00 μg/mL [IQR 4.30–5.00] vs 0.12 μg/mL [0.10–1.79], p=0.003) and these higher levels were sustained at 12 and 24 weeks. Anti-adalimumab antibodies were detected in 25% of non-responders (2/8 patients, mean follow-up 22.5 weeks) and not in any responders (n=23, mean follow-up 26.1 weeks). There was no significant association between etanercept levels and clinical response at 4 weeks (median 2.94 μg/mL [IQR 0.78–3.68] vs 1.40 [0.82–2.12], p=0.317), and no anti-etanercept antibodies were detected.

Interpretation Adalimumab drug level monitoring at 4 weeks may be useful in predicting treatment response, in contrast to etanercept drug levels. The majority of adalimumab non-responders did not have antidrug antibodies; however, lack of serum trough levels and assay limitations may have underestimated their prevalence. Larger studies are required to investigate other factors contributing to low drug levels and to assess the usefulness of these drug and antidrug assays in personalising therapy in psoriasis.

Funding National Institute for Health Research.
Diabetes and cardiovascular events in women with polycystic ovary syndrome: a 20-year retrospective cohort study

Hamidreza Mani, Miles J Levy, Melanie J Davies, Danielle H Morris, Laura J Gray, John Bankart, Hannah Blackledge, Kamlesh Khunti, Trevor A Howlett

Abstract

Background Polycystic ovary syndrome (PCOS) is the most common endocrine problem in women of reproductive age with a reported prevalence of up to 15%. Women with PCOS are potentially at increased risk of cardiovascular (CV) diseases from well-established risk factors, including insulin resistance, obesity, and type 2 diabetes. However data showing excess CV events in this population are still lacking.

Methods We investigated the incidence and prevalence of type 2 diabetes and cardiovascular events (myocardial infarction, angina, heart failure, stroke and CV death) in a retrospective cohort of women with PCOS (total follow-up >12000 person-years) The cohort consisted of 2301 women attending a specialty clinic from 1988 to 2009 in Leicestershire, UK (mean age 29·6 years [SD 9·1]).

Findings Incidence of type 2 diabetes, myocardial infarction, angina, heart failure, stroke, and CV death was respectively 3·6, 0·8, 1·0, 0·3, 0·0, and 0·4 per 1000 person-years. At the end of follow-up, prevalence of myocardial infarction in the age groups 45–54, 55–64, and older than 65 years were, respectively, 1·9%, 6·0%, and 27·3%, and of angina were 2·6%, 6·0%, and 27·3%. Age-group-specific odds ratios for prevalence of myocardial infarction and angina compared with the local female population (n=434 859) ranged between 2·6 (95% CI 1·0–6·3) and 12·9 (3·4–48·6) with the highest ratio being for myocardial infarction in the over-65 age group. Age, history of hypertension, and smoking had significant correlations with CV outcomes in women with PCOS (adjusted odds ratio 1·08 [95% CI 1·03–1·12], p<0·01 vs 9·94 (3·77–26·19), p<0·01 vs 3·33 [1·23–8·59], p<0·01).

Interpretation We have shown a high incidence and age-group-specific prevalence of type 2 diabetes, myocardial infarction, and angina in women with PCOS, with more than a quarter of those aged over 65 years having had a myocardial infarction or angina. These findings should be considered in treatment strategies, long-term planning, and CV risk reduction programmes for women with PCOS.

Funding British Endocrine Society, National Institute for Health Research, and University of Leicester.
Depot-specific and sex-specific secretion of leptin and interleukin 6: higher leptin release in women and lower interleukin-6 release from femoral adipose tissue in vivo

Konstantinos N Manolopoulos, Fredrik Karpe, Keith N Frayn

Abstract

Background The female fat distribution pattern of gluteofemoral fat mass accumulation is associated with protection from cardiovascular disease and diabetes. Furthermore, women are known to have higher systemic leptin concentrations than men. Differential adipokine secretion from subcutaneous adipose tissue depots may be responsible for these differences. We aimed to study the physiological release of leptin and interleukin (IL) 6, a pro-inflammatory adipokine, from abdominal and femoral adipose tissue in vivo.

Methods Depot-specific leptin and IL-6 release were measured in 42 healthy volunteers (23 men and 19 women), matched for age and body-mass index (mean 25.4 kg/m²). Measurements were carried out after an overnight fast. Leptin and IL-6 release were studied with the arteriovenous difference technique across abdominal and femoral adipose tissue.

Findings Leptin release showed a strong sex dichotomy with 3.5-fold higher systemic plasma concentrations (p<0.001) and a 3-fold higher leptin release rate per unit fat mass across abdominal and femoral adipose tissue in women than in men (p<0.05). In both men and women, abdominal and femoral leptin release were positively correlated with each other (r=0.54, p<0.001), and negatively correlated with waist-to-hip ratio (p<0.001, controlled for sex). Abdominal IL-6 release was higher than femoral IL-6 release (p<0.001), which was a consistent finding in both men and women.

Interpretation Adipose tissue is characterised by differential adipokine secretion between sexes and subcutaneous fat depots. The widely observed higher systemic leptin concentration in women is a result of a high leptin production rate and large gluteofemoral fat mass compared with men. Femoral adipose tissue is characterised by a lower IL-6 release rate, which may suggest that gluteofemoral fat is resistant to low-grade inflammation.

Funding Higher Education Funding Council for England.
Sensitivity to cuteness in baby faces is not influenced by pregnancy

K F M Marwick, K J Rhodes, S Serghiou, R M Steel, A Campbell, J Hall, R Sprengelmeyer

Abstract

Background The ability to discriminate cuteness may aid caregivers in prioritising care to the neediest child. This biologically important ability has been indirectly linked to higher levels of female reproductive hormones via studies of hormonal contraception and menopausal status. Pregnancy provides an opportunity to further investigate the role of reproductive hormones in cuteness discrimination since it is a time of substantial natural hormonal fluctuation.

Methods Pregnant (n=23) and matched non-pregnant women (n=11) were assessed four times over 8 months (at 20 weeks of gestation, 32 weeks of gestation, 2 weeks postpartum, 12 weeks postpartum). At each visit, cuteness sensitivity, cuteness intensity ratings, and basic visuospatial perception were assessed. Cuteness sensitivity was assessed by presenting two versions of the same face side by side, with one subtly altered by graphics software to be more or less cute than the other; women were asked to select the cuter face. Cuteness intensity was rated on a seven-point Likert scale. Results were analysed with repeated measures ANOVA.

Findings There was no difference between pregnant/postpartum mothers and control women in cuteness sensitivity, cuteness intensity ratings, or basic visuospatial perception. There was no change in these abilities across time.

Interpretation This result is not what we hypothesised. It seems that the link between female reproductive hormones and cuteness sensitivity is more indirect and complex than initially thought. Possibly female reproductive hormones other than those elevated in pregnancy are important in determining cuteness sensitivity.

Funding Wellcome Trust.
Characterisation and therapeutic potential of endothelial progenitor cells

Sandra E McAllister, Reinhold Medina, Christina O’Neill, Alan W Stitt

Abstract
Endothelial progenitor cells (EPCs) may have great potential for use as a cellular therapy for promoting vascular repair of ischaemic tissues, such as complex wounds. The term EPC, however, has been used to describe a diverse variety of cells. This ambiguity has led to conflicting results when comparing results of EPC studies from different research laboratories. Recently, there is consensus that when selective in-vitro cell culture techniques are used, two distinct EPC subpopulations are generated—namely, myeloid angiogenic cells (MACs) and outgrowth endothelial cells (OECs). This study provides a full phenotypic and functional characterisation of MACs and OECs and compares their potential therapeutic role in wound healing.

EPC subtypes were isolated from human peripheral blood and umbilical cord blood. MACs appeared early in culture (~7 days) as elongated cells with multiple fine dendritic processes and low proliferative potential. OEC colonies appeared later in culture (3–5 weeks), and formed a cobblestone-shaped monolayer. Genome-wide transcriptomics demonstrated that MACs and OECs belong to the haemopoietic and endothelial lineages, respectively. MACs cultured in vitro were further analysed, and were found to be phenotypically very similar to M2 alternative activated macrophages.

Analysis of functional characteristics showed that MACs have low proliferative potential. However, OECs possess high proliferative potential, enabling them to be single-cell cloned, with an average 60 population doublings in 80 days for cord-derived OECs.

Using in-vitro angiogenesis assays, OECs demonstrated de-novo tubulogenesis capacity, and were capable of interacting with mature endothelial cells through adherens and tight junctions. MACs did not form tubes and did not differentiate into endothelial cells, but significantly promoted tubulogenesis of mature endothelial cells by releasing paracrine factors such as interleukin 8 and monocyte chemoattractant protein 1.

Funding Northern Ireland Medical and Dental Training Agency.
Signatures of CD4 T-cell help and CD8 exhaustion predict clinical outcome in autoimmunity, infection, and vaccination


Abstract
Autoimmune diseases are common and debilitating, but their severe manifestations could be reduced if biomarkers were available to allow individual tailoring of the potentially toxic immunosuppressive therapy required for their control. Gene expression-based biomarkers have been identified and translated into clinical practice in cancer, but not autoimmunity. We show that transcriptional profiling of purified CD8 T cells, which avoids the confounding influences of unseparated cells, identifies two distinct patient subgroups predicting long-term prognosis in four distinct autoimmune diseases: anti-neutrophil cytoplasmic antibody (ANCA)-associated vasculitis, systemic lupus erythematosus, ulcerative colitis, and Crohn’s disease.

While the correlation of this signature with prognosis raises the prospect of individualised therapy in autoimmunity, the biological mechanism underlying its association with relapsing disease may indicate novel therapeutic targets. Acute exposure to foreign antigen results in persistent antigen-specific cellular immunity, but during chronic viral infection persistent antigen exposure may exhaust that response. This exhausted CD8 T-cell phenotype may itself be defined by a distinct transcriptional signature. We show that the same signature seen in chronic viral infection can be seen in chronic self antigen exposure during autoimmunity, with one exception: whereas exhaustion results in viral persistence it predicts favourable outcome in autoimmunity. Provision of adequate CD4 help can prevent or overcome the development of CD8 exhaustion resulting in clearance of chronic viral infection. Consistent with this observation, we show that the CD8 exhaustion signature in autoimmunity is inversely correlated with that of concurrent CD4 help and can be robustly identified in mixed cell populations. This has allowed independent validation of this marker on published datasets, confirming prediction of clinical outcome in a total of 1070 samples from 429 individuals spanning infection (HIV, dengue virus, hepatitis C), vaccination (yellow fever, malaria, influenza), and autoimmunity (type 1 diabetes, ANCA–associated vasculitis, systemic lupus erythematosus, Crohn’s disease, and ulcerative colitis).

Funding Wellcome Trust.
Incidence of eating disorders in the UK: findings from the UK General Practice Research Database

Nadia Micali, Katrina W Hagberg, Janet L Treasure

Abstract

Background Few studies have investigated time-trends in the incidence of eating disorders; important questions about changes in incidence over time and case detection remain unanswered. We aimed to determine changes in the incidence of the eating disorders, anorexia nervosa, bulimia nervosa, and eating disorder not otherwise specified (EDNOS) between 2000 and 2009 in the UK.

Methods We identified all patients with a first time diagnosis of an eating disorder from the General Practice Research Database (GPRD). Annual incidence rates (IR) were calculated by age group (10–14, 15–19, 20–29, 30–39, 40–49 years), gender, and eating disorder subtype.

Findings We identified 9062 patients with a first time diagnosis of eating disorder recorded in the GPRD during the study period (2000–2009). Annual IR of all eating disorders for ages 10–49 years changed from 33·0 per 100 000 in 2000 to 36·8 per 100 000 in 2009. In female patients, incidence of anorexia nervosa and bulimia nervosa was stable; however, incidence of EDNOS and the overall incidence of eating disorders increased. Between 2000 and 2009 there was a non-significant increase in the annual IR of eating disorders in male patients.

Interpretation The incidence of anorexia nervosa and bulimia nervosa remained stable between 2000 and 2009. Eating disorders increased in incidence in female patients in the decade under study. There was a significant increase in new diagnoses of EDNOS among female patients. EDNOS is the most common eating disorder in primary care.

Funding National Institute for Health Research.
Novel in-vitro model to study first responses of airway epithelial cells to allergen and pro-inflammatory stimuli at birth

D Miller, S Turner, D Spiteri-Cornish, N McInnes, A Scaife, P J Danielian, G Walsh, G Devereux

Abstract

Background The airway epithelium is increasingly being implicated in the pathogenesis of asthma. Although believed to be important, little is known about how the neonatal airway epithelial cell (AEC) phenotype impacts on respiratory disease in later life. The aim of this study was to establish a methodology for culturing neonatal nasal AEC and to describe AEC response in vitro.

Methods AECs were sampled from healthy, unsedated infants during the first week of life by brushing both nostrils with an interdental brush. Sampled AECs were used for cytopin preparation or grown to confluence before subculture. Cultured cells were characterised morphologically and by immunocytochemistry. Interleukin-8 concentrations were measured in supernatants from monolayers at rest and after exposure to concentration ranges of interleukin 1β and tumour necrosis factor α or house dust mite extract.

Findings Primary cultures were successfully established in 109 (92%) of 117 neonates sampled, with 93 (80%) successfully cultured to confluence at third passage. The epithelial lineage of the cells was confirmed by morphological analysis and immunocytochemistry. Constitutive interleukin-8 secretion was observed and was upregulated by both stimuli in a dose dependent manner.

Interpretation We describe a safe, minimally invasive method of culturing AECs from neonates suitable for functional cell analysis and amenable to large population based studies. This novel technique offers a unique opportunity to study naive AECs not yet exposed to the modifying effects of environmental pollutants and viral pathogens and may prove useful in elucidating the early origins of asthma.

Funding Chief Scientist Office of the Scottish Government.
Endocrine disruption in the human fetal testis: use of a xenograft system to assess effects of exposure to environmental agents and pharmaceutical drugs

R T Mitchell, R A Anderson, C J H Kelnar, W H B Wallace, C McKinnell, R M Sharpe

Abstract
Background Many environmental chemicals have been proposed as endocrine disruptors in the fetal testis. Studies in rats have demonstrated reproductive abnormalities after in-utero exposure to environmental chemicals (eg, phthalates) or pharmaceutical drugs such as paracetamol. Whether such effects also occur in the human fetal testis has been difficult to determine. We have recently demonstrated that xenografting of human fetal testis tissue results in normal seminiferous cord formation and cellular development/function. We aimed to determine the effects of proposed endocrine disruptors (eg, phthalates, paracetamol) on the human fetal testis using a xenograft approach.

Methods Human fetal testes (14–20 weeks’ gestation, n=17) obtained from elective terminations were xenografted into nude mice. Host mice received di-n-butyl phthalate (500 mg/kg/day), paracetamol (350 mg/kg/day), or vehicle during the grafting period. Testosterone production was determined by measurement of host animal seminal vesicle weight. Morphological and immunohistochemical analysis with a range of markers was performed to investigate seminiferous cord structure, steroidogenesis, and cellular development.

Findings Exposure to paracetamol resulted in a significant reduction in seminal vesicle weight (mean 13·6 mg [SD 8·5] vs 10·0 [5·4], p<0·01). Expression of 3β hydroxysteroid dehydrogenase (3β-HSD) was also decreased in paracetamol compared with vehicle treated grafts. Exposure to di-n-butyl phthalate did not result in a reduction in testosterone or 3β-HSD expression in human xenografts (in contrast to rats); however, testosterone independent germ cell effects were demonstrated with a reduction in gonocytes in di-n-butyl phthalate exposed grafts compared with vehicle (mean 13% [SD 11·6] vs 23 [11·3], p<0·05).

Interpretation These results suggest that paracetamol may impair testosterone production in the human fetal testis, whereas phthalates do not. This highlights important differences between rat and man in terms of the effects of chemical exposure on the developing testis.

Funding Wellcome Trust and British Society for Paediatric Endocrinology and Diabetes.
TIE2-expressing monocytes regulate revascularisation of the ischaemic limb

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Abstract

Background Monocytes (CD14+ cells) expressing the receptor TIE2 are a highly angiogenic subset that are pivotal to neovascularisation in the tumour environment. We hypothesised that TIE2-expressing monocytes (TEMs) are also important in neovascularisation of ischaemic tissues.

Methods Flow cytometry was used to quantify circulating TEMs in 40 patients with critical limb ischaemia (20 age-matched and 20 healthy controls). RT-PCR was used to confirm TIE2 expression in FACS-sorted TEMs. ELISA was used to measure circulating levels of the TIE2 ligand angiopoietin 2 (ANG2). Mice were subjected to hindlimb ischaemia and TEMs quantified. In an additional study, haemopoietic stem/progenitor cells, isolated from Pgk-rtTA-miR-126T transgenic mice, were transduced ex vivo with a TRE-miR-Tie2-OFP lentiviral vector and used to reconstitute lethally irradiated mice. These mice were treated with alternate daily doses of doxycycline to silence Tie2 expression on TEMs, and hindlimb ischaemia (HLI) was induced. Conversely, bone-marrow-derived macrophages (BMDMs) were enforced to express Tie2 using a Pgk-Tie2 lentivirus and delivered into the ischaemic hindlimb. Recovery of ischaemia was measured with laser Doppler.

Findings Flow cytometry revealed a ten-fold higher number of circulating TEMs in patients with critical limb ischaemia than in matched controls (mean 3.52% [SE 0.28] vs 0.39 [0.09], p<0.0001). Revascularisation or amputation resulted in a fall in TEM numbers to control levels (p<0.005). Analysis by RT-PCR confirmed TIE2 mRNA expression in TEMs. Circulating ANG2 levels were two-fold higher in patients with critical limb ischaemia than in controls (mean 4354 pg/mL [SE 661] vs 1973 [247], p<0.05). Circulating CD115+/Tie2+ monocyte numbers were higher following hindlimb ischaemia in mice than in sham controls (p<0.05) and Tie2 expression was upregulated in CD45+/CD11b+/F4/80+ macrophages isolated from ischaemic hindlimbs (p<0.05). Tie2 gene knockdown in TEMs inhibited neovascularisation of the ischaemic hindlimb (p<0.0001). Delivery of Tie2-expressing BMDMs into the ischaemic limb accelerated the recovery of blood flow compared with treatment with control BMDMs.

Interpretation Our studies suggest that TEMs are mobilised following ischaemia and contribute to the revascularisation of ischaemic tissue, and that TIE2 is important for the proangiogenic function of TEMs. TEMs may represent a promising, novel therapeutic target for cell therapy in critically ischaemic tissues.

Funding British Heart Foundation and Royal College of Surgeons of England.
Hepatic inflammation and fibrosis biomarkers are associated with cardiovascular risk factors but not cardiovascular disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study

J R Morling, R M Williamson, C M Robertson, I N Guha, J A Fallowfield, M W J Strachan, J F Price

Abstract

Background Both cardiovascular disease and liver disease are particularly common in people with type 2 diabetes and it is possible that the two conditions are inter-related. Non-invasive biomarkers are increasingly used to estimate liver inflammation and fibrosis. In this study the association of these biomarkers with cardiovascular risk factors and disease was explored in a large, representative population of people with type 2 diabetes mellitus.

Methods Cytokeratin 18 (CK18, biomarker of hepatic inflammation) and the European Liver Fibrosis panel (ELF, biomarker of hepatic fibrosis) were measured in a random subgroup of 564 adults, aged 60–75 years at recruitment, participating in the Edinburgh Type 2 Diabetes Study (ET2DS). Data on conventional CV risk factors (body-mass index [BMI], waist circumference, blood pressure, total cholesterol, triglycerides, smoking status) and prevalent cardiovascular disease (validated myocardial infarction, angina, stroke and transient ischaemic attack events) were also available.

Findings Median CK18 was 102 U/L [IQR 76–137, range 29–993] and mean ELF was 8·9 U/L [SD 0·8, range 6·9–11·6]. After adjustment for age and sex, increased CK18 was significantly associated with higher triglyceride levels ($r=0·157$, $p=0·002$). Increased ELF score was associated with higher BMI ($r=0·202$, $p<0·001$), waist circumference ($r=0·139$, $p=0·008$), and diastolic blood pressure ($r=–0·045$, $p=0·025$). Despite these associations, neither biomarker was significantly associated with prevalent cardiovascular disease (prevalent cardiovascular disease vs no cardiovascular disease, mean CK18 108·1 U/L [SD 26·2] vs 105·5 [22·6], $p=0·473$ and mean ELF 8·94 [0·77] vs 8·89 [0·76], $p=0·442$).

Interpretation In people with type 2 diabetes, non-invasive biomarkers of hepatic inflammation and fibrosis are associated with a number of cardiovascular risk factors but do not appear to associate with pre-existing vascular disease. Further investigation is required to determine whether liver biomarkers predict incident cardiovascular disease in this high risk group.

Funding Diabetes UK.
Preventing dedifferentiation of human exocrine enriched pancreatic cells in culture

K R Muir, M J Lima, K Docherty

Abstract

Background A cell-based therapy offers hope for a cure for type 1 diabetes. Attempts to generate a sizeable population of insulin producing cells from pancreatic cells in vitro have however been largely unsuccessful. This is partly related to beta cells and the exocrine component undergoing rapid dedifferentiation in standard culture conditions. Endocrine and exocrine markers are rapidly lost and the cells take on a mesenchymal phenotype. Because of developmental similarities this exocrine fraction holds potential for transdifferentiation towards a beta-cell lineage. We hypothesise that maintaining an epithelial phenotype will enhance this process. Here we look at the individual and combined effect of transforming growth factor β1 (TGFβ1) and rho-kinase inhibition on dedifferentiation of exocrine enriched pancreatic cells (EEPCs).

Methods EEPCs left over from islet transplantation were cultured in RPMI medium with 10% FBS and left for 48 h to attach followed by addition of rho-kinase inhibitor (Y27632), a TGFβ1 inhibitor (SB431542), or both for a further 7 days. Gene expression was measured by real-time quantitative PCR.

Findings After treatment with both Y27632 and SB431542, expression of amylase showed a 334% increase compared with untreated control and a 168% increase compared with baseline at 48 h in culture. Insulin showed a 571% increase compared with control but a decrease of 69% compared with baseline. Glucagon expression was 535% higher than control and 4% lower than baseline. Epithelial marker E-cadherin showed a 29% increase compared with control and 12% increase compared with baseline. All these markers were elevated at a statistically significant level compared with untreated controls.

Interpretation The data suggest that the addition of Y27632 and SB431542 to EEPCs may slow down this dedifferentiation process. Maintenance of an epithelial morphology may result in cells that are more amenable to transdifferentiation towards beta cells.

Funding Wellcome Trust.
Adverse impact of heart failure on the electrophysiological response to ischaemia-reperfusion in human myocardium

Fu Siong Ng, Deborah Janks, Andrew L Wit, Nicholas S Peters, Igor R Efimov

Abstract

Background Acute ischaemia and reperfusion (I-R) are associated with pro-arrhythmic electrophysiological changes such as action potential duration (APD) shortening and conduction velocity (CV) slowing, though data from human myocardium are sparse. We studied electrophysiological changes during I-R in intact human myocardium, comparing differences between failing and non-failing hearts.

Methods We optically mapped coronary-perfused left ventricular wedge preparations from six human hearts with end-stage heart failure (HF) and six non-failing hearts from donors rejected for transplant (NF). At baseline, the preparations were subjected to steady-state pacing across a range of cycle lengths, and then subjected to 30 min of global ischaemia, followed by 30 min of reperfusion. Restitution pacing protocols were repeated after reperfusion.

Findings At baseline, HF hearts had longer APD80 and slower transmural CV compared with NF hearts across a range of cycle lengths (both ANOVA p<0·001). APD80 and CV were reduced with ischaemia (at cycle lengths of 1000 ms, baseline vs 10 min ischaemia: mean HF APD 375 ms [SE 23] vs 324 [5], p<0·01; NF APD 308 [14] vs 271 [28], p<0·05; HF CV 29 cm/s [4] vs 16 [6], p<0·05; NF CV 40 [2] vs 23 [2], p<0·001), and were restored with reperfusion. APD shortening was greater in HF hearts during ischaemia (ΔAPD80 at 8 min ischaemia: mean HF 75 ms [SE 11], NF 25 [5]; p<0·01). Recovery of electrical excitability after reperfusion was delayed in HF (4·8 min [1·8] vs NF 1·0 [0], p<0·05). APD was restored to pre-ischaemic levels within the first minute of reperfusion in NF hearts, but restoration of APD was incomplete in HF early after reperfusion.

Interpretation In human myocardium, acute ischaemia was associated with APD shortening and CV slowing, which were reversed with reperfusion. In end-stage HF, these changes were accelerated during ischaemia, and recovery was slower following reperfusion. This may enhance the spatial gradients of repolarisation during acute I-R in failing hearts, and thus increase arrhythmia susceptibility. Further work is needed to elucidate the metabolic mechanisms underlying the adverse electrophysiological response to I-R in human heart failure.

Funding National Institute for Health Research, US National Institutes of Health, and British Heart Foundation.
Does muscle inflammation influence recovery of muscle strength and function in patients undergoing total hip replacement?

T Okoro, C Stewart, N Al-Shanti, A Lemmey, P Maddison, J G Andrew

Abstract

Background Genetic markers of muscle inflammation (eg, tumour necrosis factor α [TNFα] and interleukin [IL] 6) are downregulated following repeated transient increases after bouts of exercise. Total hip replacement (THR) typically resolves preoperative pain, although strength deficits of 10–21% persist in the affected hip at 1 year postoperatively. This study assessed whether mRNA expression of TNFα and IL6 in the vastus lateralis (VL) of the operated leg was related to changes in the strength of the operated leg quadriceps in patients following THR.

Methods Ten patients were recruited prospectively after ethical approval. Distal VL (5 cm proximal to lateral suprapatellar pouch) biopsy samples were obtained intraoperatively and at 6 weeks postoperatively, with maximal voluntary contraction of the operated leg quadriceps (MVCOLQ) in Newtons (N), assessed preoperatively and at 6 weeks postoperatively. RT-PCR was used to assess mRNA expression in the biopsy samples and associations evaluated with Spearman's correlation coefficient.

Findings Mean mRNA relative quotient (RQ) for comparison of 6 week intraoperative VL samples was 6.23 [SD 1.285] for TNFα and 17.10 [47.46] for IL6. Preoperatively, mean MVCOLQ was 188.90 N [76.84] and at 6 weeks it was 217.00 N [53.91]. There was no significant relation between TNFα or IL-6 RQ and absolute MVCOLQ at 6 weeks (r=0.115 [p=0.376] and –0.491 [p=0.075], respectively). No statistically significant relation existed between TNFα mRNA RQ and the improvement in MVCOLQ at 6 weeks (r=–0.498, p=0.071) nor with IL6 and the same measure (r=0.091, p=0.401).

Interpretation There is a trend to correlation that exists for improvement in MVCOLQ with a reduction in TNFα mRNA expression, as well as between absolute MVCOLQ and reduction in IL-6 mRNA expression at 6 weeks postoperatively. Improvement in muscle strength may be mediated by reduced muscle inflammation and the associated reduction in pain in patients with severe osteoarthritis.

Funding Wales Deanery and Betsi Cadwaladr University Health Board Small Grants Scheme.
CXCR6 and CXCL16 in liver disease

Richard Parker, C J Weston, D H Adams

Abstract

Background Chemokines are small molecules that act through G-protein coupled receptors to mediate primarily lymphocyte migration. CXCL16, which interacts with only one receptor (CXCR6), can mediate lymphocyte recruitment and has been implicated in various disease conditions. Steatohepatitis, caused by metabolic syndrome or alcohol misuse, is the commonest cause of liver disease in the UK. We investigated the role of CXCL16 and CXCR6 in the development of steatohepatitis.

Methods Expression of CXCL16 in whole liver and isolated cells was investigated with real-time PCR and immunohistochemistry. Serum and supernatant concentrations of soluble CXCL16 were measured with ELISA. Expression of CXCR6 on lymphocytes was investigated with flow cytometry. Lymphocyte adhesion was assessed with freshly isolated lymphocytes from liver or peripheral blood flowed over confluent layer of isolated human hepatic sinusoidal endothelium (HSEC).

Findings Whole liver expression of CXCL16 was increased relative to normal liver in fatty liver disease with increasing expression seen with increasing steatohepatitis and fibrosis. Immunohistochemistry showed CXCL16 expressed throughout regenerative nodules in both alcoholic and non-alcoholic liver disease. Isolated HSEC, biliary epithelial cells, and hepatoma cell lines increased expression of CXCL16 and released soluble CXCR6 in response to pro-inflammatory cytokines, particularly the combination of tumour necrosis factor α and interferon γ. Peripheral blood lymphocyte CXCR6 expression was confined to CD4 cells; however in the liver CD8+ cells and CD56+ cells more commonly expressed CXCR6. Inhibition of CXCR6 or CXCL16 inhibited transmigration of lymphocytes across HSEC.

Interpretation CXCL16 is expressed in diseased liver where it has a role in the transmigration of lymphocytes across endothelium. This may represent a new therapeutic target in liver disease.

Funding UK Medical Research Council.
Endothelial progenitor cells in smokers are dysfunctional because of increased DNA damage and senescence

Koralia E Paschalaki, Richard D Starke, Nicolas Mercado, Vassilis G Gorgoulis, Peter J Barnes, Anna M Randi

Abstract

Background Cardiovascular disease (CVD) is a major cause of death in smokers, especially in patients with chronic obstructive pulmonary disease (COPD). The molecular pathways that lead to endothelial dysfunction and CVD due to cigarette smoke remain unclear. DNA damage has been recognised as an important contributor in ageing disorders, including CVD. Circulating endothelial progenitor cells (EPC) are required for endothelial homoeostasis, and their dysfunction contributes to CVD. This study aimed to examine whether circulating EPC (also called blood outgrowth endothelial cells [BOEC]) from smokers and COPD patients are dysfunctional, and to investigate the role of DNA damage pathways in mediating endothelial dysfunction in these patients.

Methods BOEC were isolated from peripheral blood samples received from 16 healthy non-smokers (five men, 11 women; mean age 57 years [SE 2.7]), ten healthy smokers (five men, five women; 57 years [2.6]), and 16 COPD patients (11 men, five women; 67 years [1.6]). Endothelial senescence was measured by senescence-associated β-galactosidase (SA-β-gal) activity. Protein levels of sirtuin 1 (SIRT1) were measured by western blotting, expression of p16, γ-H2AX, and 53BP1 by immunofluorescence, and p21 by western blotting and immunofluorescence. SIRT1 activity was measured with a SIRT1 fluorescent activity assay kit.

Findings BOEC from smokers and COPD patients showed evidence of increased DNA double-strand breaks (increased γ-H2AX, 53BP1) compared with non-smokers. BOEC from healthy smokers and COPD patients displayed increased senescence (measured by SA-β-gal activity, p16, and p21) and decreased SIRT1 expression and activity compared with controls. SIRT1 protein levels and activity negatively correlated with senescence, indicating a regulatory role of SIRT1 on senescence. Interestingly, treatment of BOEC from COPD patients with a SIRT1 activator (resveratrol) rescued the senescent phenotype.

Interpretation The results from our study demonstrate that BOEC from smokers and COPD patients display increased DNA damage and senescence, associated with reduced SIRT1 expression and activity. These defects may contribute to endothelial dysfunction and cardiovascular events in people who smoke and could potentially constitute therapeutic targets for intervention.

Funding Imperial College London.
Intrahepatic natural killer cell NKp46 expression drives HCV-associated liver inflammation and viral resistance to treatment with interferon alpha

Tom Pembroke, Awen Gallimore, Andy Godkin

Abstract

Background There is evidence that natural killer (NK) cells help control persistent viral infections including hepatitis C virus (HCV). HCV infection is treated with interferon (IFN) alpha, which stimulates the immune system, and is successful in 40–80% of patients. Detailed comparison of the phenotype and function of blood and intrahepatic NK cells in chronic HCV infection and in response to treatment with IFN alpha has not been elucidated.

Methods We performed a comparison of NK cells derived from blood and intrahepatic compartments in multiple paired samples from 24 HCV infected patients pretreatment and 22 patients with non-viral chronic liver disease (CLD). NK phenotype (CD16, NKp30, NKp46, NKG2D, and NKG2A) and functional profile (CD107a, IFNγ, and granzyme B) were assessed with flow cytometry. In a separate cohort of 17 patients with HCV, who had completed treatment, rate of viral clearance was calculated and pretreatment peripheral blood NK phenotype and CD107a expression (degranulation) in response to increasing stimulation was measured.

Findings NK cells in the liver demonstrate a distinct phenotype compared with blood, manifested as downregulation of the NK cell activation receptors CD16, NKG2D, and NKp30. By contrast, NKp46 expression was not downregulated. Intrahepatic NK cells appeared to be more activated with increased spontaneous degranulation (reduced granzyme B, p<0.001 and increased CD107a, p=0.006) and production of IFNγ (p=0.05). NKp46 expression correlated with NK-cell activation, and correlated closely with the severity of liver inflammation (p=0.035). The rate of viral clearance during treatment with IFN alpha inversely correlated with NKp46 expression at baseline (p=0.01). However, the ability to increase cytotoxic NK function in response to increasing stimulation ex vivo correlated with viral clearance (p=0.029) and inversely with NKp46 (p=0.005).

Interpretation These findings indicate that NKp46 marks out pathologically activated NK cells in HCV, which are unlikely to be involved in viral control in IFN alpha-treated individuals.

Funding Welsh Assembly.
Calprotectin has a pathogenic pro-inflammatory role in anti-neutrophil cytoplasmic antibody associated vasculitis and glomerulonephritis

R J Pepper, S Hamour, K M Chavele, N Rasmussen, S Flint, P A Lyons, K G C Smith, C D Pusey, H T Cook, A D Salama

Abstract

Background Calprotectin, an endogenous toll-like receptor 4 (TLR 4) agonist that is expressed in neutrophils, monocytes, and infiltrating macrophages, promotes endothelial activation and transcription of pro-inflammatory cytokines. We investigated calprotectin in renal biopsy samples and serum of patients with anti-neutrophil cytoplasmic antibody associated vasculitis (AAV) and in mice deficient in calprotectin (cal-/-), and we assessed the interaction of calprotectin with macrophages and endothelial cells in vitro.

Methods We examined renal biopsy samples with immunohistochemistry. Serum calprotectin levels were measured with ELISA, and cell surface expression with flow cytometry. Accelerated nephrotoxic nephritis experiments were performed on both wild-type (WT) and cal-/- mice. Macrophages were isolated from WT, TLR4-/-, and cal-/- mice, and kidney endothelial cells from WT mice, and stimulated with calprotectin and supernatants harvested. Phagocytosis with opsonised beads was compared between WT and cal-/- macrophages.

Findings Patients with active AAV glomerular lesions demonstrated the most calprotectin positivity in renal biopsy samples, sclerotic lesions the least (p<0·05), linking calprotectin with disease activity. Serum levels in patients were significantly higher than in controls. In limited systemic disease, calprotectin levels assessed at 1 and 6 months after treatment predicted relapse (sensitivity 78·6%, specificity 92·3%). Patients had persistently higher monocyte and neutrophil cell surface calprotectin expression than did healthy controls suggesting a persistently activated state. Cal-/- mice were protected from renal disease with less macrophage and T-cell infiltration, less thrombosis, and preserved renal function. Calprotectin stimulation of WT macrophages and endothelial cells increased production of tumour necrosis factor α, interleukin 6, and interleukin 8 (p<0·05), an effect abrogated in TLR4-/- and cal-/- macrophages. Additionally, cal-/- macrophages had decreased phagocytosis ability compared with WT (p<0·005). Together these data demonstrate a positive amplification of inflammation mediated by calprotectin.

Interpretation Serum calprotectin is a potential biomarker in AAV, and may predict relapse. Calprotectin contributes to pathogenesis by promoting leukocyte and endothelial cell activation in a positive feedback loop.

Funding UK Medical Research Council.
Vitamin D treatment reduces inflammatory cytokine secretion by pollution-stimulated bronchial epithelial cells

P E Pfeffer, F J Kelly, C M Hawrylowicz

Abstract

Background Environmental factors have a strong causal role in the development of asthma. Vitamin D insufficiency and particulate matter (PM) air pollution are two environmental factors associated with airways disease. We hypothesise that vitamin D will reduce production of inflammatory mediators by bronchial epithelial cells stimulated with PM, protecting the airways from inflammation that would otherwise promote Th2/Th17 responses in asthma.

Methods Primary human bronchial epithelial cells from healthy and asthmatic donors were cultured with standardised PM with or without vitamin D. Activated 1,25(OH)D3 was used in preliminary experiments and the precursor 25(OH)D3 in later experiments. A transcription microarray was conducted to highlight differentially expressed inflammatory mediators for further investigation. Expression of target genes was measured by quantitative real-time PCR and levels of inflammatory cytokines in culture supernatants by Cytometric Bead Array.

Findings PM caused increased production of multiple cytokines and chemokines by bronchial epithelial cells. Vitamin D decreased production of a range of cytokines and chemokines, including interleukin (IL) 6 (47·8% [95% CI 5·3%–90·3%] reduction in gene expression with 1,25(OH)D3, 46·7% [35·6%–57·8%] decrease in supernatant protein) and IL24 (61.3% [95% CI 36·0%–86.5%] reduction in gene expression). This reduction was not due to loss of cell viability. Other cytokines were not affected by vitamin D—for example, granulocyte-macrophage colony-stimulating factor (12·9% [95% CI –19·5% to 45·4%] reduction in gene expression, 1·9% [–16·7% to 20·5%] in supernatant protein).

Interpretation The decrease in IL-6 production on treating PM-stimulated bronchial cells with vitamin D, either active 1,25(OH)D3 or the circulating precursor 25(OH)D3, is important given that IL6 inhibits regulatory T-cell responses and promotes Th17 responses. IL24 promotes Th1 cytokine secretion. Functional studies investigating the effect of epithelial-conditioned medium on T cells are in progress.

Funding Wellcome Trust and National Institute for Health Research.
Investigation of the feasibility of clinical trials in breast reconstruction

Shelley Potter, Simon Cawthorn, Nicola Mills, Jane Blazeby

Abstract

Background Breast cancer affects one in eight women and approximately 40% will require a mastectomy. The loss of a breast can dramatically impact upon quality of life. Breast reconstruction is offered to improve outcomes. Making decisions about reconstructive surgery, however, is challenging, and women and health-care professionals need to assess the likely benefits of surgery against the risks of adverse outcomes. Decisions are informed by published outcomes and surgeon and patient preferences. Well-designed studies, such as multicentre, randomised, controlled trials (RCTs) provide the best evidence, but few RCTs have been undertaken in breast reconstruction. The aim of this study was therefore to explore the need for and the feasibility of RCTs in breast reconstruction.

Methods Three systematic literature reviews (SRs) were done to critically appraised and summarise the quality of outcome reporting in breast reconstruction. Semistructured qualitative interviews were conducted with women and health-care professionals to explore decision making and attitudes to RCTs in breast reconstruction. The clinical outcomes SR included 123 observational studies and 11 RCTs. The majority were at high-risk of bias, and outcome reporting was heterogeneous. The cosmetic outcome SR included 122 studies. Cosmetic results were assessed by patients and health-care professionals, but the methodology was inconsistent. The patient-reported outcome SR included 62 studies, only 60% of which were considered methodologically robust.

Findings 62 interviews with women and health-care professionals showed that decision making in breast reconstruction was complex. A third of women reported decisional regret, and insufficient time and information were identified as barriers to decision making. Inequalities in access to care, however, emerged as the most significant determinant of women’s reconstructive experience. Both women and health-care professionals accepted RCTs in breast reconstruction in particular circumstances.

Interpretation There is an urgent need for well-designed studies with standardised outcome assessment in breast reconstruction. Selected randomised trials may be feasible, but they are unlikely to address the key questions. Service reorganisation and interventions to improve decision making are needed to improve outcome for women considering breast reconstruction in the UK.

Funding University of Bristol.
Transcription factor T-bet regulates intestinal inflammation mediated by innate lymphoid cells with the interleukin-7 receptor


Abstract

Interactions between the innate immune system and the intestinal microbiota have an important role in the maintenance of mucosal homeostasis. Indeed, impaired regulation of innate immune pathways are now considered to be a key driver of gut inflammation and may lead to the emergence of inflammatory bowel disease, such as ulcerative colitis or Crohn’s disease. Mice lacking the transcription factor T-bet in the innate immune system develop microbiota-dependent colitis that resembles ulcerative colitis. Here, we show that inflammatory bowel disease in this model is mediated by a newly discovered population of innate immune cells termed innate lymphoid cells (ILCs) that selectively produce the inflammatory cytokine interleukin (IL) 17A. Depletion of intestinal ILCs, or blockade of the IL23/IL17 axis significantly attenuated chronic inflammatory bowel disease in these mice.

We also show that elevated colonic production of tumour necrosis factor α (TNFα), a key cytokine involved in the pathogenesis of inflammatory bowel disease, was produced by CD103–CD11b+ intestinal dendritic cells (DCs). TNFα synergised with IL23 to drive IL-17A production by ILCs, demonstrating a previously unrecognised layer of cellular crosstalk between DCs and ILCs.

Other cytokines implicated in pathogenesis of inflammatory bowel disease, such as TL1A and IL6, also promoted IL-17 production by ILCs, indicating that this novel intestinal innate immune cell population is responsive to other recognised inflammatory signals to drive gut inflammation. By sequencing bacterial rRNA genes we also identify a particular component of the intestinal microbiota of TRUC mice that drives excess TNF-α production and triggers colitis. Lastly, we show that T-bet is a transcriptional repressor of IL-7R expression, a key molecule involved in controlling intestinal ILC homeostasis. The importance of IL-7R signalling in TRUC disease was highlighted by the dramatic reduction in intestinal ILCs and attenuated colitis following IL-7R blockade.

Taken together, these data demonstrate the mechanism by which T-bet regulates the complex interplay between mucosal DCs, ILCs, and the intestinal microbiota.

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Poster Abstracts

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Myeloid-derived suppressor cells in non-malignant and non-infectious liver disease
Yazid J Resheq, Henning W Zimmermann, David H Adams, Stuart M Curbishley

Abstract
Background Myeloid-derived suppressor cells (MDSC) have been described as potent immunosuppressive cells in malignant and infectious liver disease. However, little is known about their role in non-infectious or non-malignant disease. We sought to characterise MDSC in patients with chronic non-infectious or non-malignant liver disease.

Methods Explants obtained from 12 patients undergoing liver transplantation and blood from 30 patients treated for haemochromatosis at the Queen Elizabeth Hospital, Birmingham, were analysed for the frequency of functional CD14+ HLA-DR– monocytic MDSC. Functional capacity was defined as the capability to suppress proliferation of maximally stimulated, CFSE-labelled CD4 T cells using CD3/CD28-beads (Dynabeads, LifeTechnologies, UK) at a ratio of 1:1. Additionally, MDSC were analysed for their capacity to induce CD4 regulatory T cells (assessed by FoxP3 expression) in cells activated with CD2/CD3/CD28-beads (Miltenyi, Germany). Both MDSC and CD4 cells were isolated by magnet-activated cell-sorting using a combination of depletion steps and positive-selection-steps. Analysis of frequency and immunotyping of MDSC was performed with flow-cytometry.

Findings CD14+ HLA-DR– MDSC obtained both from liver tissue and peripheral blood were able to suppress proliferation of CD4 T cells and to induce FOXP3-expression in CD4 T cells, typical of regulatory T cells. No such findings were observed when using CD14+ HLA-DR+ monocytes. Moreover, MDSC depleted of CD16+ monocytes showed weaker immunosuppressive capacity. In patients with haemochromatosis, the frequency of CD14+ HLA-DR– MDSC in peripheral blood ranged from 0.5% to 79% and in the liver of cirrhotic patients from 9.1% to 75.5%.

Interpretation CD14+ HLA-DR– MDSC are fully functional in patients who have non-infectious or non-malignant liver disease. Similar to HLA-DR+ monocytes, CD16 expression may identify subtypes of monocytic MDSC with distinct immunoregulatory properties. Given the varying frequency of MDSC in the patients analysed, the clinical relevance of MDSC in non-malignant and non-infectious liver-disease has to be further analysed since they may influence the course of disease in these patients.

Funding Deutsche Forschungsgemeinschaft and Liver Foundation Trust Fund.
Interferon α impairs survival and function of circulating angiogenic cells in vitro: a model of failed endothelial repair in systemic lupus erythematosus

John A Reynolds, David W Ray, Terence O’Neill, M Yvonne Alexander, Ian N Bruce

Abstract
Background Patients with systemic lupus erythematosus have an increased risk of cardiovascular disease. Interferon α may impair endothelial repair mechanisms in lupus. Patients with systemic lupus erythematosus have fewer circulating angiogenic cells (CAC) and endothelial progenitor cells (EPCs). Mixed EPC and CAC populations have been shown to be sensitive to interferon α (IFNα). We aimed to investigate the effects of IFNα2b on an in-vitro model of angiogenesis and vascular repair.

Methods Peripheral blood mononuclear cells from healthy individuals were cultured on human fibronectin for 7 days. CAC phenotype was confirmed by low-density lipoprotein (LDL) uptake, lectin binding, and cell surface marker expression by RT-PCR. Cell survival in response to IFNα2b (0·01–10 ng/mL) was determined by the number of LDL-uptake-positive cells. To study CAC function, supernatant from CACs in the presence or absence of 10ng/mL IFNα2b was added to human aortic endothelial cells on Matrigel. Tubule formation was assessed at 14 h.

Findings CACs expressed markers of endothelial (CD31) and myeloid lineage (CD14, CD45), and strongly expressed the markers CD68, CD163, and CD206 suggesting an alternatively activated (M2) macrophage phenotype. IFNα2b significantly reduced the number of CACs at day 7 in a dose-dependent manner (r²= –0·769, p<0·0001). In co-culture with endothelial cells on Matrigel, CACs co-localised to the endothelial tubules but did not form tubule networks alone. CAC supernatant significantly increased tubule network density in terms of total pixel area (mean 27 781 [SE 1469] vs 36 283 [1804], p=0·0065), number of branches (340·2 [11·0] vs 510·6 [70·2], p=0·0434), junctions (162·4 [5·9 vs 241·1 [28·0], p=0·0104), and closed loops (21·8 [1·9 vs 38·3 [2·2], p=0·005). IFNα2b significantly reduced the number of closed loops (38·2 [2·2] vs 24·1 [3·5], p=0·0094). All other network parameters were reduced by IFNα2b but did not reach statistical significance.

Interpretation CACs are of myeloid lineage, and CAC supernatant contains potent angiogenic factors which augment endothelial tubule networks. IFNα2b dramatically reduces the survival of CACs in vitro, resulting in reduced tubule network formation and may be a mechanism by which IFNα promotes vascular damage in systemic lupus erythematosus.

Funding North West England Medical Research Council Fellowship Scheme in Clinical Pharmacology and Therapeutics.
Exploration of functional brain networks in neurodegenerative disease

Timothy Rittman, Boyd Ghosh, James Rowe

Abstract

Neurodegenerative diseases target specific anatomical and functional brain networks. A number of intrinsic functional brain networks can be identified in individuals at rest, that correspond to networks found in task-based functional MRI studies. However, the impact of pathological changes and relation to disease severity remains unclear.

We examined three networks of interest in patients with progressive supranuclear palsy (PSP) and the neurodegenerative corticobasal syndrome (CBS). These two diseases share features of cognitive decline and a movement disorder, although they have important phenotypic differences. They are both associated with accumulation of tau protein in neuronal and glial cells. We examined the default mode network, which is deactivated during tasks and has been consistently implicated in Alzheimer’s disease; the salience network, often activated during tasks and affected in frontotemporal dementia; and the basal ganglia network, since both PSP and CBS pathology affects the basal ganglia.

Using resting state functional MRI scanning, we applied independent component analysis and template matching to identify networks of interest. Spatiotemporal group differences in network architecture were identified with dual regression to extract spatial maps for each network in individuals and perform group-wise t tests. In addition, clinical test scores were added as covariates to group comparisons.

Increased functional connectivity was seen within all three networks in disease groups. Decreased connectivity was seen between the basal ganglia network and cortical regions in PSP. Network changes correlated with worse scores on clinical measures of disease.

Increased connectivity in relevant functional brain networks identify neurodegenerative diseases and mirror clinical disease features.

Funding UK Medical Research Council.
Comparative phylogenetics of ICEHin1056 family reveals deep evolutionary associations of mobile genetic elements responsible for transfer of antibiotic resistance genes

Esther Robinson, Xavier Didelot, Derek Hood, Derrick Crook

Abstract

Background Integrating and conjugating elements (ICEs) are self-transmissible mobile genetic elements. ICEs are composed of modules of conserved genes, with accessory genes at hotspots. Antibiotic resistance genes are often encoded on ICEs, leading to rapid intraspecific and interspecific spread of resistance. Our aim was to study ICEs with homology to ICEHin1056 in Haemophilus influenzae using the large number of whole genome sequences now available.

Methods Members of the ICEHin1056 family were identified with tBLASTx searches on the National Center for Biotechnology Information genome database. The query sequences were concatenated core genes from ICEHin1056. Alignments were performed with the Artemis Comparison Tool. Sequences were stored in a BIGS (Bacterial Isolate Genome Sequence) database and homologues of core genes identified. Alignments were performed in ClustalW and phylogenetic trees drawn with MEGA (Molecular Evolutionary Genetics Analysis). Ancestral sequences were predicted with GASP (Gapped Ancestral Sequence Prediction). Predicted ancestral sequences were used as BLAST inputs to find further possible members of the ICE family and more distant relatives.

Findings We identified over 100 whole or partial sequences in the ICEHin1056 family in a-proteobacteria, b-proteobacteria, and g-proteobacteria. This is the largest comparative phylogenetic study of ICEs performed to date and demonstrates extensive lateral gene transfer across the whole phylum. The three core ICE modules encode replication, type IV secretion, and excision/integration. The conservation of synteny implies a powerful selective advantage of the ICE. GC content of the core modules mirrors that of the host chromosome, suggesting coexistence deep in evolutionary history. Absence of core genes or modules represents lifestyle adaptations of the mobile genetic element. Absence of an integrase and presence of a replicative DNA helicase are markers of a plasmid lifestyle. A variety of accessory genes are found at hotspots; they confer a survival advantage in the ecological niche of the organism, which ranges from eukaryotic pathogens to extreme environments.

Interpretation This large comparative phylogenetic study of ICEs allows inference about evolutionary associations within the ICEHin1056 family. This evolutionary history is so ancient that it may link all mobile genetic elements transferred by conjugation in proteobacteria. This provides important insights into the mobile gene pool and may have implications for prediction of spread of antibiotic resistance and pathogenicity.

Funding UK National Health Service.
Personality, wellbeing, and quality of life in patients with tooth wear

Jose M Rodriguez, Harpoonam J Kalsi, Mohammad A Khan, Deborah I Bomfim, Georgios Tsakos, Ailbhe McDonald

Abstract

Background The aim of this study was to investigate, in a cohort of patients with pathological levels of tooth wear, the effect of personality and general psychological wellbeing on generic and condition specific (CS) quality of life.

Methods Patients with pathological levels of tooth wear aged 18–70 years referred to the Eastman Dental Hospital for advice on the management of their tooth wear were invited to participate. Participants completed a CS oral impact on daily performances (OIDP) quality of life questionnaire, a NEO five-factor inventory personality questionnaire, and the general health questionnaire-12 (GHQ). Tooth wear severity was measured using the basic erosive wear examination (BEWE).

Findings 102 individuals were recruited. Increased BEWE scores were positively correlated with increasing age (p=0.046) and decreased generic and CS quality of life (p=0.017 and 0.031, respectively). Increased neuroticism values were positively correlated with increased generic OIDP score (p=0.007), CS OIDP score (p=0.003), generic and CS eating scores (p=0.025 and p=0.035, respectively), CS smiling score (p=0.029), and CS carrying out major work score (p=0.039). Increased general psychological wellbeing scores were positively correlated with increased generic and CS OIDP scores (p=0.009), generic and CS eating scores (p=0.009 and p=0.003, respectively), CS speaking scores (p=0.018), generic and CS cleaning scores (p=0.002 and p=0.004, respectively), generic relaxing scores (p=0.003), generic and CS smiling scores (p<0.0001 and p<0.0001, respectively), and generic emotional state score (p=0.015). Multivariate linear regression analyses revealed that increased levels of neuroticism and decreased levels of general psychological wellbeing both had an independent and significant effect on generic and CS OIDP scores when adjusted for tooth wear severity (p<0.05).

Interpretation In this cohort of patients with pathological tooth wear, higher levels of neuroticism and decreased levels of general psychological wellbeing had a significant negative effect on quality of life. For patients with tooth wear, treating their condition solely may not help to improve quality of life because other factors may affect their perception.

Funding University College London and National Institute for Health Research.
Hepatitis C virus kinetics after liver transplantation to study the role of a small molecule inhibitor of viral entry

Ian A Rowe, Matthew Armstrong, Richard Parker, Kathy Guo, David Adams, Peter Balfe, David Mutimer, Jane A McKeating

Abstract

Background After liver transplantation in patients infected with hepatitis C virus (HCV), reinfection of the transplanted liver is universal. The targeting of viral entry at the time of transplantation is therefore an attractive strategy. An understanding of the mechanisms and kinetics of this process will allow rational studies of small molecule inhibitors or neutralising antibodies that target HCV entry.

Methods As part of a proof of concept study of ITX5061 (a small molecule antagonist of the HCV entry receptor, scavenger receptor B-I), we have studied detailed HCV kinetics. HCV RNA was quantified in plasma before and after liver transplantation. This study is registered with ClinicalTrials.gov, number NCT01292824.

Findings 13 patients were studied (median age 57 years, 11 male). Seven patients were infected with genotype 1, four with genotype 3, and one each with genotypes 2 and 4. During the period when no blood flowed through the liver there was a slow decline in plasma HCV RNA. After implantation of the donor liver there was a rapid decrease in HCV RNA in the plasma indicating entry of viral particles into the liver. Typically, there was a 90–99% decrease in HCV RNA at 4 h after implantation. This decrease continued until 12 h after implantation in all patients, and the initial rapidity of HCV RNA decline suggests the presence of specialised clearance systems in the liver. After this decrease, five patients showed a steady increase in plasma HCV RNA, which returned to baseline within 96 h indicating a rapid establishment of productive infection in the allograft. In the remaining eight patients there was a prolonged nadir for up to 21 days after transplant suggesting innate immune control in the transplanted liver. No differences in kinetics were observed between patients infected with different viral genotypes, or in the degree of liver injury at the time of transplantation.

Interpretation These marked differences in viral kinetics have major implications for trial design and probably have important biological significance in innate control of infection.

Funding National Institute for Health Research.
Label-free proteomics: a potential method for identifying protein biomarkers in pancreatic cancer

F Runau, L Norris, J Isherwood, M Metcalfe, K Brown, A R Dennison

Abstract

Background Plasma is ideal for early detection of cancer because samples are easily available by less invasive methods. The considerable complexities of clinical samples (high-abundant proteins, protein concentration, and dynamic range) make it extremely difficult to identify proteins of interest. Present plasma protein profiling strategies use immunodepletion to remove the top 14–20 abundant plasma proteins. However, even with up to 99% of high-abundant proteins removed, most disease biomarkers are in the low ng/mL to pg/mL range. Using label free proteomics, we developed a strategy for the identification and quantification of plasma proteins in control samples.

Methods Heparinised plasma from three healthy volunteers (two women, one man) was obtained. 40 µL plasma was immunodepleted with high performance liquid chromatography (HPLC) Multiple Affinity Removal System (Agilent Technologies, USA). Samples were filtered and buffered exchanged before measurement of protein concentration. Samples were reduced, alkylated, and digested with trypsin overnight. High pH reverse phase HPLC was used to fractionate samples at the peptide level into 85 individual fractions. Each fraction was analysed with ion mobility using Waters G2 high resolution TOF coupled to nanoLC system. Each fraction was analysed with Protein Lynx Global Server software for protein identification and quantification, and Non-Linear Progenesis software for relative protein expression.

Findings After fractionation and mass spectrometer analysis, we identified 318 individual proteins that might be potential biomarkers.

Interpretation This method enables more protein biomarkers to be measured compared with unfractionated samples. This method will be used to analyse plasma samples from patients with unresectable pancreatic cancers who receive intravenous omega-3 fatty acids (with a control arm without omega-3 fatty acids). Potential biomarkers identified will be verified with the aim of translating to clinical use for response to treatment.

Funding National Institute for Health Research.
Prediction model for rheumatoid arthritis: modelling 46 genetic risk variants with smoking

Ian C Scott, Sophia Steer, Rachael Tan, Paola Forabosco, Ann W Morgan, Anne Hinks, Wendy Thomson, Anne Barton, Jane Worthington, Andrew P Cope, Cathryn M Lewis

Abstract

Background Improved characterisation of the risk factors for rheumatoid arthritis raises the possibility that they could be combined to identify individuals at high risk of disease in whom preventive strategies can be evaluated. Our aim was to develop a prediction model capable of identifying such individuals with sufficient accuracy to enable the assessment of preventive treatments.

Methods Our prediction model combines odds ratios for 15 HLA-DRB1 alleles, 31 non-HLA single nucleotide polymorphisms (SNPs), and smoking status to classify an individual’s risk of seropositive rheumatoid arthritis. Our novel modelling technique employs confidence intervals to classify disease risks using a computer-simulated population. We developed several models (HLA, HLA-10/20/31 SNP, HLA-smoking models) to evaluate the impact of different factors on prediction. The ability of each model to discriminate between rheumatoid arthritis and controls was evaluated in two European cohorts: the Wellcome Trust Case Control Consortium (WTCCC: 1542 cases, 1226 controls) and the UK Rheumatoid Arthritis Genetics Consortium (UKRAG: 2623 cases, 1503 controls).

Findings HLA-DRB1 alleles conferred most prediction: the WTCCC HLA-only model classified 50% antibodies to citrullinated protein antigens (ACPA)-positive rheumatoid arthritis versus 17% controls as high risk and 60% controls versus 25% ACPA-positive rheumatoid arthritis as reduced risk. Adding smoking information improved prediction (p=0.00033); SNPs provided no significant benefits. The highest area under the curve was 0.81 (95% CI 0.78–0.85). Only a minority had substantially elevated risks of rheumatoid arthritis: 6.9% ACPA-positive cases and 0.31% controls in WTCCC had an odds ratio for rheumatoid arthritis of more than 20 when evaluated with the HLA-31 SNP model.

Interpretation Combined information on HLA-DRB1 alleles and smoking provides informative risk prediction for rheumatoid arthritis. Since only a minority of individuals are at substantially elevated risks, modelling may be best focused in a-priori high-risk groups such as those with family histories of rheumatoid arthritis. Further work is needed to define risk factors with large effect sizes for incorporation within our modelling framework.

Funding Arthritis Research UK.
Expression of toxic shock syndrome toxin-1 by epidemic meticillin-resistant Staphylococcus aureus 16

Hema Sharma, Debra Smith, Angela Kearns, Shiranee Sriskandan

Abstract

Background Staphylococcal toxic shock syndrome (TSS) is a fatal illness attributed to Staphylococcus aureus toxic shock syndrome toxin-1 (TSST-1), encoded by the tst gene. No epidemiological data on TSS exist from the UK. Strains of S aureus may carry the tst gene but not cause TSS, such as the UK strain epidemic meticillin-resistant SA-16 (EMRSA-16). The aim of this study was to determine the epidemiology of TSS in the UK and investigate the expression of TSST-1 by EMRSA-16 and tst+ meticillin-sensitive S aureus (MSSA).

Methods Cases of TSS were identified at the UK Health Protection Agency (HPA) and epidemiological features analysed. A selection of clonal complex (CC)-30 EMRSA-16 and TSS-associated MSSA strains was chosen for investigation. The tst gene and promoter region were sequenced in all strains. The staphylococcal pathogenicity island (SaPI) harbouring tst was determined by PCR. Growth of strains in brain heart infusion broth was monitored by optical density (OD600). TSST-1 in supernatants was quantified by western blot using densitometry according to a TSST-1 standard curve. RNA was isolated for tst transcript analysis by quantitative reverse-transcription-PCR (qRT-PCR). Whole genome sequencing was performed on all strains to identify potential mutations in known regulators of toxin synthesis.

Findings 150 cases of TSS were reported to the HPA over a period of 4 years. Six cases (4%) were attributable to MRSA and the remainder to MSSA. There was no difference in the tst gene or promoter sequence among the strains and tst was carried by SaPI2. TSST-1 production by EMRSA-16 strains commenced earlier in growth than did MSSA strains and was greater in TSS-associated MSSA than in EMRSA-16 strains throughout growth. These results were compared with tst transcription by MSSA and EMRSA-16 using qRT-PCR.

Interpretation EMRSA-16 produces less TSST-1 than does MSSA of the same lineage; this may be due to differences in global gene regulators, perhaps as a result of changes in PBP2a expression, or the carriage of a large SCCmec element by EMRSA-16. This difference in production may explain the paucity of TSS cases caused by MRSA.

Funding UK National Centre for Infection Prevention and Management.
Common lymphatic endothelial and vascular endothelial receptor-1 mediates the transmigration of regulatory T cells and B cells across hepatic sinusoidal endothelium

Shishir Shetty, Christopher Weston, Tony Bruns, Ye Htun Oo, Nina Westerlund, Zania Stamataki, Janine Youster, Stefan Hubscher, Marko Salmi, Sirpa Jalkanen, Patricia F Lalor, David H Adams

Abstract

Background Adult inflammatory liver diseases are driven by lymphocyte infiltration of liver tissue. Lymphocyte recruitment occurs within the unique low shear environment of the hepatic sinusoids. These channels are lined by hepatic sinusoidal endothelial cells (HSEC) which lack conventional adhesion receptors such as selectins, leading us to investigate the role of unconventional adhesion molecules. The common lymphatic endothelial and vascular endothelial receptor-1 (CLEVER-1) is a C-type lectin that has been shown to mediate lymphocyte recruitment to lymphatic endothelium.

Methods We studied the expression of CLEVER-1 in human liver tissue by immunohistochemistry and immunofluorescence. We proceeded to confirm the expression of CLEVER-1 in isolated HSEC in vitro. Flow based adhesion assays were performed with adhesion blocking antibodies to elucidate the functional role of CLEVER-1. These studies were extended to visualise lymphocyte recruitment and transendothelial migration with immunofluorescence staining and confocal microscopy. Migration of lymphocyte subsets was also studied with tracking software.

Findings CLEVER-1 was expressed at sites of lymphocyte trafficking within the inflamed human liver, particularly the hepatic sinusoids, neovessels of the fibrous septum, and tertiary lymphoid follicles. Furthermore, CLEVER-1 was also detected in the sinusoids and supplying vessels of hepatocellular carcinomas. Functional assays with HSEC showed that CLEVER-1 mediated the transendothelial migration of peripheral blood lymphocytes with preferential activity for regulatory T cells and B cell subsets. Detailed confocal analysis visualised a novel transcellular route of migration by regulatory T cells. B cells also demonstrated altered migratory capacity compared with T cells.

Interpretation These results suggest that CLEVER-1 has a role in modulating the recruitment of regulatory T cells and B cells to the human liver. This C-type lectin could be a potential therapeutic target for chronic inflammatory liver disease and hepatocellular cancer.

Funding Wellcome Trust.
Improving team training in acute health care: critical synthesis of seven mixed-methods studies

Dimitrios Siassakos, Robert Fox, Katherine Bristowe, Jo Angouri, Helen Hambly, Timothy Draycott

Abstract

Background Research had previously shown that practical team rehearsals in acute health care are beneficial, but subsequent work suggested that further improvement is possible. We critically synthesised seven studies aiming to identify the characteristics of effective teams and inform and guide better team training.

Methods Two studies aimed to identify successes and challenges in a unit with improvements in outcome after the introduction of team training. The studies were a staff safety attitudes survey and an interrupted time-series of the effect of training on the management and outcome of an emergency. Mixed-methods research was used in five further studies to identify the characteristics of effective maternity teams in simulation and experiences of real life.

Findings The introduction of team training improved the management and outcome in the index emergency, but there remained persistent variation in team performance managing an emergency. The staff survey demonstrated a positive safety culture yet identified a perceived need for improved senior presence. Analysis of simulation established that some teams were significantly better than others in managing the emergency, and this variation was correlated with their teamwork, not their individual knowledge, skills, or attitudes. Declaring the emergency early, structured handover, and closed-loop communication were associated with significantly better team performance. Better teamwork, including the clear verbalisation of crucial information, was also associated with better patient perception of care. The focus groups corroborated these findings and agreed that the behaviours of a leader are more important than seniority rank. Simple practical methods for teaching those behaviours were explored and agreed. Triangulation of focus group and simulation data using an established framework revealed similarities (convergence and complementarity), differences (dissonance), and issues requiring further research to corroborate or refute findings.

Interpretation The effectiveness of acute health-care teams is related to simple behaviours that can be taught, with methods appropriate for different learners.

Funding National Institute for Health Research.
Sociodemographic determinants of place of death in dementia: whole population cross-sectional analysis in England, 2001–10

Katherine Sleeman, Yuen King Ho, Julia Verne, Myer Glickman, Wei Gao, Irene Higginson

Abstract

Background The in-hospital death rate from dementia in England is among the highest in Europe. The personal, regional, and temporal factors influencing place of death in dementia are unknown.

Methods Office for National Statistics mortality data were used for whole population cross-sectional analysis of deaths from dementia in England, 2001–10. Place of death (home, hospice, care home, hospital) was the outcome variable. Explanatory variables included demographic, social, and regional factors. Multivariable Poisson regression analysis was used to provide an independent relative risk of place of death for each of the variables studied. The change in place of death over time was studied using linear regression.

Findings 387 004 deaths were included. Most patients died in care homes (55·3%) or hospital (39·6%). Home deaths (4·8%) and hospice deaths (0·3%) were rare. Hospital deaths were less likely with increasing affluence (relative risk [RR] 0·83, 95% CI 0·81–0·85), and increasing provision of a care-home bed (0·61, 95% CI 0·58–0·63). Lack of social support (marital status) reduced home deaths (RRs 0·45–0·60). Hospice deaths were increased in areas with greater hospice bed provision (RR 4·23, 95% CI 2·14–8·35) and in patients whose underlying cause of death was cancer (19·0, 95% CI 16·2–22·3). Deaths in care homes were more common with increasing affluence (RR 1·28, 95% CI 1·24–1·32) and increased provision of a care-home bed (2·02, 95% CI 1·97–2·08). Time-trend analysis showed a recent decrease in hospital deaths and reciprocal increase in care-home deaths.

Interpretation Governments and policy makers recognise the urgent need for public health planning in dementia. Reducing the number of deaths in hospital, and increasing those at home, in dementia has both societal and economic benefits. Social support, deprivation, and service provision are key targets for policy.

Funding National Institute for Health Research.
Relaxin is a renal vasodilator in experimental models of cirrhosis and a potential novel therapy for hepatorenal syndrome in man

Victoria K Snowdon, Antonella Pellicoro, Prakash Ramachandran, William Mungall, Maurits Jansen, Ross Lennen, Rebecca Aucott, Timothy Kendall, Jeremy Hughes, John Iredale, Jonathan Fallowfield

Abstract

Background Hepatorenal syndrome is a feared complication of cirrhosis, with a high mortality rate and limited treatment options. The hallmarks of the disorder are functional renal failure, normal kidney histological findings, and profound renal vasoconstriction. The hormone relaxin mediates haemodynamic adaptations to pregnancy including increased renal blood flow and glomerular filtration rate (GFR). We hypothesised that relaxin could be used to modulate renal blood flow in cirrhosis.

Methods Cirrhosis was induced in male rats by 16 weeks of carbon tetrachloride (intraperitoneally) and decompensated biliary cirrhosis by bile duct ligation. The effect of acute intravenous or extended (72 h) subcutaneous relaxin versus placebo was determined by analysis of systemic haemodynamics, renal blood flow, GFR, and organ histology. The effect of co-treatment with L-NG-nitroarginine methyl ester (L-NAME) was measured in subgroups of relaxin or placebo treated rats. Blood oxygen dependent-MRI (BOLD-MRI) was used to non-invasively detect changes to renal oxygenation. Hepatic and renal expression of RXFP1 (relaxin receptor) was determined by IHC and quantitative PCR (qPCR). A qPCR array was used to quantify changes in renal vasodilator/vasoconstrictor gene expression in response to relaxin.

Findings RXFP1 was detected in glomerular podocytes, renal pericytes, and renal, segmental, and interlobar arteries of cirrhotic rats. In carbon tetrachloride (CCL) induced cirrhosis, acute intravenous relaxin (4 μg) induced a 50% increase in renal blood flow after 60 min (p<0.01 vs placebo, n=6). BOLD-MRI showed statistically significant (p<0.05) increased tissue oxygenation at the same timepoint in renal cortex and medulla. Extended subcutaneous relaxin increased renal blood flow by 54% in CCL (p<0.01 vs placebo, n=8) and 87% in bile duct ligated animals (p<0.05 vs placebo, n=3) and increased GFR by 138% in CCL (p<0.01 vs placebo, n=8) and 70% in bile duct ligated animals (p<0.05 vs placebo, n=3). Mean arterial pressure was unaffected by relaxin. L-NAME (250 mg/L) orally abrogated the effect of relaxin on renal blood flow and GFR. Relative expression of vasoconstrictor genes in kidney was markedly reduced by relaxin treatment.

Interpretation Relaxin increases renal blood flow in experimental models of cirrhosis. Crucially, relaxin also improves renal function and oxygenation and does not induce systemic hypotension even in advanced disease. These effects are modulated via augmentation of nitric oxide and downregulation of vasoconstrictors, pivotal in the pathogenesis of hepatorenal syndrome. Relaxin has potential as a novel therapy for hepatorenal syndrome, and further translational studies are warranted.

Funding Wellcome Trust through the Scottish Translational Medicine and Therapeutics Initiative.
The Rheumatoid Arthritis and Falls (RAF) study: a prospective study of fall risk factors in adults with rheumatoid arthritis

Emma K Stanmore, Jackie Oldham, Dawn A Skelton, Terence O’Neill, Mark Pilling, A John Campbell, Chris Todd

Abstract

Background Rheumatoid arthritis is linked to an increased risk of falls resulting in osteoporotic fractures, which may involve lower limb joints, leading to impaired mobility, impaired balance, and postural instability. This study aimed to investigate the association between potential risk factors and falls in community dwelling adults with rheumatoid arthritis.

Methods Adults with rheumatoid arthritis were recruited from four outpatient clinics in the northwest of England and followed for 1 year after clinical assessment, using monthly falls calendars and telephone calls. Outcome measures included fall occurrence, reason for fall, type and severity of injuries, fractures, fall location, lie-times, use of health services, and functional ability. Risk factors for falls included lower limb muscle strength, postural stability, number of swollen and tender joints, functional status, history of falling, fear of falling, pain, fatigue, and medication. Data on demographics, vision, co-morbidities, history of surgery, fractures, and joint replacements were also recorded.

Findings 559 adults with rheumatoid arthritis (386 women, 173 men, aged 18–88 years) had baseline measurements taken. 535 (96%) participants completed 1-year follow-up. Univariate logistic regression showed that falls risk was independent of age and gender. Multivariate logistic regression revealed that a history of multiple falls in the previous 12 months was the most significant predictive risk factor (odds ratio 5.3 [95% CI 2.3–12.3], p<0.001). The most significant modifiable risk factors were swollen and tender lower limb joints (odds ratio 1.7 [95% CI 1.1–2.7], p=0.03), psychotropic medication (1.8 [1.1–3.1], p=0.03), and fatigue (1.13 [1.02–1.2], p=0.01).

Interpretation Adults of all ages with rheumatoid arthritis are at high risk of falls. In clinical practice, patients with rheumatoid arthritis at high risk of falls can be identified by asking whether they have fallen in the past year. The management of swollen and tender lower limb joints, fatigue, and consideration of psychotropic medicines may be the most effective strategy to reduce falls in this group of patients.

Funding Arthritis Research UK.
Fn14 is expressed on neoplastic cholangiocytes in intrahepatic cholangiocarcinoma and promotes necrosis after interaction with TWEAK

Barnaby Stephenson, Elizabeth Humphreys, Linda Burkly, David Adams, Simon Afford

Abstract

Background Cholangiocarcinoma is the second most common primary hepatic malignancy worldwide. The incidence of intrahepatic cholangiocarcinoma in the UK has steadily increased over the past 40 years. The main treatment is chemotherapy, and 5-year survival after radical surgery is only 25%. The carcinogenic mechanisms involved in cholangiocarcinoma remain elusive. The fibroblast growth factor-inducible 14 (Fn14)/tumour necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) receptor-ligand system has been shown to be of importance in cellular proliferation and tumour angiogenesis in hepatocellular carcinoma. The aim of this study was to demonstrate the expression of Fn14 and TWEAK in cholangiocarcinoma and determine the functional significance of Fn14/TWEAK interaction on neoplastic cholangiocytes.

Methods Human liver samples were obtained with consent from the Queen Elizabeth Hospital liver transplant programme. Sections were stained for Fn14 with immunohistochemical techniques. Expression of Fn14 on a cholangiocarcinoma cell line (CC-LP-1) stimulated with TNFα, interferon γ and fibroblast growth factor (FGF) basic was established quantitatively with flow cytometry. Stimulated CC-LP-1 were exposed to different concentrations of TWEAK for 24h. Apoptosis, necrosis, autophagy, and reactive oxygen species production at 24 h were determined by flow cytometry using annexin, 7AAD, dansylcadaverine, and dichlorofluorescein assays, respectively. Proliferation was determined with Ki69 nuclear staining.

Findings Immunohistochemistry revealed Fn14 on the intrahepatic malignant small bile ducts in cholangiocarcinoma. Exposure of neoplastic cholangiocytes to TWEAK for 24h induced necrosis and reduced apoptosis in FGF-activated neoplastic cholangiocytes.

Interpretation Fn14 is expressed on neoplastic cholangiocytes in intrahepatic cholangiocarcinoma. Activation of the Fn14/TWEAK receptor-ligand system induces necrosis. The role of Fn14/TWEAK in cholangiocarcinoma needs further investigation to ascertain the mechanisms involved and outcome on overall tumour viability.

Funding UK Medical Research Council.
Investigation of the pathogenesis of uromodulin-related genetic disease

Andrew P Stewart, Fiona E Karet, Richard N Sandford, J Michael Edwardson

Abstract
Uromodulin (Tamm-Horsfall protein) is the predominant protein in human urine. Although its function is currently unclear, genetic mutations in UMOD result in a group of allelic chronic renal diseases, such as familial juvenile hyperuricaemic nephropathy (FJHN). Furthermore, a genome-wide association study has shown that UMOD variants are crucial in determining the progression overall of chronic kidney disease.

We generated constructs expressing wild-type protein and two pathogenic mutations causing FJHN, indel and C150S, and expressed them in cell lines. Immunofluorescence imaging revealed an accumulation of intracellular aggregates within the endoplasmic reticulum (ER) of mutant-expressing cells. Immunoblots showed a decrease in secretion of the mutants relative to wild-type. This effect, which is consistent with the imaging, can be accounted for by the retention of unprocessed protein within the cell, seen when intracellular uromodulin is purified and immunoblotted.

Atomic force microscopy (AFM) imaging of native uromodulin purified from human urine revealed a large fibrous protein. Imaging of protein purified from the media of uromodulin-expressing cells showed that all three forms of the protein generated similar structures. AFM imaging of uromodulin purified from within the cell demonstrated that, whereas the wild-type protein has a globular structure, the pathogenic proteins form intracellular fibres, similar to those normally formed only after secretion.

Retention of protein within the ER probably results in toxicity via an ER stress mechanism, evidenced by the finding that BiP expression is upregulated by expression of the pathogenic mutants.

Using a variety of independent techniques we have demonstrated the unexpected and novel finding that premature intracellular polymerisation is probably the cause of uromodulin-related genetic disease. These results allow the grouping of this disease alongside other protein aggregation disorders, and identify uromodulin-related genetic disease as the first such disease specific to the kidney.

Funding Jean Shanks Foundation.
A strong correlation between expression of Wntless and of human epidermal growth factor receptor 2 in gastric, ovarian, and breast cancers suggests a novel-signalling pathway involving NFkB and STAT3

Jonathan Stewart, Jacqueline James, Glenn McCluggage, Stephen McQuaid, David Boyle, Kenneth Arthur, Paul Mullan, Claire Kidson, Nuala McCabe, Richard Kennedy, Darragh McArt, Anne Carson, Benedict Yan, Lei Zhengdeng, Patrick Tan, David Virshup, Manuel Salto-Tellez

Abstract

The involvement of Wnt signalling in cancers including ovarian, gastric, and breast cancer is well characterised. Wntless (Wls) (also known as GPR177, Evi, and Srt) is a key modulator of Wnt protein secretion, and was recently found to be highly overexpressed in malignant astrocytomas, promoting proliferation, survival, and migration of glioma cells. It was hypothesised that this molecule may be aberrantly expressed in other cancers known to possess aberrant WNT signalling.

Using immunohistochemical analysis of ovarian, gastric, and breast cancer tissue microarrays, we found that Wls was overexpressed in a subset of tumours from each cancer subtype. Wls overexpression was associated with poorer clinical outcomes in gastric cancer.

Interestingly, a strong correlation was observed between Wls expression and human epidermal growth factor receptor 2 (HER2) expression. Eight of eight (100%) HER2-positive intestinal gastric carcinomas, five of five (100%) HER2-positive serous ovarian carcinomas, and 41 of 64 (64%) HER2-positive breast carcinomas expressed Wls. This finding has clear biological and clinical implications. HER2 is an important predictive biomarker in breast and gastric cancer for selection of patients likely to respond to trastuzimab. However, a substantial proportion of HER2-positive patients do not respond to this therapy.

Recent literature suggested a possible pathway in which Wls and HER2 may be involved via the known mediator of trastuzimab and chemotherapy resistance NFkB, which may explain the low response rate of patients with HER2-positive breast, ovarian, and gastric cancers to agents that target HER2. An in-vitro study has been commenced to try to confirm the existence of this pathway. This study should provide significant insights into the mechanistic association between these important signalling molecules.

Funding Queens University Belfast.
Molecular effects of UVA1 in human skin in vivo

A Tewari, K Grys, D Dafou, R Sarkany, A R Young

Abstract

The carcinogenic potential of UVA1 (340–400nm) is increasingly being recognised as evidence accumulates of its ability to induce cyclobutane pyrimidine dimers (CPD) ex vivo and in vivo in human beings which if unrepaired may lead to skin cancer. Despite widespread use in phototherapy, tanning lamps and its abundance in terrestrial UVR (ultraviolet radiation), we lack data on its effects on gene expression in human skin. 12 volunteers with skin type I/II were tested for UVR sensitivity: five were given 1 minimal erythema dose (MED) of UVA1 and biopsy samples taken at 6 h and 24 h; an unirradiated control sample was also taken. A further four participants were given 1 MED of UVB and UVA1 and biopsy samples taken at 6 h and 24 h, RNA extracted, converted to cRNA, and hybridised to agilent 44K oligomicroarray plates. After rosetta resolver software analysis, the data were analysed using GeneGo metacore v7 and DAVID.

UVA1 upregulated 301 genes at 6 h and 264 genes at 24 h (p<0.05, ≥2 fold change). At 6 h, key gene expression pathways upregulated were inflammation (p=3.46×10^{-17}), apoptosis (p=2.148×10^{-8}), and response to oxidative stress (p=6.457×10^{-7}). CD83 was also dramatically upregulated (positively regulates interleukin 10). At 24 h the top pathway enriched was extracellular matrix remodelling (p=5.549×10^{-7}). A pathway analysis demonstrated that UVB induced the same pathways at 6 h and 24 h as did UVA1; however, functional studies shows that UVB induces a greater upregulation of apoptotic proteins and in terms of genes, similar levels of immunomodulatory genes (CD83, IL10) upregulated.

Erythemally equivalent doses of UVA1 induce less apoptosis than UVB but there are similar levels of immunosuppression. Our data suggest that UVA1 might be inducing less of a photoprotective response in vivo in human beings than UVB, which might explain the mechanism of some of our other new findings—that UVA1 CPDs are slower to be repaired at the basal epidermis than UVB CPDs. These findings are important for our understanding of skin cancer.

Funding UK Medical Research Council, La Roche-Posay, and Biomedical Research Council.
Analyses of blood outgrowth endothelial cells reveal an endothelial HOX gene signature in human beings

Mark Toshner, Ben Dunmore, Eoin McKinney, Mark Southwood, Mark Ormiston, Gerard Nash, Amer Rana, Paul Upton, Nicholas Morrell

Abstract

Endothelial cells have a remarkable ability for subspecialisation, adapting to the needs of a variety of vascular beds. It is not known how much of this subspecialisation is related to developmental programming versus the environment in which the endothelial cell finds itself. In transcriptomic studies of endothelial cells we have noted a hierarchy of HOX gene expression which predicts endothelial cell specification and fate. HOX genes are well described as master-regulators of positional identity, predominately in the developing embryo.

Initial studies in human cells compared blood outgrowth endothelial cells (BOECs), a circulation-derived endothelial progenitor cell, with mature adult pulmonary artery endothelial cells (PAECs). We confirmed the endothelial phenotype of BOECs with a combination of traditional cell surface markers, electron microscopy, vacuolisation and network formation, ligand stimulation studies, leucocyte transmigration assays, and transcript expression profiling.

In microarray analysis of mRNA transcripts from BOECs and PAECs only 0.005% of genes were differentially expressed. Developmental processes dominated these differentially regulated genes when analysed by gene ontology. In particular we identify a BOEC HOX gene signature, particularly in the B and D HOX gene clusters. These differences in HOX gene expression were confirmed by quantitative PCR. Furthermore, in analyses of three independent datasets of microarrays from 56 adult cell and tissue arrays (both human and mouse), HOX genes discriminated endothelial cells from other cell types. According to these analyses, HOX gene expression clustered endothelial cells into hierarchies based on their anatomical location. In particular the HOXD cluster, identified as highly expressed in BOECs, was observed in microvascular endothelial cells and in angiogenesis. Adult and embryonic tissue staining of HOXD proteins confirmed this pattern of expression.

Since microvascular cells have been shown to be capable of repopulating the entire endothelial hierarchy, they have been posited as an angiogenic progenitor niche. Together these observations suggest a specific HOX signature involved in angiogenesis, endothelial cell differentiation and fate.

Funding Wellcome Trust.
Investigation of the role of B lymphocytes and tertiary lymphoid tissue in a murine model of renal chronic allograft damage

George Tse, David Gray, Lorna Marson

Abstract

Background Nodular B-lymphocyte rich infiltrates have been identified in chronically rejected renal allografts and biopsies of acute transplant rejection, and this has been associated with the development of tertiary lymphoid tissue (TLT). However their significance is unclear, with conflicting published data. We aim to investigate the role and significance of B-lymphocyte infiltrates and the development of TLT in a murine model of renal chronic allograft damage.

Methods We used congenic strains with donor C57bl/6-BM12 mice kidneys transplanted into C57bl/6 recipients, this being a single MHC-II mismatch. Using immunohistochemistry we investigated the presence of B lymphocytes within the allograft. In addition we investigated other markers of chronic allograft damage in this model including lymphatic expansion, microvessel rarefication, and fibrosis.

Findings Nodular aggregates of B cells appearing to be TLT developed over 12 weeks; however, in some allografts we observed a scattered B-cell pattern. The B-cell infiltrate of the allograft cortex increased progressively with a significant increased density by 12 weeks compared with 5 days after transplantation. Microvessels were counted with a 25-point graticule, and there was a significant difference between allograft and native kidney cortex (p<0.01) with both time and transplant kidney being responsible for the effect. There was a significant difference in the number of lymphatic vessels at 12 and 8 weeks compared with 5 days (p<0.05). Similar to findings in chronic allograft nephropathy, the expanded lymphatics were seen both in the tubulointerstitium and in the perivascular regions. The B-lymphocyte phenotype was explored and shown by immunofluorescence to form germinal centres and IgG-positive plasma cell (CD138+) differentiation.

Interpretation We have shown that this strain combination closely models that of chronic rejection of the renal allograft. Furthermore, we have identified the progressive infiltration and expansion of the B-lymphocytes compartment within the allograft cortex. This work has provided the basis for further investigation of B-lymphocyte depletion and the prospects of identifying a regulatory B lymphocyte.

Funding Kidney Research UK.
The first inborn error of manganese metabolism caused by mutations in SLC30A10, a newly identified manganese transporter


Abstract

Background We have identified an autosomal recessively inherited disorder of manganese metabolism that causes manganese accumulation in liver and brain with characteristic MRI brain appearances of hyperintense basal ganglia on T1-weighted sequences. Most affected individuals present in childhood with difficulties walking and fine motor impairment due to dystonia. Movement disorder is accompanied by liver cirrhosis, and some patients have died at a young age following complications of cirrhosis. An adult-onset form of parkinsonism associated with hepatomegaly and hypermanganesaemia has also been described. Further characteristics of both phenotypes include polycythaemia and features of iron depletion.

Methods Homozygosity mapping was performed using an Illumina CytoSNP-12. The candidate gene was sequenced on an ABI sequencer. Functional studies in the manganese-sensitive yeast strain Δpmr1 were performed using Gateway technology (Invitrogen).

Findings Homozygosity mapping identified SLC30A10 as the affected gene, and homozygous sequence changes were found in all affected individuals. SLC30A10 had previously been presumed to belong to a class of zinc transporters. However, expression of human wildtype SLC30A10 in Δpmr1 rescued growth in high manganese conditions confirming its role in manganese transport. The presence of missense and nonsense mutations in SLC30A10 failed to restore manganese resistance.

Interpretation Evidently, evolutionary changes in the aminoacid sequence have altered the substrate specificity of this transporter from zinc in yeast to manganese in mammalian cells. SLC30A10 is the first recognised human manganese transporter that, when defective, causes two distinct phenotypes—childhood onset dystonia and adult onset parkinsonism—that are associated with hepatic cirrhosis and polycythaemia. Present treatment strategies, including chelation therapy with disodium calcium edetate and iron supplementation, lead to significant improvement of clinical symptoms and blood manganese levels.

Funding Action Medical Research.
Parallel pathways of glutamate and ATP-mediated excitotoxicity cause significant neural cell death during ischaemia: potential for novel neuroprotective strategies

Philipp Vermehren, Robert Fern

Abstract
Neural cell death during cerebral ischaemia is correlated with various human pathological conditions leading to significant levels of mortality and morbidity. Despite decades of intense research there remains a paucity of clinically effective non-vascular neuroprotective measures. We present data supporting the theory that parallel pathways of ATP and glutamate excitotoxicity operate during ischaemia, which require the cooperation of both astrocytes and neurons, and that concomitant blockade of both pathways at the receptor level provides synergistic neuroprotection by preventing both astrocyte and neuronal death during the initial phase of a severe ischaemic insult.

Investigations were carried out with primary neuron and astrocyte cell cultures obtained from embryonic mice. Astrocytes and neurons expressed a range of functional P2 and glutamate receptors. 90 min of oxygen-glucose deprivation (OGD), used to simulate ischaemia, induced significant release of glutamate from both monocultures of neurons or astrocytes while astrocytes alone were responsible for ATP release. OGD induced significant amounts of both astrocyte and neuronal death, with neuronal death being enhanced in the presence of astrocytes. In co-cultures of astrocytes and neurons AMPA/kainate and NMDA receptor antagonist prevented only neuronal death, whereas broad-spectrum P2 receptor antagonists were significantly protective of both neurons and astrocytes. Astrocytes and neurons were also protected by a selective P2Y1 receptor antagonist. Combining AMPA/kainate, NMDA, and P2 receptor antagonists, even at greatly reduced concentrations, was significantly protective of all cell types during OGD.

These results provide further evidence for the existence of both ATP and glutamate mediated excitotoxicity during ischaemia in the brain and highlight the important role of astrocytes in this process. Furthermore, the synergistic protective effect of inhibiting both pathways suggests that future effective neuroprotective strategies may benefit from a multipronged approach aimed at the large number of cell death pathways known to lead to eventual cell death and dysfunction.

Funding None.
Hypoxia modulates the expression and secretion of inflammation-related adipokines in differentiated human adipocytes

Bohan Wang, Paul Trayhurn

Abstract

Background Obesity is characterised by a state of chronic low-grade inflammation. Recently, we proposed that hypoxia may occur in enlarged adipocytes distant from the vasculature as adipose tissue mass expands, and that this drives the inflammatory response through dysregulation of inflammation-related adipokines. We have now examined the effects of low oxygen tension and chemically induced hypoxia on the production of key adipokines in differentiated human adipocytes.

Methods Cultured human adipocytes (15 days post differentiation) were exposed to 1% oxygen or 100 μM cobalt(II) chloride for up to 24 h; control cells were maintained in normal levels of oxygen only. mRNA levels of key adipokines were quantified by real-time PCR. Cellular levels of the hypoxia-sensitive transcription factor HIF-1α and the secretion of adipokines into the medium were measured with ELISAs.

Findings A large (7·8 fold) increase in HIF-1α protein was induced in human adipocytes after 4 h of hypoxia. The mRNA level of the facilitative glucose transporter, GLUT1, increased 14 fold by 24 h and there were increases (by 24 h) in the level of the mRNAs encoding major adipokines, including leptin (28 fold), fasting-induced adipose factor (11 fold), vascular endothelial growth factor (23 fold), interleukin 6 (4·5 fold), and migration inhibitory factor (2·5 fold). By contrast, adiponectin mRNA level fell (3 fold). Changes in mRNA level were accompanied by parallel alterations in adipokine secretion into the medium. Similar results were obtained when hypoxia was induced chemically with cobalt(II) chloride.

Interpretation Hypoxia dysregulates the production of key adipokines in human adipocytes, leading to an inflammatory state. Hypoxia may underlie the development of inflammation in adipose tissue in obesity.

Funding None.
Selective recruitment and retention of regulatory T cells in human colorectal cancer

S T Ward, E Hepburn, K Li, S M Curbishley, R Hejmadi, T Ismail, R Bicknell, A Rot, D H Adams

Abstract

Background Colorectal cancer (CRC) is the third most common malignancy in the UK, and lymphocytic infiltration is associated with improved survival. Analysis of lymphocytic infiltration yields a prognostic ability rivaling the TNM tumour staging system. Regulatory T cells (Treg) are known to be enriched in CRC, and it is postulated that they promote immunological tumour escape by suppression of effector T-cell responses. Little is known about the signals controlling entry of Treg into CRC.

Methods Matched CRC, distal colonic tissue, draining lymph node, and blood were obtained from patients undergoing resection of CRC. Tumour-infiltrating lymphocytes were isolated and phenotyped for chemokine receptors with flow cytometry. The presence of tissue chemokines was analysed with real-time PCR and western blotting. Standard chemotaxis and suppression assays were performed. Peripheral blood lymphocytes were co-cultured with tumour supernatant, and effects on lymphocyte phenotype were assessed.

Findings The proportion of T cells with a Treg phenotype was significantly increased in CRC compared with that in colonic tissue (14.8% vs 5.1%, p<0.01). More than 95% of this cell population expressed the transcription factor FOXP3. CCR5 was found to be markedly upregulated on Treg compared with other T cells. The ligands for CCR5 were overexpressed in CRC compared with those in colonic tissue. CCL4 was found to localise to the tumour endothelium. Tumour Treg migrated in response to CCL4, and blockade of CCR5 inhibited migration. Co-culture experiments demonstrated that CCR5 was upregulated on peripheral blood lymphocytes, especially Treg, in mixed lymphocyte reactions. This effect could be augmented by the addition of tumour supernatant.

Interpretation Conditions exist to recruit CCR5+ Treg into human CRC. CCR5 upregulation is promoted by the tumour microenvironment, providing a retention signal. CCR5 inhibition may prove to be a novel immunotherapy for CRC by blocking the recruitment and egress of suppressive Treg, promoting an anti-tumour immune response.

Funding UK Medical Research Council.
Mutations of TCF12, encoding a basic-helix-loop-helix partner of TWIST1, are a frequent cause of coronal craniosynostosis


Abstract

Background Craniosynostosis, the premature fusion of the cranial sutures, is the second most common craniofacial malformation. A genetic aetiology can be identified in about 21% of cases, including mutations of TWIST1 that cause Saethre-Chotzen syndrome and are associated with coronal synostosis. By contrast, the cause of non-syndromic craniosynostosis is largely unknown.

Methods We undertook exome sequencing of seven individuals with bilateral coronal synostosis, identifying mutations of TCF12 in three samples. We sequenced TCF12 in a further 347 patients with craniosynostosis. We performed mutation testing in extended families and determined the effects of mutations on mRNA expression. We examined the genetic interaction between loss-of-function mutations of Tcf12 and Twist1 in mice.

Findings Heterozygous TCF12 mutations were present in 38 of the 347 unrelated patients with craniosynostosis. These included 22 (32%) of 69 with isolated bilateral coronal synostosis, and 14 (10%) of 141 with unilateral coronal synostosis, but none with premature fusion of only the metopic, sagittal, or lambdoid sutures (p<10$^{-4}$). An additional two patients had either bilateral or right coronal synostosis in addition to sagittal synostosis. 14 cases arose de novo, but vertical transmission was demonstrated in 23 families. 16 mutation-positive individuals (47%) had craniosynostosis or suspicious clinical features, but 19 (53%) of 35 mutation-positive relatives were non-penetrant for craniosynostosis. TCF12 mutations were associated with diminished mRNA expression, indicating haploinsufficiency. With appropriate surgical correction the clinical outcome was usually good; six of 66 individuals had learning disability. Mice doubly heterozygous for Twist1 and Tcf12 mutations had severe bilateral coronal synostosis.

Interpretation Mutations of TCF12 are a frequent and specific cause of coronal craniosynostosis; mutation testing is indicated in the assessment of these patients. Genetic interaction of Tcf12 and Twist1 is crucial for coronal suture development.

Funding National Institute for Health Research Biomedical Research Centre, Oxford (BRG)/Oxford University Clinical Academic Graduate School (OUCAGS), and Oxfordshire Health Services Research Committee (OHSRC), Oxford Craniofacial Unit Charitable Fund; Thames Valley Comprehensive Local Research Network; The Dutch Center for Translational Molecular Medicine; Carolien Bijl Foundation; US National Institutes for Health; Wellcome Trust.
Defining the functional role of laminin isoforms in the adult hepatic progenitor cell response


Abstract
During chronic liver injury, regeneration occurs through hepatic progenitor cells (HPCs), which can generate both hepatocytes and biliary cells. Understanding the regulation of HPCs may offer therapeutic opportunities to augment liver regeneration. HPCs are associated with an increase in laminins in the extracellular matrix, leading to speculation that cell-matrix interactions may regulate HPC behaviour. Laminins are heterotrimeric proteins composed of an α, β, and γ chain. There are five α chains, with different cell surface receptor binding properties. We aimed to describe the laminin α chains associated with the HPC response, and to define the effect of different laminin chains on HPCs in vitro.

We found that the laminin α chains are differentially regulated during HPC activation. There were significant increases in α2 and α5 chains in two independent murine models of regeneration, with downregulation of the α3 chain. Using dual immunofluorescence, we showed that HPCs are most closely associated with the laminin α5 chain.

To look at the functional effects of matrix components on cell behaviour, we have used a line of spontaneously immortalised HPCs (bmols). Laminin α5 promotes HPC adhesion, spreading, and migration, compared with other α chains. These effects are partly blocked by antibodies against β1 integrin. Small-interfering RNA knockdown of laminin α5 results in hepatocytic differentiation, as demonstrated by increased albumin synthesis.

Laminin α5-containing matrix is deposited around HPCs during regeneration and this supports cell attachment, migration, and maintenance of an undifferentiated phenotype in vitro.

Funding UK Medical Research Council.
Trimodal pattern of C9ORF72 GGGGCC normal allele repeat number in sporadic amyotrophic lateral sclerosis and lack of association with disease risk and age at onset

Ione Woollacott, Aleksey Shatunov, Ashley Jones, Karen E Morrison, P Nigel Leigh, Pamela J Shaw, Christopher E Shaw, Ammar Al-Chalabi

Abstract

Background Recent research has discovered a non-coding hexanucleotide repeat expansion mutation (HREM) of the chromosome 9 open reading frame 72 (C9ORF72) gene, which produces hundreds of pathological GGGGCC repeats on one allele in up to 40% of familial and 10% of patients with sporadic amyotrophic lateral sclerosis (ALS). We studied patients with sporadic ALS who had a normal number of repeats (up to 23) on both alleles (non-HREM cases), patients with the expanded repeat on one allele (HREM cases), and healthy controls, to determine whether a higher normal number of GGGGCC repeats increases the risk of developing sporadic ALS, and whether it is associated with an earlier age at symptom onset.

Methods We used PCR techniques and amplified fragment length polymorphism analysis to characterise repeat numbers on each allele in 397 white UK patients with sporadic ALS and 235 white UK controls. This method identified 357 non-HREM cases and 40 HREM cases. We then performed logistic regressions of repeat number against disease status (cases vs controls) and linear regressions of repeat number against age at onset of symptoms (cases), all adjusted for sex. All four models of allelic effect (recessive, dominant, additive, and multiplicative) were evaluated for each analysis.

Findings Normal repeat numbers on each allele were not significantly associated with disease risk or age at onset of symptoms (p>0.05 for all analyses). These factors may therefore be determined by other environmental or genetic influences in patients with sporadic ALS. We found a trimodal pattern of repeat number expression, with two, five, or eight GGGGCC repeats on each allele in most of the cases and controls. This pattern may relate to the normal structure or function of this region or of C9ORF72 mRNA transcripts.

Interpretation Determination of the effects of repeat number pattern on C9ORF72 gene expression and function, and an understanding of what increases the risk of developing the expansion mutation, will be vital in elucidating the pathological mechanisms of the C9ORF72 mutation in ALS.

Funding National Institute for Health Research.
Mitochondrial DNA damage promotes atherosclerosis and is associated with vulnerable plaque

Emma Yu, Lauren Baker, James Harrison, Nichola Figg, John Mercer, Patrick Calvert, Antonio Vidal-Puig, Michael Murphy, Martin Bennett

Abstract
Mitochondrial DNA (mtDNA) damage is associated with atherosclerotic disease in man. However, when mtDNA damage occurs, whether it promotes atherogenesis and whether the damage is associated with plaque volume or vulnerability are unknown.

To assess the role of mtDNA defects in atherosclerosis, we first performed a time-course study in apolipoprotein E deficient (ApoE−/−) mice. MtDNA damage was present at the earliest stages of atherogenesis, before histological evidence of disease, with mitochondrial dysfunction occurring in advanced disease. We then studied ApoE−/− mice that were doubly deficient for a proof reading deficiency of mitochondrial DNA polymerase (PolG−/−ApoE−/− mice). PolG−/−ApoE−/− mice had increased plaque burden and hypercholesterolaemia, despite a marked reduction in adiposity and no increase of reactive oxygen species. PolG−/−ApoE−/− mice had increased aortic mtDNA damage and decreased expression and respiration of complexes that have mtDNA-encoded subunits. PolG−/−ApoE−/− smooth muscle cells showed reduced ATP content, impaired proliferation, and increased apoptosis.

To determine whether MtDNA damage correlates with human disease we studied 1096 plaques in 170 patients who had undergone three-vessel virtual histology intravascular ultrasound of their coronary arteries at Papworth Hospital. mtDNA damage correlated strongly with the number of vulnerable lesions but not plaque volume.

Our results indicate that mtDNA damage occurs early in atherosclerosis and leads to respiratory dysfunction without increased oxidative stress. mtDNA damage causes impaired bioenergetics, changes cell proliferation and apoptosis, and promotes hypercholesterolaemia and atherosclerosis. mtDNA damage may also be a novel marker for unstable atherosclerosis in man.

Funding British Heart Foundation.
The contact electrogram and its architectural determinants in atrial fibrillation

Junaid A B Zaman, Sayed Al-Aidarous, Pravina M Patel, Michael T Debney, Caroline Roney, Eugene T Y Chang, Rasheda A Chowdhury, Nicholas Peters

Abstract

Background The basis for the contact electrogram, the basic unit of cardiac electrophysiology, is only partly understood, especially in atrial fibrillation, the commonest sustained cardiac arrhythmia. We aimed to characterise the determinants of electrogram formation by electrophysiological investigation and by tissue level substrate characterisation in human and rat atrial tachycardia (AT) and atrial fibrillation (AF) using high density epicardial electrode arrays.

Methods Human intraoperative epicardial pacing was performed in nine patients from the right atrial wall using a high density AFocusII 20 electrode catheter at 500 ms and 200 ms cycle lengths to assess AF inducibility. None of the patients had had atrial fibrillation and were operated on using cardiopulmonary bypass. Burst pacing was conducted and resulting electrograms recorded using BARD software in real time. Ten brown Norway rats and ten spontaneous hypertensive rats were culled at 12–14 weeks of age and hearts removed rapidly for ex-vivo Langendorff experiments. Bilateral epicardial atrial appendage electrograms were recorded with a high-density microelectrode array consisting of 32 50 μm electrodes at 300 μm spacing. 10 s burst pacing was performed from the right atrium using decreasing cycle lengths from 150 ms to 30 ms and atrial arrhythmia susceptibility, electrogram duration, and interatrial conduction time measured. Ventricular programmed electrical stimulation with an S1-S2 protocol was performed before tissue was frozen for histological analysis.

Findings Human bipolar electrograms recorded intraoperatively were significantly prolonged with shortened cycle lengths of pacing but were no different between those who developed AF for more than 30 s (n=6) and those who did not (n=3). Rat atrial electrogram duration was no different between groups during intrinsic rhythm but mean conduction velocity was statistically significantly different between both species and right and left atrial appendages, as was interatrial conduction time. Brown Norway rats were significantly more likely to have a 30 s episode of AT or AF recorded than were spontaneous hypertensive rats, but no difference was seen in ventricular arrhythmias. The heart weight:body weight ratio confirmed that spontaneous hypertensive rats had cardiac sequelae of hypertension.

Interpretation Local electrogram prolongation is witnessed in human AF, and AF induction using a standard pacing protocol confirmed those capable of developing arrhythmia. There is an overt electrophysiological phenotype of AF susceptibility in brown Norway rats compared with spontaneous hypertensive rats, which is unaccompanied by underlying changes in mean electrogram duration. Further work is required to investigate the underlying substrate for these findings, especially with respect to electrogram determination and formation.

Funding British Heart Foundation.
Loss of CaMKKβ attenuates endotoxin induced hypotension

Jiexin Zhao, David Carling, Zhen Wang, Phillip Muckett, James Leiper, Angela Woods

Abstract

Septic shock is a syndrome defined by persistently reduced blood pressure despite fluid resuscitation leading to multiple organ failure resulting from overwhelming infection. It is a major cause of intensive care admission and is associated with a high mortality rate. The phosphorylation state of myosin light chain (MLC) in smooth muscle cells directly controls the contractility of blood vessels and the maintenance of blood pressure. Activation of AMP-activated protein kinase (AMPK) by calcium/calmodulin dependent protein kinase kinase beta (CaMKKβ) has previously been reported to inhibit MLC phosphorylation, and induce vasodilation in mice.

We investigated the involvement of CaMKKβ in blood pressure control using a model of endotoxin induced septic shock. Using implantable telemetry probes, we recorded aortic blood pressure in age-matched male wild-type (WT) and CaMKKβ knockout (CaMKK KO) mice under both basal conditions and during endotoxin induced sepsis. We showed that in mice with global deletion of CaMKKβ, basal blood pressure is increased compared with their wild-type littermates. In addition, when treated with intraperitoneally injected bacterial endotoxin, the lack of CaMKKβ appeared to confer protection against hypotension, with significantly higher blood pressure seen in CaMKK KO mice, as well as improved mobility and general physiological state, compared with WT mice. We found no indication of altered inflammatory response between CaMKK KO and WT mice after intraperitoneal endotoxin injection. We are addressing the role of CaMKKβ in vascular smooth muscle MLC phosphorylation and the potential for AMPK involvement in the signalling pathways.

Our findings suggest that loss of CaMKKβ in mice protects against septic shock through the maintenance of blood pressure. Inhibition of CaMKKβ could be an alternative strategy to assist with the maintenance of blood pressure during sepsis.

Funding

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<table>
<thead>
<tr>
<th>Poster abstract number</th>
<th>Presenting author (corresponding author if different)</th>
<th>Page number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reza Aghamohammadinzadeh</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>Mark Davies</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>Nigel Drury</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>Vimal Gokani</td>
<td>45</td>
</tr>
<tr>
<td>5</td>
<td>David Hutchings</td>
<td>53</td>
</tr>
<tr>
<td>6</td>
<td>Oliver Lyons</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>Fu Siong Ng</td>
<td>81</td>
</tr>
<tr>
<td>8</td>
<td>Koralia Paschalaki</td>
<td>84</td>
</tr>
<tr>
<td>9</td>
<td>Ashish Patel (Bijan Modani)</td>
<td>78</td>
</tr>
<tr>
<td>10</td>
<td>Elaine Teh (David Chambers)</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>Ashleigh Wilcox (Daniel Espino)</td>
<td>41</td>
</tr>
<tr>
<td>12</td>
<td>Emma Yu</td>
<td>117</td>
</tr>
<tr>
<td>13</td>
<td>Junaid Zaman</td>
<td>118</td>
</tr>
<tr>
<td>14</td>
<td>Saima Ehsan</td>
<td>39</td>
</tr>
<tr>
<td>15</td>
<td>Andreas Kyriacou</td>
<td>62</td>
</tr>
<tr>
<td>16</td>
<td>Mark Toshner</td>
<td>108</td>
</tr>
<tr>
<td>17</td>
<td>Michael Ibrahim</td>
<td>54</td>
</tr>
<tr>
<td>18</td>
<td>Abdul Hameed</td>
<td>47</td>
</tr>
<tr>
<td>19</td>
<td>Satveer Mahil</td>
<td>69</td>
</tr>
<tr>
<td>20</td>
<td>Angela Tewani</td>
<td>107</td>
</tr>
<tr>
<td>21</td>
<td>Veronika Kinsler</td>
<td>58</td>
</tr>
<tr>
<td>22</td>
<td>Hamidreza Mani</td>
<td>70</td>
</tr>
<tr>
<td>23</td>
<td>Konstantinos Manolopoulos</td>
<td>71</td>
</tr>
<tr>
<td>24</td>
<td>Rod Mitchell</td>
<td>77</td>
</tr>
<tr>
<td>25</td>
<td>Kenneth Muir</td>
<td>80</td>
</tr>
<tr>
<td>26</td>
<td>George Tse</td>
<td>109</td>
</tr>
<tr>
<td>27</td>
<td>Andrew Stewart</td>
<td>105</td>
</tr>
<tr>
<td>28</td>
<td>Nick Powell</td>
<td>89</td>
</tr>
<tr>
<td>29</td>
<td>Tom Pembroke</td>
<td>85</td>
</tr>
<tr>
<td>30</td>
<td>Jexin Zhao</td>
<td>119</td>
</tr>
<tr>
<td>31</td>
<td>James Bluett</td>
<td>24</td>
</tr>
<tr>
<td>32</td>
<td>Johnathan Cooper-Knock</td>
<td>32</td>
</tr>
<tr>
<td>33</td>
<td>Vikram Sharma (Andrew Wilkie)</td>
<td>114</td>
</tr>
<tr>
<td>34</td>
<td>Karin Tuschl</td>
<td>110</td>
</tr>
<tr>
<td>35</td>
<td>Tracy Briggs</td>
<td>27</td>
</tr>
<tr>
<td>36</td>
<td>Matthew Armstrong</td>
<td>20</td>
</tr>
<tr>
<td>37</td>
<td>David Bantlett</td>
<td>21</td>
</tr>
<tr>
<td>38</td>
<td>Chiara Bracci</td>
<td>26</td>
</tr>
<tr>
<td>39</td>
<td>Matthew Hoare</td>
<td>49</td>
</tr>
<tr>
<td>40</td>
<td>Ka-Kit Li</td>
<td>64</td>
</tr>
<tr>
<td>41</td>
<td>Yazid Resheq</td>
<td>90</td>
</tr>
<tr>
<td>42</td>
<td>Ian Rowe</td>
<td>95</td>
</tr>
<tr>
<td>43</td>
<td>Victoria Snowdon</td>
<td>102</td>
</tr>
<tr>
<td>44</td>
<td>Barney Stephenson</td>
<td>104</td>
</tr>
<tr>
<td>45</td>
<td>Michael Williams</td>
<td>115</td>
</tr>
<tr>
<td>46</td>
<td>Tom Bird</td>
<td>23</td>
</tr>
<tr>
<td>47</td>
<td>Shishir Shetty</td>
<td>99</td>
</tr>
<tr>
<td>48</td>
<td>Claire Booth</td>
<td>25</td>
</tr>
<tr>
<td>49</td>
<td>Esther Gathogo</td>
<td>43</td>
</tr>
<tr>
<td>50</td>
<td>Julie Glaville</td>
<td>44</td>
</tr>
<tr>
<td>51</td>
<td>Maria Longhi</td>
<td>65</td>
</tr>
<tr>
<td>52</td>
<td>Richard Parker</td>
<td>83</td>
</tr>
<tr>
<td>53</td>
<td>Bohan Wang</td>
<td>112</td>
</tr>
<tr>
<td>54</td>
<td>Eoin McKinney</td>
<td>74</td>
</tr>
<tr>
<td>55</td>
<td>Paul Collini</td>
<td>31</td>
</tr>
<tr>
<td>56</td>
<td>Esther Robinson</td>
<td>93</td>
</tr>
<tr>
<td>57</td>
<td>Hema Sharma</td>
<td>98</td>
</tr>
<tr>
<td>58</td>
<td>Shabnam Ali</td>
<td>19</td>
</tr>
<tr>
<td>59</td>
<td>Jose Rodriguez</td>
<td>94</td>
</tr>
<tr>
<td>60</td>
<td>Michelle da Silva Lodge</td>
<td>33</td>
</tr>
<tr>
<td>61</td>
<td>Ruth Pepper</td>
<td>86</td>
</tr>
<tr>
<td>62</td>
<td>Harry Haynes (Kathreena Kunian)</td>
<td>61</td>
</tr>
<tr>
<td>63</td>
<td>John Jacob</td>
<td>55</td>
</tr>
<tr>
<td>64</td>
<td>Timothy Rittman</td>
<td>92</td>
</tr>
<tr>
<td>65</td>
<td>Philipp Vermehren</td>
<td>111</td>
</tr>
<tr>
<td>66</td>
<td>Ione Woollacott</td>
<td>116</td>
</tr>
<tr>
<td>67</td>
<td>Poster withdrawn</td>
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</tr>
<tr>
<td>68</td>
<td>Chern Lee</td>
<td>63</td>
</tr>
<tr>
<td>69</td>
<td>Francois Runau</td>
<td>96</td>
</tr>
<tr>
<td>70</td>
<td>Jonathan Stewart</td>
<td>106</td>
</tr>
<tr>
<td>71</td>
<td>Stephen Ward</td>
<td>113</td>
</tr>
<tr>
<td>72</td>
<td>Jasmina Cehajic-Kapetanovic</td>
<td>29</td>
</tr>
<tr>
<td>73</td>
<td>Dimitrios Siassakos</td>
<td>100</td>
</tr>
<tr>
<td>74</td>
<td>David Carr</td>
<td>28</td>
</tr>
<tr>
<td>75</td>
<td>Katherine Sleeman</td>
<td>101</td>
</tr>
<tr>
<td>76</td>
<td>Suzy Hope</td>
<td>51</td>
</tr>
<tr>
<td>77</td>
<td>Salim Elyas</td>
<td>40</td>
</tr>
<tr>
<td>78</td>
<td>Jessica Eccles</td>
<td>38</td>
</tr>
<tr>
<td>79</td>
<td>Katie Manwick</td>
<td>72</td>
</tr>
<tr>
<td>80</td>
<td>Nadia Micici</td>
<td>75</td>
</tr>
<tr>
<td>81</td>
<td>Rina Dutta</td>
<td>37</td>
</tr>
<tr>
<td>82</td>
<td>Ivan Koychev</td>
<td>59</td>
</tr>
<tr>
<td>83</td>
<td>Joanne Morling</td>
<td>79</td>
</tr>
<tr>
<td>84</td>
<td>Alexander Basran</td>
<td>22</td>
</tr>
<tr>
<td>85</td>
<td>Michele Horns</td>
<td>50</td>
</tr>
<tr>
<td>86</td>
<td>David Miller</td>
<td>76</td>
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