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Second Annual Scientific Sessions

27th April 2007

Programme and Abstracts

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Shiromi Handunnetti
27th April 2007

Our Vision

" Fostering a conducive culture for research in molecular life sciences
and high quality human resources training to
facilitate national development "

PROGRAMME

09.00 h - Lighting of the Traditional Oil Lamp

09.10 h - Welcome address by Director, IBMBB

09.20 h - Address by the Vice-Chairman, University Grants Commission - Prof Gunapala Malalasekera

10.55 09.30 h - Address by the Vice Chancellor, University of Colombo

09.40 h - **Professor Stanley Wijesundera Memorial Lecture -**

Challenges in University Education in Sri Lanka

by Prof. W. D. Lakshman, Senior Professor of Economics

University of Colombo

10.40 h -Vote of thanks

10.45 h - Reception

11.30-12.00 h: **Plenary Lecture - Chairperson Prof. E R Jansz**

Molecular Biotechnology for Conservation and Breeding of Tea (*Camellia sinensis*)

by Dr. I. S. B. Abeysinghe, Director, Tea Research Institute

12.00 h onwards: Research Presentations

Oral Communications

Session 1: Cancer

Chairperson: Prof E R Jansz

12.00 -12.15 h (Abstract No. 1) Prevalence of two recurrent germline polymorphisms in exon 16 of *BRCA-1* gene in Sri Lankan breast cancer patients and at risk individuals
De Silva JWN, Karunanayake EH, Tennekoon KH, Amarasinghe I, Angunawala P

12.15 – 12.30 h (Abstract No. 2) Screening of Sri Lankan breast cancer patients for germ line mutations in exon 8 of TP53 gene
Gunaratne AD, De Silva JWN, Tennekoon KH, Karunanayake EH, Angunawala P, Amarasinghe I

Session 2: Reproduction and Development

Chairperson: Prof Eric H. Karunanayake

14.00 – 14.15 h (Abstract No. 03) Expression of the short isoforms of the leptin receptor in the rat uterus during the estrus cycle
Eswaramohan T, Tennekoon KH, Karunanayke EH

14.15 – 14.30 (Abstract No. 04) Leptin receptor gene polymorphism in relation to lactational amenorrhoea
Sandanayake HI, Muller A, Tennekoon KH, Karunanayake EH

14.30 – 14.45 h (Abstract No. 05) Maternal plasma leptin and soluble leptin receptor concentrations in preeclampsia/pregnancy induced hypertension
Sugathadasa BHKR, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundera APDS

14.45 – 15.00 h (Abstract No. 06) Maternal and cord blood levels of insulin-like growth factor (IGF)-I and IGF-binding protein-1: Contribution to the variation in normal birth weight
Jayanthiny P, Ziard MH, Tennekoon KH, Karunanayake EH, Kumarasiri JM, Wijesundra APDS

Session 3: Medicinal Plants, Plant Biology/Molecular Biology

Chairperson: Prof Kamani H Tennekoon

15.00 – 15.15 h (Abstract No. 07) Anti inflammatory activity of methanol leaf extract of *Ixora coccinea* in rats
Kumara RR, Nanayakkara AA, Deraniyagala SA, Ratnasooriya WD, Handunnetti SM

15.15 – 15.30 h (Abstract No. 08) Micro propagation of Papaya (*Carica papaya*) through auxiliary bud and shoot tip culture (Variety Red Lady)
Warakagoda PS, Kumari DLC and Subasinghe S

15.45 – 16.00 h (Abstract 09) Preliminary study of the suitability of commercial canola spring varieties to the local environment
Peiris PKD, Weerakoon SR, Weerasena OVDSJ

15.30 – 15.45 h (Abstract No. 10) Early detection techniques for *Corynespora* leaf fall disease resistance clones using Resistant Gene Analog (RGA) markers.
Jayakody A, Silva WPK, Karunanayake EH

Poster Presentations (16.00 – 17.00 h)

Parasitic Diseases

PP 01 (Abstract No. 11) Hypothetical proteins of filarial parasite *Setaria digitata*
Wickramanayake MN, Karunanayake EH

PP 02 (Abstract No. 12) Immunoepidemiology of *Plasmodium vivax* Merozoite Surface Protein – 4 (PvMSP-4) in Sri Lanka: A preliminary study
Dissanayake MU, Dias KVGS, Premaratne, PH, Longacre S, Udagama-Randeniya PV

PP 03 (Abstract No.13) Natural antibody responses to *Plasmodium vivax* Merozoite Surface Proteins-1 and 4 in Sri Lanka
Dissanayake MU, Dias KVGS, Premaratne, PH, Wickramarachchi WTA, Handunnetti SM , Longacre S, Udagama-Randeniya PV

Reproduction and Molecular Techniques

PP 04 (Abstract No. 14) Expression of leptin and short and long forms of the leptin receptor in the rat oviduct
Eswaramohan T, Tennekoon KH, Karunanayke EH

PP 05 (Abstract No. 15) Establishment of expression microarray analysis: A preliminary report
Tennekoon KH, Premakumara KS, Wijesinghe S, Ziard MH, Perera A, Karunanayake EH, Kumarasiri JM

Medicinal Plants and Plant Molecular Biology

PP 06 (Abstract No. 16) Inhibition of rat peritoneal phagocytic cell functions by aqueous leaf extract of *Ixora coccinea*
De Silva SSP, Kumara RR, Nanayakkara AA, Deraniyagala SA, Ratnasooriya WD, Handunnetti SM

PP 07 (Abstract No. 17) Isolation and characterization of potential disease resistance gene analogs from traditional, wild and weedy rice of Sri Lanka
Samaranayake DP, Weerasena OVDSJ, Karunanayake EH

Professor Stanley Wijesundera Memorial Lecture

Prof. Stanley Wijesundera served the University of Colombo as its first Vice-Chancellor from 1978 to 1987. He also held the Chair of Biochemistry during the same period. Prof. Wijesundera's vision as an administrator was unparallel and the infrastructure development that he initiated in the University stands in testimony. He also strongly believed in human resource development at frontier disciplines and capacity building in science as essential requirements for national development. In tribute to the key role he played in the establishment of the discipline of molecular biology in the University of Colombo, the Institute of Biochemistry, Molecular Biology and Biotechnology established the Stanley Wijesundera Memorial Lecture as an annual event with generous sponsorship of Wijesundera family. The second Stanley Wijesundera Memorial Lecture will be delivered by Professor W D Lakshman, Senior Professor of Economics and former Vice Chancellor, University of Colombo.

Eric Karunanayake

Chairman – Organising Committee, Second Annual Scientific Sessions

Abstract No: 01

Prevalence of two recurrent germline polymorphisms in exon 16 of *BRCA-1* gene in Sri Lankan breast cancer patients and at risk individuals

De Silva JWN[1], Karunanayake EH[1], Tennekoon KH[1,2], Amarasinghe I[3], Angunawala P[4],

[1]Institute of Biochemistry Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

[3]National Cancer Institute, Maharagama

[4]Department of Pathology, Faculty of Medicine, University of Colombo

Breast Cancer is the most commonly diagnosed cancer among Sri Lankan women. Germline mutations in *BRCA-1* and *BRCA-2* predispose to hereditary breast cancer. Numerous genetic variations in *BRCA-1/-2* genes have been reported worldwide and we have previously reported a novel mutation in exon 21/*BRCA-1* from Sri Lanka. This study describes the prevalence of two recurrent germline polymorphisms in Sri Lankan breast cancer patients and at risk individuals. A total of 126 patients with (N=64) and without (N=62) a family history of breast cancer, 69 unaffected (at risk) individuals with a family history of breast cancer and 38 control subjects without a personal or a family history of any cancer were studied. Genomic DNA was isolated from peripheral blood lymphocytes and exon 16/*BRCA-1* was amplified. The PCR products were screened by combination of single strand conformation analysis (SSCP) and heteroduplex analysis, and visualized by silver staining. Those giving abnormal patterns in SSCP were sequenced using the MegaBACE 1000 automated DNA sequencer. Two recurrent polymorphisms 4931A>G (Codon-1604) and 4956A>G (Codon-1613) in exon 16/*BRCA1* were found. Both polymorphisms were found in two breast cancer patients with a family history of breast cancer. The prevalence of 4956A>G polymorphism was 81.3% among patients with a family history of breast cancer, 87.1% among patients without a family history of breast cancer, 86.9% in at risk individuals and 63.2% among the controls. Thus among Sri Lankans 4956A>G polymorphism in exon 16/*BRCA-1* is very common compared to other populations.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 02

Screening of Sri Lankan breast cancer patients for germline mutations in exon 8 of TP53 gene

Gunaratne AD[1], De Silva JWN[1], Tennekoon KH [1, 2], Karunanayake EH[1], Angunawala P[3], Amarasinghe I[4]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology and [3]Department of Pathology, Faculty of Medicine, University of Colombo

[4]National Cancer Institute, Maharagama

The tumor suppressor gene TP53 is located on the short arm of chromosome 17. TP53 gene encodes a protein known as 'P53' which plays a pivotal role in the cell cycle regulation. TP53 gene mutations are seen in almost 50% of all human cancers. Most mutations accumulate in the hotspot region of exons 4-8 of the gene. Our study was focused on screening exon 8 for germline mutations. Breast cancer patients with a family history (N=45) and without a family history (N=50) were investigated. Patients were selected within the high risk age group of 40 – 65 years. DNA was extracted from peripheral blood. Exon 8 of TP53 gene was amplified by polymerase chain reaction using exon specific primers under optimized conditions. Primers used amplified a product of approximately 200bp. Single strand conformation analysis was carried out to screen the amplified samples for mutations. The results did not show any evidence of germline mutations in the exon 8 of TP53 gene in breast cancer patients with or without a family history. DNA sequencing of samples is currently being carried out to confirm absence of mutations.

Supported by SAREC Grant for Molecular Biology and Biotechnology.

Abstract No: 3

Expression of the short isoforms of the leptin receptor in the rat uterus during the estrus cycle

Eswaramohan T[1], Tennekoon KH[1,2], Karunanayke EH[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

Leptin is now considered to have diverse effects on reproduction. Previously we reported that leptin (Ob) and the long form of the leptin receptor (Ob-Rb) are expressed in the rat uterus throughout the estrus cycle. This study examined the effect of estrus cycle stage on expression of short isoforms of the leptin receptor in the rat uterus. Total RNA extracted from uteri at different stages of the estrus cycle (N=6 per cycle stage) was reverse transcribed using M-MLV reverse transcriptase and random primers. Complementary DNA was PCR amplified using specific primers. Hypoxanthine-guanine phosphori-bosyltransferase was used as the internal control. PCR products were resolved in 2% agarose gel and intensity of bands normalized to internal control (BIO RAD quantity One quantitation software). One way ANOVA with Bonferroni multiple comparison was used to detect the effect of estrus cycle stage. All three isoforms tested (ie: Ob-Ra, Ob-Rc, Ob-Re) were detected throughout the estrus cycle. Relative expression of Ob-Rc and Ob-Re (mean ratio±SEM) was significantly higher at estrus (Ob-Rc: 0.73±0.01, Ob-Re: 0.96±0.02) and metestrus (Ob-Rc: 0.84±0.06, Ob-Re: 1.05±0.05) than at proestrus (Ob-Rc: 0.42±0.07, $P<0.05$ to 0.01; Ob-Re: 0.50±0.12, $P<0.05$ to 0.01) and diestrus (Ob-Rc: 0.41±0.03, $P<0.05$; Ob-Re: 0.45±0.10, $P<0.05$ to 0.01). Ob-Ra expression though lowest at diestrus did not significantly differ between estrus cycle stages. Thus the rat uterus appears to be a target for leptin action via short receptor isoforms. The cyclical variation in the expression of leptin receptor isoforms observed is probably mediated by the cyclical variation of reproductive hormones.

Supported by SAREC Grant for Molecular Biology and Biotechnology and National Research Council Grant 00-07

Abstract No: 04

Leptin receptor gene polymorphism in relation to lactational amenorrhoea*

Sandanayake HI[1], Muller A[1], Tennekoon KH[1,2], Karunanayake EH[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

Leptin receptor is a trans-membrane protein with several isoforms. In humans leptin receptor gene (LEPR) is located at chromosome 1p31 region. In addition to the isoforms, single nucleotide polymorphisms have also been described in the coding region of LEPR. Some of these polymorphisms are reported to modulate biological effects of leptin. Among its many other functions, leptin serves as a metabolic signal to the reproductive axis conveying information on the organism's nutritional status to the hypothalamic-pituitary-ovarian axis. Lactational amenorrhoea, the interval between the birth of a child and subsequent return of menstruation in breast feeding women, is inversely associated with maternal nutritional status. However, our previous studies found no difference in serum leptin levels between women with short duration (<24 weeks) and long duration (≥24 weeks) of lactational amenorrhea. This study examined the prevalence of the polymorphisms in exons 4, 6, 14 and 20 of LEPR gene in women for whom data on the duration of lactational amenorrhea was already available (N=31). Male samples (N=10) were taken for comparison. DNA was extracted from peripheral venous blood. Exons 4, 6, 14 and 20 were PCR amplified using specific primers. PCR products were screened by single strand conformation polymorphism and restriction fragment length polymorphism. Results were confirmed by direct sequencing. Prevalence of these four polymorphisms did not significantly differ between women with short and long duration of lactational amenorrhoea. Thus the leptin system is unlikely to be an important determinant of the duration of lactational amenorrhea.

Supported by SAREC Grant for Molecular Biology and Biotechnology

*Part of this work has been presented at the Annual SLAAS Sessions 2005

Abstract No: 05

Maternal plasma leptin and soluble leptin receptor concentrations in preeclampsia/pregnancy induced hypertension

Sugathadasa BHKR[1], Tennekoon KH[1,2], Karunanayake EH[1], Kumarasiri JM[3], Wijesundera APDS[3]

[1]Institute of Biochemistry Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine University of Colombo

[3]Castle Street Hospital for Women, Colombo

Leptin is a 16-kDa polypeptide implicated in the regulation of reproduction. During pregnancy, leptin is produced by the placenta, concentrations in the maternal blood increase particularly in the second trimester and decline postpartum. Leptin concentrations are reported to be elevated in established preeclampsia. Soluble leptin receptor is thought to reduce bio-availability of leptin. We examined circulating leptin and soluble leptin receptor (SLR) concentrations in patients with preeclampsia (PE)/ pregnancy induced hypertension (PIH) (N=33) and in women with uncomplicated normal pregnancy (N=40). Non-fasting venous blood samples were obtained in the third trimester. Leptin and SLR concentrations were measured using enzyme immunoassays. Data were compared using Mann-Whitney U test. Maternal plasma leptin concentrations were significantly higher [geometric mean, (95% CI): 54.20 (47.32, 61.94) vs. 35.24 (28.97, 42.85) ng/ml; $P<0.05$] and SLR concentrations were significantly lower [geometric mean, (95% CI): 21.04 (18.66, 23.77) vs. 30.06 (27.01, 33.42) ng/ml; $P<0.05$] in PE/PIH than in normal pregnancy. Free leptin index (ie: leptin/SLR concentration) and leptin values corrected for body mass index were also significantly higher ($P<0.05$) in PE/PIH than in normal pregnancy. Thus we confirm that the leptin system is altered in PE/PIH. Prospective studies are in progress to assess the feasibility of developing biomarkers for early detection of PE/PIH based on the leptin system.

Supported by UGC (RPC/2005/2i/06/CMB) and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 6

Maternal and cord blood levels of insulin-like growth factor (IGF)-I and IGF-binding protein-1: Contribution to the variation in normal birth weight

Jayanthiny P[1], Ziard MH[1], Tennekoon KH[1,2], Karunanayake EH[1], Kumarasiri JM[3], Wijesundra APDS[3]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

[3]Castle Street Hospital for Women, Colombo

Insulin like growth factor (IGF) system is implicated in fetal growth. However our previous longitudinal studies did not show a significant correlation between maternal or cord blood IGF-I levels and birth weight in normal pregnancies. This may have been partly due to the small number of subjects studied. In this cross sectional study maternal and cord blood levels of IGF-I (N= 76) and IGF binding protein (IGFBP)-1 (N= 65) were measured by enzyme linked immunosorbant assay in women with normal uncomplicated singleton pregnancies delivering babies with birth weights between 2.5 - 3.5 kg at term. Relationships of IGF-I and IGFBP-1 to birth weight were analyzed by Spearman rank correlation test. Cord blood IGF-I and IGFBP-1 [geometric mean (95% CI)] levels were 34.67 (31.26, 38.46) ng/ml and 52.97 (39.08, 71.78) μg/ml respectively. Maternal IGF-I and IGFBP-1 [geometric mean (95% CI)] levels were 253.51 (222.33, 289.73) ng/ml and 92.469 (72.61, 118.03) μg/ml respectively. Cord blood IGF-I levels showed a weak but a statistically significant positive correlation with birth weight ($r=0.266$, $P=0.0203$). However this explains only 7.07 % of the variation in birth weight in term babies with normal birth weight. Maternal IGF-I and maternal and cord blood IGFBP-1 did not show a significant correlation with birth weight in this group. Further studies are in progress to assess the relative contribution by other components of the IGF system on birth weight.

Supported by National Research Council Grant 05-28 and SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 07

Anti inflammatory activity of methanol leaf extract of *Ixora coccinea* in rats

Kumara RR[1], Nanayakkara AA[1], Deraniyagala SA[2], Ratnasooriya WD[3], Handunnetti SM[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Chemistry, [3]Department of Zoology, Faculty of Science, University of Colombo.

Aqueous leaf extract of *Ixora coccinea* has been shown to possess anti inflammatory activity previously. In this study, we investigated the potential anti inflammatory activity of methanol leaf extract (MLE) of *Ixora coccinea* in rats using carrageenan induced rat paw edema model with three doses (1500, 1000 and 500 mg/kg). Different doses of MLE were orally administrated to rats and inhibition of paw edema was calculated. Maximum inhibition of rat paw edema was recorded as 64%, 46% and 37% ($P<0.01$) respectively with 1500, 1000 and 500 mg/kg. The extract dose dependently inhibited paw edema ($r^2=0.967$) in rats. Further, MLE significantly inhibited the infiltration of phagocytic cells/macrophages in rats (58% at 1500 mg/kg; $P=0.005$) and impaired production of nitric oxide intermediates in rat peritoneal cells (58% at 1500 mg/kg; $P=0.005$) and lipid peroxidation in TBARS method (45% at 15.6 μg/ml). MLE also possessed significant *in vitro* anti-oxidant activity as determined by DPPH method (85% at 100 μg/ml) and anti histamine activity *in vivo* (55% at 1500 mg/kg; $P=0.005$). However, MLE did not have a membrane stabilizing activity in the heat-induced haemolysis assay. These results suggest that inhibition of NO intermediates, inhibition of phagocytic cell/macrophage infiltration, anti histamine effect, scavenging of free radicals and lipid peroxidation activity may be involved in the anti-inflammatory activity of *Ixora coccinea.*

Supported by the National Science Foundation Grant RG/2005/HS/15

Abstract No: 08

Micro Propagation of Papaya (*Carica papaya* – Var. Red Lady) through auxiliary bud and shoot tip culture

Warakagoda PS, Kumari DLC and Subasinghe S

Department of Crop Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya

Red Lady is a variety of papaya, producing quality fruits for export market. It is a vital requirement to identify an efficient vegetative propagation method for large scale production of this economically valuable species. Applying tissue culture techniques to mass propagation of papaya enables not only maintain clonal uniformity but also produce disease free plants. Experiments were carried out to develop an *in vitro* protocol for micro propagation of auxiliary buds and shoot tips of variety Red Lady. Four concentrations of NaOCl (5%, 10%, 15% and 20%), four exposure time durations (5, 10, 15 and 20 min.) with and with out 70% ethanol were tested in MS basal medium with five modifications. Modified MS media with three BAP levels (0. 2, 0. 4, and 0.6 mg/l) with and without 0.1 mg/l NAA were tested as proliferation media. Half strength MS medium with two IBA (1, 2 mg/l) and NAA (0, 0.5 mg/l) levels were tested as rooting media. Three potting media (sand 1: coir dust 1, sand 1: cow dung 1, sand 1: coir dust 1: cow dung 1) were tested to acclimatize the rooted plantlets. Results revealed that 10% NaOCl for 10 minutes with 70% ethanol is the best surface sterilization procedure where ex- plants were well established in MS medium containing 0.15 g/l Amoxaline, 0.1 g/l $AgNO_3$ and 1g/l Casein hydrosilate. Highest proliferation rate was obtained in MS medium containing 0.1 g/l $NaH_2PO_4.2H_2O$, 0.05 g/l Adenine SO_4 and 0.2 mg/l BAP. *In vitro* rooting occurred in half strength MS medium with 2 mg/l IBA and 0.5 mg/l NAA. Rooted plantlets were well acclimatized in Sand 1: Coir dust 1: Cow dung 1 medium placed in a humid chamber for first four weeks.

Abstract No: 09

Preliminary study of the suitability of commercial canola spring varieties to the local environment

Peiris PKD[1], Weerakoon SR[2], Weerasena OVDSJ[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo ,

[2]Department of Botany, Open University of Sri Lanka.

Brassica species are grown in many countries as important oil, vegetable and condiment crops and contribute considerably to the world economy, health and environment. In Sri Lanka, mustard *(Brassica juncea)* is cultivated as a subsidiary crop and the seeds used as a condiment in cooking. In the Indian sub-continent, it is an important oilseed crop used as a source of vegetable oil. However mustard contains over 45% erucic acid (C22:1) which is nutritionally unfavorable for human consumption. Canola (*Brassica napus*) is used as a major oil seed crop in Canada, Australia, China and U.S.A. Canola oil contains low amount of erucic acid (0.2% to 0.5%). Therefore it is more suitable as an edible oil seed crop. Hybrids between *B. napus* and *B. juncea* may yield improved *B. juncea* varieties with low amount of erucic acid. Six commercial spring varieties of *Brassica napus* grown in Western Australia designated Narendra, Monty, Oscar, Outback, Hyola and Karoo were planted in a net house. A preliminary study of the possibilities of growing and producing hybrids with *Brassica juncea* (AC: 7700) were observed under local environmental conditions. Out of above six spring varieties Narendra, Monty, Outback, and Hyola could be grown in local environment. Narendra was the most suitable spring canola variety to the local environment. Karoo was grown very weakly and Oscar did not flower during the grown period. Crosses *(B.napus* x *B.juncea*) and reciprocal crosses (*B.juncea* x *B. napus*) were carried out in the same environment. Although reciprocal crosses failed among all *B.napus* species, *B.juncea* (AC: 7700) with *B.napus* crosses were successful and indicated the possibility of producing mustard hybrids with canola quality.

Supported by the National Science Foundation Grant RG/2006/AG/04

Abstract No: 10

Early detection techniques for Corynespora leaf fall disease resistance clones using Resistant Gene Analog (RGA) markers: A preliminary report

Jayakody A[1], Silva WPK[2], Karunanayake EH[1]

[1]Institute of Biochemistry Molecular Biology & Biotechnology, University of Colombo

[2] Rubber Research Institute, Dartonfield, Agalawaththa

Corynespora leaf fall (CLF) caused by the fungus *Corynespora cassiicola* is one of the most important diseases of *Hevea brasiliensis* in several rubber growing countries including Sri Lanka. With regard to the management of the disease it has been agreed by the rubber pathologists that the use of resistant genetic materials is the only hope as chemical control is not cost effective. From the past experience it seems that the field clearing should be at least 3-5 years old to determine whether a particular clone can tolerate the disease or not. In the light of this situation the present study was designed to identify possible disease resistance markers in rubber with the view of developing an early detection system for CLF resistance in potential clones. Many disease resistance genes in plants are found to have similar sequences that are presumably involved in pathogen recognition and signal transduction. Using these sequences PCR markers are being designed referred to as RGA markers. In this study 5 RGA primer combinations were used to amplify the genomic DNA to obtaine DNA sequences that correspond to conserved motifs of resistance genes. Primer S1-AS3 gave specific bands for 6 resistant samples of *Corynespora* DNA. Investigations are underway to develop a specific primer to amplify DNA of resistant clones, using the above specific bands.

Supported by SAREC Grant for Molecular Biology and Biotechnology and CARP Grant No. 12/497/370.

Abstract No: 11

Hypothetical proteins of filarial parasite *Setaria digitata*

Wickramanayake MN, Karunanayake EH

Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

Lymphatic filariasis is caused by the parasitic nematodes, *Wuchereria bancrofti, Brugia malayi, and Brugia timori*. It is an important cause of physical and social disability that affects over 100 million people in 83 countries of the developing world. The diagnostic tools for lymphatic filariasis are very important for any global programme aimed to eliminate lymphatic filariasis. This study describes the preliminary findings from an investigation of the bovine filarial parasite, *Setaria digitata,* the model organism used to study *W. bancrofti*. One hundred and twenty three randomly selected clones of *S. digitata* cDNA library were sequenced. Among these sequences, 8 hypothetical proteins, myosin light chain protein, ubiquitin protein, and calmodulin binding proteins were identified. Studies are in progress to ascertain the potential of these novel hypothetical proteins as diagnostic or therapeutic targets.

Supported by SAREC Grant for Molecular Biology and Biotechnology

Abstract No: 12

Immunoepidemiology of *Plasmodium vivax* Merozoite Surface Protein – 4 (PvMSP-4) in Sri Lanka: A Preliminary Study

Dissanayake MU[1], Dias KVGS[2], Premaratne PH[2], Longacre S[3], Udagama-Randeniya PV[2]

[1]Institute of Biochemistry Molecular Biology & Biotechnology, University of Colombo

[2]Department of Zoology, University of Colombo

[3] Department of Parasitology, Institute of Pasteur, Paris, France.

Molecules involved in erythrocyte invasion by malaria merozoites are attractive targets for effective immune intervention. Seroepidemiology of vaccine candidates is important to gain insights into vaccine development. Recombinant protein PvMSP-4, expressed in the baculovirus vector, representing native *Plasmodium vivax* MSP-4, was used in an indirect ELISA to assay total immunoglobulin (IgG + IgM) responses of patients with acute vivax malaria infections from two endemic areas, Anuradhapura (N=68) and Kataragama (N=87), and from a non-endemic area, Colombo (N=69). Healthy individuals (N=30) with no history of malaria served as controls. PvMSP4 appeared to be immunogenic both in endemic and non-endemic populations. Prevalence (responding proportions) was 80%, 62% and 62% from Colombo, Anuradhapura and Kataragama, respectively. A significantly higher (Chi square test, $P<0.05$) prevalence and antibody magnitudes (Kruskal-Wallis Test, $P<0.05$) was recorded for non-endemic Colombo. Prevalence and antibody magnitudes between individuals previously not exposed to malaria in Colombo (PNE) and their previously exposed counterparts did not significantly differ. Antibody magnitudes but not the prevalence was significantly higher in the PNE group (Kruskal-Wallis Test, $P<0.05$) than in the two endemic areas. Antibody magnitudes of the three test populations were independent of age, previous exposure to malaria and parasitaemia.

Supported by National Science Foundation (NSF/RG/2005/HS/06)

Abstract No: 13

Natural antibody responses to *Plasmodium vivax* Merozoite Surface Proteins-1 and 4 in Sri Lanka

Dissanayake MU[1], Dias KVGS[2], Premaratne, PH[2], Wickramarachchi W T A [2], Handunnetti S M [3#], Longacre S[4], Udagama-Randeniya PV[2]

[1]Institute of Biochemistry Molecular Biology & Biotechnology, [2]Department of Zoology, [3]Malaria Research Unit, Department of Parasitology, University of Colombo

[4]Department of Parasitology, Institute of Pasteur, Paris, France

Current Affiliation - Institute of Biochemistry Molecular Biology & Biotechnology

Subunit vaccines composed of multiple antigen constructs may gain greater protection against malaria. Therefore, the natural antibody response to three asexual vaccine candidates of *Plasmodium vivax* using the same battery of sera samples from patients with acute vivax malaria infections from endemic areas, Anuradhapura (N=45) and Kataragama (N=84) and from Colombo (N=30) a non-endemic area was analyzed. Recombinant proteins PvMSP-4, p42 and p19, the first representing native merozoite surface protein (MSP)-4 and the other two representing MSP-1 of *P.vivax* were used in an ELISA to assess the total immunoglobulin (IgG+IgM) responses of these patients. All proteins were immunogenic during natural vivax malaria infections in both malaria endemic and non-endemic areas. 66%, 47% and 44% of the test populations from Colombo, Anuradhapura and Kataragama respectively, responded to all three antigens. More than 80% from each test area responded to at least one of the three antigens tested. The antibody prevalence in all test areas for p42 was significantly higher (McNemar test $P<0.05$) than for the other two antigens. 7%, 11% and 4% of individuals from Colombo, Anuradhapura and Kataragama respectively, preferentially recognized PvMSP-4 (Chi square test, $P<0.05$). Responding proportions to three antigens did not significantly differ among the study areas. 45% of previously unexposed individuals from Colombo responded to all antigens, 9% did not respond to any. Previously exposed individuals from Colombo showed a higher prevalence (Chi square test, $P<0.05$) to all three antigens compared to endemic areas. Number of responders were higher for paired combination of MSP-1p42 + p19 than for MSP-1 with MSP-4 (Chi square test, $P<0.05$).

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Abstract No: 14

Expression of leptin and short and long forms of the leptin receptor in the rat oviduct*

Eswaramohan T[1], Tennekoon KH [1,2], Karunanayke EH[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

Leptin receptors (Ob-R) have been detected in the oviduct of women and in a few animal species. Leptin and leptin receptor expression in the rat oviduct has not been previously investigated. We studied the expression of leptin and leptin receptor isoforms (Ob-Ra and Ob-Rb) at the mRNA and protein levels in the rat oviduct throughout the estrus cycle. Immunohistochemistry was performed with formaldehyde fixed oviduct sections to localize the presence of leptin and Ob-R proteins. Semi-quantitative RT-PCR with normalization to internal controls (beta-actin for leptin, hypoxanthine-guanine phosphori-bosyltransferase for Ob-Ra and Ob-Rb) was used to investigate the relative mRNA expression of leptin, Ob-Ra and Ob-Rb. Intensity of each PCR band was measured and normalized to the internal control (BIO RAD quantity one quantitation software for normalization). Immunohistochemical studies showed the presence of Ob-R protein during all estrus stages, but a difference in the intensity of staining was not discernible between stages. Both Ob-Ra and Ob-Rb mRNAs were detected by RT-PCR. The relative expression of Ob-Ra was significantly higher at metestrus than at estrus [mean ratio±SEM: 1.23±0.11 vs 0.73±0.01; $P<0.01$ (one way ANOVA with Bonferroni's multiple comparison test)]. Ob-Rb expression was highest at diestrus and lowest at estrus but the difference was not statistically significant. Leptin was not detected in the oviduct either at mRNA or at protein level. Thus the rat oviduct appears to be a target tissue for leptin but not a source. Leptin may play an important role in the oviductal function.

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Abstract No: 15

Establishment of expression microarray analysis: A preliminary report

Tennekoon KH[1,2], Premakumara KS[1], Wijayasinghe S[1], Ziard MH[1], Perera A[1], Karunanayake EH[1], Kumarasiri JM[3]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

[2]Department of Physiology, Faculty of Medicine, University of Colombo

[3]Castle Street Hospital for Women, Colombo

Microarray is a high throughput technology which allows simultaneous analysis of thousands of genes. In order to exploit this technology to assess spatial and temporal variations in gene expression, microarray analysis was established. Placental tissue was washed in phosphate buffered saline and either snap frozen in liquid nitrogen or preserved in RNALater (Ambion, UK). Total RNA was extracted using TRIzol method (Invitrogen). RNA quality and quantity were assessed using Agilent Bioanalyzer 2100. Complementary DNA was synthesized and labelled with Cy3 and Cy5 using Cyscribe post labeling kit (GE Helathcare Biosceinces) and hybridized to 46 K human cDNA arrays (KTH, Stockholm, Sweden). Preservation with RNALater protected RNA from degradation whereas snap freezing in liquid nitrogen was not suitable for placental tissues. Despite obtaining high quality RNA as detected by Agilent Bioanalyzer, hybridization was not successful when RNA was used without purification. cDNA synthesis and subsequent labeling with Cy3 and Cy5 were successful with Cyscribe post labelling kit when RNA was column purified. Experiments are in progress to compare gene expression under different conditions using microarray technology.

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Abstract No: 16

Inhibition of rat peritoneal phagocytic cell functions by aqueous leaf extract of *Ixora coccinea*

De Silva SSP[1], Kumara RR[1], Nanayakkara AA[1], Deraniyagala SA[2], Ratnasooriya WD[3], Handunnetti SM[1]

[1]Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

Departments of [2]Chemistry & [3]Zoology Faculty of Science, University of Colombo

The anti-inflammatory action of aqueous leaf extract (ALE) of *Ixora coccinea* has been shown previously using carrageenan induced rat paw edema model. In the present study, the mechanisms of anti-inflammatory action such as inhibition of cell infiltration and production of NO intermediates production were investigated. Four groups of adult rats were selected and treated orally with ALE of *Ixora coccinea* (1500mg/kg; n=6), indomethacin (5mg/kg; n=3), prednisolone (10mg/Kg; n=6) and distilled water (1ml; n=6). After 1 hour, all animals were injected intraperitoneally with 5mg/kg body weight of carrageenan, peritoneal cells were collected into PBS 2 hours later and cell counts were performed. In the *Ixora coccinea* group, the mean percent inhibition of total immune cell infiltration and phagocytic cell infiltration were 36% and 30% respectively ($P<0.05$). Indomethacin failed to inhibit peritoneal cell infiltration, whereas prednisolone significantly inhibited cell infiltration (73%; $P<0.05$). To assess the effect of ALE of *Ixora coccinea* on production of NO intermediates, peritoneal cells were cultured *in vitro* for 24 hours and NO intermediates in the culture supernatants were assessed using Griess reagent. Oral treatment of rats with ALE of *Ixora coccinea* had significantly inhibited NO production (39%; $P<0.001$) which was comparable to that by indomethacin (32%; $P<0.05$). Rats treated with prednisolone showed higher inhibition of NO production (87%; $P<0.05$). It is concluded that the inhibition of immune cell infiltration and production of NO intermediates play an important role in the anti-inflammatory activity of ALE of *Ixora coccinea.*

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Abstract No: 17

Isolation and characterization of potential disease resistance gene analogs from traditional, wild and weedy rice of Sri Lanka

Samaranayake DP*, Weerasena OVDSJ, Karunanayake EH

Institute of Biochemistry, Molecular Biology & Biotechnology, University of Colombo

Rice is the staple food crop for nearly half of the world's population. The rice genome has been sequenced and has 430 million base pairs and about 50,000 genes. Out of these, the resistance genes (R genes) have evolved to recognize direct or indirect products of the pathogens avirulence gene, activate pathogen resistance mechanisms and make the plants resistant to pathogens. Plant disease R genes were found to encode proteins with similar structural motifs and domains. Potential disease resistance traits of rice, amplified by PCR using primers designed from these conserved domains can be used to develop molecular markers directly linked to disease resistance loci. Furthermore, such amplified fragments can be sequenced and characterized to ascertain the presence of potential disease resistance genes in rice plants. Rapid advances in molecular genetics now provide opportunities for development of new rice varieties with sustainable resistance to pathogens by transferring such genes to susceptible varieties. In this study traditional, weedy and wild rice plant genomic DNA was amplified by PCR using Resistance Gene Analog (RGA) primers. The products were separated by polyacrylamide gel electrophoresis, specific bands re-amplified and sequenced in order to identify novel or conserved R genes in the genome of the chosen plants.

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*Undergraduate student, Faculty of Science, University of Colombo

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Programme and abstract book edited and compiled by Prof. Kamani Tennekoon and Dr.Niroshani Epitawalage and designed by Mr. Kanchana Senanayake on behalf of the organizing committee.

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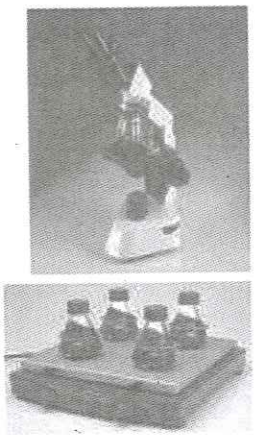

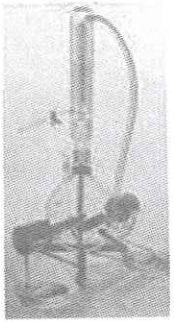

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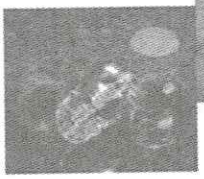

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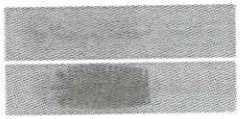

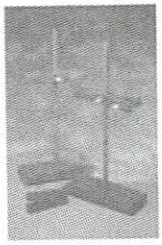

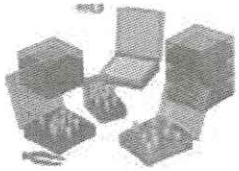

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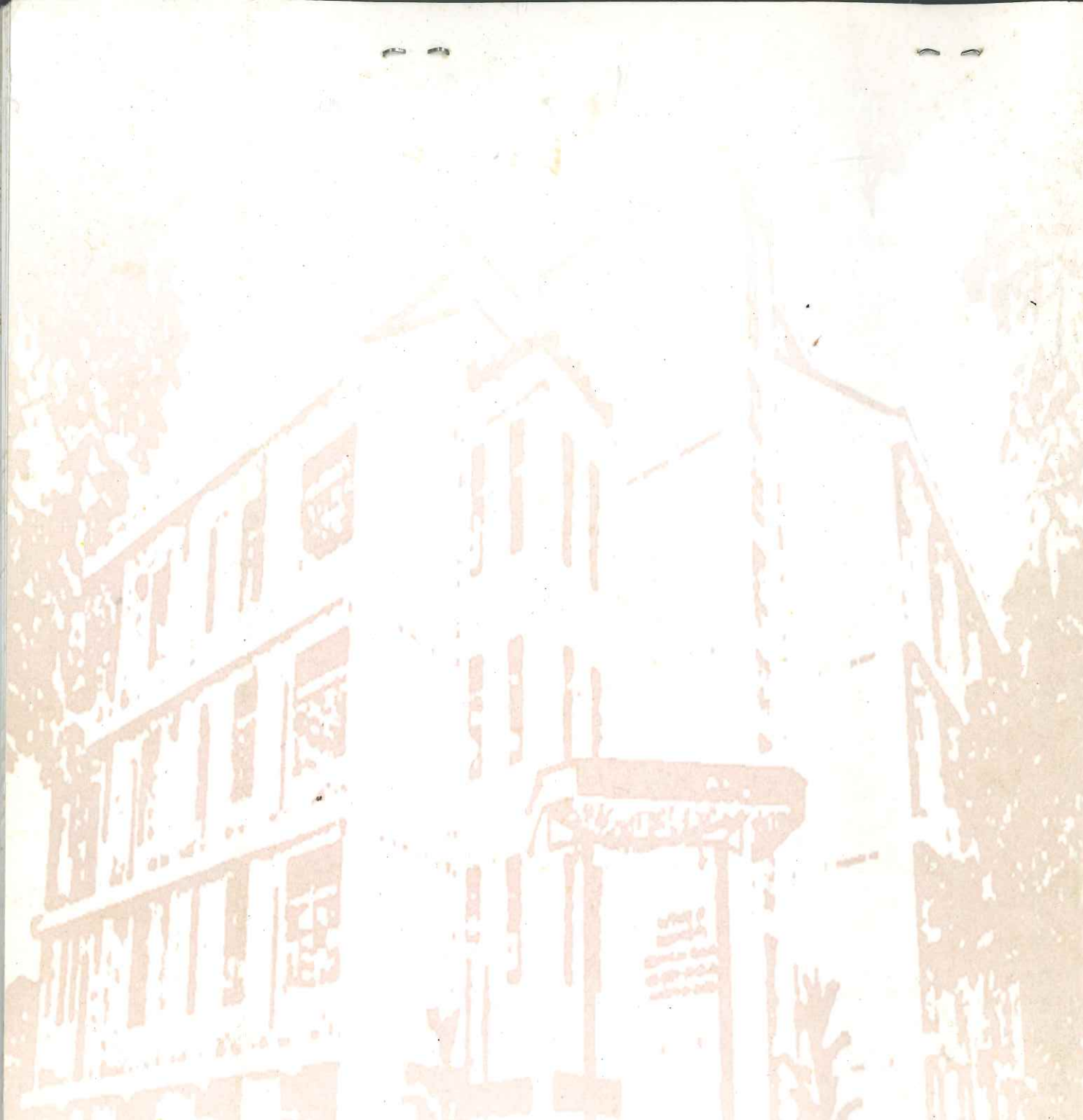

Institute of Biochemistry, Molecular Biology and Biotechnology
90, Cumaratunga Munidasa Mawatha
Colombo 03
Sri Lanka

Tel : +94-11-2552528 / +94-11-2552534 Fax : +94-11-2553683
Website : www.ibmbb.lk