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The 7th Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia was held at the Swiss Garden Hotel, Kuantan, Pahang from 2 to 4 June 2006. Abstracts of original paper and poster presentations follow:

ORAL FREE PAPERS

1. 16S ribosomal DNA sequences of gut bacteria in human and rabbit stool metagenomes

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Stool metagenomes, comprising genomic DNA, were extracted from two stool samples, S1 (human) and S2 (rabbit), and used as templates to amplify bacterial 16S ribosomal DNA (16S rDNA) sequences by the polymerase chain reaction with a pair of 16S rDNA universal primers. The amplified 1.5 kb 16S rDNA fragments were ligated to pGEM-T Easy Vector and the ligation mixture was electroporated into competent *Escherichia coli* cells. Recombinant plasmids containing 16S rDNA sequences were extracted from 37 randomly selected transformants and digested with *Eco*RI to screen for variations in restriction patterns among them. Four S1 or S2 derived recombinant plasmids showing different *Eco*RI restriction patterns were selected for DNA sequencing. Two of the eight clones had identical partial 16S rDNA nucleotide sequences and they were different from the other six clones with unique sequences. All of them were shown by the BLAST search algorithm to be from cultured and uncultured bacteria. A rooted phylogenetic tree divided the eight clones into three groups. This preliminary investigation confirmed that stool metagenomes can be used to investigate the diversity of gut microbes in human and rabbit. This approach can also be used to compare the bacterial communities present in the guts of healthy and sick individuals as reported by other researchers.

2. Drug susceptibility testing for *Mycobacterium tuberculosis* isolates in the National Public Health Laboratory, Sungei Buloh

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In 2005, a total of 5493 *Mycobacterium tuberculosis* (MTB) isolates were received by the National Public Health Laboratory (MKAK) for drug susceptibility testing by the agar-based Absolute Concentration method. Only 9.6% of the isolates were found to be resistant to at least one of the 4 drugs routinely tested i.e. streptomycin, isoniazid, rifampicin and ethambutol. Streptomycin monoresistance was the most common (6.8%) followed by isoniazid monoresistance (1.2%). MDRTB (resistant to at least rifampicin and isoniazid) accounted for only 0.3% of total MTB and 3.6% of drug-resistant MTB examined. To determine the reliability of the Absolute Concentration method, a random selection of isolates were re-tested by the BACTEC MGIT 960. Discordant results were resolved by repeat testing and PCR detection of gene mutations associated with drug resistance.

3. Non-tuberculous mycobacteria in clinical specimens : species distribution and drug susceptibilities

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From January to December 2005, a total of 6321 mycobacterial isolates were received by the National Public Health Laboratory (MKAK) for species identification. Using the Accuprobe Culture Identification Test (Gen Probe, CA), 89.2% of the isolates were identified as members of the *Mycobacterium tuberculosis* complex, 0.3% *M. avium*, 0.3% *M. intracellulare*, 0.5% other members of the *M. avium* complex (MAC) and 0.2% *M. kansasii*. The remaining non-tuberculous mycobacteria (NTM) were classified into Runyon groups 1, 2, 3 and 4 by growth characteristics and biochemical tests. The majority (82.7%) of these were identified as group 4 non-*chelonae* and non-*fortuitum* mycobacteria. As for *M tuberculosis* which was mostly (93.9%) isolated from respiratory specimens, the large majority (91.4%) of NTM isolates were also from sputum and other respiratory secretions. Less frequent sources of NTM included urine, tissue biopsies, gastric lavage, peritoneal fluid, and cerebro-spinal fluid. A random selection of MAC and *M kansasii* isolates were tested for susceptibility to clarithromycin and rifampicin by an in-house broth macrodilution method. The results showed no *in vitro* resistance to either drug.

4. Meningococcal carriage among students and staff in a residential secondary school

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Following the report of a suspected case of meningococcal disease in a thirteen year-old student from a residential secondary school, a total of 1055 students, teachers and other staff at the school were screened for nasopharyngeal meningococcal carriage. Throat swabs were transported to the National Public Health Laboratory, Sungai Buloh, in Amies transport medium and cultured onto MTM and chocolate agar plates for incubation at 36ºC in candle jars. Oxidase positive colonies were identified as meningococci by gram-staining, biochemical reactions in API NH tests and oxidation patterns in CTA sugars. A total of 109 meningococcal isolates were obtained of which 8 were from the 16 children who shared the same dormitory with the suspected case. Serogrouping was carried out on 30 isolates by slide agglutination using antisera (Difco and Remel) against serogroups A, B, C, D, X, Y, Z and W135. None were groupable by the antisera used. PCR amplification of species and serogroup-specific genes confirmed the isolates as meningococci but not any of the common serogroups (A, B, C, Y and W135). PFGE with *Not*1 carried out on the isolates from the suspected case's dorm-mates and classmates showed 4 distinctive patterns, with one pattern occurring in half of the strains isolated from the dorm-mates.

5. Correlation of human papillomavirus dna detection and immunofluorescence staining of minichromosome maintenance 2 (mcm-2) protein in women with cervical neoplasia

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Human papillomavirus (HPV) has been implicated in virtually all cervical cancers and is believed to be the primary etiological factor in the development and progression of cervical cancer. In cancerous cells, the integration of viral DNA into the host genome causes abnormal expression of E6 and E7 oncoproteins and disruption of the E2 gene. Recently, minichromosome maintenance proteins (MCMs) that are expressed during all phases of the cell growth cycle but not after exit from the cycle, have been identified as indicators of abnormal proliferation in tissues where the integration of HPV has occurred. This study aims to evaluate the potential use of the novel proliferation marker, MCM-2, in the diagnosis of HPV associated epithelial lesions of the uterine cervix. Cervical cells were obtained from asymptomatic women with no history of neoplasia who attended GP clinics for routine PAP smears, as well as women attending gynaecology clinics who had PAP smear reports indicating abnormal cervical cytology. Each sample was examined for the presence of high-risk HPV genotypes by a pan-HR HPV PCR. Those with positive pan-HR HPV PCR results were further tested in a multiplex PCR assay for the detection of HPV DNA types 16, 18, 31, 33, 45 and 52, and in a real-time PCR assay for E2 and E6, to obtain the E2/E6 ratio used to determine the HPV physical status. All samples were also examined for the presence of MCM-2 by indirect immunofluorescence staining of cell monolayers prepared by centrifuging onto glass slides. At least 500 epithelial nuclei per slide were analyzed to obtain the labeling index (LI), which represents the percentage of epithelial nuclei stained positively. Preliminary immunostaining of MCM-2 on exfoliated cervical cells obtained from 10 women aged 24-70 years, with normal cytology and negative HPV DNA, showed no significant nuclear staining while a similar examination of HeLa cells showed diffuse nuclear staining, highlighting the potential of MCM as a biomarker for the identification of exfoliated dysplastic cells from the uterine cervix.

6. Establishment of Tupaia hepatocytes cell line and evaluation of its suitability as an *in-vitro* experimental model for hepatitis B virus infection studies

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The hepatitis B Virus (HBV) has a strict host tropism. Most researchers use the chimpanzee animal model for studying HBV infection but these animals are expensive and difficult to obtain. Hence, there is a need to develop a more economical and practical small animal model for the study of HBV infection and pathogenesis. The tree shrew (Tupaia belangeri) is a small squirrel-like mammal that has been recently shown to be susceptible to HBV infection as well as infection by many other human viruses like hepatitis A, C and D virus, rotavirus, TT virus and herpes simplex virus. This study aims to establish an immortalized non-tumorigenic hepatocyte cell line from Tupaia that can be continuously grown *in-vitro* in unlimited quantities, while retaining much of the characteristics of differentiated hepatocytes, and evaluate its susceptibility to HBV infection. Tupaia primary hepatocytes were isolated using a two-step collagenase perfusion technique, and maintained up to three weeks. Their susceptibility to HBV infection was demonstrated by analyzing intracellular HBV-DNA and HBV-RNA by southern and northern blotting techniques and by PCR and RT-PCR. The cells were examined for their reaction to viral genes, including those of the Simian virus 40 (SV40) T antigen, and human papillomavirus (HPV) E6 and E7, and/or for expression of the telomerase reverse transcriptase protein (TERT). The use of E6, hTERT and SV40 large T antigen

as single agents, or a combination of E6/E7, E6/E7/hTERT or hTERT/SV40 led to the successful immortalization of Tupaia hepatocytes. However cells transformed with E7 alone died after only the second passage. Immortalized hepatocytes exhibited cell morphologic changes and lost their characteristic cell morphology after a few passages. Tumorigenic transformation of the cells was tested with respect to their anchorage dependence when grown on soft agar surface. The results show a growth of colonies of all immortalized Tupaia hepatocytes.

7. Mycobacterial speciation using PCR-RFLP assays targeting *hsp65* and *rpoB* genes

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With increasing incidence of tuberculosis and non-tuberculous mycobacterial infections, rapid species differentiation of mycobacteria has become more important for patient management. While conventional methods using growth characteristics and biochemical tests are time-consuming, laborious and sometimes not able to give a definite identification, genotypic and amplificationbased molecular techniques enable more rapid and accurate species identification. In this study, two PCR-RFLP assays (PRA), targeting the *hsp65* gene and *rpoB* gene respectively, were evaluated and compared for suitability for application on local mycobacterial isolates. Sixty five isolates, comprising 43 rapid-growers and 22 slow-growers (11 nonchromogens, 8 scotochromogens and 3 photochromogens), were tested. A 439bp segment of *hsp65* gene and a 360bp segment of *rpoB* gene were amplified and subjected to restriction endonuclease digestion using 2 enzymes for each amplification product. The resulting patterns were visualized using gel electrophoresis and interpreted according to published algorithms. Isolates with discordant results were subjected to 16S rRNA sequence analysis. Except for 5 isolates, concordant results were obtained for both assays. All *M. tuberculosis* complex isolates were correctly identified. The *hsp65* PRA was found to have a superior differentiation power for rapid-growers. Moreover, the *hsp65* based database is more established with a much wider representation of species than the relatively new *rpoB* database. However, in the *rpoB* PRA, most species can be identified with the use of only one restriction enzyme whereas the *hsp65* PRA often required digestion with two enzymes for a definitive identification. These results suggest that the *hsp65* PRA can be used as an initial speciation test with confirmation by the *rpoB* PRA if necessary.

8. Study of HBV precore and core promoter mutations in chronic hepatitis B

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Variants in the precore (G1896A) and core promoter (A1762T, G1764A) regions of hepatitis B virus are reported to be common in chronic HBV infections. This study aims to determine the frequency of theses mutations in subjects chronically infected with the virus and to determine whether there is an association between these HBV variants and development of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). A total of 53 serum samples from patients with chronic complications of HBV infection (22 with HCC and 31 with LC) and 49 serum samples from chronic hepatitis B patients without any specific pathological changes were studied. Precore and core promoter mutations were identified using polymerase chain reaction coupled with restriction fragment length polymorphism (PCRRFLP) in separate reactions. The HBV precore mutant was found in 27/49 (50.9%) sera of chronic hepatitis B patients who did not have LC or HCC and 32/53 (65.3%) of samples from chronic hepatitis patients with LC or LCC. Statistical analysis showed that there is no difference in the

frequency of precore mutations in these 2 groups of patients. On the other hand, the core promoter double mutations (A1762T, G1764A) were found to be significantly (p=0.006) more frequent in subjects with LC and HCC (71.1%) than in those without (39.6%). Therefore, the presence of the core promoter double mutations is associated with liver cirrhosis and hepatocellular carcinoma in chronic hepatitis infection, and may be a possible risk factor and/or surrogate marker for the development of these complications in chronic hepatitis B infection.

9. A rare case of congenital atonic ureter

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We report an extraordinary case of primary atonic ureter in a 16-year-old boy who presented with an abdominal mass. Clinical examination and subsequent imaging studies resulted in a diagnosis of massive left sided hydronephrosis. A left nephrectomy was consequently performed. Grossly the specimen was a cystic mass measuring 160x110x25 mm and weighing 269 g. There was very little residual renal parenchyma. The pelvicalyceal system was dilated, but no evidence of physical obstruction to the lumen of the renal pelvis either by calculi, tumours or any other structures was found. A thin tubular structure was noted on the side of the renal pelvis, possibly the ureter. It measured 50mm in length and 4 mm in diameter and seemed to have an intact lumen. Histopathological examination revealed minimal residual renal parenchyma with the cortex and medulla largely replaced by fibrous tissue. An occasional tubule was seen. The ureter appeared normal. Immunohistochemical study of the ureter to locate the nerve cells were performed. The results and review of literature on this interesting case is presented.

10. Recent advances in the undergraduate medical education in United Kingdom with reference to MBChB, University of Sheffield

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We present five years of personal and group experience on the philosophy and concept of undergraduate MBChB medical curriculum, highlighting the outcome based integrated programme. In this curriculum the students are motivated and monitored throughout the course by the lecturers and phase co-ordinators. Student problems are identified early in the course and remedial measures are taken from the beginning. The students are assessed in a total of 27 times during the period of 5 years. More emphasis is given on concept delivery and students are encouraged to build their knowledge around it. The teaching learning process revolves round patients. A brief discussion on the standard setting for qualitative and quantitative assessment of the students is presented and discussed which follows the best practice guidelines. Newer innovative case based (patient's complaints) curriculum is also briefly mentioned. A highly motivated student centred integrated curriculum in our setting has produced successive batches of final year students with approximately above 95% pass rate with 4-5 distinctions and 2-3 honours per batch. An interactive integrated undergraduate medical curriculum using modern method of teaching and assessment can be implemented in the Malaysian setting.

11. Isolation of mesenchymal stem cells from cornea

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Mesenchymal stem cells (MSC) have many potential applications in cellular therapy, such as regenerative medicine and gene therapy. Studies have shown that these multipotent stem cells could be isolated from bone marrow, umbilical cord blood, adipose tissue and amniotic fluid. These studies suggested that MSC could possibly be found in different parts of the body. Thus, we investigated the possibility of MSC locating in cornea, a source that have yet to be discovered. This is the first preliminary study ever conducted to isolate MSC from cornea.
The cornea tissues were supplied by one of the co-authors who obtained them as a discarded tissue post-operation and informed consent was obtained for scientific studies from the patients. They were then chopped to fine pieces and cultured in DMEM supplemented with 10% FBS. After a few days, the crude pieces of cornea were removed. Cells were grown till confluency before subculture. Immunophenotype was evaluated by flow cytometry. Differentiation assays were performed to differentiate cultured cells into adipocytes, osteocytes and neuron-like cells.
Cultured cells featured a fibroblastoid morphology and expressed CD29, CD13, CD90, CD147, CD44, CD54, CD10, CD105, CD 73 and CD133. However, these cells are negative for HLA-DR (HLA class-II), CD34, CD117 and CD45. These morphology and expressions are similar to bone marrow-MSC. For the differentiation assays, we were able to observe the ability of cultured cells to differentiate into adipocytes, osteocytes and neuron-like cells.
Our results indicate that MSC could be cultured from cornea and discarded cornea post-operation may be an alternative source of supply for future cellular therapy.

12. Molecular Study of the Hepatitis C Virus (HCV) in Haemodialysis Patients.

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Background: Hepatitis C virus (HCV) is a single stranded RNA virus belonging to the *Flaviviridae* family. It has been estimated that approximately 170 million people are chronically infected with HCV worldwide. In Malaysia, the seroprevalence of anti-HCV among adult blood donors is estimated to be between 1.49% to 3.0%. HCV infection occurs frequently in haemodialysis (HD) patients. This is a result of their frequent exposure to blood from transfusions or exposure to HCV-contaminated medical equipment during haemodialysis or at the time of renal transplantation. In Malaysia, the prevalence of anti-HCV antibody in HD patients is 17% (12th Report of the Malaysian Dialysis and Transplant Registry, 2004). Hepatitis C is an important complication in HD patients as it increases morbidity and mortality in HD patients. **Objective:** The objective of this study is to look at the molecular aspects of HCV in HD subjects in particular the HCV genotype and viral load. **Methods and Result:** A total of 35 HD subjects were studied and 67 HCV patients with no known renal disease served as control. Hepatitis C virus genotype was studied using polymerase chain reaction coupled with restriction fragment length polymorphism (PCR-RFLP). This method is able to unambiguously distinguish major genotypes and subtypes: 1a, 1b, 2a, 2b, 3a, 3b, 4, 5 and 6. The results showed that all the HD patients were infected by HCV of genotype 1a, 1b or 3a. Sixty five out of 67 control subjects were also infected by HCV of genotype 1a, 1b or 3a; the remaining 2 subjects were infected by HCV of genotype 2b and 3b respectively. There was no significant difference in HCV subtypes distribution between the control and HD group (p=0.420). The HCV viral load was studied using an in-house quantitative real-time PCR (RTm-PCR). The assay was optimized for primer concentration,

annealing temperature, specificity and precision. The detection limit of the in-house quantitative RTm-PCR was ~$5x10^2$ IU/ml, which is comparable to that of commercially available quantitation kits. The specificity of the RTm-PCR was 100% as no DNA amplification was observed using DNA from Hepatitis B Virus, Human Immunodeficiency Virus, Cytomegalovirus, Japanese Encephalitis Virus, Dengue Virus 1-4, *Candida albicans*, *Corynebacteria sp*. and *Staphylococcus sp*. The mean viral load for the HD and control group were $2.46x10^5$ and $2.59x10^5$ IU/ml respectively. There was no difference in HCV RNA concentration between these two groups by t-test analysis (p=0.902). **Conclusion:** The results from this study showed that the HCV genotypes and viral load were similar in HD patients and HCV patients with no known renal disease.

POSTERS

1. Isolation of purified autologous peripheral blood CD34+ cells with low T cell content using CliniMACS device – a local experience.

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Peripheral blood stem cells (PBSC) mobilized with growth factor and / or chemotherapeutic regimens are used increasingly in both autologous and allogeneic transplantation. PBSCs that were used directly without ex vivo manipulation were contaminated with tumour cells that may contribute to subsequent relapses post transplantation. Purging of malignant cells from the harvest has initiated a variety of methods to reduce tumour cell contamination of the graft by the positive selection of CD34+ progenitor cells or negative selection of tumour cells using other cell-specific antigens. We report here our local experience with the CliniMACS (magnetic-activated cell separation system) in 8 adults patients with haematologic malignancies. To evaluate the purity, recovery and viability of CD34+ cells selected from harvested peripheral blood stem cells using the CliniMACS device, as well as to evaluate the T and B cell contents of these products, 8 adult patients with malignant haematological diseases (5 non-Hodgkin's lymphomas in 2nd CR and 3 acute myeloid leukaemias in 1st CR) were mobilized with granulocyte colony-stimulating growth factor with or without chemotherapeutic regimens. A total of 10 leukaphereses for peripheral blood stem cell harvest using the Cobe Spectra cell separator (Cobe BCT Lakewood, CO) were performed. The harvested PBSC were then positively selected for CD34+ cells using the CliniMACS device (Milteny Biotech, Germany). A total of 10 leukapheresis products from 8 adults with a median pre-selection CD34+ cell count of 4.31x106/kg body weight (BW) (range 1.82 -16.05x106/kg BW) were positively selected with CliniMACS. The median post-selection CD34+ cell count was 1.57x106/kg BW (range 0.15 – 7.22x106/kg BW) with a median recovery of CD34+ cells of 63% (range 2 – 94%) and a median purity of 81% (range 18 – 98%). The median total T cell count was reduced dramatically from 1.2x109 pre-selection to 1.4x107 post-selection. The selection did not affect the viability of selected cells that was tested with Trypan Blue Exclusion method with a median viability of 98% (range 95-98%). 3/7 patients whom had undergone PBSCT remain alive and well, 3 relapsed and died and 1 undergone allogeniec PBSCT and remain well. *Conclusion:* We conclude that the CliniMACS device yielded highly purified CD34+ cells with an acceptable recovery rate for PBSC harvests.

2. Experimental production of clinical-grade leukemic vaccine

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Dendritic cells (DC) are professional antigen presenting cells of the immune system playing a crucial role in the induction of anti-tumour responses. The use of DC vaccines, cryopreserved in single-use aliquots is an attractive immunotherapeutic strategy. In this paper we describe the *invitro* attempt to scale-up production of clinical-grade DC-based vaccines from leukemic cells. Blast cells of two relapsed AML patients were harvested through bone marrow aspiration using aseptic techniques. The bone marrow aspirate was then subjected to Ficoll gradient centrifugation. The interphase was used for DC generation using serum-free culture medium following good manufacturing practice guidelines. Clinical-grade cytokines including GM-CSF, IL-4 and TNFalpha were used. One harvest was cultured in culture bag; another was cultured in T-75 flask. The numbers of seeding cells were 2.24 x 108 and 8 x 107 respectively. DC yields were 10 x 106 and 29.8 x 106 cells, giving a conversion rate of 4.7% and 37% respectively. Prior to cryopreservation, these DC were to be 90% viable and shown to have characteristic phenotypic markers of DC by flow cytometry. Subsequently, these leukemic vaccines were washed and resuspended in saline and cryopreserved in approximate one million cells per vial with 20% fresh frozen AB plasma and 10% DMSO. After one year, the leukemic vaccines were thawed, and their viability and phenotype were studied. Viability was found to be 50% and 70% with respect to bag-cultured and flaskcultured cryopreserved DC. In phenotypic studies, both vaccines exhibited mature DC phenotypic markers in which CD1a, CD83 and HLA-DR were positive and haemopoietic markers were negative. Further studies such as functional studies are to be conducted on these cryopreserved DC over time. These experiments show that it is possible to produce clinical-grade DC-based leukemic vaccines from patients' blast cells *in-vitro*, which could be cryopreserved for use in clinical trial.

3. *In vitro* expression of erythropoietin in transfected human mesenchymal stem cells

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Mesenchymal stem cells (MSC) are pluripotent progenitor cells that can be found in human bone marrow, umbilical cord blood and adult peripheral blood. These cells have low immunogenicity and could suppress alloreactive T cell responses. In the current study, mesenchymal stem cells were tested for their capability to carry and deliver therapeutic gene such as erythropoietin (EPO) gene *in vitro*. The MSC samples were obtained commercially or isolated from the bone marrow of a patient with non-malignant blood disorder. The isolated MSC was characterized based on their morphology, cytochemistry, immunochemical properties by flow cytometry, and differentiation into adipocytes, chondrocytes and osteoblasts. Both samples were then transfected with EPOencoded plasmid pMCV1.2 and EPO-encoded MIDGE vector by electroporation. Following transfection, pMCV1.2-transfected MSC expressed up to 4779.4 mU/mL EPO per 1.0 μg of vector per 1 x 105 cells on Day 1 and the yield dropped sharply to 2.45 mU/mL EPO per 1.0 μg of vector per 1 x 105 cells on Day 23. However the cells maintained the expression for 4 months in culture. Meanwhile the MIDGE vector-transfected MSC expressed highest amount of EPO on Day 6, up to 2683.76 mU/mL EPO per 1.0 μg of vector per 1 x 105 cells, and the yield dropped to 641.56 mU/mL EPO per 1.0 μg of vector per 1 x 105 cells after 3 months post-transfection. The results showed that MIDGE vector is more effective and stable than the plasmid (pMCV1.2) in delivering EPO gene into MSC. Further studies showed that the supernatants containing EPO obtained from the transfected cell culture were able to induce the differentiation of hematopoietic stem cells into

erythroid colonies. In conclusion, MSC hold promise to be a cell factory for the production of biological molecules and MIDGE vector is superior to plasmid in transfection experiments involving erythropoietin gene *in-vitro*.

4. Clinical and laboratory features between CD 38 positive and negative B-CLL diagnosed in HUKM

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Chronic B-lymphocytic leukaemia (B-CLL) is a disease of elderly and has an extremely variable clinical course and prognosis. Age, white cell count, serum LDH and staging have been used to assess patient outcome and prognosis. Studies also had shown that B-CLL with CD 38 expression had poor prognosis. Recently, study had shown that CD 38 expression has relationship with Ig V_H status on B-CLL patients. Thus CD 38 can be used to act as a surrogate marker for Ig V_H gene status. To compare clinical and laboratory features between CD 38 positive and negative B-CLL diagnosed in HUKM, retrospective analyses of all cases diagnosed as B-CLL in HUKM were done. The patients' data were retrieved from the patients' files as well as the laboratory reports. From 1993 to 2005, there were 43 analyzable cases of B-CLL diagnosed in HUKM. Based on surface expression of CD38 marker, 18/43 (41.9%) were positive and 25/43 (58.1%) were negative. There was no different in the median age of presentation for these two groups of patients (66 and 65 years respectively). However, CD38 positive patients showed higher percentages of elderly patients, >70 years old (44.4% vs 33.3%). Both groups have a male predilection (77.8% vs 57.1%). 27.8% of CD38 positive and 38% of CD38 negative patients were of Rai stage II. (No lympadenopathy or splenomegaly but with hepatomegaly). The median lymphocyte count was 43.3 x 109/l vs 32.7 x 109/l in these two groups respectively with 5/18 (28%) of CD38 positive cases showing marked lymphocytosis of >100 x 109/l. This study showed no significant difference in the clinical aspects of B-CLL patient with or without CD38 surface expression except for the tendency of patient with CD38+ being more elderly, and presented with marked lymphocytosis which may indicate more advanced disease.

5. Differentiation of human mesenchymal stem cells into mesangial cells in renal glomeruli post glomerulonephritis in a murine model

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Adult human bone marrow contains a population of mesenchymal stem cells (MSC) that contributes to the regeneration of tissues such as bone, cartilage, muscle, ligament, tendon and fat. It has recently been shown that functional stem cells exist in the adult bone marrow and they can reconstitute damaged tissues in other parts of the body including the kidneys. The purpose of this study is to examine the ability of MSC isolated from human bone marrow to differentiate into mesangial cells *in vivo* in athymic BALB/c mice. MSC were isolated from human bone marrow mononuclear cells based on plastic adherent properties and expanded *in vitro* in the culture medium. MSC were characterized using microscopy, imunophenotyping and their ability to differentiate into adipocytes. MSC were then injected into athymic BALB/c mice, which had induced glomerulonephritis (GN). Control mice (infused MSC but without induced GN) and test mice (infused MSC and induced GN) were shown to have anti-human CD105+ cells present in the kidneys. However, cells positive to

anti-human desmin, a marker for mesangial cells, were observed within the glomeruli of test mice only. Furthermore, immunofluorescence assays also demonstrated that anti-human desmin+ cells in the glomeruli of these test mice were in proliferation stage, being positive to anti-human Ki-67. These findings indicate that human MSC found in renal glomeruli differentiated into mesangial cells after kidney injury.

6. Proliferative index of hepatocellular carcinoma: its association with raised liver enzymes

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Alanine (ALT) and aspartate aminotransferase (AST), serological markers of liver cell damage, are often raised in hepatocellular carcinoma (HCC). A study was conducted at the Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur to determine the association of these enzymes with the proliferative index of the tumour, as determined by Ki67. 31 cases of HCC who were histologically diagnosed and underwent surgical resection were retrieved from the files of the department. All were histologically re-confirmed. 4 μm sections were cut from the paraffinised tumour tissue block of each case and were immunohistochemically stained with Ki67 (DAKO monoclonal MIB-1) using the commercially available DakoCytomation EnVision+System-HRP kit. The latest ALT and AST levels, prior to date of tumour resection, for each case were retrieved from the patients' case records. Raised levels of one or both enzymes were seen in 77.4% of HCC cases. Ki67 immunopositivity was semi-quantitated according to percentage of tumour nuclei unequivocally stained into 2 groups viz low (<75%) and high immunopositivity (>75%) respectively. High Ki67 immunoreactivity was noted in 9 of 24 (37.5%) HCC cases with raised ALT and/ or AST compared with 1 of 7 (14.3%) with normal ALT and AST. Therefore it appears that HCC with high proliferative index are, as would be expected, associated with liver damage more than those with lower proliferative index.

7. Association of Ki67 with alphafetoprotein in hepatocellular carcinoma

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Alphafetoprotein (AFP) is one of the most commonly used serological markers in screening for hepatocellular carcinoma (HCC). Although AFP is frequently used, it is unable to detect all HCC (false negatives) and is expressed at varying levels by different cases. This prompted our interest to assess whether the proliferative index, as determined by Ki67, is correlated with rise of serum AFP in HCC. 25 HCC histologically diagnosed at the Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur and which had undergone surgical resection, were retrieved from the department archives. All cases were histologically reviewed and re-confirmed. 4 μm sections were cut from the paraffin block of the tumour and immunostained for Ki67 (DAKO monoclonal MIB-1) using the commercially available DakoCytomation EnVision+System-HRP kit. Immunopositivity was semi-quantitated according to percentage of tumour nuclei unequivocally stained into 2 groups viz low (<75%) and high immunopositivity (>75%) respectively and correlated with AFP levels arbitrarily divided as < 500 ng/ml and > 500 ng/ml. 16 (64.0%) of HCC in this study had AFP levels < 500 ng/ml while 18 ((72.0%) showed low Ki67 immunopositivity. High Ki67 immunopositivity (4/9 cases, 44.4%) was more frequently encountered in HCC with AFP levels > 500 ng/ml compared with those having AFP levels < 500 ng/ml (3/16 cases, 18.8%)). Granted that the number of cases is too small for proper statistical analysis and other parameters that may have

contributed in raising AFP were not assessed in this study, it is nonetheless interesting that a high proliferative index may have a role in determination of AFP levels.

8. An abdominal mass post bone marrow aspiration and trephine biopsy

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Bone marrow aspiration and trephine biopsy is generally a safe procedure. It is widely performed and is a relatively simple technique with a low risk of morbidity. Adverse events are rarely reported but nevertheless occur. We describe a 56-year-old Malay lady who presented with headaches and two episodes of loss of vision of the right eye. She was previously well and an active lady. On examination, she had bilateral eye redness and her Traube's space was dull to percussion. Her full blood count on admission revealed a haemoglobin level of 22g/dL, a haematocrit level of 68%, a white cell count of 13 x 109/L and a platelet count of 338 x 109/L. She was started on aspirin in view of her high risk in developing thrombosis. She was venesected and a diagnostic bone marrow aspiration and trephine biopsy was performed. The following day, her blood pressure dropped and she developed and acute abdomen. It was noted that she had a palpable mass on the right side of the abdomen extending from the right hypochondriac region till the right iliac fossa. An urgent ultrasound of the abdomen was performed which revealed a large retroperitoneal mass and a CT scan of the abdomen done after that confirmed a retroperitoneal haematoma. Bone marrow aspiration and trephine biopsy are generally safe procedures but are not free of risks. Adverse events such as haemorrhage do occur and risk factors that contribute to them need to be carefully sought for before performing the procedure.

9. A mediastinal mass series with clinicopathological and radiological correlations

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In surgical pathology, mediastinal mass biopsies or excised specimens are uncommon. In addition, diagnostic dilemmas are sometimes encountered when faced with small biopsies as the mediastinum house an array of pleuripotent cells able to differentiate into a number of tumours which may be confusing histologically. We review the clinical, histological and radiological characteristics of a series of mediastinal masses with the purpose of gaining more knowledge of the various characters of the tumours for future gain Mediastinal biopsies or specimens received within the last 4 years in the University Sains Malaysia Hospital Pathology Department were retrieved and reviewed. The case notes and imaging films were traced and studied. The cases were then analysed on these characteristics.

A total of 7 cases were collected from the stated period. All of the patients were male. The age range was from 5 to 64 years. The most common clinical presentation was shortness of breath. One patient was asymptomatic; two had anterior chest wall swelling as the main presentation. Radiological imaging studies ranged from widening of the mediastinum to complete infiltration and obliteration of one side of the lung. Histological evaluation resulted in a conclusive diagnosis in 4 patients and a less definite in 3 cases. Immunohistochemical studies of these cases are also presented Clinicopathological and radiological collaboration is of utmost importance in the diagnosis of a mediastinal mass.

10. A comparison of p53 and pRB immunohistochemical expressions in tissue microarrays of hepatocellular carcinoma and hepatoblastoma

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Mutations of the tumour suppressor genes, p53 and pRb, are mooted to play important roles in neoplastic transformation. While molecular routes to the uncontrolled growth of hepatocytes, leading to primary liver cancer have generated considerable interest, the roles of p53 and pRb mutations in hepatocellular carcinoma (HCC) and hepatoblastoma (HB) remain to be clarified. We examined the immunohistochemical expression of p53 and pRb gene products in 26 HCC and 9 HB, sampled into a tissue microarray block. 10 (38%) of 26 HCC showed nuclear staining for p53 protein. 3 of the 10 positively-staining HCC were also HbsAg positive. Conversely, none of the 9 HB expressed nuclear p53 staining. 24 (92%) HCC and 8 (89%) HB showed loss of pRb nuclear expression. Two of the 26 HCC and one of the 9 HB showed nuclear staining for pRb protein.
Our results suggest that p53 gene mutation occurs in a substantial proportion (38%) of HCC but does not appear to have an important role in the development of HB. There is also loss of pRb expression in the majority of HCC and HB, supporting a role for pRb gene deletion and mutation in hepatocarcinogenesis. However, a comparative study of the staining pattern of p53 and pRb in the HCC and HB cases did not detect a consistent staining pattern to differentiate between the two types of tumours immunohistochemically. Hence the use of p53 and pRB expression has no contribution in the situation where there is a diagnostic difficulty in deciding between HCC and HB.

11. Extracellular enzymatic profiles of *Cryptococcus species* in Malaysia and PCR-RFLP analysis of phospholipase genes of *Cryptococcus neoformans*

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Determination of the enzymatic profiles of *Cryptococcus neoformans* is important for epidemiology purposes and understanding of the pathogenesis of the organism. In this study, the extracellular enzymatic activity of *C. neoformans* and other *Cryptococcus spp.* was investigated using the API ZYM commercial system. A total of 21 clinical *C. neoformans,* 19 *C. gattii*, 5 environmental *C.neoformans* and 8 reference strains of *C. neoformans* produced esterase C4, esterase lipase C8, leucine arylamidase, acid phosphatase and Naphthol-AS-BI-phosphohydrolase. *C. neoformans* were distributed into 13 enzymatic patterns, while *C. gattii* and the reference strains were distributed into 6 patterns respectively. Four different enzymatic profiles were obtained for 5 environmental *C. neoformans* strains. The extracellular proteinase and phospholipase activities of various *Cryptococcus* spp. were determined by nutrient gelatin and protein agar clearance assays. More than 50% of the strains in each *Cryptococcus* species were positive for the nutrient gelatin and proteinase assay. A significance difference in the phospholipase production was noted when the organisms were incubated at 28°C and 37°C on egg-yolk agar plates. The clinical *C. neoformans* strains were further differentiated by PCR-restriction fragment length polymorphism (RFLP) analysis of the phospholipase gene (*PLB1*) into molecular type VNI (15 strains), VNI* (34 strains) and VNII (3 strains). A total of 14 molecular type VGI and 7 VGII* were also identified. All the 16 environmental strains of C.neoformans were molecular type VNI*. The difference noted in the RFLP patterns of VNI* and VGII* molecular types of the Malaysian strains as compared to the reference strains, is possibly an indication of *PLB1* gene variation of the organisms.

12. Sequence analysis of the internal transcribed spacer (ITS) gene of *Cryptococcus neoformans* and *Cryptococcus gattii* from Malaysia

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Cryptococcus neoformans and *C. gattii* are two pathogenic yeasts that cause life-threatening infections that differ in their biochemical properties, ecology, epidemiology, and infection preference. Internal transcribed spacer (ITS) gene sequencing was performed in this study to determine the sequence variation among the Malaysian clinical and environmental isolates. The sequences were aligned and searched for variety or molecular type-sequence signatures. Four known ITS types were identified in this study. Of 45 isolates, 18 were ITS type 1 (ATACTAGCT), 3 were ITS type 3 (GCGCTGGCT), 14 were ITS type 7 (ACGCTGGCT) and 2 was ITS type 4 (ACACTGACT). A new ITS type ITS type 4* (ACACTGACC) were recognized in 8 Malaysian isolates. All the bird dropping isolates were ITS type1. Phylogenetic analysis showed that all the Malaysian isolates of *C. neoformans* were grouped in a single subtype. *C. gattii* were differentiated into 3 subtypes; with ITS type 3 and 7 in subtype 1, ITS type 4 in subtype 2 and the new ITS type 4* in subtype 3. This study demonstrated the genetic diversity of C. *neoformans* and *C. gattii* in Malaysia. The information obtained in this study contributes to the understanding of epidemiology and surveillance of cryptococcosis in this country.