

## THE THIRD COMBINED SINGAPORE-MALAYSIA PATHOLOGY CONFERENCE

The Third Combined Scientific Meeting of the Singapore Society of Pathology and the Malaysian Society of Pathologists was held at the Boulevard Hotel, Singapore on 5-6 September 1992. Abstracts of the Free Paper communications follow:

### 1. Structural changes in the kidney in renal wrap hypertension in rabbits

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Wrapping the kidneys in cellophane causes the development of scar tissue around the kidney and blood pressure to increase gradually over 6 weeks, and renal function is reduced concomitantly. The aim of this study was to correlate the changes in renal structure and function in renal wrap hypertension. Rabbits underwent a bilateral renal cellophane wrap or sham operation and were studied 2, 4 and 6 weeks later. Then they were anaesthetised and the kidneys perfused with 2.5% glutaraldehyde. The kidneys were then prepared for light and transmission electron microscopy. The renal capsule had thickened markedly 4-6 weeks after wrapping. Electron microscopy revealed that this capsule was composed predominantly of fibroblasts at 2 weeks after wrapping, but had changed to predominantly myofibroblasts at 4-6 weeks. Cortical changes were seen 4-6 weeks after wrapping. The glomerular capillary tufts appeared to be shrunken, and some tubules were collapsed, with proteinaceous casts and areas of calcification. The glomerular and tubular changes are consistent with the decrease in renal function observed 4-6 weeks after wrapping. Compression of the kidney by the thickened renal capsule may contribute to the development of the hypertension.

### 2. Proteinase inhibitors (PIs) and tubulointerstitial damage: An immunohistochemical and electron microscopic study

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Proteinase inhibitors (PIs) have been demonstrated immunohistochemically in renal tissues. Their presence is interpreted as defence mechanism against the proteinases. We studied the role of two PIs,  $\alpha_1$ -antitrypsin ( $\alpha_1A$ ) and  $\alpha_1$ -antichymotrypsin ( $\alpha_1X$ ) in relation to an enzyme, lysozyme (LZM) which is normally freely filtered and almost completely reabsorbed by the tubules, and membrane attack complex (C5b-9) in renal tubular damage. Forty-five renal biopsies from various glomerular diseases and 10 controls were studied using immunoperoxidase, double labelling techniques, immunofluorescence and standard electron microscopy. The patients were divided into two categories on the basis of the presence or absence of tubulointerstitial lesions (TIL) by light microscopy; one group with TIL (30 cases), and the other without (15 cases). Significant antiproteinase response was observed in both disease groups compared to the controls ( $\alpha_1A$ ,  $p$  value  $<0.001$ ;  $\alpha_1X$ ,  $p$  value  $<0.001$ ), indicating that the tubules were subject to proteolytic attack by various proteases, and the renal tubules showed an appropriate response to maintain the proteinase-antiproteinase balance. This response was seen in proximal tubules only.  $\alpha_1A$  showed the predominant staining. There were significant correlations between PIs staining score, LZM score and proteinuria.

Increased-deposition of C5b-9 was observed in the disease group with TIL ( $P$  value  $<0.01$ ) indicating direct damage to cell membranes. C5b-9 may also generate oxygen species, potent inhibitors of PIs, which allow the proteinase to cause tubular damage. On electron microscopy all the cases (20 with and 10 without TIL), showed varying degrees of lytic damage to the tubular basement membrane indicating proteolytic lysis.

### 3. An immunohistochemical study of ras oncoprotein expression in gastric carcinoma

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*Background:* Previous studies have shown that ras oncoprotein is overexpressed in gastric carcinoma. It is unsettled whether there is differential expression of this oncoprotein in the two major subtypes of gastric carcinoma - intestinal-type and diffuse-type.

*Methods:* Forty-four cases of gastric carcinomas (24 intestinal-type; 18 diffuse-type and two mixed according to the Lauren classification) were analyzed for ras oncoprotein expression using commercially available antibodies in routinely formalin-fixed paraffin-embedded tissue sections.

*Results:* Altogether, thirteen cases stained unequivocally and these appeared as granular cytoplasmic staining in tumor cells. Only five were intestinal-type carcinomas; the remaining eight were diffuse-type and included two intramucosal carcinomas. When the comparison between the two subtypes was limited only to the poorly differentiated carcinomas, the difference is statistically significant ( $p < 0.05$ ). Ras oncoprotein was also strongly expressed in normal duodenal mucosa as well as areas of gastric intestinal metaplasia.

*Conclusion:* Our results suggest that the strong expression of ras oncoprotein in gastric intestinal metaplasia may be merely a reflection of metaplastic change to an intestinal epithelial phenotype. The differential expression of ras oncoprotein between diffuse-type and poorly differentiated intestinal-type gastric carcinoma implies that these are two distinct subtypes of gastric carcinoma.

### 4. Cytomorphological aspects of benign breast lumps

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A detailed cytomorphologic study was done at the Department of Pathology, Maulana Azad Medical College, New Delhi (India) on fine needle aspiration (FNA) smears from 650 benign breast lumps. Cytologic categorization enabled the distinction of proliferative from non-proliferative and infective lesions in the majority of cases. Lumpectomy provided the histologic diagnosis in 584 cases, most of which were proliferative lesions. Gross cystic disease (GCD) and fibroadenoma (FA) were the commonest lesions encountered. Microcysts with apocrine change, sclerosing adenosis, proliferative disease without atypia, atypical ductal hyperplasia, atypical lobular hyperplasia, and lobular neoplasia in situ were associated with the dominant lesion in many of the cases. In all these cases, retrospective analysis of the cytologic smears was done in an attempt to identify cytologic features which may indicate these lesions.

### 5. A novel finger protein in lysosome recognized by an autoantibody present in the serum from a patient with SLE

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Autoantibodies have been used extensively by investigators in molecular and cell biology studies. It is fairly clear that many autoantigens are proteins or subcellular particles which are involved in important or even essential biological functions in the cell. For instance, autoantibodies to centromeres, to nuclear lamins and to topoisomerase have been used for molecular cloning of the corresponding proteins, and autoantibodies to UI RNP and to Sm have given us important insights into the splicing of pre-messenger RNA. However, the functions of a number of these cloned nuclear autoantigens still remain unclear. With the introduction of cultured cell lines for the detection of antinuclear autoantibodies by immunofluorescence, autoantibodies to cytoplasmic components can also be readily visualized. However, these anti-cytoplasmic autoantibodies have been largely neglected.

In this study, we demonstrate an autoantibody to the structure of cytoplasmic vesicles by immunofluorescence; this autoantibody has been shown to react with a 180 Kd protein by immunoblotting. Using this autoantibody as a probe, we have identified, cloned and sequenced a corresponding cDNA from a HeLa cell cDNA expression library. Based on the cDNA sequence obtained, the primary sequence of the finger protein motif was deduced. A comparison of the obtained sequence with data in the literature led to the conclusion that it is a novel finger protein.

#### **6. Prevalence of enterococcal high-level aminoglycoside resistance in Japan: comparative detection by three methods**

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A total of 250 strains of enterococci isolated in Kumamoto University Hospital, Japan during the period from January to March 1992 were tested for high-level aminoglycoside resistance. Brain heart infusion agar plates supplemented with 1000 ug/ml of gentamicin and 2000 ug/ml of streptomycin detected 164 (66%) isolates resistant to either gentamicin or streptomycin alone, or both, comprising of 107 (43%) resistants to gentamicin and 96 (38%) resistants to streptomycin. The Vitek Gram-Positive Susceptibility cards (GPS-TA) revealed high correlations with those by agar screens, the results indicating 100% and 99% sensitivities to gentamicin and to streptomycin, respectively and 100% specificities to both. Also, the microdilution tests of National Committee for Clinical Laboratory Standards (NCCLS) showed 100% and 92% sensitivities to gentamicin and to streptomycin, respectively, and no false resistant (100% specificity) when compared with the results by agar screens.

#### **7. Resistance patterns of commonly isolated bacteria in the National University Hospital, Singapore**

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The commonly isolated organisms *S.aureus*, *Ps aeruginosa*, *Pseudomonas* sp., *Acinetobacter* sp., *E. coli*, *Klebsiella* sp., *Proteus* sp. and *Enterobacter* sp. from clinical material were analysed for their incidence of resistance to the commonly used antimicrobials. Identification was done by using conventional tests and rapid kits and antimicrobial sensitivity was performed by the Kirby - Bauer method. *S. aureus* was tested against penicilin (PG), methicillin (CLX), erythromycin (E), gentamicin (GN), and co-trimoxazole (SXT); *Ps aeruginosa* against amikacin (AK), ceftazidime (CAZ), GN, piperacillin (PIP) and cefsulodin (CFS); and Gram negative bacilli against ampicillin (AM), SXT, GN, nalidixic acid, nitrofurantoin, cefalexin (CF), cefuroxime (CXM) and ceftriaxone (CRO). A high degree of resistance was noticed among the commonly isolated organisms to the commonly used antimicrobials.

#### **8. Use of interactive multi media in the archival storage and self instructional learning of histopathology**

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Archival use of Data base software has been widely used in the histopathology laboratories while the actual biopsy report is linked to the patient record through word processing file. Study or review of Histopathology of a particular lesion therefore would involve sorting, sifting through the files and actual examination of the slides under the microscope. In order to offset these time consuming steps as well as to encourage independent learning a computer programme has been developed in our school using interactive multi media, whereby a student can learn or review histopathological and

clinicopathological correlations of a particular lesion in a series of patients by linking different files containing patient data base, histopathology report, graphic images of gross and microscopic appearances of the specimens etc using chooser manual. The programme uses Macintosh 4D software for a powerful data base capable of rapid multi-parameter sorting and analysis, which is linked to the word processing file containing detailed clinical history, gross and microscopic appearance of the lesion and finally linking these files with graphic images of gross and microscopic pictures of the lesion digitised through the use of a videographic card and photomicroscope fitted with a CCD camera. The data base of text and graphic information can be compressed into a CD-ROM for mass storage. Insertion of self assessment questions, "Pop up" buttons for further explanation or reading references, "zooming" of specified graphic areas for detailed morphology or identification skill can be introduced to make the programme more interactive as well as self evaluation of skill acquired. The same programme therefore can be used for:

- (a) archival storage of data for the Department
- (b) self instructional learning for the students
- (c) clinical referencing