



Division of Anatomical Pathology

Head: Prof Dhiren Govender

Diagnostic services

The Division of Anatomical Pathology provides comprehensive diagnostic histopathological, cytopathological and autopsy services to Groote Schuur, Red Cross Children's, Somerset and Victoria hospitals, which belong to the University of Cape Town's academic hospital complex. There are separate laboratories at Groote Schuur and Red Cross Children's hospitals. The latter has been SANAS-accredited since 2005. In addition, diagnostic services are offered to the University of Cape Town Private Academic Hospital and consultative and referral services to laboratories in East London, Port Elizabeth, Cape Town, Durban and Windhoek.

The Groote Schuur histopathology laboratory received 26,124 surgical pathology cases (including many cases with multiple specimens) during 2008, and the Red Cross laboratory received 2,658 cases. The cytopathology laboratory dealt with 54,284 cases, of which 44,263 were cervical smears, and 10,021 non-gynaecological cases. The division obtained a new transmission electron microscope. The electron microscopy unit processed 503 specimens in total and the immunohistochemical laboratory performed 13,709 tests. A consultative service for muscle biopsies is based at the Red Cross Children's Hospital; during the reporting period 87 muscle biopsies were processed, 78 of these were referrals from external laboratories.

During the reporting period 140 adult autopsies (only 24 of these cases were from Groote Schuur Hospital), 64 paediatric autopsies and 100 neonatal autopsies were performed. The foetal and perinatal service examined 72 fetuses and 727 placentas.

Consultants and registrars participated in 50 clinicopathological meetings per month held at Groote Schuur and Red Cross Children's hospitals.

Research projects

- Thioredoxin and human papillomavirus in oropharyngeal carcinoma
Researchers: Dr M Locketz, Prof D Govender
Collaborators: Dr K Pekkari (Danderyds Hospital, Sweden), Dr M Pekkari (Karolinska University Hospital, Sweden), Prof J Fagan (UCT/Groote Schuur Hospital (GSH))
- Mechanisms of fibrosis in pulmonary tuberculosis
Researchers: Dr CM Dittrich, Dr ML Locketz, Dr HC Wainwright
Collaborators: Dr L Bekker (Desmond Tutu HIV Institute/UCT), Dr G Walthar (GSH), Dr G Kaplan (Public Health Research Institute, USA), Dr J McKinney (The Rockefeller University, USA), Dr D Russell (Cornell University, USA)
Funding: NHLS Research Trust
- An efficient method for the detection of Microsporidia in formalin-fixed duodenal biopsies
Researchers: Dr C Maske, Dr C Walker
- Markers of oxidative stress in human placentas that have been exposed to excess alcohol *in utero*
Researchers: Dr S Malaka, Dr H Wainwright, Dr C Maske

- Collaborators:* Foundation for Alcohol-Related Research, Rondebosch
Funding: NHLS Research Trust
- Studies on skeletal dysplasias detected at postmortem
Researchers: Dr H Wainwright, Prof Beighton (UCT)
- Postmortem studies of hyperthermia/heatstroke in neonates and young children in the absence of exercise
Researchers: Dr C Warner, Dr H Wainwright
Collaborators: Prof L Martin (UCT Forensic Medicine), Prof T Noakes (UCT Sports Medicine)
- Prevalence of causes of arterial vascular disease in young South Africans
Researchers: Ms R Alexander and Dr G Gunston (UCT Human Biology), Dr L Liebenberg (UCT Forensic Medicine), Dr H Wainwright
Funding: Department of Human Biology, UCT
- Dysadherin expression in breast carcinoma: relationship to lymphangiogenesis and tumour microenvironment
Researchers: Prof D Govender (UCT), Dr AI Motala
Collaborators: Dr E Murray (UCT, Groote Schuur Hospital)
Funding: NHLS Research Trust
- Expression of TKTL-1 in favourable and unfavourable histology neuroblastoma
Researchers: Dr H-T Wu, Dr D Govender (UCT)
Funding: NHLS K-funding
- Wnt signalling pathway in Ewings sarcoma/PNET: an immunohistochemical investigation
Researchers: Dr H-T Wu, Prof D Govender (UCT)
Funding: NHLS Research Trust
- Histopathology and immunohistochemical expression of cell cycle regulators, mismatch repair gene proteins and MUC proteins in colorectal carcinoma: a comparative study
Researchers: Dr S Khosa, Prof D Govender (UCT)
Collaborators: Prof R Ramesar (UCT), Prof A Mall (UCT)
Funding: NHLS Research Trust
- Role of melanoma cell adhesion molecule expression in intermediate trophoblast invasion in pre-eclamptic and non pre-eclamptic placentae
Researchers: Dr D Maartens, Dr H Wainwright, Dr C Maske
- A study to investigate the immunoprofile of large cell lymphomas and correlation with HIV status and prognosis
Researchers: Dr S Pather, Dr K Pillay, Dr Z Mohammed (UCT), Prof N Novitzky (UCT)

Teaching and training

Undergraduate

The consultant staff are responsible for delivering undergraduate teaching in anatomical pathology to MBChB students during semesters 3-5 in an integrated, problem-orientated (case-based) course, with computer-based tutorials and small-group teaching (i.e. museum and mortuary demonstrations). A limited number of students gain access to a special study module in anatomical pathology in semester 4, currently the best and earliest opportunity of attracting future anatomical pathologists into the discipline. Third year BSc (Occupational Therapy) and BSc (Physiotherapy) students are taught by anatomical pathology consultants as an integral part of the clinical sciences course for the allied health sciences.

Postgraduate

There were 11 registrars, one supernumerary registrar and one forensic medicine registrar in training during the reporting period. One registrar was successful in the FCPath (Anat) Part 1 examination. The two registrars who were successful in the FCPath (Anat) Part 2 examination accepted consultant posts in the division. The forensic medicine registrar who rotated through the division during the year was successful in the FCForPath Part 1 examination. The consultant staff also participated in teaching in the BSc (Honours) course.

Medical technologists

The division provides training for student histotechnologists and cytotechnologists. Two student histotechnologists were successful in the Board examinations.

Research output

Publications published: 13
Conference presentations:
International: 3
National: 8

Division of Chemical Pathology

Head: **Prof T Pillay**

Diagnostic services

The **Integrated Clinical Pathology Laboratory** at Groote Schuur Hospital (GSH) (comprising chemical pathology, haematology, immunology, allergology, virology and microbiology) has held SANAS accreditation since 2006. This laboratory passed a functional audit with four stars in February 2009, being assessed by the leading SANAS assessor as the best run laboratory in the region. The laboratory provides a routine diagnostic service to GSH and other provincial healthcare facilities in the Western Cape region, as well as to UCT Private Academic hospital. In addition, it also offers a clinical trials service.

The chemical pathology service is highly automated, with 95% of all requested tests performed using the Roche MODULAR™ analyser. New tests recently added to the automated test repertoire include CEA, PCT and CA15-3 and acetylcholine receptor antibody. Urinalysis was automated this year with the introduction of the Siemens Clinitec Analyser. The laboratory has also experienced an increased workload in the last year due to the expansion of antiretroviral rollout in the primary health clinics, thus leading to the need for an after hours service.

The radioimmunoassay (RIA) laboratory offers a unique routine diagnostic service. A new gamma counter and data analysis software were acquired to perform this service. Four of the tests offered (active renin, aldosterone, 17OH progesterone, acetylcholine receptor autoantibody) are not available elsewhere in the Western Cape, and one (11-deoxycortisol) is not offered elsewhere in the country. In addition, the laboratory performs any esoteric RIA/IRMA assays requested. These include: leptin, adiponectin, plasma renin activity, and atrial natriuretic peptide. The training of registrars in RIA principles and methodology has continued to be successful.

The clinical chemistry laboratory located at **Red Cross Children's Hospital** forms part of the registrar training platform at the University of Cape Town. The laboratory received its second SANAS accreditation in July 2008 and subsequent to that two new instruments have been placed in the laboratory, i.e. a Beckman DXC replaced the old CX9 with smooth transfer of the routine diagnostic test menu and an Abbott AxSYM was placed to continue therapeutic drug monitoring assays that will be discontinued on the old TDX instrument. The test volumes for the inherited metabolic disease (IMD) diagnostic service increased as more laboratories within and outside the NHLS have commenced utilising the service. The laboratory has also instituted external quality control schemes for seven IMD methods, including urine organic acid analysis and amino acid analysis and is currently gearing towards SANAS accreditation of these methods. New methods implemented during the year include quantitative enzymatic ketone body and pyruvate analysis, red cell pyruvate kinase activity, red cell cytochrome B5 methaemoglobin reductase activity as well as a comprehensive development and validation of an isotope dilution GCMS method for leucocyte cystine.

Frontline GC/MS technology has been established within the **Inherited Metabolic Disease Laboratory**. This caters for the analysis of tissue organic and amino acids and various derivatives of these compounds. This division now offers critical ward/laboratory liaison, versatile cell culture facilities, highly adaptable enzyme assay expertise for organelle function, expert nucleic acid analytical expertise and bioinformatics and a metabolomics capability for small molecule profiling of metabolic disorders.

The DNA diagnostics section was accredited in April 2008. The tradition of introducing new diagnostic tests continued, with nine additional genetic assays being offered. These tests either deal with single common mutations or with mutation screening of all the exons of the gene in question. The new listings are alpha-1 anti-trypsin deficiency, AMP deaminase deficiency, Bath syndrome (TAZ), carnitine palmitoyltransferase 2 (CPT2) deficiency, glutaryl-co-enzyme A dehydrogenase deficiency (GA-1), ornithine transcarbamylase deficiency, primary hyperoxaluria (AGTX), pyruvate dehydrogenase alpha E1 deficiency and SURF 1 deficiency.

Another highlight was the part acquisition of an automated DNA extractor for small numbers of samples. This ideally suits the IMD laboratory as low sample numbers is the norm, with extensive testing often done on individual samples. The automated DNA extractor will be shared between IMD, and the genetics, haematology and tissue immunology laboratories.

Research projects

Molecular genetics of the IL12p40 gene, of the IFN γ pathway, in the resistance or susceptibility to TB in children

Researcher: Ms S Pienaar (for MSc)

Supervisors: A/Prof H Henderson, Dr B Eley

Funding: Bristol-Meyers Squibb Secure the Future grant

Sequence analysis of the promoter region of the IL12p40 gene in a large cohort of children with TB has uncovered two interesting polymorphisms (SNPs). The functional significance of these SNPs is being determined in reporter gene assays.

C5/6 gene frequency of mutations in the local population

Researchers: Dr EP Owen, A/Prof H Henderson

Collaborator: Dr A Orren (Cardiff University)

Funding: Medical Research Council

C5: Two patients previously diagnosed as suffering complement component C5 deficiency (C5D) have been examined and three out of the four mutations were found. Two pathological mutations of the C5 gene have previously been described in three American black families. The researchers intend establishing the mutation frequency in blood spots extracted as part of this project as they may be important defects responsible for C5D.

C6: DNA has been extracted from 1,000 newborn blood spots from both mixed ancestry and African race groups. Four common genetic defects found in the Western Cape and responsible for complete C6D are 821delA, 828delG, 1138delC and 1879delG. Initial results show that heterozygotes are at an incidence of 6/100 in neonates of mixed ancestry. This makes the frequency C6D equal to that of other common diseases such as cystic fibrosis. Therefore, this genetic disease, which dramatically increases susceptibility to *Neisseria meningitidis* infections, may play a far bigger role in influencing incidence and outcome of meningococcal disease in South Africa than has been anticipated. Allele frequency in African neonates still needs to be determined.

Screening for single nucleotide polymorphisms (SNPs) and mutations in the XPNPEP2 gene in black South Africans with angioedema induced by angiotensin I-converting enzyme inhibitors

Researcher: Dr EP Owen

Students: R Moholisa (MSc), Dr J Stark (4th year elective student from UK)

Collaborators: Prof B Rayner, Prof E Sturrock

Funding: NHLS

The condition of angioedema (AEi) and low serum APP (chromosome 10) has been linked to aminopeptidase P membrane bound (APPM) (X-linked) and the C-2399 A SNP, thought to be an area where HNF-4 and PPAR/RXR bind. To date, samples have been collected from 140 hypertensive patients of which 38 had a known history of AEi caused by enalapril and 102 control patients all on enalaprin for at least two years with no history of AEi. Initial results indicate no link between the C-2399A SNP and AE1 in patients. X-inactivation patterns showed no skewing in females. ACE-ID genotype showed that the majority of black AEi patients showed ID and DD genotypes giving a higher D allele frequency of 75% vs 53% in controls, which may be associated with incidence of AEi. ACE-DD genotype expresses increased levels of ACE activity which may contribute to hypertension and require high dosages of ACE inhibitors. Future work includes ACE, APP enzyme activities, and investigations into DPPIV genotyping and activity.

Trial to evaluate Xpert *Mycobacterium tuberculosis* test using a Cepheid instrument compared to smear, culture and nucleic acid amplification test

Researcher: Ms F Leisengang

Collaborators: Dr C Boehme, Dr G Hussey, Dr M Nicol

Funding: FIND Diagnostics

The study involves an evaluation carried out by the Foundation for Innovative New Diagnostics, Geneva, Switzerland. This organisation has a developmental agreement with the Cepheid instrument company to deliver a fully automated molecular platform for TB case detection and drug resistance testing in high-endemic countries. The trial seeks to achieve the following objectives: evaluate the performance characteristics of the 'new generation' Xpert *Mycobacterium tuberculosis* (MTB) test compared to smear and culture results; evaluate the performance characteristics of the Xpert MTB test compared to conventional drug susceptibility testing, and evaluate the performance of Xpert compared to standard nucleic acid amplification testing.

Domain A of sarcoplasmic reticulum Ca²⁺-ATPase forms part of the ATP binding site

Researchers: Prof D McIntosh, Mr D Woolley

Collaborator: JP Andersen (Aarhus University, Denmark)

Funding: National Research Foundation and UCT

Crystal structures of sarcoplasmic reticulum Ca²⁺-ATPase suggest that *domain A* could form part of the ATP site in certain conformations. Site-directed mutagenesis of relevant amino acid residues in *domain A*, combined with the unique ATP binding assay using the radio-labelled ATP analogue, [γ -³²P]TNP-8N₃-ATP serves to confirm that ATP interacts with *domain A* in E2 conformational states.

An *in vitro* study using a liver cell line (hep-G2) to elucidate the biochemical mechanism for alcohol-induced hypoglycaemia

Researcher: Dr P Berman

Collaborator: Miss I Baumgarten

The working hypothesis is that NAD-dependent acetylation (by sirtuin-1) of PCG-1 α is required for induction of gluconeogenic gene transcription, and that alcohol metabolism interrupts this pathway by transiently transforming cytosolic NAD to NADH. Strategies including the use of the alcohol dehydrogenase inhibitor fomipazole, and the general sirtuin inhibitor nicotinamide will be employed to substantiate this hypothesis.

Rapid sensitive measurement of leucocyte cystine by isotope dilution gas chromatography-mass spectrometry

Researchers: Dr G van der Watt, B Bahar, F Omar, B Bergstedt

Funding: NHLS K-fund

This project was instituted and completed within the laboratory to develop and validate a novel gas chromatography-mass spectrometry method for the determination of leucocyte cystine, an analyte used to diagnose and monitor patients with nephropathic cystinosis. Many of the known patients countrywide and farther afield with this disease are managed by the specialised renal and transplant services based at Red Cross Hospital and as such this method has greatly enhanced the level of patient care offered by this institution.

Evaluation of thyroid hormone autoantibody interference on automated free-T4 immunoassay platforms

Researchers: Dr G van der Watt, Dr D Haarburger, Dr P Berman

Funding: NHLS K-Fund

This recently completed project described the interference of specific autoantibodies on the Advia Centaur FT-4 immunoassay platform and demonstrated that this type of interference could be elucidated and quantitated in patient serum samples using a simple polyethylene glycol precipitation protocol. The method described can therefore be used by laboratories to exclude this type of interference in test samples with non-concordant thyroid function test results.

Development and assessment of a DNA multiplex screening assay for detection of glutaric aciduria type 1 and galactosaemia

Researchers: Dr G van der Watt, A/Prof H Henderson, Dr EP Owen

Funding: NHLS Development Grant

This research project has been initiated based on the fact that all known African patients diagnosed with galactosaemia or glutaric aciduria type 1 (GA 1) in South Africa have been homozygous for a single point mutation in each disease, respectively. Available data suggest that the carrier frequencies of these mutations may be much higher than previously thought and that these treatable diseases may be significantly underdiagnosed. This project entails the development of a robust multiplexed molecular assay for detection of these mutations in low volume samples such as dried blood spots and the subsequent utilisation of this assay to screen for the mutations in a large cohort of previously obtained dried blood spots. These data will be used to predict the expected disease burden for these disorders in the African population. The last leg of this study will entail screening a large cohort of patients with cerebral palsy for the GA1 mutation.

Development of an isotope dilution gas chromatography-mass spectrometry method for analysis of amino acids in body fluids

Researchers: Dr D Haarburger, Dr G van der Watt

Funding: NHLS K-fund

Current gas chromatography-mass spectrometry methodologies utilising a single internal standard for amino acid quantitation are hampered by poor reproducibility and accuracy when compared to routine high-performance liquid chromatography methods. In an attempt to improve these weaknesses, a method is being developed whereby a unique algal-derived physiological mixture of deuterated aminoacids is being utilised to develop a novel simple and accurate method for amino acid quantitation

Glucocorticoid receptor haplotypes and cortisol sensitivity

Researcher: Dr I Ross (Endocrinology)

Supervisor: Prof T Pillay

Collaborators: Dr J Dave, Prof N Levitt

This project examines the role of glucocorticoid receptor polymorphisms and the influence on cortisol sensitivity. Specifically, it aims to test the hypothesis that the common polymorphisms are to some extent influencing the metabolic parameters among patients with Addison's disease by virtue of their increasing or decreasing sensitivity to glucocorticoids.

The relationship between vitamin D, calcium and parathyroid hormone

Researcher: Dr D Haarburger

Supervisor: Prof T Pillay

Collaborators: Dr M Hoffman, Prof R Erasmus

The aim of this study was to test the hypothesis that vitamin D deficiency is associated with abnormal levels of calcium and the parathyroid hormone (PTH). The study has revealed that hypovitaminosis D may co-exist with a blunted PTH response. Therefore, assumptions about vitamin D status should not be made based on PTH and calcium values. 25-hydroxy-vitamin D measurements should be requested when vitamin D deficiency is clinically suspected, irrespective of biochemical results.

Quality of teaching in chemical pathology and the ability of interns to request and interpret laboratory tests

Researcher: Mr J Macauley (medical student)

Supervisors: Prof T Pillay, Dr J Stanfliet

The study investigated the ability of interns to order and interpret the results of common laboratory tests. The results of the study indicate that institutions need to develop strategies to combat the attrition in the depth of pathology teaching in medical schools in South Africa. The decrease in the amount of pathology teaching has had a knock-on effect on the ability of junior doctors to use laboratory tests efficiently.

Teaching and training

Undergraduate

Undergraduate teaching in chemical pathology to medical students in the 3rd, 4th, and 5th semesters consists of formal lectures, large group tutorials, problem-based learning facilitation and online sessions on Vula, the e-learning interface at the University of Cape Town.

Postgraduate

The DNA section of the Inherited Metabolic Disease Laboratory supervised two PhD candidates and one MSc candidate.

Registrars in chemical and clinical pathology receive formal teaching in tutorials by the consultants. These consist of issues of assay methodology, mechanisms of disease and result interpretation. Registrars also receive weekly training in various aspects of laboratory management, tutorials in molecular biology and diagnostics.

Medical technologists

A 10-lecture series was delivered to medical technology students in preparation of their clinical pathology board exams. All five intern technologists passed the Medical Technology Board Examination.

Honours

The UCT Chemical Pathology website has continued to be rated as the number 1 website, out of nine million websites, using the search term 'Chemical Pathology'.

Prof T Pillay was awarded a Fellowship by peer review of the College of Pathologists of South Africa. The Fellowship by peer review is intended as an important token of recognition of the Colleges of Medicine of South Africa, and by its constituent colleges, of the scholarship, clinical expertise and high professional standing of the recipient.

Dr F Omar, a senior registrar in Chemical Pathology, received the best Masters poster award at the Faculty of Health Sciences research day for her presentation titled 'Protease inhibitors decrease HMW: total adiponectin ratios in Xhosa female patients with HIV'. Dr Omar also received the best poster award for her poster at the 48th Congress of the Federation of South African Societies of Pathology, titled 'Reference range for non-esterified free fatty acids in neonates'."

Research output

Publications published: 11

Conference presentations

International: 7

National: 10

Division of Haematology

Head: **Prof Nicolas Novitzky**

Diagnostic services

The haematology section at the Groote Schuur Hospital site is a comprehensive 24-hour diagnostic service that has become an established referral centre for the academic hospital as well as for other state and private facilities in the province including the Eastern Cape.

The diagnostic functions are carried out at two main sites - one where the routine laboratory functions are performed within the 24 hour service laboratory and the other where the cytogenetics and molecular work is performed.

The laboratory offers routine tests to the local hospitals and outlying clinics, with volumes increasing, partly due to the continued antiretroviral rollout. The total numbers of all haematology tests during the reporting period were 744,995. The test repertoire includes the normal routine haematology as well as the functional thrombophilia tests and routine haemophilia testing for both factor assays and inhibitor screening. As part of the diagnostic workup, the cytometry panels have been expanded to aid in better diagnosis, classification and management of various blood disorders. This laboratory receives numerous samples from other centres including the private sector. The exponential increase in pan-leukocyte for CD4 evaluation (74,498) has continued. The antenatal section of the laboratory receives a large number of samples for Hb, ABO, Rhesus and antibody detection.

Haematology molecular genetics was again accredited by SANAS and BCR/ABL p210 quantitative and Jak2V617F were added to the accreditation schedule. Two new assays are now online: Flt3 ITD detection and qualitative p190 BCR/ABL detection, with validations and standard operating procedures completed for both tests. Four new assays are currently in development: BCR/ABL kinase mutation detection, PML/RAR α (t15:17) diagnostic assay, Terc/Tert mutation detection and STR analysis.

In the cytogenetics unit, fluorescence *in situ* hybridisation (FISH) has been developed on site, which has improved the turnaround time to the clinicians. FISH is offered on a limited panel and includes BCR/ABL (chronic myeloid leukaemia), del(13q14) and del p53(17p13) for chronic lymphoid leukaemia and myeloma; t(8;21)(q22;q22), 11q23, and inv(16) for acute myeloid leukaemia; 14q32 for lymphoma. Methodology for the culture and chromosome analysis on chorionic villus biopsies has also been added which allows earlier detection of chromosome abnormalities, and aids in the provision of more acceptable treatment. Cultures and chromosome analysis on fibroblast cultures, allowing post mortem chromosome analysis have also been developed.

Research projects

Prof N Novitzky directs the UCT Leukaemia Unit, supervises the laboratory component of the stem cell transplantation programme and is involved in a number of projects.

Search for a chronic lymphoid leukaemia antigen

Student: Mr S Njikan (Masters)

Supervisor: Dr K Shires

Funding: NHLS Research Trust, Cancer Association of South Africa

The primary aim of the project is to identify unique changes on the BCR/ABL transformed cell surface that can be used for vaccine development in chronic lymphoid leukaemia or targeted drug therapy. The first step is to establish a stable primary cell line transfected with BCR/ABL. Four plasmids have been characterised that will be used for transfection of the BCR/ABL oncogene. NIH3T3 cells have also been transfected with the MIGR1 and MIGp210 plasmids to test the methodology. Assays have been established to confirm BCR/ABL expression in transfectants, including reverse transcriptase polymerase chain reaction of BCR/ABL and PRAME, mitochondrial apoptotic pathway analysis, western analysis of BCR/ABL and p27 expression and actin filament staining.

Unraveling apoptosis malfunctions in myelodysplastic syndrome

Student: Ms S Rossouw (Masters)

Supervisor: Dr K Shires

Funding: Medical Research Council

The main aim of the project for the last year was to establish all the assays that were needed to reliably detect the three caspase-dependent apoptotic pathways in myeloid cell lines. This part of the project is now complete. The main finding, which was tested through the analysis of pathway-specific drug testing on HL60, was that only caspase assays, detecting the release of AMC substrates with a fluorimeter provides a reliable, reproducible and sensitive assay that can be utilised in a myeloid setting. This information will now be used in the analysis of apoptosis in myelodysplastic syndrome samples.

Diagnosis, prognosis and molecular monitoring in acute promyelocytic leukaemia

Student: Dr T Gerdener (MMed)

Supervisor: Dr K Shires

Funding: NHLS Research Trust

In order for improvements to occur in the treatment protocols for acute promyelocytic leukaemia, it is necessary to develop reliable and sensitive methods for detecting and monitoring the levels of PML-RAR α , as well as prognosis indicators such as Flt3 mutations. A routine assay was developed to detect the three major isoforms of PML/Rar α , as well as several bcr1 sub-isoforms using real-time PCR. This assay should shortly be on-line in the diagnostic laboratory. A sensitive detection method for Flt3ITD mutations has been established which has been implemented as a routine assay since September 2008. The quantitative PML/Rar α assay is still under development.

Bone marrow transplant stromal damage: identification of the damaged signaling pathways involved in stem cell homing and self renewal

Student: Mr A Morris (Masters)

Supervisor: Dr K Shires

Funding: NHLS Research Trust, Medical research Council

In order to improve patient prognosis following BM radiation ablative therapy, it is imperative to define the stromal/haematopoietic stem cell (HSC) signaling pathways that are affected by this treatment. Radiation-induced stromal damage is being assessed by microarray analysis, concentrating on expression of genes specifically involved in HSC homing and self renewal. All of the assays needed to monitor the cellular response to radiation have been defined. The response of fibroblasts to radiation has been tested, looking at the expression of Intg1, FasLG and Jag1, where definite changes in transcript levels have been observed.

Cancer-testis antigen expression in myeloma

Researcher: R Mohamed

Supervisor: Dr K Shires

Using a restricted cancer-testis antigen (CTA) panel, the expression of 15 CTAs is being investigated simultaneously in newly diagnosed/untreated myeloma patients. In addition to confirming the expression of PRAME, SSX-2 and Spanxb as other researchers have found, two novel discoveries have been made, the first being the observation that CTA expression is also found in the peripheral blood of these patients, implicating a circulating cell in the malignant clone; the second being the unexpected high expression of Rbf1, a newly described protein involved in ribosome binding and translation. The expression of this gene is being investigated further.

The diagnostic utility of bone marrow examination performed for the investigation of fever and/or cytopenias in HIV-infected adults at Groote Schuur Hospital

Researchers: W van Schalkwyk, J Opie, N Novitzky

A retrospective review of the medical and laboratory records of HIV-positive adults who underwent bone marrow biopsy for the investigation of fever and/or cytopenias at Groote Schuur Hospital from January 2006 to March 2007 is being done to evaluate the diagnostic yield of bone marrow samples taken from HIV-infected patients with respect to histopathological and microbiological diagnoses and to determine the most common diagnoses made and how often the bone marrow biopsy results in a change of patient management. The most frequent diagnosis is disseminated mycobacterial infections, particularly in patients with advanced immunodeficiency infection.

Teaching and training

The department is a primary teaching and training site for technologists, technicians, and includes both the undergraduate and postgraduate rotations. In addition, training has been undertaken for students from other laboratories covering mainly the special haematology investigations.

The training of registrars is a high priority with haematology pathology, clinical pathology and clinical haematology trainees accommodated. One candidate passed clinical haematology and a number of other registrars are writing their final exams in the coming year.

Participation in the undergraduate and postgraduate programmes of the University of Cape Town continues with input into the undergraduate problem-based learning facilitation and lecture modules, as well as BSc Honours and Masters programmes.

Research output

Publications published: 5

Conference presentations

International: 1

National: 12

Local: 3

Division of Immunology

Head: **Dr Muazzam Jacobs**

Diagnostic services

Studies investigating the establishment of a diagnostic test to assess steroid insensitivity in cases of 'difficult-to-control' or refractory asthma are ongoing in collaboration with Prof Motala, Head: Allergy and Asthma Service, Red Cross Children's Hospital. There is currently no such laboratory test available and the successful outcome of this study will have wide spread clinical applicability. A whole blood mitogen assay has been characterised in normal subjects and in children with 'difficult-to-control' asthma. The assay will further be evaluated by comparing the responses of known steroid responsive and steroid insensitive asthmatic children.

Research projects

UCT/NHLS-European/African Consortium: assessing immune modulation and pathogenesis of visceral leishmaniasis, schistosomiasis and African trypanosomiasis induced by HIV, malaria and TB co-infections

Researcher: Dr M Jacobs

Collaborators: Prof S Magez (Flanders Institute for Biotechnology, Belgium)

Funding: European Union (pending)

An international consortium, consisting of 19 international institutions, was established under the directorship of Prof S Magez, Flanders Institute for Biotechnology, Belgium, to generate a large scale integrating project proposal for submission under the FP7 programme to the European Union. The outcome of the application is pending.

Vaccine against TB: exploratory visit

Researcher: Dr M Jacobs

Collaborator: Dr J Birchall (Cardiff University)

Funding: National Research Foundation/The Royal Society

A successful application was made under the South African-United Kingdom Networks Program, NRF/The Royal Society to host Dr Birchall to explore the potential application of microtechnology in the delivery of a vaccine against tuberculosis in preclinical evaluation.

Preclinical evaluation of a nano-drug delivery system for TB treatment

Researcher: Dr M Jacobs

Collaborators: Dr H Swai (Council for Science and Industrial Research), Prof P Smith (Department of Pharmacology, University of Cape Town)

A preclinical evaluation of the potential for treating tuberculosis with nano-drug delivery systems is being undertaken. Ethical approval for such studies has been obtained from the Animal Ethics Research Committee, UCT and sample material exchanged.

Effects of medroxyprogesterone acetate on TB

Researcher: Dr M Jacobs

Collaborator: Dr K Ronacher (Department of Molecular Biology and Human Genetics, University of Stellenbosch)

This collaborative study investigates the impact of the contraceptive medroxyprogesterone acetate on susceptibility to tuberculosis in a preclinical *in vivo* model.

Investigation of the levels and role of CD4⁺ IL10⁺⁺ FOXP3⁻ and CD8⁺ IL10⁺⁺ FOXP3⁻ regulatory T cells in drug-responsive and extensively drug-resistant TB

Researcher: Dr B Nurse

Collaborator: Dr K Dheda (University of Cape Town)

This is a sub study of the protocol: Multidrug-resistant and extensively drug-resistant tuberculosis in the Western Cape Province. The first phase of the laboratory work has been started. Pilot studies for the next phase of the project is planned.

Tumour necrosis factor neutralisation therapy for inflammatory diseases: strategies to prevent TB reactivation

Researcher: Dr M Jacobs

Collaborators: Dr V Quesniaux and Prof B Ryffel (Centre National de la Recherche Scientifique Research (CNRS), France), Dr I Garcia (University of Geneva), Prof G Kollias (Alexander Flemming Institute), Prof S Nedospasov (German Rheumatology Research Center)

Funding: European Union

The project has addressed the role of tumour necrosis factor (TNF) in both acute and chronic TB infection studies using novel genetically modified mice. Highlights include the generation of a novel TNF mutant mouse strain, the importation and expansion of TNF/TNF receptor mutant strains from our European counterparts for local research

application. Six manuscripts and one book chapter have been generated under the programme.

The International Centre for Indigenous Phytotherapy Studies (TICIPS) evaluating the efficacy of *Artemisia afra*-derived compounds for anti-tuberculous activity *in vivo*

Researcher: Dr M Jacobs

Collaborators: Prof W Folk (University of Columbia), Prof Q Johnson (University of the Western Cape)

Funding: NCCAM (NIH)

This is an ongoing programme which forms part of a larger international collaborative research effort that includes the universities of Missouri-Columbia (USA), Texas (USA), Western Cape, Cape Town and KwaZulu-Natal. The UCT/NHLS component of the project has been concluded and final reports submitted.

TNFRp75 as a potential target for immunotherapy during *Mycobacterium tuberculosis* infection

Researcher: Dr M Jacobs

Collaborators: Dr V Quesniaux and Prof B Ryffel (CNRS)

Funding: National Research Foundation, NHLS, UCT

The study addresses the role of TNFRp75 as a signalling conduit and regulator of TNF-mediated immunity. This study shows that TNFRp75 inhibits immune function during exposure to *M. tuberculosis*.

Drug discovery of novel antituberculosis agents from South African natural products

Researcher: Dr M Jacobs

Collaborator: Prof P Folb

Funding: National Research Foundation

This is a South African-based project being investigated by a consortium consisting of the University of Cape Town, the SA Medical Research Council and the SA National Biodiversity Institute. It aims to investigate the efficacy of identified lead compounds *in vitro* and *in vivo*. Derivatives of lead compounds have been synthesised and evaluated in culture-based assays. Cooperative agreements with a Singapore Institute have been signed.

Tuberculosis of the central nervous system

Researcher: Dr M Jacobs

Collaborators: Dr M Combrinc and Dr L Kellaway (UCT)

Funding: National Research Foundation, Medical Research Council, NHLS

The project is an ongoing collaboration between the divisions of Immunology, Neurosurgery and Physiology at the University of Cape Town. It models immunity of the brain during challenge with virulent *M. tuberculosis*. The study forms the basis for a post doctoral project, a PhD thesis and a Masters thesis. Results generated have yielded novel findings in both tumour necrosis factor signalling in immune function in the brain in the context of TB infection.

The role of macrophage/neutrophil tumour necrosis factor in host immunity against *M. tuberculosis*

Researcher: Dr M Jacobs

Collaborators: Dr V Quesniaux and Prof B Ryffel (CNRS), Prof S Nedospasov (German Rheumatology Research Center)

Funding: National Research Foundation, NHLS

This study investigates the specific contribution of macrophage/neutrophil tumour necrosis factor (TNF) in host immune function during *M. tuberculosis* challenge. A critical dependence on TNF to control bacilli replication in gene deficient murine models has previously been shown. In this study a transient dependence on macrophage/neutrophil TNF during early innate infection is demonstrated but which does not compromise adaptive immunity during late infection. The study has been extended to address the effects macrophage/neutrophil TNF in latent TB.

Studies completed during the year include:

- Immuno-modulation: immune changes underlying immunotherapy for atopic rhinitis status
Researcher: Dr B Nurse
Collaborator: Dr R Fadel (Stallergenes, France)
- Inappropriate TH2 cytokine responses to mTB proteins in atopic asthmatic South African children
Researcher: Dr B Nurse
Collaborator: Prof PC Potter (UCT)
Funding: Medical Research Council

Teaching and training

The division provides lectures, tutorial and practical instruction at MBChB and BSc Honours level and offers research projects at BSc Honours, Masters, doctoral and post doctoral levels. The division participates in the problem-based learning programme offered to undergraduate students.

Research output

Publications published: 5
Conference presentations
 International: 1
 National: 1

Professional development

Candidates registered: 30 (13 post doctoral fellows, 10 doctoral, 4 Masters, 3 BSc Hons)
Candidates graduated: 5 (2 PhD, 3 BSc Hons)

Division of Tissue Immunology

Head: **Prof Ernette du Toit**

Diagnostic services

During the reporting period, the Laboratory for Tissue Immunology (LTI) continued rendering an HLA (tissue) typing service to Groote Schuur and Tygerberg hospitals as well as to other clinical institutions and doctors in private practice throughout the Western Cape. In addition, the service is also provided to the rest of South Africa and other countries on the African continent, particularly for haematopoietic stem cell transplantation. The LTI is still the only laboratory on the African continent to be accredited to the European Federation for Immunogenetics. This international accreditation enables the LTI to provide the level of tissue typing acceptable to the World Marrow Donor Association (WMDA) and other international institutions, for HLA typing of patients and potential unrelated and related stem cell donors, as well as solid organ transplantation.

During 2008 the LTI was also inspected for ISO 15189 accreditation with SANAS. This South African accreditation body is a recognised international accreditation body. In August 2008, after a successful external audit, the laboratory gained this status.

The South African Bone Marrow Registry (SABMR)

The SABMR was established in 1991 in the LTI, then residing under the Provincial Administration of the Cape. When the NHLS took over all laboratory services country wide, the SABMR continued to function within the LTI. The SABMR provides unrelated donors for all South African patients requiring stem cell transplantations. The SABMR is registered as a non-profit Public Benefit Organisation.

The objective of the SABMR is to be a national organisation with the responsibility of processing requests for haematopoietic stem cells from unrelated donors from local and overseas sources. Thus, the registry maintains a directory of HLA (tissue-typed) volunteers who are prepared to donate stem cells anonymously and without payment. The registry promotes expansion, both in number and ethnic diversity, of the database of haematopoietic stem cell donors in South Africa. It thus makes matched unrelated donor (MUD) transplantation available to a wider spectrum of the South African population.

For the optimal functioning of the SABMR, it is essential to have an internationally accredited HLA typing facility at its disposal. Fortunately for South Africa this is provided by the LTI. This symbiosis has resulted in two members of the LTI receiving training in HLA immunogenetics, being responsible for all administrative aspects of the SABMR. This involves numerous functions such as selection of the best HLA matched donor for a particular patient, co-ordinating the harvesting and transportation of the stem cells between the transplant and donor centre etc. The SABMR serves all South African patients with blood diseases such as leukaemia who require MUD stem cell transplantation.

This service costs considerably less than stem cell donations from international sources. To date, the SABMR has facilitated stem cell transplants in 150 patients in South Africa, in four transplant centres. Thirty-seven patients received stem cells from local donors and 113 patients had international donors. The total number of donors registered with the SABMR is now just over 64,000. Unfortunately this growth represents only HLA-AB typed donors and at present only 5,334 (8%) of the SABMR donors are HLA-ABDR typed compared to the international average of 75%.

Bone Marrow Donors Worldwide (BMDW), an organisation based in Leiden, The Netherlands, co-ordinates the collection and distribution of HLA phenotypes of the more than 13 million donors worldwide. The donor data on the BMDW website are used to track down possible donors for an individual patient. Last year in excess of 10,000 patients received stem cell transplants from an unrelated stem cell or cord blood donor. The BMDW now has 695 users from 454 organisations that are authorised to access the online BMDW services.

The WMDA is a global association of 69 registries and 49 cord blood banks worldwide. The WMDA was established in 1994 in order to provide guidelines and promote the use of haematopoietic cells from volunteer donors for transplant in different countries.

As the Hub centre for the whole of South Africa, the SABMR is a member of the WMDA and the BMDW.

When considering unrelated individuals as donors, HLA matching has to be as close as possible. Due to the extreme polymorphism of the HLA system, the chance of finding an HLA-matched unrelated donor is approximately 1:100,000, necessitating the establishment of very large panels of HLA-typed individuals. No single registry is entirely self-sufficient; all registries worldwide are dependent on each other.

Research

The Collaborative Transplant Study (CTS)

The CTS is an international registry of solid organ transplantation including renal, heart, heart-lung, lung, liver and pancreas transplants run by Prof G Opelz, Head of the Transplantation Immunology Unit, University of Heidelberg, Germany since 1982. The LTI has participated in this since its inception. The CTS collects all relevant clinical, HLA and other immunological data on transplant patients worldwide. To date 1,202 centres have contributed data on 307,773 kidney transplants and 105 centres on 43,920 heart, heart-lung and lung transplants. A total of 52,851 liver and 9,000 pancreas transplants have also been reported. The LTI is the only laboratory in South Africa participating in this study and has reported on 2,543 kidney, 527 heart and 61 liver transplants. A monthly newsletter is sent to all the participants with up-to-date scientific analysis of the data, including survival graphs etc. In addition, each centre has access to the international as well as its own data on a sophisticated CTS website allowing for confidentiality with individual passwords for centres.

Teaching and training

Postgraduate

Special training in laboratory methods as well as the immunogenetics of the HLA system is provided for consultants and registrars in haematopathology, oncology and similar disciplines at Tygerberg and Groote Schuur hospitals.

Medical technologists

Two LTI medical technologists were awarded degrees in BTech Medical Biotechnology and another received a degree in BTech Business Administration.

Research output

Publications published: 1
Conference presentations
International: 1
National: 2

Division of Medical Microbiology

Head: **Prof Mike Nicol**

Diagnostic services

The diagnostic laboratory has had a major thrust to improve efficiency over the last year. Both the blood culture and tuberculosis sections have adopted a paperless system. Apart from the positive environmental implications, this has also resulted in better utilisation of technologist time by eliminating unnecessary paperwork.

The rapidly expanding tuberculosis section has also acquired two additional MGIT machines due to an increase in specimen numbers and tuberculosis clinical trials. The Hain MTBDR_{plus} assay has been adopted for the identification of positive cultures and for rapid genotypic drug susceptibility testing with significantly improved turnaround times. In selected cases this assay has been used for direct drug susceptibility testing on sputum samples to provide an even more rapid result for the clinician.

The VITEK2 system has been interfaced directly with the laboratory information system to facilitate rapid identification of cultures and provision of drug susceptibility results. The introduction of direct inoculation of the VITEK2 cards from positive blood cultures has similarly improved turnaround times to provide early culture results for critically ill patients.

The drive for efficiency continues with the evaluation of two automated methods for urine microscopy, one of which will be introduced during the next year.

The laboratory played a major role at the height of the cholera epidemic, as the local reference laboratory for confirmation of the *Vibrio cholerae* isolates.

The diagnostic laboratory had its accreditation with SANAS renewed and received excellence awards for achieving external quality assurance targets, confirming the consistently high standards achieved. The laboratory has developed a reputation for excellence in tuberculosis diagnostics in which capacity it hosted and trained visiting scientists from across Africa during 2008.

Research

Shiga-toxin containing *Escherichia coli* from cattle and diarrhoeic children in the pastoral systems of south-western Uganda

Researchers: Dr S Majalija, Dr H Segal, A/Prof G Elisha

Funding: Rockefeller and Carnegie Foundations through the University Science, Humanities, and Engineering Partnerships in Africa programme

Dr Majalija completed his research on the characterisation of shiga-toxin containing *Escherichia coli* (STEC) from cattle and diarrhoeic-children, living in a pastoral community in Uganda. Serotyping of the STEC identified groups not previously identified in clinical isolates of this organism. Additionally, the intimin gene (*eae*), which is associated with the pathogenicity of STEC was detected in previously *eae*-negative serogroups. In summary, the genetic content of the STEC suggested that they have the potential to cause illnesses ranging from mild to bloody diarrhoea and haemolytic uraemic syndrome in children.

Molecular characterisation of methicillin-resistant *Staphylococcus aureus* from hospitals in the Western Cape

Researchers: Dr A Whitelaw, Dr E Madikane, A/Prof G Elisha

Funding: NHLS Research Trust

The epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals in Cape Town is poorly understood. Fifty-six clinical MRSA strains from five hospitals were characterised using pulsed-field gel electrophoresis (PFGE) and Staphylococcal Chromosomal Cassette *mec* (SCC*mec*) typing. PFGE identified intra- and inter-hospital-related MRSA, suggesting patient-to-patient transmission, either directly or, more probably, via the hands of healthcare workers, who circulate through the hospitals. The predominant MRSA SCC*mec* type (26 strains) was resistant to multiple antibiotics, including co-trimoxazole and rifampicin. This raised the question of whether the frequent use of co-trimoxazole and rifampicin for management of *Pneumocystis* infections and treatment of tuberculosis, respectively, has selected for MRSA able to survive these antibiotics.

The epidemiology of *Staphylococcus aureus* in the Western cape

Principal investigator: Prof BG Elisha

Co-investigators: Dr A Whitelaw, Dr E Madikane, Ms M Jansen van Rensburg

The study examines both the molecular epidemiology of MRSA isolates from Western Cape hospitals, as well as the mechanisms involved in the acquisition/development of resistance to other antibiotics, and how antibiotic pressure may influence the molecular epidemiology of *S. aureus*.

Performance outcomes of LED technology (Lumin) for microscopic detection of mycobacteria in a high HIV sero-prevalence setting in SA

Investigators: A Whitelaw, M Pai, D Viljoen, K Dheda

The aim of this study is to investigate the utility of a LED-based fluorescent microscopy for reading auramine-stained smears for the diagnosis of TB. The LED microscope is being compared to a mercury vapour lamp (current gold standard), as well as to Ziehl-Neelsen stained smear read with a light microscope.

Investigation of the prevalence and role of mobile genetic elements in *Acinetobacter baumannii*

Researchers: Ms T Jongwe, Dr H Segal

Funding: Faculty of Health Sciences, UCT

IS*Aba-1* contains promoter sequences for regulating transcription of a number of associated genes, including antibiotic resistance genes, under various stress conditions. Regulation and expression of genes located downstream of IS*Aba-1* are being investigated. All strains with IS1133 carry a portion of IS*Aba-1* associated with *aacC2*. Strains not carrying IS1133 did not contain *aacC2*. PFGE genotyping of strains from Groote Schuur Hospital over a 23-year period indicated that strains harbouring both IS1133 and *aacC2* were closely related, suggesting clonal dissemination of these strains. This genetic arrangement was shown to include a novel IS1422-like element. Using genomic DNA libraries and Southern hybridisation experiments, sequences flanking this genetic arrangement is being investigated to determine its association with pathogenicity islands in *A. baumannii*.

Characterisation of mixed infections of *Mycobacterium tuberculosis* in a sample of South African TB patients by spoligotyping

Researchers: Mr M Stead, Ms J Evans, Dr H Grewal (University of Bergen, Norway), Dr H. Segal

Funding: Medical Research Council, Faculty of Health Sciences, UCT

The prevalence of mixed infections of *M. tuberculosis* in a collection of samples has been investigated. The eight samples obtained consecutively from each patient over an eight-week period were included in the study if they were culture-positive. Spoligotyping of the isolates was carried out to determine the lineages present in each patient. Of the 77 patients genotyped, 48% were of the W-Beijing lineage and 18% were LAM 3. The remaining isolates included LAM 5, LAM 9, X3, X2, X1, T1, T4 and Haarlem. A total of five of the 77 patients have suspected mixed infections. A PCR assay designed to detect Beijing and non-Beijing strains was carried out to confirm the mixed infections in these patients.

Investigation of the genetic basis of multidrug resistance in *M. tuberculosis* from Groote Schuur Hospital

Researchers: Ms J Evans, Dr H Segal

Funding: Medical Research Council, NHLS Research Trust, National Research Foundation

Several rapid molecular assays for detection of drug resistance in *M. tuberculosis* have been designed, including MAS-PCR and GenoType MTBDR*plus* assays. MAS-PCR assays were performed on 224 *M. tuberculosis* isolates from Groote Schuur Hospital in 2006. These isolates were used to evaluate the efficacy of the GenoType MTBDR*plus* assay. Both assays produced comparable results. Notably, both assays failed to detect isoniazid (INH) resistance in a large proportion of INH mono-resistant strains, suggesting that these assays be used with caution in this setting. Spoligotyping indicated that W-Beijing is overrepresented amongst multidrug-resistant (MDR) strains, while 8/11 rifampicin (RIF) mono-resistant isolates are of the LAM3/F11 lineage. Mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) showed clustering of MDR W-Beijing strains harbouring *inhA* C-15T, and clustering of RIF mono-resistant LAM3/F11 strains, indicating that these strains may represent two distinct clonal lineages.

Integrated microanalytical extraction-amplification system for detection of tuberculosis in low resource settings

Researchers: Prof M Nicol, Dr D Kelso (Northwestern University, USA)

Funding: Center for Point-of-Care Diagnostics for Global Health, PATH

This study aims to develop and test a low-cost, robust, point-of-care device for the diagnosis of tuberculosis in low resource settings. This involves the development of an integrated specimen collection/processing container and integration into a novel real-time PCR platform.

Diagnosis of tuberculosis in HIV-infected children - development of microbiological and immunological strategies

Researchers: Prof M Nicol, Prof H Zar, Prof B Eley, Prof G Hussey, Prof R Wilkinson, Dr T Connell

Funding: NHLS Research Trust, National Institutes of Health (USA), MRCI

This study evaluates a range of novel molecular and immunological tests for their performance in the diagnosis of tuberculosis in children, particularly those infected with HIV. Induced sputum, nasopharyngeal aspirates, urine and site-of-disease samples are collected and used in a range of novel assays, including GeneXpert real-time PCR, MODS, site-specific ELISpot, urinary LAM.

The use of modern molecular tools to understand and intervene in the spread of multidrug-resistant tuberculosis

Researchers: Prof M Nicol, Prof G Hussey, Prof R Wilkinson, Prof T Victor, Prof R Warren, Dr D Theron, Dr S Gagneux

Funding: Wellcome Trust, EDCTP

The aim is to assess the impact of novel rapid diagnostic tests for multidrug-resistant (MDR)-TB (GeneXpert, Hain MTBDR*plus*) on treatment outcome of MDR patients and transmission of MDR-TB in the community. To assess the impact of the novel methods, two studies are being conducted: one using a contact-tracing design and the other a clinic-based intervention.

Innate immunity to TB is lineage- and host-dependent

Researchers: Prof M Nicol, Mr R Sarkar, Prof R Wilkinson, Dr K Wilkinson, Mrs K Wood

Funding: National Research Foundation

The aim was to determine whether different lineages of *M. tuberculosis* induce lineage-specific innate immune responses in macrophages. Spoligotyping, MIRU-VNTR and large sequence polymorphism analysis were performed to classify isolates into major strain lineages. A primary human monocyte-derived macrophage model was used to study characteristic innate cytokine profiles and *in vitro* growth rate. The data suggest that there are indeed lineage-specific patterns of cytokine induction and that clinical strains may be better adapted to intracellular growth than laboratory-adapted strains.

Cepheid Xpert MTB evaluation study

Researchers: Prof M Nicol, Prof G Hussey, Dr C Boehme

Funding: FIND

The aim is to determine the performance of a novel integrated real-time PCR device (GeneXpert) for the rapid detection of tuberculosis and rifampicin resistance amongst tuberculosis suspects. GeneXpert was performed in parallel to routine culture and drug susceptibility testing on sputum samples from patients with suspected TB

presenting to a peripheral clinic.

Teaching and training

Undergraduate

The division participates in the facilitation of problem-based learning for MBChB students as well as in integrated teaching now adopted by the faculty for senior medical students. Teaching activities include lectures, tutorials, seminars, practicals and computer-based learning. The division offers a 'Bugs and drugs' module and contributes to teaching a molecular techniques course for the BSc (Med) honours programme in the School of Biomedical Sciences. In addition, training is provided for student technicians and technologists.

Postgraduate

The division has an active postgraduate programme, including training at Masters and doctoral level. There are currently six registrars in training, five specialising in medical microbiology and one in clinical pathology. The division also provides a six-month laboratory training period for sub-specialist registrars in infectious diseases. Consultants also offer a range of seminars for postgraduate students in other departments and contribute to refresher courses in other clinical specialties. Members of the division have been involved as external examiners in other African countries.

Honours

Dr M Moodley (senior registrar) was awarded a Discovery Foundation Academic Fellowship Award to support her studies towards a PhD degree.

Mr R Sarkar (PhD student) was awarded the prize for the best poster presentation at the University of Cape Town, School of Biomedical Sciences Research Day and a Global Health Travel Award to attend a Keystone Tuberculosis meeting in Colorado.

In recognition of the quality of research conducted within the division, members received substantial research funding (totalling over R20 million) from the following organisations during 2008: National Institutes of Health of the United States, The Wellcome Trust, European and Developing Countries Clinical Trials Partnership, PATH, National Health Laboratory Service Research Trust, Medical Research Council and the National Research Foundation.

Research output

Publications published: 14
Conference presentations:
 International: 7
 National: 2

Professional development

Postgraduate candidates graduated: 4 (1 PhD, 1 MMed, 2 BSc (Med) honours)
Postgraduate candidates enrolled: 20 (2 postdoctoral, 5 PhD, 8 MMed (including 1 clinical pathology), 3 MSc, 2 BSc (Med) honours)

Division of Medical Virology

Acting head: **Prof Anna-Lise Williamson**

Diagnostic services

The diagnostic virology laboratory at Groote Schuur Hospital provides a comprehensive service to two of the three tertiary hospitals in the Western Cape Province. In addition, it is the referral laboratory for a large proportion of the regional virology testing in the province. It offers a wide repertoire of tests in the fields of viral serology, cell culture and molecular diagnostics. The laboratory has a special interest in molecular diagnosis of human viral diseases and performs an extensive range of in-house and commercial molecular assays. The laboratory is poised to switch from culture-based detection of respiratory viruses to a commercial multiplex PCR. This move will expand the range of respiratory pathogens detectable in clinical samples to include human meta-pneumovirus and rhinovirus A, increasingly recognised as important causes of lower respiratory tract infections in infants. Close collaboration between pathologists and scientists led to the recent identification of novel parvovirus variants in clinical samples from immuno-compromised patients with severe anaemia. Genome sequencing and phylogenetic analysis revealed that all three parvovirus genotypes co-circulate in the Western Cape as well as novel genotype 1 viruses. Further characterisation of these novel genotypes is on-going.

The laboratory has again obtained SANAS confirmation of good laboratory practice (GLP) compliance. This laboratory was established to test vaccine potency of candidate HIV vaccines. It is the only GLP-compliant laboratory in a South African university and one of five in the country.

Research

A highlight of the year was the approval by the Medicines Control Council of phase 1 clinical trials to test two candidate HIV vaccines (SAAVI DNA-C2 and SAAVI MVA-C) developed by the UCT/NHLS team headed by Prof A-L Williamson. The vaccines had previously been approved for phase 1 clinical trials by the FDA in the USA at the end of 2007. The clinical trials started in Boston (USA) in February 2009 and are scheduled to start in South Africa during 2009.

Staff are involved in the following research projects:

Does HIV infection enhance the hepatocarcinogenic potential of chronic hepatitis B virus infection?

Researchers: Dr H Smuts, Ms A Stewart

Collaborator: Prof MC Kew (Liver Research Centre, Department of Medicine, University of Cape Town)

Funding: NHLS Research Trust

Increasing numbers of patients co-infected with HIV and hepatitis B virus (HBV) have been reported to develop hepatocellular carcinoma (HCC). Two possible explanations are that the prolonged survival of HIV patients receiving highly active antiretroviral treatment now allows sufficient time for hepatitis virus-induced HCC to develop, or that HIV acts synergistically with the hepatitis virus in causing the tumour. The prevalence of HIV antibodies in the serum of 144 southern African black males with HBV-induced HCC and 121 closely matched apparently healthy black African HBV carriers from the same time period was compared. HIV antibodies were present in 5/144 patients with HCC (3.46%) and in 0/121 controls. The failure to find a statistically significant greater incidence of HIV infection in patients with HCC than in matched controls argues against HIV acting synergistically with HBV in the aetiology and pathogenesis of HCC.

Optimisation of rBCG as an HIV vaccine vector

Researchers: A-L Williamson, H Stutz, R Chapman, GK Chege

Collaborator: EG Shephard

Funding: National Institutes of Health, National Research Foundation

The primary aim of this research is to generate an effective HIV 1 subtype C vaccine using recombinant *Mycobacterium bovis* BCG as a vector. To achieve this, a number of variables have to be optimised. These can be divided into two broad categories: i) vaccination and immunology (i.e. optimal immune response) which would include dose, route, sacrifice times, immunological assay, boosting and pre-immunisation strategy; ii) the vaccine which would include the vector design, the antigen (folding and toxicity), antigen targeting, expression control signals and bacterial strain.

A comparison of vaccine vector platforms developed in Cape Town with others using standardised immunogens and immunogenicity tests

Researchers: A-L Williamson, EP Rybicki, EG Shephard

Post-doctoral fellow: N Chin'ombe

Funding: European Union

Funding was received to participate in the Compuvac programme entitled 'Rational design and standardised evaluation of novel genetic vaccines'. Vaccines based on DNA, virus-like particles, BCG and modified vaccinia Ankara were constructed and are being tested.

Natural history of human papillomavirus infection in South African men and women recruited for a study on HIV discordant couples

Researchers: A-L Williamson, D Marais

Collaborators: M Hoffman, D Coetzee, J Moodley

Funding: Polio Research Foundation, SIDA, Cancer Association of South Africa, NHLS Research Trust, Medical Research Council

The aim of the study was to investigate the natural history of genital human papillomavirus (HPV) infection in HIV-positive and HIV-negative men and women and HPV transmission between HIV-concordant negative, HIV-concordant positive and HIV-discordant (where one partner is HIV-positive) heterosexual couples in South Africa within a six-month period. The prevalence of genital HPV infection was significantly higher in both HIV-positive women (99/145; 68%) and men (67/93; 72%) compared to HIV-negative women (33/107; 31%) and men (65/150; 43%). HIV-negative men showed a significantly higher prevalence of HPV than HIV-negative women (65/150; 43% compared to 33/107; 30%); when comparing HPV prevalence between HIV-negative men and women the difference was not significantly different (67/93; 72%) compared to 99/145; 68%. HIV-positive women and men showed a significantly higher prevalence of multiple (>1) HPV genotypes (69/145; 48% and 51/93; 55% respectively) compared to HIV-negative women and men (10/107; 9% and 32/150; 21% respectively).

Typing and molecular characterisation of human papillomavirus

Researchers: A-L Williamson, I Hitzeroth, EP Rybicki

Collaborator: J Moodley

Funding: National Research Foundation, Polio Research Foundation, Medical Research Council

This project focuses on characterisation of variants of genital human papillomavirus (HPV) in specimens from African women, with and without simultaneous HIV infection. HPV genotyping was done on 109 cervical samples from HIV-positive women. HPV was detected in 89% of the samples. The most common HPV types were HPV61 (24%), HPV66 (18%), HPV53 and 58 (17%), HPV18, 45 and HPV70 (16%), HPV35 (25%), HPV16 (14%) and HPV51 (13%).

Development of Penguinpox virus as a vaccine vector

Researchers: A-L Williamson, N Douglass

Funding: National Research Foundation

The initial aims of this project are to determine the DNA sequence of the PEPV genome and to further characterise the virus with respect to growth in different cells. Interest in the Avipox viruses, notably Fowlpox virus and Canarypox virus has increased due to their successful use as vaccines on commercial flocks and their extensive use and testing as vaccine vectors. Penguinpox virus, a novel Avipox virus, isolated from African penguins (*Spheniscus demersus*), is an excellent candidate for use as a vaccine vector.

Impact of HIV-1 and sexually transmitted infections on inflammatory cytokine and chemokines in the female genital tract and in plasma: implications for HIV transmission and disease progression

Principal investigator: Dr J-A Passmore

Co-investigators: A/Prof C Williamson, Prof S Karim

Funding: Poliomyelitis Research Foundation, CHAVI

The study investigated the impact of acute HIV-infection on female genital tract inflammatory responses and the role of genital mucosal inflammation on subsequent disease progression (CD4 counts at 12 months and viral load at setpoint). The investigators hypothesised that inflammation at the female genital tract during early HIV-1 infection plays a pivotal role in driving recruitment of HIV-specific T cells to the genital mucosa and impacting on HIV pathogenesis and disease progression. Their results led to the conclusion that immune activation in the female genital tract during early HIV-1 infection is associated with greater CD4+ T cell decline during the first year of infection, and lower CD4+ T cell counts and higher viral load at 12 months post infection.

Mucosal and blood T-cell responses in the control of HIV infection

Principal investigator: Dr J-A Passmore

Co-investigators: Dr W Burgers, A/Prof C Williamson, Prof C Gray

Funding: Wellcome Trust Project Grant, SAAVI HIV present at mucosal surfaces has been shown to differ phenotypically and genotypically from those circulating in blood. The impact of distinct virus populations on the specificity and function of T cells circulating in blood and at mucosal surfaces is unclear. The investigators proposed to determine if compartmentalisation occurs in early and chronic HIV infection by comparing the phenotypic and functional characteristics of T cells in the female genital tract with those in blood. Two major aims were proposed: first, to develop methods to evaluate genital T cell responses and second, to determine if compartmentalisation occurred in early and chronic HIV infection. To define mucosal immune cells sampled by cervical cytobrushing and to validate this approach for local immunity studies, they investigated the impact of HIV and inflammation on yield and composition of cervical cytobrush specimens. HIV-infected women had significantly higher yields of CD3+, CD45+, CD19+, CD14+, Langerin+ and CD24+ cells than uninfected women. Cytobrush T cells from uninfected women were predominantly CD4+ while CD8+ T cells were predominant in HIV-infected women. T cell counts and IL-1 β , TNF- α and IL-12 significantly correlated suggesting that inflammation at the cervix and HIV infection are likely to be key determinants in the absolute number of mucosal immune cells recovered by cervical cytobrushing. They then investigated whether *ex vivo* HIV-specific CD8 T cell-mediated immune responses could be detected in the genital mucosa of HIV-infected women. They demonstrated that CD8+ T-cell IFN- γ responses to Gag were detectable at the cervix of HIV-infected women but found no correlations between cervix and blood in the magnitude or presence of these responses. They showed that inflammatory cytokines are associated with increased levels of HIV-specific CD8 effector cells at the genital mucosa but that these were not able to control genital HIV shedding. They then investigated whether cervical Gag-specific T cells could be expanded *in vitro*. Cytobrush-derived cervical cells were expanded with anti-CD3 and rIL2 and investigated for Gag-specific responses by IFN- γ ELISPOT. In contrast to the *ex vivo* findings, they found that both the magnitude and specific Gag regions targeted by cervical T cell lines correlated significantly with those detected in blood following *in vitro* expansion. With one exception, cervical IFN- γ -T cell responses to Gag were detected only in HIV-infected women with blood Gag-specific response >1000 SFU/10⁶ cells. They concluded that cervical Gag-specific T cell responses in expanded lines are most easily detectable in women who have corresponding high magnitude Gag specific T-cell responses in blood.

Characterisation of mucosal and peripheral T-cell responses associated with early control of HIV replication

Principal investigator: Dr L Bekker

Co-investigators: A/Prof C Williamson, Dr W Burgers, Dr H Jaspan, Prof C Gray

Funding: International AIDS Vaccine Initiative

During heterosexual HIV transmission, the female genital mucosa is the initial site of viral challenge and early replication. Primate studies have shown that responses to simian immunodeficiency virus following vaginal infection are of greater magnitude in genital tissue as compared to blood. The aim of this study was to investigate the magnitude of HIV T-cell responses at the cervix and in blood of acutely HIV-infected women and compare these with responses during chronic infection. Six women with acute and 51 women with established HIV infection were recruited. Blood and cervical specimens were obtained from all acutely HIV-infected women at enrolment and then longitudinally for 18 months. Cervical and blood specimens were obtained from chronically infected women at a single time point. *Ex vivo* intracellular IFN- γ responses to HIV subtype C Gag and Nef peptides were evaluated in cervical cytobrush-derived T cells and blood by flow cytometry. The magnitude of HIV-specific IFN- γ CD8⁺ T cell responses to Gag and Nef peptides at the cervical mucosa of acutely HIV-infected women were comparable to those detected in blood. No correlation between the magnitude of HIV-responses in blood and at the mucosa of acutely HIV-infected women was observed. Longitudinal analysis of HIV responses at the cervix and in blood of the six acutely infected women showed that cervical responses to Gag and Nef were generally higher than those in blood in acute and early infection, and that this trend is not maintained in chronic infection. Therefore, HIV-specific IFN- γ responses by T cells in the female genital tract are not enhanced compared to blood at early time points.

Characterisation of HIV-specific responses in cervical and blood compartments in discordant and HIV-positive couples

Principal investigator: Dr J-A Passmore

Co-investigators: Prof A-L Williamson, Dr D Coetzee, Prof M Hoffman

Funding: Wellcome Trust, Medical Research Council

Women who remain uninfected despite repeated exposure to HIV (such as women in discordant relationships) provide a unique opportunity to evaluate local events in the female genital tract associated with protection or resistance to HIV-infection. The overall objective of this proposal is to evaluate innate and cellular immunity in the female genital tract of women in discordant relationships that may be associated with protection from or susceptibility to HIV infection. The investigators propose to do this by i) evaluating the frequency and quality of T-cell immunity in the female genital tract in uninfected women in discordant relationships and comparing T-cell specific function with women in concordant HIV+ and HIV- relationships (proliferative capacity, polyfunctionality, and maturational status in CD4+ and CD8+ T-cell subsets), and ii) assessing the level of immune activation present at the cervix of women in discordant relationships and comparing expression of activation markers (HLA-DR, CD38, CCR5, Ki67) on CD4+ and CD8+ T cells with women in concordant HIV+ and HIV- relationships. The association between genital tract inflammation, T-cell functionality and immune activation in HIV- women who have been exposed will inform on the vaginal immune profile conducive to protection against HIV infection at the genital mucosa.

Other studies:

- Investigating the occurrence, diagnostic implications and characterisation of parvovirus variants in the local population
Researchers: Dr C Corcoran, Dr H Smuts
Funding: NHLS Research Trust
- Bacterial 16s ribosomal DNA amplification and characterisation in cerebrospinal fluid specimens to improve diagnostic yield in suspected central nervous system infections in adults
Researchers: Dr C Corcoran, Dr M Mendelson, A Deffir
Funding: NHLS Research Trust
- Development and validation of an in-house PCR assay for the detection and identification of clinically relevant fungi
Researchers: K Bonorchis, C Corcoran
Funding: NHLS Research Trust
- Evaluation of an acute HIV infection diagnosis, behavioural counselling, and partner notification programme in a public health service for youth in Cape Town
Investigators: B Wolpaw, C Mathews, M Chopra, D Hardie, V Azevedo, K Jennings

Teaching and training

The diagnostic laboratory is a registered training laboratory with the HPCSA and is also affiliated with the Department of Virology at the University of Cape Town. Pathologists and scientists are joint university staff and are involved in the teaching and training of medical students and BSc (Med) Honours students as well as registrars and medical technologists. MSc and PhD students are trained in the laboratories of the Institute of Infectious Disease and Molecular Medicine at UCT.

Honours

Prof A-L Williamson was appointed as a member of Global HIV Vaccine Enterprise Science Committee and served as a member of the NIH Scientific Review Group for HIVRAD applications.

Research output

Publications published: 21

Conference presentations:

International: 23

Local: 2