European Medical Research Group (Meeting held on 26 November 1991)

The European Medical Research Group met at the Medical Society of London, Lettsum House, on 26 November 1991. The guest speaker was Dr Nicholas D. Carter who gave a lecture on 'The genetics revolution – dreams and realities'.

Dr N. Mellado (Barcelona, currently working at Charing Cross Hospital) gave a short talk on 'Medicine in Spain'. Both talks were followed by questions and discussion. A poster session was held demonstrating the research in progress of some members of the Group. Their abstracts are published below.

Presystolic sounds due to flow reversal in the superior vena cava

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The classical S_4 is due to atrial flow into a diseased ventricle, but for some presystolic sounds (PSS), venous origin has been postulated. We therefore recorded phonocardiograms over the precordium, upper right sternal edge and jugular veins with simultaneous electrocardiograms, Doppler flow in the superior vena cava (SVC), and atrioventricular (AV) valve flow in all patients referred for echocardiography over a 6 month period. In 21 patients (10 pulmonary hypertension, three dilated cardiomyopathy, three pericardial constriction, one aortic stenosis, one subaortic stenosis, one hypertrophic cardiomyopathy, one cardiac amyloid and one post Mustard operation) PSS were recordable from a localized area (only over the SVC or jugular veins). Each was associated with a rapid blood flow reversal in the SVC, evident on Doppler. PSS occurred at a point of change in acceleration (mean change = $16.7 \pm 6.4 \text{ m/s}^2 = 1.7 \text{ g}$). The onset of the sound coincided with peak retrograde velocity in 13 cases, peak forward velocity in 5, and with the onset of retrograde flow in 3. In 16 cases no atrial flow across either AV valve could be detected, excluding a classical S_4 . In five cases with atrial tricuspid flow, the sounds could only be recorded over the jugular veins.

Conclusions: Presystolic sounds may be recorded over the SVC and jugular veins from patients with ventricular disease. They appear to originate from a rapid blood flow reversal in the SVC rather than AV flow, and are thus quite different from the classical S_4 .

Retinoic acid-induced differentiation of human neuroblastoma cell lines results in sequentially increased expression of messenger RNAs coding for interleukin-6 and corticotrophin releasing hormone

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Neuroblastomas (NB) are known to produce several neuropeptides, which may exert paracrine effects on tumour-infiltrating inflammatory cells of the host. Scarce data exist on the capacity of NB to secrete conventional cytokines, such as interleukins (ILs) and the interrelationship of ILs with *in situ* expressed neuropeptides. Some NB cell lines can be induced by retinoic acid (RA) to differentiate either towards a neuronal or towards an epithelioid phenotype. In the present study, we have investigated the effects of differentiation on the expression of IL-6 mRNA in the NB parent cell line SK-N-BE(2) and its derivative clones SK-N-BE-2C(2C) and SK-N-BE-M17(M17). We have also attempted to relate these effects temporally to the modulation of the expression of corticotrophin releasing hormone (CRH) mRNA that also accompanies differentiation.

SK-N-BE(2) cells and their clones, 2C and M17 were grown in monolayer cultures in a 1:1 mixture of MEM and Hams F-12 media supplemented with 15% heat-inactivated FCS, NaHCO_3 (1.2 mg/ml), Hepes buffer (15 mmol/l), L-Gln (2 mmol/l) and non-essential amino acids (1% v/v). Cells were fed every 3–4 days, detached using trypsin/EDTA (0.025%: 0.02%) and were diluted between 1/5 and 1/10 every week. Cells were routinely fed 24 h before harvest for experiments. In the differentiation experiments, 5,000 to 10,000 cells/cm² were treated with medium containing 5 mmol/l RA. Fresh RA-containing or control medium was replaced daily. Total mRNA was prepared using the glyoxal-thiocyanate method and Northern blotting was performed, loading 20 µg of total RNA per lane, using Gene-Screen Plus membranes. Blots were hybridized with nick-transplanted ³²P-labelled cDNA probes for huIL-6 and huCRH.

Very little expression of CRH mRNA was detected in the absence of RA in the NB cell lines. Following RA treatment, there was a dramatic increase in CRH mRNA accumulation in the clone M17 by day 3 of the culture, which was further increased on day 5. However, in the clone 2C only a very slight increase in CRH mRNA accumulation was observed on days 3 and 5 post-RA. In the parent cell line, we were unable to detect CRH mRNA expression in the presence or absence of RA. IL-6 mRNA was present in untreated cultures of both parental and cloned cells, with a stronger signal from the uncloned parental line. Following addition of RA to the cloned cells, the amount of IL-6 mRNA increased by 4 h and 8 h, declined towards control levels by day 1 and thereafter increased again by days 3 and 5. These changes were more marked with clone M17 than with clone 2C. No change in accumulation of IL-6 mRNA was seen in parental cells.

The major finding of this study is that human NB cell lines express IL-6 mRNA and that IL-6 mRNA accumulation is influenced by the induction of differentiation with RA. The production of cytokines and neuropeptides by tumours and by the inflammatory cells that may infiltrate them suggests that a complex molecular dialogue exists between tumour cells and anti-tumour immunity. A complete understanding of this dialogue, and its therapeutic manipulation, is one of the most important tasks in neuroimmunomodulation.