

# INFECTION AND IMMUNITY DIVISION



*Professor Graham Brown  
Division Head  
Professor of Infectious  
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Health, The Royal Melbourne  
Hospital*



*Dr Robin Anders*



*Dr Alan Cowman*



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## OVERVIEW

*The long term goal of our Division is to improve current methods for preventing or treating two important parasitic diseases – malaria and leishmaniasis, by investigating the basic mechanisms of immunology, cell biology and genetics that are key determinants of the outcome of the relationship between the host and invasive organisms.*

*Many malaria antigens have been identified but little is known of their function. Using gene knock out technology developed in the laboratory, we are investigating the role of candidate vaccine molecules in parasite survival by studying the defects produced by removing these genes.*

*From our studies of the molecules expressed on the surface of infected erythrocytes, we have localised the sites of binding to chondroitin sulfate that is critical for developing strategies for prevention of placental malaria.*

*We are defining the role of the malaria toxin glycosylphosphatidylinositol (GPI) in causing severe disease and the role in parasite immunology of GPI-specific CD1d-restricted NK T cells. The role of a Leishmania virulence factor, the proteophosphoglycan (PPG) is also being investigated; much progress has been made with the cloning of the PPG gene.*

*Genetic studies of host resistance to malaria and leishmaniasis are carried out in collaboration with the Genetics and Bioinformatics Group.*

*In collaboration with colleagues of the Papua New Guinea Institute of Medical Research vaccine combinations from our malaria team have been tested in young children, and clinical trials have continued with AMA-1, another promising candidate. We are investigating the mechanism and control of the protective immune responses induced by the Leishmania candidate vaccine, Parasite Surface Antigen 2.*

## Research Focus

**Malaria**

**Leishmania**

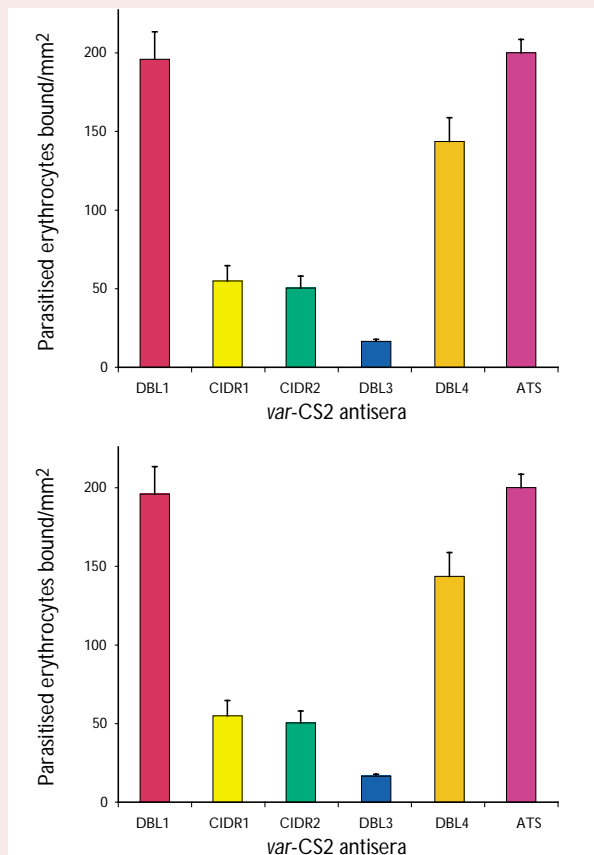
**Identification of regions of PfEMP1 responsible for binding of malaria infected cells to chondroitin sulfate A**

*JC Reeder, GV Brown, JG Beeson, AF Cowman, R Noviyanti, KM Davern, A Thaus, T Byrne*

Maternal malaria affects millions of women each year. A prominent feature of this infection is the adherence of *Plasmodium falciparum*-infected erythrocytes in the placenta and our field studies have shown that chondroitin sulfate A (CSA) is a major host receptor in this interaction. Targeting CSA binding thus appears to be a rational therapeutic strategy for the disease, but an essential pre-requisite is the elucidation of the mechanism of adhesion.

Last year we reported the cloning and characterisation of a *var* gene associated with adhesion to CSA. We are now able to inhibit adhesion of infected erythrocytes to CSA by antibodies raised to recombinant fusion-proteins corresponding to the major domains of the gene and thereby identifying *P falciparum* erythrocyte membrane protein 1 (PfEMP1) as the major parasite ligand. The specific region containing CSA binding sites can be inferred from the adhesion inhibition experiments (Figure 1).

*Figure 1 Inhibition of binding of CS2 parasites to immobilised CSA with antibodies to recombinant proteins from the CS2 var-gene*



**Characterising the adhesive and antigenic properties of *Plasmodium falciparum* infections during pregnancy**

*JG Beeson, GV Brown in collaboration with SJ Rogerson, ME Molyneux of The Wellcome Trust Research Laboratories and Malaria Project, and C Mhango of the Department of Obstetrics and Gynaecology, College of Medicine, University of Malawi, Blantyre, Malawi*

*P falciparum* malaria during pregnancy is an important cause of maternal and infant morbidity and mortality. Results from field studies conducted in Africa suggest that the accumulation of large numbers of *P falciparum*-infected erythrocytes in the placenta is mediated by parasite adhesion to chondroitin sulfate A, present on the placental lining. *P falciparum* isolates that were collected directly from infected placentae could bind to chondroitin sulfate A in the majority of cases, whereas there was little or no adhesion to other parasite receptors CD36 and ICAM-1. An evaluation of the presence of variant-specific antibodies among different groups of adults to *P falciparum* isolates from pregnant and non-pregnant individuals suggests that pregnant women are infected with a distinct sub-set of *P falciparum* variant types that rarely occur outside pregnancy.

Thus, immunity to these sub-types does not exist prior to pregnancy, a finding that may account, in part, for the increased susceptibility to infection at first pregnancy. A detailed understanding of the pathogenesis of maternal malaria and of the immune responses that develop may lead to specific therapies and preventive interventions.

**Structural requirements of chondroitin sulfate A for *P falciparum* adhesion**

*JG Beeson, GV Brown in collaboration with W Chai, AM Lawson, Imperial College, Harrow, UK*

Following the finding of an association between adhesion of *P falciparum*-infected erythrocytes to chondroitin sulfate A (CSA) and the development of placental malaria infection, investigations aimed at identifying unique motifs in CSA that constitute the parasite binding domain are in progress. We have previously determined that the minimum chain length of CSA oligosaccharides required for parasite binding consists of 14 monosaccharide units. Oligosaccharide sub-fractions of different sulfate content and sulfation patterns are being isolated and tested for parasite binding with a view to identifying the precise sequence(s) of CSA involved in adhesion.

## **Australia/Indonesia Medical Research Initiative (AIMRI)**

The second year of this Australian Government (AusAID) funded initiative has seen expansion to a full scientific program with Australians working along side Indonesian colleagues at the Eijkman Institute for Molecular Biology in Jakarta. Working closely with scientists from the Division of Infection and Immunity (and Dr Terry Spithill from Monash University), a malaria research program has been developed in Jakarta and Indonesian colleagues have established links to colleagues working in field sites. Mutations conferring resistance to antimalaria drugs have been identified in malaria infected cells. Analysis of adherence to specific substrates and expression of *var* genes has been done at individual cell level. Two students from the Eijkman Institute continue their doctoral studies at this Institute.

## **Targeted mutagenesis of *Plasmodium falciparum* erythrocyte membrane protein 3 disrupts cytoadherence of malaria-infected red blood cells**

*JG Waterkeyn, ME Wickham, KM Davern, JC Reeder, AF Cowman in collaboration with JG Culvenor Department of Pathology, University of Melbourne, RF Waller, Department of Botany, University of Melbourne, BM Cooke, Department of Microbiology, Monash University*

The protozoan parasite *P falciparum* causes lethal malaria and adhesion of parasite-infected red cells to vascular endothelium is believed to be important in the pathogenesis of this disease. Adherence is mediated by the variant erythrocyte membrane protein 1 (PfEMP1). A second protein, erythrocyte-membrane-protein-3 (PfEMP3), is deposited under the membrane of the parasite-infected red cell and its function is unknown. We mutated PfEMP3 and disrupted transfer of PfEMP1 onto the outside of the *P falciparum*-infected red cell. The truncated PfEMP3 accumulates in structures apparently associated with the erythrocyte membrane. We suggest that the transfer of PfEMP1 onto the outside of the parasitised red cell surface occurs through an erythrocyte membrane-associated compartment.

## **The vaccine candidate RAP1 controls rophtry localisation of RAP2 in *P falciparum***

*DL Baldi, AF Cowman in collaboration with BS Crabb, Department of Microbiology and Immunology, University of Melbourne, KT Andrews, Queensland Institute of Medical Research, RF Waller, Department of Botany, University of Melbourne, DS Roos, Department of Botany, University of Pennsylvania, USA*

Rhoptry associated antigen 1 (RAP1) and 2 (RAP2) are leading vaccine candidates for the control of malaria. These molecules form a complex and are localised in the rophtry organelles of the extracellular blood-stage form of the parasite known as the merozoite. We have disrupted the *RAP1* gene by gene targeting and this led to the expression of severely truncated forms of RAP1. In these parasites, the RAP1/RAP2 interaction was blocked resulting in aberrant localisation of RAP2. We conclude that RAP1 has a chaperonin-like function that controls the localisation of RAP2 to the rophtries. Clearly, the function of both RAP1 and RAP2 are not required for invasion of human

RBCs *in vitro*. These results have important implications for both RAP1 and 2 as vaccine candidates.

## **A YAC contig and high resolution restriction map of chromosome 4 and 5 from *P falciparum***

*JK Thompson, S Caruana, AF Cowman*

The development of two technologies has enabled physical mapping studies which have greatly assisted in understanding the genome of *P falciparum*. Pulsed field gel electrophoresis (PFGE) resolved chromosomes of *P falciparum* and allowed the construction of macro-restriction maps of individual chromosomes. Cloning of *P falciparum* DNA in yeast artificial chromosomes (YACs) has provided a means of generating high resolution restriction maps of *P falciparum* chromosomes. We have obtained physical maps of contiguous YACs that correspond to *P falciparum* chromosomes 4 and 5. These maps have also provided information about the structure of the subtelomeric regions of *P falciparum* chromosome size polymorphism as well as the organisation of genes and gene families within the genome.

## **Prevention of malarial pathology by antagonists of the GPI toxin**

*L Schofield, SD Tachado and S Gerondakis in collaboration with N Hyams, RT Schwarz, Phillips University, Marburg Germany*

Much of the morbidity and mortality associated with malaria is thought to arise from the actions of a malarial toxin. The toxic basis of malarial pathology was recognized as early as 1888 by the great Italian physiologist Camillo Golgi but the molecular identity of the malaria toxin has long remained obscure. Recently, we have identified a molecule with the properties of such a toxin; the glycosylphosphatidylinositol (GPI) of *P falciparum* origin. We have shown that gross malarial pathology and even death due to the infection can be specifically blocked by diverse pharmacological antagonists of the GPI-induced signalling pathway. These findings may help rationalise the clinical management of malaria in humans.

## **Recognition of Glycosylphosphatidylinositol by CD1-Restricted NK T Cells**

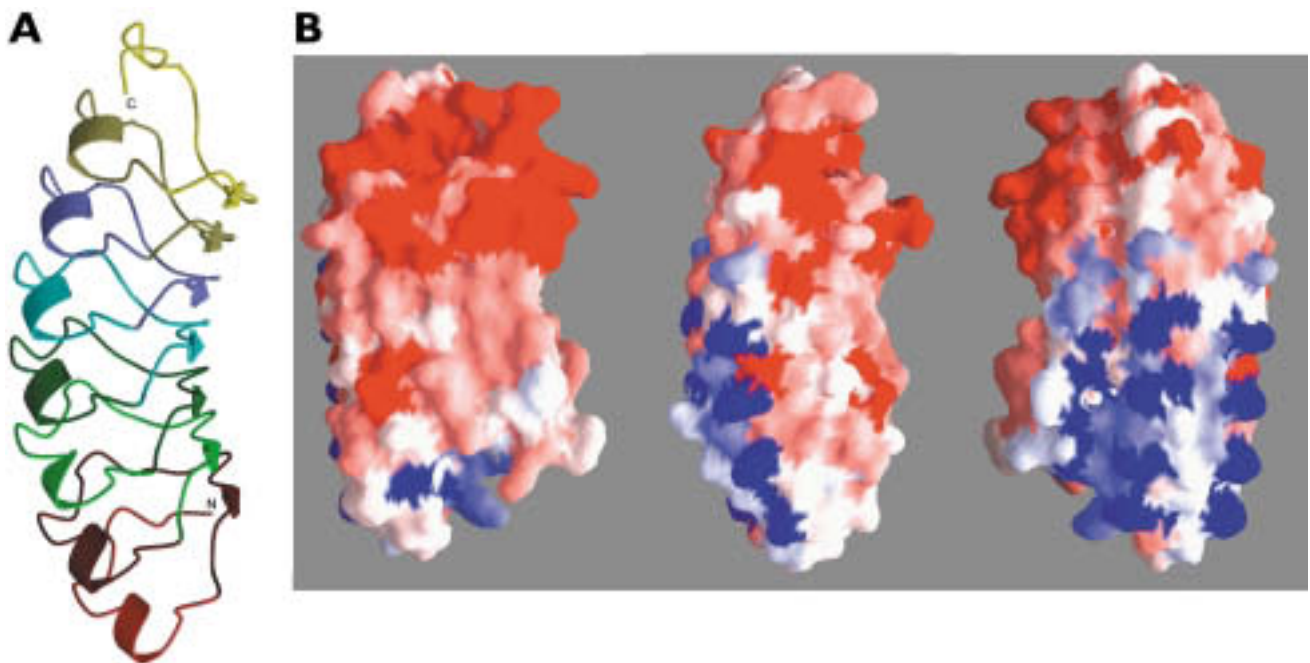
*L Schofield, D Hansen and SD Tachado in collaboration with M McConville, University of Melbourne, S Campbell and B Fraser-Reid, Natural Products and Glycotechnology, NC USA, and MJ Grusby, Harvard School of Public Health, Boston, MA USA*

NKT cells are unusual CD4+, NK1.1+ lymphocytes with a very skewed TCR repertoire, suggesting they are selected by a limited range of ligands. Dysregulation of this population is implicated in many autoimmune diseases, including type I diabetes. However, the natural ligand and functional significance of NKT cells in immune responses *in vivo* remains unclear. We have found that GPIs of self and non-self origin are recognised by CD1d-restricted NKT cells, identifying for the first time a natural ligand for this important and unusual T cell population. Recognition of both self and non-self GPIs and related ligands may be involved in the aetiology of autoimmune states and the regulation of immunity to bacterial and parasitic pathogens.

### Identification and predicted structure of a Leucine Rich Repeat motif shared by *Leishmania major* proteophosphoglycan and Parasite Surface Antigen 2

*J Montgomery and E Handman in collaboration with T Ilg, Max-Planck-Institut für Biologie, Tübingen, Germany, and B Kobe, St Vincent's Institute of Medical Research, Melbourne Australia*

The newly discovered membrane-anchored *Leishmania major* proteophosphoglycan shares significant sequence similarity with the unrelated membrane glycoproteins of the Parasite Surface Antigen 2 family. This similarity is restricted to a region of multiple Leucine Rich Repeats and places both molecules in the Leucine Rich Repeat superfamily, all of which are involved in protein-protein interactions and many in signal transduction processes. The availability of the crystal structure of porcine ribonuclease inhibitor and the spliceosomal protein U2A' allowed the construction of a three dimensional model of the proteophosphoglycan motif. The model suggests that the conserved amino acids provide a scaffolding for an array of surface exposed residues most likely involved in ligand binding. Moreover, the similarity of PPG and PSA-2 may also indicate a similar function and interaction with a common ligand.



**Figure 2** (A) a ribbon diagram of the model of the leucine rich repeats of PPG and (B) the molecular surface of the model of leucine rich repeats colour-coded according to the similarity between PPG and PSA-2 from red (0) to white (50%) to blue (100%), and the three views are related by consecutive 90° rotations; the middle view corresponds to the orientation in (A).

### **A new potential virulence factor for *Leishmania***

Protozoan parasites of the genus *Leishmania* are the causative agent of a variety of human diseases in the tropics, the subtropics and the Mediterranean region. They possess a digenetic life cycle that includes several forms of flagellated, extracellular promastigotes in the gut of their sandfly vector and a nonflagellated intracellular amastigote form that resides in macrophages in the mammalian hosts. Much of our work over the past year has focused on a mucin-like proteophosphoglycan that we have isolated and cloned.

### **Filamentous proteophosphoglycan secreted by *Leishmania* promastigotes forms gel-like three dimensional meshworks that obstruct the digestive tract of infected sandfly vectors**

*E Handman and J Curtis in collaboration with T Ilg and Y-D Stierhof, Max-Plank Institut für Biologie, Tübingen, Germany, PA Bates, Liverpool School of Tropical Medicine, Liverpool, UK, and RL Jacobson and Y Schlein, The Hebrew University, Jerusalem, Israel*

One of the factors which determine parasite virulence in the disease-transmitting insect is the formation of a filamentous gel loaded with parasites which blocks the mouth parts. This gel is thought to promote parasite transmission by forcing the fly to feed repeatedly and thus deposit infectious promastigotes into the wound. We have isolated a parasite filamentous proteophosphoglycan which is extremely resistant to proteolysis and have now shown that it is the major component of the plug that obstructs the insect's digestive tract. The secretion of this glycoconjugate by promastigotes in the sandfly is therefore a virulence factor promoting the transmission of the parasite.

### **Molecular cloning and characterization of a novel repeat-containing *L major* gene, *ppg1*, that encodes a cell surface membrane-associated form of proteophosphoglycan with a putative glycosylphosphatidylinositol anchor**

*T Ilg, J Montgomery, J Curtis and E Handman in collaboration with Y-D Stierhof from the Max-Plank Institute for Biology, Tübingen, Germany*

We have cloned a gene encoding a membrane-associated proteophosphoglycan (mPPG), a member of a family of related mucins, including filamentous PPG described above. The function of mPPG is the focus of current work. mPPG with its 100 repeats of proline, alanine and serine may extend more than 300  $\mu\text{m}$  above the plasma membrane making it highly accessible for binding partners. It provides many potential receptor and complement binding sites, since it may be modified with up to 800 serine-linked phosphoglycan chains. In addition, mPPG contains a leucine rich repeat region at its amino terminus, also suggestive of a role in protein-protein interactions (see box p. 49).

### **Development of a prototype *Leishmania* vaccine**

*A Hadjinoormohammadi, J Curtis, T Baldwin and E Handman*

A major focus of the laboratory has been the development of a prophylactic vaccine against cutaneous leishmaniasis based on the Parasite Surface Antigen 2, a glycosylphosphatidylinositol-anchored membrane protein present on most *Leishmania* species. Having shown that a DNA vaccine protects mice from infection because it induces an exclusive Th1 immune response, we are currently attempting to target the expression of this DNA vaccine to a Th1 cytokine environment by using IL-12 as adjuvant. For this purpose, we have designed a single chain IL-12 gene in which the p40 and p35 subunits are linked. Vaccination using this DNA encoding bioactive IL-12 has commenced.

## **Acknowledgements**

The Infection and Immunity Division acknowledges the generous gifts and bequests listed elsewhere in this Report and also thanks the following organisations, trusts and persons whose major grants supported research in this Division.

### **Australia:**

National Health and Medical Research Council  
Australian Research Council Fellowships Scheme  
Victorian State Government  
Cooperative Research Centre for Vaccine Technology  
Australia Agency for International Development (AusAID)  
Australia/Indonesia Medical Research Initiative  
BHP Community Trust  
Rotary Against Malaria Programme  
Australia India Council  
Harold and Cora Brennan Benevolent Trust  
Hazel and Pip Appel Fund

### **Overseas:**

UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases  
The Wellcome Trust (UK)  
Human Frontiers Science Program  
Hoffman La Roche (Switzerland)  
Papua New Guinea Institute of Medical Research (PNG)  
National Institute of Health - NIH (USA)

### **Income from the following Estates also supported this Division:**

JW Ballantyne Estate  
Jean C Burns Estate  
Isabella A Brown Estate  
Catherine Carlson Estate  
John PG Claridge Estate  
GC Graves Estate  
Joan Henderson Estate  
M Hamilton Estate

Catherine M Jackson Estate  
Alexia Lyell Bequest  
Trevor Geoffrey Mansfield Estate  
G Mitchell Fund  
Ida Alice Moon Estate  
Alan Ambrose Murray Estate  
EM Newton Estate  
Jessie Reeves Estate  
Agnes T Robertson Estate  
Stanley L Spencer Estate  
John T Tomasetti Estate  
Tropical Diseases Fund  
Dorothy Hope Walker Estate  
Ita E Westcott Estate

## Staff List

**Graham V Brown, Division Head** (to 4/99), Consultant to AIMRI Project (from 4/99)

Heather Saunders, Secretary (to 6/99)

Ross Coppel, MB BS PhD *Melb DTM&H London*, Sabbatical Visitor (from 3/99)

**Graham V Brown**, MB BS PhD *Melb MPH Harvard* FRACP FAFPHM (to 4/99)  
John Reeder, MSc *Salford* PhD *Manchester*

Tim Byrne, BAppSc *Swinburne*  
Kathy Davern, BAppSc *RMIT MSc Melb*  
Anne Thaus

James Beeson, BMedSc(Hons) MB BS *Mon*, PhD student  
Rintis Noviyanti, BSc(Hons) *Indonesia*, PhD student

Australia Indonesia Medical Research Initiative  
Michael Duffy, BSc(Hons) PhD *Melb*  
Nagesh Hadya, BVSc MVSc *Bangalore* PhD *Melb*

Gerard Casey, BSc(Hons) *Mon* (from 10/98)  
Vicki Doherty, BSc(Hons) *LaT* (to 8/98)  
Soula Krejany, BSci *Melb* (from 6/99)  
Ann Seward, BSc(Hons) *Melb* (to 1/99)

**Robin F Anders**, BAgSc PhD *Melb*  
Adrian Batchelor, BA(Hons) *Cambridge* PhD *London* (from 9/98)  
Anthony Hodder, BSc(Hons) PhD *Mon*

Pauline Crewther, BSc(Hons) MSc *Melb*  
Vince Murphy, BSc(Hons) *Melb* (from 9/98)

Eva Bucsu, BSc(Hons) *Melb*, PhD student  
Andrew Pearce, BSc(Hons) *ANU*, PhD student

**Alan F Cowman**, BSc(Hons) *Griffith* PhD *Melb*  
Tim-Wolf Gilberger, B Biology *Hamburg*, Visiting Scientist (from 1/99 to 3/99)  
Julie Healer, BSc(Hons) *Glasgow* M Phil *London* PhD *Edinburgh* (from 3/99)  
Michael Reed, BSc(Hons) *Mon* PhD *Melb*  
David Roos, PhD *Rockefeller*, Sabbatical Visitor (to 7/98)  
Ngo Viet Thanh, (from 4/99)  
Till Voss, PhD *Basel*, Visiting Scientist (to 9/98)  
Jacqui Waterkeyn, BSc(Hons) *UNSW* PhD *Melb*

Sonia Caruana  
Jennifer Thompson, BSc *Melb*  
Tony Triglia, BSc(Hons) MSc *Melb*

Deborah Baldi, BSc(Hons) *Melb*, PhD student  
Kerry Mills, BSc(Hons) *ANU*, PhD student  
Mark Wickham, BSc(Hons) *Melb*, PhD student

**Emanuela Handman**, MSc PhD *Jerusalem*  
Amir Hadjinoormohammadi, DVM(MS) *Teheran* PhD *Melb* (from 8/98)  
Sandrine Henri, PhD *Marseille* (from 6/99)

Joan Curtis

Anja Jensen, MSc *Copenhagen*, Overseas Research Trainee (from 3/99)  
Jacqui Montgomery, BSc(Hons) *Melb*, PhD student

**Louis Schofield**, BSc MSc *Lond* PhD *Old*  
Diana Hansen, BBiologicalSc *Buenos Aires* PhD *Uppsala* (from 9/98)  
Ramin Mazhari-Tabrizi, PhD *Marburg*

## Staff Notes

### Arrivals

Adrian Batchelor, Postdoctoral Fellow from the Department of Biophysics at the Johns Hopkins School of Medicine, Baltimore  
Ross Coppel, Sabbatical Visitor from the Department of Biochemistry, Monash University  
Tim-Wolf Gilberger, Visiting Scientist from the Bernhard Nocht Institute for Tropical Medicine, Hamburg  
Amir Hadjinoormohammadi, Postdoctoral Fellow from the Department of Veterinary Science, University of Melbourne  
Diana Hansen, Postdoctoral Fellow from the Department of Biochemistry and Molecular Biology, Monash University  
Julie Healer, Postdoctoral Fellow from Edinburgh University  
Sandrine Henri, Postdoctoral Fellow from the University of Marseille-Luminy, France  
Ngo Viet Thanh, Visiting Trainee Scientist, from the Institute of Malariology and Parasitology, Vietnam

### Departures

David Roos, Sabbatical Visitor, to the University of Pennsylvania  
Till Voss, Visiting Scientist, returned to the Swiss Tropical Institute, Basel, Switzerland

## Major Invited Lectures and Exchanges

### Professor Graham Brown

Papua New Guinea Institute of Medical Research, Goroka, Wewak, Maprik, 8/98. *Field trip*  
Menzies School of Health Research, Darwin, 10/98. *Review Panel Member*  
Associated Society for Blood Transfusions, Melbourne, 10/98  
Global Programme for Vaccines and Immunization (GPV), "Novel Adjuvant Currently in Human Clinical Testing", Ancey, France, 11/98. *Co-chairman*  
Malariology Centenary Conference "The Malaria Challenge after One Hundred Years of Malariology", Rome Italy, 11/98  
Eijkman Institute for Molecular Biology, Jakarta, Indonesia, 2/99. *Site visit*  
7th Commonwealth Pharmaceutical Association Conference, Melbourne, 3/99  
Discipline Panel, National Health and Medical Research Council, Canberra, 4/99. *Chair*  
Vaccine Discovery Research, Steering Committee Meeting, World Health Organization Palawan Philippines 5/99  
World Health Organization/TDR Meeting on Malaria and Schistosomiasis Vaccine Research in Asia, Manila, Philippines, 6/99

### Dr Alan F Cowman

IXth International Congress of Parasitology, Makuhari, Japan, 8/98. *Invited Speaker and Session Chairperson*  
Workshop on Functional Analysis of the Malaria Genome, Rockville MD, 11/98  
WHO/TDR Strategic Research Steering Committee, Jakarta, Indonesia, 9/98  
Eijkman Institute for Molecular biology, Jakarta, Indonesia, 9/98. *Site visit*  
The Novartis Foundation Symposium, London, UK, 1/99  
World Health Organization Workshop on Molecular Tools for the Functional Analysis of the Malaria Genome. London, UK, 1/99  
Strategies for Exploiting the Malaria Genome Sequence Data. Wellcome Trust, London, UK, 1/99  
Pathogenic Protozoa: Molecules, Structures and Mechanisms, Heidelberg, Germany, 4/99

### Dr Emanuela Handman

University of Melbourne, Department of Immunology and Microbiology, 8/98  
LaTrobe University, Department of Biochemistry, 9/98  
Pasteur Institute, Paris, France, 10/98. *Manlio Cantarini Visiting Professorship*  
Keystone Symposium on Macrophage Biology, Keystone, CO USA, 1/99.  
Washington University, St Louis, MO USA, 1/99  
University of New South Wales, Department of Biochemistry, 3/99

### Dr John Reeder

WHO/TDR Strategic Research Steering Committee, Jakarta, Indonesia, 9/98  
Eijkman Institute for Molecular Biology, Jakarta, Indonesia, 9/98. *Site visit*

### Dr Louis Schofield

Human Frontiers of Science Programme Workshop, Institut für Medizinisch Mikrobiologie, Marburg, Germany, 7/98  
Biennial International Symposium on Molecular Medicine, Brisbane, 8/98  
IXth International Congress of Parasitology, Chiba, Japan, 8/98  
Second Louis Pasteur Conference on Infectious Diseases, Paris, France, 11/98  
British Society for Immunology Annual Symposium, Harrogate, UK, 12/98  
5th International Symposium on Biochemical Roles of Eukaryotic Glycoconjugates, Bangalore, India, 1/99  
Conference on NK T Cells and CD1-restricted Antigen Presentation, La Jolla Institute of Allergy and Immunology, San Diego, CA USA, 4/99  
8th Annual Conference of the International Centre for Tropical Disease Research of the National Institute of Allergy and Infectious Diseases, Washington, DC USA, 4/99  
Centres for Disease Control, Chamblee, Atlanta, USA, 4/99  
Naval Medical Research Institute, Bethesda, MD USA, 5/99

*New staff and students: from left, Julie Healer, Sandrine Henri, Amir Hadjinoormohammadi, Diana Hansen. Absent: Gerard Casey, Adrian Batchelor.*

