(i) a systematic review of the literature, identifying seven categories of patient-level outcome domain and principles for implementing routine outcome assessment; and
(ii) a testable model which suggests that completing therapeutic process and clinical outcome measures and receiving regular feedback may itself be of benefit to individual patients.

The purpose of routine outcome assessment is to improve outcome through empowering patients and changing the service culture. An MRC-funded randomised controlled trial to evaluate routine outcome assessment is currently underway in London.

M115

Characterisation of a Rare Inherited Disorder of Pain Sensation - Familial Rectal Pain

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Objective: To elucidate the pathophysiology underlying the autosomal dominant condition familial rectal pain. Understanding the patho-physiology of this condition may enable a more tailored approach to the management of these patients.

Methods: Characterisation of familial rectal pain has been undertaken in a large pedigree ascertained with 25 individuals, 13 of whom are affected. Detailed clinical histories and examination have been carried out and responses to neuropathic pain and general health (SF-36) questionnaires have been evaluated using the statistical package SPSS. In two affected adults detailed quantitative sensory testing has taken place. This included vibration and perception thresholds, monofilament (touch) and thermal thresholds, pin prick, axon reflex vasodilatation induced by both histamine and capsaicin and the sweating rate in the sole and dorsum of the foot and the palm. Finally, histological analysis of rectal biopsy material has been undertaken. Besides routine histological staining, antibody labelling of neuronal channels specifically involved in nociception has been undertaken in biopsy material and compared to age-matched control specimens.

Results: We provide further clinical information about familial rectal pain. Responses to the questionnaires indicate that although the pain experienced by individuals varied in severity between individuals, the quality of the pain was consistently described as hot and sharp and never as cold or itchy. Quantitative sensory testing was within normal limits. Detailed staining of rectal biopsy material revealed no abnormality as compared to the control tissue.

Conclusions: We have further characterised familial rectal pain and can make tentative inferences about the pathophysiological basis of this disease. We postulate that the abnormality underlying this condition is most likely to be central in origin as both histological data and the results of sensory testing indicate that peripheral innervation is normal. We anticipate that we may be able to offer patients improved treatment which includes novel pharmacological agents and other pain management strategies.

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M116

A novel whole blood model to investigate immunogenicity of the BCG vaccine in neonates in a tuberculosis-endemic setting in South Africa

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Introduction: BCG remains the world’s most widely used vaccine, yet its efficacy varies considerably in different populations for reasons not understood. Major research efforts are currently directed towards the development of a “better” anti-mycobacterial vaccine, but new candidate vaccines will have to be evaluated against the existing “gold standard”. It is therefore vital to understand, which immune responses are elicited by BCG vaccination, and how those compare with potential new candidates.

Progress in the field of TB-vaccine development has been hampered by the lack of human in vitro models to assess vaccine immunogenicity and efficacy. We have developed a whole blood model which enables us to measure immune responses to mycobacteria employing reporter-gene tagged BCG (BCG lux). In previous studies, we have shown differences in immune responses in tuberculosis-positive and -negative adults and recently in HIV-infected children (Kampmann et al, JID 2000, 182:895). Here we extend the use of this model to study immune responses to BCG vaccination in a group of neonates.

Methods: 50 HIV-negative neonates were recruited from a Maternity Obstetric Unit (MOU) in Cape Town, South Africa, prior to receiving BCG inoculation. 3 ml of venous blood was drawn from these infants and inoculated in vitro with BCG lux. Growth of BCG lux and accompanying production of TNFα and IFNγ were studied as well as responses to PPh (a mitogen) and PPD (a crude preparation of mycobacterial antigens). All laboratory investigations were repeated 3-6 months later and a Mantoux test was also applied at that stage.

Results: There was significantly less growth of BCG lux in the whole blood assay following BCG vaccination (p<0.001). This was accompanied by significantly higher IFNγ production in response to BCG lux and also in response to PPD (p<0.001). Only two of the babies had developed a DTH response after 3-6 months.

Conclusion: The whole blood model reflects changes in cellular immune responses to mycobacteria following BCG vaccination. This makes the assay a useful tool to study immunogenicity of new vaccine candidates prior to large field trials assessing efficacy.

M117

Subcellular targeting of Adenovirus core polypeptides Mu and VII

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Adenovirus DNA is covalently linked to the viral terminal protein and non-covalently bound to the viral core proteins Mu, V, and VII. Protein V is believed to form a link between the viral DNA-core protein complex and the viral capsid, whilst protein VII and Mu are tightly associated with the viral DNA. Nuclear and nucleolar targeting sequences have previously been examined in protein V, and
since proteins Mu and VII have similar arginine rich regions, their putative targeting sequences were investigated. Regions of the open reading frame of Pre-Mu and Pre-VII protein were amplified from adenovirus DNA by polymerase chain reaction, and further oligonucleotide mutants of Mu and VII were designed with selected arginine codons replaced by alanine or lysine codons. Fragments were cloned into a mammalian expression plasmid to express the amino acid sequences as N-terminal fusions to enhanced green fluorescent protein (EGFP). Full length Pre-Mu and Pre-VII demonstrated exclusive nucleolar targeting. Mu protein, and a similar sequence within protein VII, demonstrated nucleolar targeting with a background distribution within the cytoplasm. Site-directed mutation of arginine to alanine residues reduced the intensity of nucleolar targeting, but this intensity remained similar if the arginines were mutated to lysines.

Our data suggests that, as with protein V, Mu protein and protein VII demonstrate nucleolar and/or nuclear targeting sequences, rich in basic amino acids. These proteins may therefore have a role in the nuclear import of adenoviral DNA during adenovirus infection.

M118

Computational Magnetic Resonance Image Processing Applied to the Developing Human Brain

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Aim. To use non-linear image registration to make brain atlases of preterm infants at term and normal controls respectively, and to quantitatively assess the groups for differences.

Introduction. Computational MR image processing techniques are used to study subtle non-localised brain changes in a range of neurological applications. Neurocognitive deficits are common among infants born prematurely (5% of births), but the nature of pre-term brain injury, the resultant neuroanatomical deficits, and their relationship with functional impairment are incompletely understood. Detailed neuroanatomical exploration of the brain at this stage of development will further knowledge about the mechanisms underlying normal and abnormal development. Rapid changes in growth and structure make computational MR analysis technically challenging in this age group.

Methods. 33 infants were studied (gestational age 24 to 32 weeks) at term equivalent (38 to 42 weeks post-conceptional age). A 1.5T MR system was used to acquire T1 weighted volume datasets. A non-rigid transformation algorithm was used to register 33 MR datasets to a segmented reference brain image. The same technique was used to register 8 normal controls. Changes in the Jacobian operator were used to quantify regional differences in tissue volume between datasets.

Results. The techniques successfully mapped datasets into the coordinate system of the reference brain, generating atlas images. By visual analysis anatomical localisation was precise using non-rigid registration. Preliminary quantitative analyses suggest localized cerebellar and cortical volume reduction in infants born prematurely at term equivalent age, compared to infants born at term.

Discussion. Atlases can be analysed to study subtle volumetric differences between groups, and to compare individuals to a reference atlas, which has immediate clinical implications. Ultimately computational image data can be fused with collateral clinical, imaging, genetic or biochemical data. This will enable testing and generation of hypotheses about structure-function relationships in normal brain development, and the origins of pre-term brain injury.

M119

Neural Differentiation of Fetal Mesenchymal Stem Cells

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Introduction. Mesenchymal stem cells (MSC) have recently been identified circulating in first trimester fetal blood. These cells have considerable proliferative capacity and have been demonstrated to differentiate down osteogenic, chondrogenic and adipogenic lineages. We have preliminary data that suggests neural cell differentiation from these MSCs is also possible.

Aims of the Project. The ultimate aim is to determine whether MSC can integrate into the damaged central nervous system and functionally improve outcome in relevant models of brain injury. In order to do this we need to: 1) define conditions for the proliferation of undifferentiated MSC, 2) define culture conditions for the differentiation of MSC into specific neural lineages, and 3) to purify MSC-derived neural progenitor cells at defined stages of development.

Methods. We have previously shown that MSC divide in DMEM with serum. In time-course and dose-response experiments we examined the effects of serum withdrawal, and whether the addition of growth factors including basic fibroblastic growth factor and platelet-derived growth factor support survival and/or promote differentiation into specific neural lineages. Finally, conditioned medium from neural cell lines was applied to MSC to determine if neural cell-derived soluble factors could influence differentiation. Neural cell phenotypes were characterised on the basis of cell morphology combined with antibody/RT-PCR analysis of lineage specific markers.

Results and Conclusions. MSC proliferated for more than 20 passages with no change in doubling time and no apparent phenotypic change. Serum withdrawal alone did not promote neural differentiation but rather triggered apoptosis. Chemical induction promoted MSC differentiation towards a neuronal phenotype, while experiments using conditioned media from neural cell lines demonstrated dramatic morphological and phenotypic differentiation of MSC along the oligodendrocyte lineage.