



INSTITUTO GULBENKIAN DE CIÊNCIA

ANNUAL REPORT 2002

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This Report can be consulted at the IGC website: <http://www.igc.gulbenkian.pt>

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**BOARD OF ADMINISTRATION
OF THE
FUNDAÇÃO CALOUSTE GULBENKIAN**

The Fundação Calouste Gulbenkian, established by Calouste Sarkis Gulbenkian by his Will dated June 18, 1953, is a private Institution of general public utility, endowed with legal personality. The aims of the Foundation are charity, art, education and science. The members of the Board of Administration in 2002 were:

President

Victor de Sá Machado (to July 2002)

Emílio Rui Vilar (from July 2002)

Honorary President

Mikhael Essayan

Executive Trustees

José Blanco

Diogo de Lucena

Isabel Mota

Eduardo Marçal Grilo

Non-Executive Trustees

André Gonçalves Pereira

Eduardo Lourenço

Artur Santos Silva

BOARD OF DIRECTORS

The Board of Directors for the Instituto Gulbenkian de Ciência (IGC) ensures that the activities at the Institute follow the guidelines and objectives defined by the Board of Administration of the Fundação Calouste Gulbenkian. The members of the Board of Directors for 2002 were:

Board of Directors

Diogo de Lucena (Chairman)

João Caraça

Manuel Rodrigues Gomes

Manuel Carmelo Rosa

Horácio Menano

António Coutinho

The Board of Directors met on 28th February 2002.

SCIENTIFIC ADVISORY BOARD

The Scientific Advisory Board of the IGC scrutinises the scientific progress and teaching programmes, as well as the recruitment and activity of personnel and research groups. The Scientific Advisory Board also advises the Board of Administration of the Fundação Calouste Gulbenkian on all matters relevant to the mission of the Institute. The members of the Scientific Advisory Board for 2002 were:

Prof. Sydney Brenner (Chairman)

Prof. Philippe Kourilsky

Prof. Nicole Le Douarin

Prof. Martin Raff

Prof. Kai Simons

Prof. Susumu Tonegawa

Prof. Hans Wigzell

Prof. Lewis Wolpert

The Scientific Advisory Board met at the IGC on 9-10 May 2002.

STAFF

DIRECTOR

António Coutinho

DEPUTY-DIRECTORS

Sérgio Gulbenkian

José Mário Leite

RESEARCH MEMBERS

The IGC is not divided into departments, and its scientific activities are organised in relatively small groups. Research is autonomously conducted by individual scientists and small groups who are free to associate in projects.

It should be noted that nearly all the scientists at the IGC are affiliated at other institutions or supported by national or international organisations; these are indicated in parenthesis. Some of those listed below were present at the IGC for only part of the year.

Jan Andersson (Univ. Stockholm/FCG)

Jörg Becker (Univ. Bielefeld/FCT)

Juan Carlos Belmonte (Salk Institute/FCG)

José António Belo (UALG/FCG)

Paula Parra Bueno (FCT)

Jorge Carneiro (Lab. Associado)

Cristina Casalou (IPOFG/FCT)

Moises Marinho Cavalcante (UE)

Pierre-André Cazenave (Univ. Paris VI/Institut Pasteur/CNRS/FCT)

Sukalyan Chatterjee (FCG)

Susana Constantino (IPOFG/FCT)

Ana Coutinho (FCT)

António Coutinho (CNRS/FCG)

Pedro Coutinho (FCT)

Ana Crespo (UE)

Jocelyne Demengeot (FCG)

Sérgio Dias (IPOFG/FCT)

Francisco Dionísio (FCG/FCT)

José Faro (Univ. Salamanca/FCT)

José Feijó (FCUL)

Lisete Fernandes (ESTSL)

Carlos Alberto Ferreira (HUSM)

Constantin Fesel (Weizmann Institute/FCT)

Carlos Penha Gonçalves (FCG)

Simone Gines (FCT)

Gabriela Gomes (FCT)
Isabel Gordo (FCG/FCT)
Christophe Gregoire (FCG)
Isabel Gregoire (FCT)
Sérgio Gulbenkian (FCG)
Werner Haas (FCG)
Matthias Haury (FCG)
Domingos Henrique (FMUL)
Dan Holmberg (Univ. Umea/FCG)
António Jacinto (FCT)
Gregory King (Univ. Warwick)
Moises Mallo (Lab. Associado)
Maria Marone (UCSC)
Maria Mota (FCT)
Maria Teresa Faria Pais (FCT)
Isabel Palmeirim (ECSUM)
Michael Parkhouse (FCG)
Leonor Parreira (FMUL/CEBIP/FCG)
Bernardo R. Peixoto (HDES/FCT)
Sylviane Pied (INSERM/FCT)
Joaquin Rodriguez (FCT)
Leonor Tavares Saúde (FCT)
Elsa Seixas (FCT)
Gabriela Silva (FCT)
Helena Soares (ESTSL)
Miguel Che Parreira Soares (Lab. Associado)
João Pedro Simas (FMUL)
John Stewart (Univ. Tech. Compiègne/CNRS)
Ana Teresa Tavares (FCT)
Álvaro Augusto Tavares (ISTUL)
Vera Lucas Teixeira (FCT)
Solveig Thorsteinsdottir (FCUL)
Maria de Jesus Trovoada (FCG)
Tatiana Vassilevskaia (Astrazeneca)
Astrid Vicente (FCG)
Luisa Mota Vieira (HDES)
Ana Margarida Vigário (FCT)
Ari Waisman (Univ. Cologne/FCT)
Andrew Waters (Univ. Leiden/FCG)

STUDENTS

Ph.D. Students

Isabel Alcobia (FMUL/FCT)
Emília Almeida (ITQBUNL)
Sílvia Almeida (FCUL/FCT)
Inês Ribeiro Martins Alves (FCUL/Salk Inst./FCT)
Paulo Alves (FMUL/FCT)
Fernanda Bajanca (FCUL/FCT)
Marta Barreto (FCUL/FCT)
Marie-Louise Bergman (Univ. Umea/IGC)
Leonor Boavida (FCUL/FCT)
Ana Cristina Borges (UALG/FCT)
Ana Sofia Cachaço (FCUL/FCT)
Dinis Calado (FMUL/FCT)
Susana Campino (FCUL/FCT)
Marta Campos (FMUL/FCT)
Iris Caramalho (ICBAS/FCT)
Daniel Carapau (FCUL/FCT)
Marta Carapuço (ITQBUNL/FCT)
Thiago Lopes Carvalho (ICBAS/FCT)
Ana Catarina Certal (FCUL/FCT)
Ângelo António Chora (FMUL/FCT)
Sofia Cordeiro (FCUL/FCT)
Vasco Vieira Correia (ICBAS/FCT)
Sílvia Costa (FCUL/FCT)
Ana Margarida Coutinho (FCUL/FCT)
Margarida Cunha (ICBAS/FCT)
Cornelia Doeblis (Univ. Berlin)
Célia Domingues (FCUL/FCT)
Nádia Duarte (FCUL/FCT)
Mariana Faria (FCUL/FCT)
Catarina Figueiredo (ITQBUNL/FCT)
Mário Rui Filipe (FCTUNL/FCT)
Cláudia Florindo (FCTUNL/FCT)
Francisca Fontes (Hospital Egas Moniz/ Ministério da Saúde)
Rita Fragoso (FCUE/IPOFG)
Catarina Freitas (FCUL/FCT)
Susana Godinho (IST/FCT)
Mário Grãos (FCUL/FCT)
Vincent Guiyedi (Inst. Pasteur)
Cátia Igreja (FCUL/FCT)
Cláudia Istrate (FMUL/FCT)
Pedro Laires (FCUL/FCT)
Patrícia Leirião (IHMTUNL/FCT)

Patrícia Madureira (FMUL/FCT)
Sofia Marques (FMUL/FCT)
Maria Hortense Matos (ITQBUNL/FCT)
Joana Monteiro (FCUL/FCT)
Kalet Leon Monzon (ICBAS/CIM/IGC)
Rute Nascimento (FMUL/FCT)
Hélia Neves (FMUL/CEBIP/FCT)
Sofia Nolasco (FCUL/FCT)
Vivian Oliveira (IGC)
Martyn Parker (Univ. Warwick/EPSRC)
Susana Pascoal (ESCUM/FCT)
Alexis Perez (CIM/IGC)
Ana Margarida Prado (FCUL/FCT)
Ana Sofia Quina (FMUL/FCT)
Alessandro Ramos (FCUL/PDEE)
Manuel Rebelo (FCUL/FCT)
Ana Luisa Reis (FMVUTL/FCT)
Maria Gabriela Rodrigues (FCUL)
Lénia Rodrigues (FMUL/FCT)
Sofia de Albuquerque Rodrigues (ECSUM/FCT)
Margarida Santos (FCUL/FCT)
Ana Cecília Seixas (FCUL/FCT)
Mark Jan Seldon (ICBAS/FCT)
João Sousa (FCUL/Inst. Rocha Cabral/FCT)
Ana Sofia Veloso (ITQBUNL/FCT)
Ana Maria Vieira (FCUL/FCT)
Santiago Zelenay (FCUL/IGC/FCT)

B.Sc. Students

Ana Alexandra Almeida (FCUL)
Lara Carvalho (FCUL/PRODEP/FCT)
Inês Conceição (FCUL/PRODEP)
Catarina Correia (FCUL)
Ana Neves Costa* (FCUL/PRODEP)
Vanessa Cristão (FCUL)
Lurdes Duarte (Univ. Évora)
Andreia Feijão (IST/UTL)
Ricardo Ferreira (FCUL)
Diogo Fonseca (UALG/FERN)
Catarina Gomes (Univ. Lusofona)
Alexandre Gonçalves (FCUL)
Susana Igreja (Univ. Lusofona)
Andreia Lino (FCUL)
Marta Luz (FCUL/PRODEP)
Sara Oliveira Marques (Univ. Évora)

José Nuno Martins (Univ. Évora)
Vera Martins (FCUL)
Bruno Mateus (ISTUTL)
Nuno Pedrosa (FCUL)
Rui Peixoto (FCTUC)
Carlos Pereira (FCUL)
Joana Ribeiro (ICBAS/FCT)
Tiago Robalo (IST)
Cristina Rodrigues (FCUL)
Gustavo Rosa (FCUL/PRODEP)
Joana Santos (FCUL/PRODEP)
Daniel Vieira (FCUL)

Laboratory Technical Support

Maria da Luz Alvim (BIC/FCT) (left Sept 2002)
Ana Água-Doce (BIC/FCT)
Paulo Almeida (Lab. Associado)
Marisa Cabrita (BTI/FCG)
Lara Carvalho (BTI/FCG)
Carl Collins (BIC/FCT)
Maria do Céu Conceição (BTI/FCG)
Sílvia Correia (BIC/FCT)
Ana Neves Costa* (BIC/FCT) (from October 2002)
Dolores Ferreira (BTI/FCG)
Lídia Fonseca (BTI/UE)
Beatriz Garcia (BIC/FCT)
Ana Cristina Gaspar (BTI/FCG)
Susana Magrito (BTI/FCG)
Sara Marques (BAI/FCT)
Miguel Monteiro (BTI/IEFP)
Filipa Moraes (BTI/FCT)
Ana Nóvoa (Lab. Associado)
Helena Nunes (BIC/FCT)
Dominique Ostler (BTI/FCG)
Miguel Casanova Parente (BIC/FCT)
Rui Rodrigues (BIC/FCT)
Nuno Sepúlveda (BIC/FCT)
Ana Cristina Silva (BI/FCT)
Catarina Silva (BTI/FCT)
Sérgio Simões (BTI/FCT)
Sofia Simões (BTI/UE)
Marta Soares (BTI/IEFP)
Marta Vitorino (BIC/FCT)

Short Term Apprentices

Ana Cláudia Marques

Joana Martins (left February 2002)

Carla Narciso

Vânia Parelho

Catarina Serrano

Susana Correia Silva

Susana Silva

ADMINISTRATIVE, SECRETARIAL AND TECHNICAL STAFF

The administrative, secretarial, and technical staff of the IGC provide support to the research and teaching activities. Everyone here worked at the IGC for all or part of 2002.

Administrative and Secretarial Staff

Manuel Carvalho
Manuela Cordeiro
Jorge Costa
Greta Martins
Fátima Mateus
Maria Matoso
Ana Carolina Maya
João Nunes
Ana Lícia Pires
Ana Maria Santos
Vítor Santos
Abílio Simões
Teresa Maria Sousa
Lurdes Torres

Laboratory Technical Staff

Maria Ressurreição Alpiarça (left November 2002)
Ana Cristina Leitão Homem
Bruce Lenhart
Júlia Lobato
Isabel Marques
Nuno Moreno
Dolores Oliveira (left July 2002)
Rosa Maria Santos

Technical Support Staff

António Gomes (left December 2002)
António C. Ligeiro
João Carlos Lopes
Paulo Martinho (left May 2002)
Severino Matias (from June 2002)
Carlos Nunes
António Sousa
Vítor Varão

UNITS AND SERVICES

The IGC has set up and runs a series of differentiated Services and research-supporting Units that are manned, operated and financed under institutional responsibility. These Services and Units provide regular scientific and technological expertise and advice, as well as personnel support, to the researchers at the IGC and elsewhere in the campus, while open to others in Portugal and abroad.

Animal Facility

Bruce Lenhart (until October 2002)

Jocelyne Demengeot (from October 2002)

Bioinformatics

Pedro Fernandes

Cell Imaging

Sérgio Gulbenkian/Matthias Haury

Genetic Manipulation of Mice and Rats

Moises Mallo

Informatics

José Mário Leite

Library and Scientific Information

Sérgio Gulbenkian

Sequencing and Genotyping

Matthias Haury

Animal Facility

The year 2002 proved that the new animal facility is fully functional. Efforts have been focused on increasing the production of conventional mouse strains such that the needs of internal users could be covered. Production for other institutions in Portugal have also been resumed. A major effort has been given to the introduction of diverse transgenic and mutant mouse strains to accommodate the work of newly installed groups and new projects at the IGC. At the closing of the year, the animal house facility hosted about 10.000 mice representing 60 different strains.

To facilitate the expansion of our mouse model collection two technical platforms are currently reinforced: embryo transfer and embryo freezing. Moreover, an in house health control service is being implemented.

Bioinformatics

In 2002 the Bioinformatics Unit has increased the level of Bioinformatics support to the scientific community.

As scientists become more aware of the available tools and methods, they also realise that expert counselling is hardly avoidable, especially to the people who cannot devote a significant amount of time learning how to use them proficiently. Also in this concern, the interest on our training courses (GTPB) is increasingly higher. The courses have an established international quality and are attended of by a large number of candidates from several countries. In 2002, from over 200 applicants, we selected 110 attendees, only 32 of which were from the IGC, and the faculty included 10 external teachers. Worth mentioning is the “Applied Malaria Bioinformatics 2002” course, held in October, organized very shortly after the release of the complete genomes of *Plasmodium falciparum* and *Anopheles gambiae* (mosquito). A course was ran a course on the Management of Biological Collection Data, a novel initiative in the field of Biodiversity Bioinformatics. The year 2002 also marked the first steps that the IGC took towards the creation of Post-Graduate education in Bioinformatics. A Post-Graduate programme (PGBIOINF) was designed and launched, in conjunction with several departments of the FCUL, the first course starting on January 6th 2003.

Cell Imaging Unit

The year 2002 was characterised by the extensive usage of the UIC Services by most IGC scientists and by many external users, as well. The only heavy equipment purchase was a new Leica Spectral Confocal Microscope in order to suit the increasing demand for confocal microscopy.

The mostly used equipment in 2002 :

Leica DMR:	3400 hours
FACSCalibur Analyser:	2600 hours
MoFlo High-Speed CellSorter:	1900 hours,
Confocal Multiphoton Microscope:	1600 hours
Confocal Leica Microscope:	1300 hours

Severly reduced early in the year due to the lack of trained personnel, highspeed cell sorter was again available from September 2002, the UIC providing cell-sorting on a regular schedule 5 days a week. The histology group produced over 3000 slides for IGC users, and over 35 monoclonal antibodies were purified and labeled with at least 3-5 different fluorochromes. Some of the most used monoclonal antibodies are consumed at a rate of over 2500 reactions a week by IGC users (including sortings).

The media preparation lab centralises media and buffer preparation for all of the IGC, and is also managing the central cleaning and sterilisation facility.

The UIC unit also took over the management and programming of the IGC Website, as well as the integration of all management databases for infrastructure ordering and human

resources management in the IGC. Moreover, all MacIntosh Computer support and most of the IGC Network Security support is provided by UIC members, who also coordinate all IGC scientific equipment, and ensure the implementation of appropriate usage and maintenance policies. Lastly, all aspects of Radiation Safety and Laboratory Risk Management are managed by UIC members.

The UIC Unit has continued serving several outside users from ITQB, IBET, Instituto Medicina Tropical, IPO, IBMC and FCUL.

Genetic Manipulation of Mice and Rats

During 2002, the UMTG went through a consolidation phase, following the transition and renovation year of 2001. Besides its own research activities, centered on development of new gene manipulation techniques, the UMTG has provided technical support to several ongoing projects at the Institute requiring the use of transgenic technology. In total, 23 transgenic mice and embryos have been generated during 2002 by pronuclear injection of fertilized oocytes.

Informatics

At an internal level, the Informatics Unit secured all the services for networks, email and maintenance of hardware and software. The equipment, infrastructure and informatic programmes were adjusted to the growth of the IGC caused by the opening of a new laboratorial wing and by the arrival of several new groups. The bandwidth of access to the Internet was doubled and the interface equipment was substituted to support this new change. An evaluation of our security systems, by the University of Minho, produced recommendations of short-term and long-term actions. Actions were taken for the reorganisation of the internal network on a short-term basis, and for the installation of firewall hardware and software. The internal administrative network connections to the intranet of the FCG headquarters were upgraded.

Library and Scientific Information

In 2002, the IGC Library received 169 subscriptions and provided local online access to 146 titles.

Improvements were also made in the seating area where data ports and outlets were installed for laptop user connection to the internet for online reading.

The IGC Library was visited by some 7500 readers in 2002.

Sequencing and Genotyping

In the year 2002 the Sequencing and Genotyping Unit has sequenced some 6200 samples for IGC users and for the following institutions: ITQB - Instituto de Tecnologia Química e Biológica da Universidade Nova de Lisboa; Centro de Genética e Biologia Molecular da Universidade de Lisboa; Faculdade de Farmácia da Universidade de Lisboa; Faculdade de Medicina da Universidade de Lisboa; Instituto de Histologia e Embriologia da Faculdade de Medicina de Lisboa; Instituto Superior Técnico da Universidade Técnica de Lisboa e Laboratório de Biologia Molecular do Instituto Português de Oncologia.

One of the sequencing machines was also used for genotyping in a "self-service" mode by IGC users.

INTRODUCTION

The first 5 years of the IGC after the 1998 reform.

This is the fifth Annual Report of the Instituto Gulbenkian de Ciência since the last institutional reform. It is pertinent, therefore, to draw a critical appraisal on its performance. On doing that, I shall attempt to review the strategies that were originally laid out in order to reach the objectives that led the Fundação Calouste Gulbenkian to reform and maintain the Institute.

When the Board of Administration of the Foundation decided on implementing the 1997 reform, it defined a set of objectives for the IGC. These reflected the Foundation's policy of contributing to the development of scientific research and education in the country in complementary manners to the public investments. Hence, the institutional model would likely be alternative to those used elsewhere, in universities and state laboratories. The independence and autonomy of the Foundation allowing for innovation and flexibility, its record of excellence contributing the necessary national and international credibility, several goals were set for this new phase of the IGC. As the reform was implemented, these had to be somewhat adjusted to the local and international conditions, and to the overall plans of the Foundation as well. With some updating that only the hindsight permits, the IGC missions were the following. (1) Maintain the Gulbenkian PhD Program in biomedical sciences that had been run by the Institute, and further develop formal educational programs, as well as activities directed at promoting science in society and establishing a "culture of science" in the general public. (2) Incubate leaderships; using worldwide competences, identify and educate the most capable students, attract (back) to Portugal promising young scientists, provide them with conditions for setting-up their groups and exercising leadership, such that other Portuguese institutions could use the IGC as a source of recruitable candidates. (3) In this process, produce internationally competitive biomedical science, particularly in areas that were poorly or not at all developed in Portugal. (4) Contribute to internationalizing Portuguese science, to transfer novel questions, approaches and technologies into the country, both in basic science and biomedical research, while promoting and catalyzing inter-institutional collaborations, in Portugal and abroad. (5) Whenever possible, broadly contribute to a "culture of innovation" in Portugal, notably by fostering notions of intellectual property and its entrepreneurial "transfer" in the country. This seemed also necessary for the survival of the Institute, as the decisions of the Board of Administration concerning the financial support to the Institute have evolved with time, making it clear that the IGC would have to generate a significant part of its budget.

The initial strategic decision was to adopt a model of "host institution" where most of the research and education is ensured by a transitory "faculty", hosted at the IGC for periods of a few days up to 5 years. The Foundations' investments should be primarily directed at ensuring three types of conditions: (1) competitive, high-quality

infrastructures and services, particularly in state-of-the-art technologies, capable to attract the best candidates; (2) an intense scientific environment in areas related to those pursued in the laboratories, thus providing “intellectual support” to small groups, often working in relative isolation in the country; (3) “seed money” for starting groups that, if limited, proved often necessary for their constitution and initial operation. All other conditions that we can offer to such candidates, however, are extremely limited with one exception. The IGC provides no positions or career, scarce laboratory space and little seed-money for setting up new groups. Yet, the IGC’s flexibility allows to offer an attractive condition at this point in career development: very young scientists are given full scientific autonomy and independence in the recruitment and management of research groups, and can thus give proof of their leadership competences, such as to attract the interest of other institutions in their recruitment. The IGC was thus turned into an “incubator” of “certified” young group leaders, and a potential source for the renewal of other institutions in the country. While “certification” can only be obtained *a posteriori*, in view of the group performance, group leaders had to be selected among many candidates, this choice representing already a statement of confidence in the final result. As the research groups represent the flesh and bones of an Institute, such crucial decisions had to be based on a solid international evaluation, and “make sense” within the general scientific strategy for development of the IGC. Hence, the Board of Administration of the Gulbenkian Foundation appointed a Scientific Advisory Board whose attention to the IGC has been one of the strong pillars of this process. Naturally, such new groups have to be kept small and, yet, competitive. Hence, the IGC’s resources had to be primarily invested in ensuring technological and intellectual support, such that small and young groups can perform well in the face of international competition, and are limited only by their own scientific creativity. In turn, the investments made in keeping excellence in the “intellectual environment” are simultaneously profitable for our educational efforts and PhD programs, as well as for the “internationalization” of local science.

The execution of these decisions, had to be contained within “fixed costs” for the Gulbenkian Foundation, thus requiring the availability of space and budgets at the IGC. Hence, the first concern was to find the conditions that would allow for the groups, which were present in the previous structure, to maintain their scientific activity, in spite of leaving the Institute. This process was successfully conducted by the Board of Administration, establishing agreements with the scientists and with the University departments to where they were moving, and resulting in the creation, by the Foundation, of a series of “endowed chair” equivalents in several Universities in the country. The research groups previously at the IGC could thus move with all their equipment and reagents, and continue their work in other laboratories (often refurbished with the support of the FCG). With buildings and facilities that were nearly 40 years old, it was thereafter necessary to build or re-do electricity and air-conditioning, fire-alarms and emergency exits, reconstruct laboratories, animal house and service areas, taking the opportunity to adapt them to more interactive work-spaces and to the necessary rationalization of common services. Financial constraints imposed a stepwise re-construction, facilitated by the architectural structure of the Institute, organized in independent wings. As the

renewed laboratories became progressively available, the Institute could host novel groups, according to a plan that, while submitted to a general strategy of scientific coherence and priorities, was obviously also influenced by the interest of candidates and the opportunities to recruit them. The experience of these 5 years, however, confirmed the notion of a “minimal viable size” for an Institute of this type, imposed not only by the appropriate use of centralized infrastructures and equipments, but by the necessary cooperativity among research groups and scientific themes.

Let us see now the IGC’s performance in the pursuit of the missions that should guide its activity, using criteria as objective as possible.

(1) “Incubate leaderships; using worldwide competences, identify and educate the most capable students, attract (back) to Portugal promising young scientists, provide them with conditions for setting-up their groups and exercising leadership, such that other Portuguese institutions could use the IGC as a source of recruitable candidates.” As many as 24 of the 29 group leaders at the IGC, moved here directly from abroad, and it can be argued that they would not have done it, had it not been for the IGC. In addition, a number of post-doctoral fellows and some PhD students have joined groups at the IGC, again directly from abroad. Of the 29 group leaders, only 6 are “established” investigators, 5 of which were generous enough to agree to come to Portugal and help with this endeavor. Clearly, most of the other 23 would have had great difficulties to find any other institution in Portugal where they could operate in full scientific autonomy and thus test their leadership competences. In summary, there is little doubt that the mission to enlarge the Portuguese scientific community, “attracting (back) to Portugal promising young scientists” has been successfully achieved. Unquestionably, on the other hand, the Gulbenkian PhD Programs have been instrumental “upstream” in “identifying and educating the most capable students”. The PGDB continues to receive nearly 200 applications/year, and the worldwide reputation of excellence of our PhD students suggests that the Program continues to attract the most capable ones. It must be underlined, however, that the IGC is not an extension of the PGDBM/PGDB programs, and it is not planned as a mechanism dedicated to help in the career of our alumni. Far from it: the IGC is open to young capable scientists from all origins, provided they are ready to assume responsibilities and risks, and to do the best they can about their own careers in science (and, therefore, about science at the Institute, in Portugal and in the world). Thus, only 6 of the 29 current leaders were educated in the frame of the PGDBM, a program that also “produced” young scientists to all other major biomedical research centers in Portugal (ITQB, IBMC, CNC, IMM).

The ultimate test of the IGC’s strategy, however, will have to be asked at two levels. First, by the number of young group leaders who are given positions elsewhere, after a period of some years at the IGC. Here, the objective data are quite favorable as well. Thus, of the 17 young investigators who came to Portugal with no stable position, a total of 11 have now been integrated into the Portuguese scientific system: 3 were recruited by Universities, 6 have favorably competed for positions in “Associated Laboratories” of the National Research Council (FCT), and 2 more were recruited by the Gulbenkian Foundation. Hence, it is fair to conclude that, in spite of its very short time of

operation, the IGC's objective to "export" leaderships and help to consolidate the national scientific system has been successful.

(2) "In this process, produce internationally competitive biomedical science, particularly in areas that were poorly or not at all developed in Portugal." The second test for our strategy must be the quality of the science produced by the young research groups at the IGC. The fact that the respective leaders are being recruited to competitive positions elsewhere gives already a good indication that they are doing well in science, and this can be confirmed by a glance over the list of papers that were published by the IGC over the last 2 or 3 years. It is now widely recognized that the IGC is second to no other institution in Portugal as to the quality of its publications. Thus, IGC's scientists have regularly published in journals with an "impact factor" above 10.0, (representing the top 1.2% of all journals), and most of the Institute's contributions have appeared in journals with an "impact" above 5.0 (4.3% of all journals). In spite of the fact that, only 5 years ago, the IGC was empty of scientists and equipments, that all research groups were started with the arrival of a single individual, and that most have been operating for less than 3 years, it is satisfactory to see that nearly all groups are producing internationally competitive science. It may be pointed out that the bulk of these contributions concern biological and biomedical fields that were "poorly or not at all developed in Portugal", often representing the work of young students and scientists who are thus enlarging the range of competences in the local scientific community. Accordingly, much of this work continues to be done in collaboration with other groups and institutions abroad, but a number of relevant publications have now been entirely produced at the IGC.

(3) "Contribute to internationalizing Portuguese science, to transfer novel questions, approaches and technologies into the country, both in basic science and biomedical research, while promoting and catalyzing inter-institutional collaborations, in Portugal and abroad." Over the last few years, the IGC did become the "meeting point", a "life sciences platform" and the "hall of entrance" to Portugal that it was expected to be. Literally hundreds of research seminars and lectures are held every year, together with several international meetings, courses and workshops. Initiatives were taken to complement the tradition of graduate education at the IGC with meetings and courses, aimed at bringing post-doctoral fellows and young scientists to Oeiras. A series of Gulbenkian Autumn Meetings on selective topics of prospective institutional interest was launched with success, already in 1998. Several EMBO laboratory courses were held in the past years, and our plans are to progressively increase their number to a hand-full every year. In addition, we were fortunate enough to attract to Oeiras/Lisbon, at small costs, a few international meetings with a reputation of excellence, such an EMBO Sectoral Meeting and a Juan March Workshop. Together with the "good name" gained over the years by the PhD students of our programs, this has contributed significantly for bringing Oeiras to the map of biomedical sciences.

In parallel, the IGC developed strong collaborations with a number of Portuguese institutions, and has given much attention to foster such relationships, attempting to mediate and consolidate the transfer of novel scientific themes and technologies to this country. For example, modern developmental biology, immunology, genetics of

“complex traits”, host-parasite interactions were poorly or not at all present in Portugal, in hand with the under-development of technologies such as imaging and cell sorting, genetic manipulation of mice, genome-wide typing and “gene chips”, etc. In all of these, the IGC has established “service units” that are open to external users and currently serve an increasing number of other institutions, such that they are under evaluation for governmental support as “national facilities”. The IGC has established formal cooperation agreements with several institutions at universities and hospitals, integrates 4 “external groups” located in different institutions and, most importantly, is now part of a Laboratório Associado to the Fundação para a Ciência e a Tecnologia (FCT - the National Research Council), together with two other institutions in the campus of Oeiras: the ITQB and the IBET. In addition, a number of the IGC’s group leaders already have positions at Universities (or are being recruited), thus reinforcing our close interactions with the Portuguese scientific community. This “internal” mission of the IGC has certainly been supported by the establishment of international cooperation agreements and programs. Other than the “European Union Networks” that groups at the IGC currently integrate, the Institute is the home for a “Laboratoire Européen Associé au CNRS”, composed by 4 other French laboratories, and one of the “nodes” of the European Mouse Mutant Archive.

(4) “Whenever possible, [the IGC should] broadly contribute to a “culture of innovation” in Portugal, notably by fostering notions of intellectual property and its entrepreneurial “transfer” in the country.” In the nearly complete absence of local tradition in these matters, and in the extremely difficult situation where the two terms of the equation (the science/technology, as well as the entrepreneurial side) are both very weak, the first things to do should be of an educational nature. First, PhD students and post-docs are systematically exposed to lectures and seminars in this topic. Second, intellectual property issues were addressed and institutionally regulated, after ample discussion with all at the IGC. Third, initiatives were taken to encourage the foundation of “start-ups” which, while independent of the IGC, are given support of various nature, not the least preferential access to the institute’s intellectual property. One such “start-up” has been launched by a visiting scientist at the IGC and an ex-student of our PhD Program, and a few others are under study, also in the context of the “associated” IBET (Instituto de Biologia Experimental e Tecnológica) in the Oeiras campus. Finally, it is our conviction that the very nature of the group structure at the IGC and of its operating rules helps to install a spirit of entrepreneurship (autonomy and responsibility for the full financing of the group) that is much lacking in other local institutions, notably at some University departments.

The “culture of innovation”, however, includes as well initiatives at a longer term, concerning the scientific education of the public at large, most particularly of those whose position in society is endowed with power, either in the media or in the political scene. In this respect, the situation in Portugal is particularly difficult, as can be concluded from the repeated assessments conducted by the EU on “scientific culture” and on the image of science in society. This is a theme of concern for the Gulbenkian Foundation and for the National Research Council as well, such that that IGC’s activities are integrated in wider plans. Besides participating in various initiatives of the Research

Council, such as “open house” days, “summer students”, and “public understanding” lectures, the IGC has taken the following specific steps. (1) We launched an annual series of seminars for secondary school biology teachers, aimed at transmitting, rather than scores of acquired conclusions, the enthusiasm for recent progresses and for the questions thus unveiled. These seminars are often held by PhD students and they have had a clear success, as measured by the number of attending teachers. (2) The IGC has maintained for several years a schedule series of site visits by biology classes of secondary schools, promoting the contact with science of some 600 youngsters annually. The conditions for this type of activities will certainly improve whenever the planned “science garden” installations will be made available in the campus. (3) From early 2002, a monthly session of “FAQ” with journalists has been regularly held at the IGC, with variable attendance but frequently in the order of some 10 or 12 professionals who are in charge of the “science sections” in the local media. (4) From 2002 as well, the IGC has started producing “press releases” accompanying the publication of major articles with our institutional address. Such press releases are often followed up by press conferences or by scheduled interviews with the authors, and they have had a definite impact in the media. While this is certainly positive for our mission, it seems that it also raises opposition from other research institutions, and the strategy must be, therefore, re-evaluated. (5) Following the initiatives of the Communication Department of the Gulbenkian Foundation, several large audience programs in national radio and TV stations were organized at the IGC, where science and science education were intensively debated. (6) In collaboration with this Department, we are planning to organize a workshop on science communication, under the responsibility of 3 ex-students of our PhD Program who have engaged in post-graduate education in this area. (7) Last but not least, again with the support of the Communication Department of the Gulbenkian Foundation and of the Research Council, we have appointed in 2002, a scientist at the IGC as responsible for all institutional communication and contacts with the media.

(5) “Maintain the Gulbenkian PhD Program in biomedical sciences that had been run by the Institute and further develop formal educational programs, as well as activities directed at promoting science in society and establishing a “culture of science” in the general public.” The priority for education continues to inform much of the IGC’s current plans. The first preoccupation was to maintain and consolidate the Gulbenkian PhD Program. This required institutionalizing the Program, modifying it according to the previous experience. After 7 years of an initial “experimental” period, a new program was launched on a permanent basis, again resulting from an agreement of cooperation between the Gulbenkian Foundation, the Ministry for Science and High Education, and the Luso-American Foundation for Development, who signed the founding protocol with no time limitations. This program has a new Direction, is entirely centered to the IGC, but it includes laboratory rotations in 4 other Portuguese Institutes. More importantly, and in contrast with the previous program, the PGDB is entitled to accept 3 foreign students every year and is already serving as an excellent contribution to our efforts of “internationalization”. As seen above, the Program continues to attract a large number of applications, now including European and non-European students, even though these are bound to conduct their thesis work in Portuguese laboratories.

As the population of PhD students in the Institute increased with the number of laboratories, it became clear that a new “internal” PhD Program had to be established at the IGC to support them and help the group leaders with their education. Thus, most of the PGDB students move abroad for their thesis work after the graduate course year, such that the population of PhD students at the IGC is largely non-overlapping with that in the ongoing program. An IGC PhD Program (PDIGC) was therefore launched, providing the students with tutoring and broad-based learning in biomedical sciences, such that the IGC can stand for the quality of their education, while ensuring their guidance and support all the way to a degree. This Program has now some 70 students, whom together with the 150 or so that have been thus far selected into the PGDBM/PGDB, make up for a considerable number of young people educated into modern biomedical sciences by the IGC initiatives.

In addition to launch this “internal” PhD Program, the IGC has also re-enforced the several years-old educational program on bioinformatics, now in collaboration with the Science Faculty at the University of Lisbon. We have definite plans for bringing in competences from abroad to help us starting, together with our own people in bioinformatics and theoretical biology, a strong program in Computational Biology. Furthermore, while our past attempts to initiate a PhD program for MDs in the context of their hospital functions have remained unfruitful, new initiatives are on the table to launch some form of post-graduate education in modern biomedical sciences, as well as other models of cooperation with university-level institutions to help in the education of medical technicians and engineers. Finally, aiming at enlarging the basis for student recruitment and teaching competences of our PhD programs, a general agreement with two Universities in Lisbon and the respective “Associated Laboratories” is being discussed, such that a “Gulbenkian Graduate School” could be founded in order to coordinate many a dispersed effort.

At the start, 5 years ago, there were three major concerns with the future of this enterprise. First, the IGC had little to offer and it could perhaps not attract young scientists of such a quality and in such numbers that would make it possible to generate a dynamic environment of excellence. Second, the hardships of setting-up a group from scratch, particularly for people with no such experience, together with the “aleas” and time-consuming duties of finding external support, would make it impossible for young scientists to produce good science within the five years or so of their presence at the IGC. This concern was all the more serious as there is little doubt that it takes more to publish from a new group at a new institution in a scientifically underdeveloped country, than from established institutes of great reputation. Third, it could well happen that the structure and size of the Portuguese scientific community would not be compatible with our wish to “export certified leaders” at a reasonable rate. In this case, even if everything else would go well, we would have failed in the mission of contributing to the renewal and consolidation of science in this country: all IGC group leaders would be stuck here for ever, or would have to return abroad. The first two concerns are today less present, for these first 5 years experience has shown that the “model works”. As to the third concern, while the recruitment elsewhere in Portugal of so many of the current group leaders in such a short time is very encouraging, only next year will we be entering the real phase of group turn-over (the two groups that already left the IGC were both special cases). One

more reason to be attentive to this aspect of the process, to strongly support the establishment of such groups in other institutions, and to hope for the sustained growth of the “science and technology system” in this country. A significant impact of this whole project requires that the “IGC spirit” is transported and consolidated elsewhere, but it also necessitates the continuous support of the Gulbenkian Foundation. All those participating in the project take their own risks, starting by the Foundation itself. We all acknowledge this and are very grateful for the constant support that we receive from its Board of Administration. Thus far, the IGC has had the privilege of receiving direct guidance and support from four members of that Board, as well as the interest of all others. Whatever was achieved certainly owes very much to that.

The last year at the IGC

In 2002, the growth of the IGC reflected previous investments in the renewal of laboratories, as the global economic situation has now allowed for engaging in further reconstruction, except for a common “fly room”. The most significant events corresponded to (1) the arrival of several new groups, previously approved by the Scientific Advisory Board, made possible by the opening of a new wing of laboratories; (2) the installation of a second confocal microscope and other improvements in the imaging facility (made necessary by the increase in the number of users), and of a “gene chip station”; (3) the foundation of the first “start-up” in close association with the Institute; (4) the launching of the “internal” PhD program; (5) the initial operation of a “communication office” at the IGC and the establishment of regular procedures in this respect; (6) last but not least, the consolidation of the Associated Laboratory, with increasing levels of scientific and technologic interactions among scientists from the three Institutes, and with a growing sense of a truly “common project” in the campus, best demonstrated by our application to the Research Council’s call for “re-equipment”.

The year of 2002 was marked by two events of intense emotion, but of opposite nature. In April 27, Dr. Victor de Sá Machado, Chairman of the Board of Administration of the Calouste Gulbenkian Foundation, passed away after a prolonged fight with a progressive, degenerative condition. Dr. Sá Machado, who had previously been the member at the Board of Administration in charge of Science and of the IGC, was responsible for the 1997/8 institutional reform that brought about the current project and operational model. All of us at the IGC will remain very grateful for his clarity of principles and objectives, for his confidence in the persons and his trust in novelty and youth, for his contagious enthusiasm and for the never-failing support he was kind enough to give us. We learned many things from him, not the least his constant preoccupation to have the interest of the Foundation above all others. If we know that Dr. Sá Machado was moved by those qualities when speaking of the IGC as “crown jewel” of the Foundation, his generous expression is a reason of both pride and great responsibility for us.

Needless to say, the very joyful event in 2002 was the attribution of the Nobel Prize in Physiology and Medicine to Dr. Sydney Brenner, Chairman of the IGC’s

Scientific Advisory Board. Very rarely, if ever, has a prize been more deserved. I would like to take this opportunity to very sincerely thank Dr. Brenner and all other members of the Board, in my own name and in that of all others at the IGC, for their never-failing attention to the project and to the solution of our problems.

Without the support of the National Research Council (Fundação para a Ciência e a Tecnologia, FCT) this project could not possibly have been brought forward, for it relies on the ability of the Institute's scientists to find external support for their research activities, including their own salaries and those of their post-docs, students and technicians. Most of such financing comes from competitive grants distributed by the FCT, which also supports the IGC through "Research Units" and the "Associated Laboratory", and has had a major role in the initiation and consolidation of the PhD programs. We want to formally acknowledge this support, and to thank the continuous interest of the FCT in our initiatives and problems. Even in a less favorable situation as the current period is, we could always count on the good will of the FCT's President and personnel. In October 2002, we were proud to receive in the Associated Laboratory the official visit of the Science and High Education Minister, Prof. Pedro Lynce, accompanied by the Secretary of State for Science, Prof. Manuel Fernandes Thomaz, and by the President of the Foundation for Science and Technology, Prof. Fernando Ramoa Ribeiro. These high officials could witness the vigor of the activities in the campus, and the manner how we are accomplishing our contracts with the Government. It has become increasingly clear that the establishment of the Associated Laboratory was of great importance to the IGC, resulting in the further integration of all research and development activities in the campus. I want to acknowledge here the unique role in that process played by Prof. Manuel Nunes da Ponte, who has now stepped down as Director of the ITQB and of the Associated Laboratory. It has been a great honor and pleasure to work with him. I am certain that our institutional and personal relations will continue to be excellent with the new Director of ITQB/Associated Laboratory, Prof. Peter Lindley.

All of us at the IGC deeply regret the recent death of Rodney Langman. For several years, Rod was a regular visiting scientist at the Institute, where he spent a few months in leave from his position at the UCSD's Faculty. Students, scientists and personnel, had rapidly learned to appreciate his spirit, generosity and intelligence. This was a great loss for science and for the IGC.

António Coutinho

RESEARCH

The IGC's scientific interests are centered on the genetic basis of development and evolution of complex systems, privileging organism-centred approaches and using experimental models that include plants, yeast, flies and mice, while working on the genetics of complex human diseases as well. A strong theoretical sector is also one of the IGC's specificities.

PROJECT REPORTS

Tradeoffs in the evolution of virulence

Members: Francisco Dionisio and Isabel Gordo

Students: Carla Narciso and Inês C. Conceição

This project aims at studying the evolution of interactions between parasites and hosts. To understand how these interactions evolve, we study bacteria (hosts) and their respective parasites. Several types of parasites infect bacteria. Plasmids, transposons and viruses are the most common ones. The experiments mainly involve plasmids.

Plasmids are double-stranded circular DNA molecules that can exist independently of host chromosome. They are present in most natural isolated bacterial strains. Some plasmids, called conjugative plasmids, are able to transfer to other cells. Conjugative plasmids have some interesting properties. Many of them carry genes that confer resistance to antibiotics to their hosts. Similarly, some conjugative plasmids carry genes that help bacteria to infect their own hosts (human tissues, for example). Another interesting property of conjugative plasmids is their ability to transfer between different species of bacteria as well as between bacteria and cells of plants, fungi or animal cells. These properties of conjugative plasmids imply that they are able to disseminate genes over different kinds of living beings. Therefore, by studying plasmids, we are also studying the evolution of bacterial resistance to antibiotics as well as bacterial virulence. This study involves both experiments and theoretical models.

Models of natural selection in populations and chromosomes with restricted recombination

Members: Francisco Dionisio and Isabel Gordo

Students: Ana Cláudia Marques and Tiago Robalo

Collaborator: Doris Bachtrog, University of Edinburgh, UK.

All natural populations have to adapt to new environments. Despite its extreme importance the process of adaptation is still poorly understood. For example: What is the rate at which beneficial mutations arise in a natural population? What is the distribution

of fitness effects throughout the adaptive process? are questions whose answer remains obscure to us.

An important feature to the understanding of the process of adaptation and molecular evolution concerns the rate at which new beneficial mutations fix in populations. We have been addressing this question in asexual populations by modelling populations subject to both beneficial and deleterious mutations. We have used both simulation and analytical methods to predict the rates of fixation of advantageous mutations and the change in the population mean fitness under such model. The results are important for understanding the evolution of Y chromosomes, highly selfing populations and asexual microbes such as bacteria. Therefore, in parallel to the theoretical approach, we are doing some experiments with bacteria of the *Escherichia coli* species.

Recombination activating genes 1 and 2 and vertebrate genome stability

Members: Jocelyne Demengeot, Moises Mallo and Antonio Jacinto

Students: Thiago Carvalho

Collaborators: Vasco Barreto, Rockefeller Institute, NY, USA and Miguel Godinho, MRC, London, England

The previous year, we reported the generation of transgenic mice expressing the Rag1 and 2 genes both continuously throughout lymphocyte development and constitutively in most non-lymphoid tissues. We showed that ectopic expression of the Rag genes is lethal, both to lymphocytes, and to the organism as a whole. These animals display growth retardation and early death reminiscent of mice deficient in double strand break repair molecules. We are now developing a novel transgenic system, where Rag1 and 2 are independently expressed to allow survival of the parents and guaranty efficient production of experimental animals. Analyses will concern the function the Rag genes *in vivo* in relation to the development immune system and to the genome stability. We are also investigating the impact of Rag-1 and 2 on the vertebrate genome evolution, by introducing these genes in invertebrate organisms.

Symmetry and pattern formation

Member: Gabriela Gomes

Student: Martyn Parker

Collaborator: Ian Stewart, Mathematics Institute, University of Warwick, UK.

Visualisation techniques require reaction-diffusion experiments to be performed in thin domains. This thinness of the domain motivated the development of two-dimensional models. Such models reproduce many of the observed patterns, but some discrepancies occur – for example, the black-eye pattern. Reaction-diffusion processes are three-dimensional, and we defend that three-dimensional effects will persist no matter how thin the domain is. Three years ago, we proposed that black-eye patterns are inherently three-

dimensional structures, generating some controversy. We developed a mathematical framework to support our arguments. Martyn Parker developed an extensive methodology to uncover three-dimensional effects in pattern formation.

The functional organization of chromatin in the nucleus

Member: Leonor Parreira

Students: Isabel Alcobia, Ana Sofia Quina and Hélia Neves

In this research line we investigate the functional implications of the 3-D organization of chromatin in the nucleus of hematopoietic cells. The spatial positions of specific genes as well as heterochromatic centromeric regions are analysed in 3D preserved cells using in situ hybridisation and confocal microscopy. We have shown that genes commonly involved in chromosomal translocations in leukaemia have an intrinsic spatial dynamics that is established early in hematopoiesis, and perpetuated differentially in distinct cell lineages, what may facilitate their collision and reciprocal recombination at subsequent stages of hematopoietic differentiation (*Neves et al, Blood, 1999, 93:1197*). This phenomenon may be mechanistically relevant for the occurrence of oncogenic gene rearrangements in human leukemia. As to the spatial organization of heterochromatic regions, we observed that chromocenters (associations of centromeres) present in quiescent lymphoid and non-lymphoid peripheral blood cells represent cell-type-specific arrangements of centromeric heterochromatin (*Alcobia et al, Blood, 2000, 95:1608*). These observations were followed by the analysis of the dynamics of these spatial arrangements during hematopoietic differentiation. The spatial patterns of association of different centromeres were analysed in CD34+ cells and compared with those in early-B, early T cells, mature B and T lymphocytes. Those patterns were shown to change during lymphoid differentiation, with major spatial arrangements taking place at different stages during T and B-cell differentiation. Heritable patterns of centromere association are observed, which can occur either at the level of the common lymphoid progenitor, or in early-T or early-B committed cells. A correlation of the observed patterns of centromere association with the gene content of the respective chromosomes, further suggests that the variation in the composition of these heterochromatic structures may contribute to the dynamic relocation of genes in different nuclear compartments during cell differentiation, what might have functional implications for cell-stage-specific gene expression (Alcobia et al, submitted Nov. 2002).

Mechanisms of plant cell growth and morphogenesis

Member: José Feijó

Students: Ana Catarina Certal, Sofia Cordeiro, Ricardo Bandarrinha Almeida, Ana Margarida Prado, Ana Maria Vieira, Sílvia Costa and Leonor Boavida

We are developing a systematic approach to the basic phenomena underlying cell growth and morphogenesis. We intend to tackle some of these issues by means of state-of-the-art biophysical approaches aimed at understanding some of the fundamental physiological regulatory loops in growing pollen tubes grown in vitro, a paradigmatic model for studying apical growth. Data gathered with electrophysiology and imaging techniques is to be integrated on a coherent theoretical background by established collaborations with *physicists and* theoretical biologists. On the other hand a systematic molecular approach will now be started to establish the molecular counterparts of the physiological models.

Pollen stigma interaction and sexual plant reproduction

Member: José Feijó

Students: Ana Catarina Certal, Sofia Cordeiro, Ricardo Bandarrinha Almeida, Ana Margarida Prado, Ana Maria Vieira, Sílvia Costa and Leonor Boavida

Sexual Plant Reproduction represents the evolutionary context in which pollen tubes evolved and fit in. We aim to apply the knowledge on the mechanisms that control growth to a better understanding of the complex communication and guidance behaviour of pollen tubes within the female tissue. This objective will imply development of a number of fluorescent tags for pollen tubes and advanced imaging inside living pistils using multi-photon microscopy. On the other end mutants of *Petunia* and *Arabidopsis* defective on reproductive steps will be screened, and characterized in terms of the inherent physiological deficiencies.

A lateral effort is being made on the establishment of sexual cycles in a number of non-studied species, especially with forestry of fruticulture interest. Besides the immediate applied interest of the results, this effort has repeatedly guided us into interesting basic research projects.

Reversible gene inactivation in the mouse

Member: Moisés Mallo

Students: Joana Martins and Vanessa Cristão

We have recently developed a new genetic strategy to be able to inactivate endogenous genes in the mouse in a reversible fashion. Our approach is based on the elements of the regulatory system of the bacterial tetracycline resistance operon. We have introduced the operator sequences (tetO) into the *Hoxa2* gene locus using a knock-in strategy. The presence of these sequences within the promoter area of the *Hoxa2* locus confers extra transcriptional regulatory possibilities. A repressor protein (tetR) is able to bind to these sequences thus blocking transcription from tetO-containing loci. The repressor protein is provided as a transgene that can be activated both ubiquitously (e.g. with an actin promoter) or in a tissue-specific fashion. The tetR-mediated repression can be modulated by the presence or not of tetracycline (or an analog of this antibiotic like doxycycline).

When no doxycycline given to the animal, tetR binds tetO and, in our case, no *Hoxa2* mRNAs are made. However, when doxycycline is administered to the animal, tetR is removed from tetO and transcription is resumed to control levels. We have proven that this strategy actually works in vivo. We are now using this system to explore the temporal and spatial requirements of the *Hoxa2* gene. In addition, we are improving this technique in order to be able to use this strategy to inactivate rather than reactivate gene expression from tetO-modified loci.

Time counting system during vertebrate embryonic development

Member: Isabel Palmeirim

Students: Catarina Freitas and Sofia Rodrigues

Collaborators: Marie-Aimée Teillet (Institut d'Embryologie Cellulaire et Moléculaire – Nogent sur marne, France)

In Human, Mouse, Chicken, Fly or any other organism the time of embryonic development is maintained and strictly controlled. Each step of embryonic development will only achieve the desired effect if it occurs at the right moment and in the right place. Somites are transient segmented embryonic structures that constitute the basis of the segmental pattern of the adult vertebrate body. They give rise to structures such as the axial skeleton, the dermis of the back and all striated muscles of the adult body, except those of the head. In the Chick embryo, a pair of somites buds off, in a highly coordinated fashion (every 90 minutes) from the cranial end of the presomitic mesoderm (PSM) while new PSM cells enter the caudal end of this tissue as a consequence of gastrulation. However, very little is known about the spatial and temporal coordination of this segmentation process in vertebrates. Recently, two avian homologues of the hairy *Drosophila* gene, “c-hairy1” and “c-hairy2”, were cloned, and the study of their expression pattern showed that these genes are dynamically and cyclically expressed in the PSM, in an autonomous manner and with a periodicity corresponding to the formation time of one somite (Palmeirim *et al.*, 1997; Jouve *et al.*, 2000). The dynamic expression pattern of these genes (“c-hairy1” and more recently the “c-hairy2”) provided the first molecular evidence for the existence of a developmental clock linked to somitogenesis.

“c-hairy” genes are expressed in prospective segmented tissues like PSM but are completely absent in presumptive unsegmented tissues such as lateral mesoderm (LM). Nevertheless, LM cells are able to form segmented structures (somites) if CHO-Noggin expressing cells are grafted in the prospective area of this tissue inhibiting the BMP4 (Bone Morphogenic Protein 4) action in the LM (Tonegawa *et al.*, 1997). In addition, if stage 4 (HH) Quail Hensen's node is grafted isochronically and heterotopically in the lateral or extraembryonic region of a Chick embryo it will induce the formation of a secondary axis with neural tube, notochord and ectopic somites (Hornbruch *et al.*, 1979). We intend to evaluate the expression of the genes implicated in the clock controlling somitogenesis (i.e. the c-hairy genes) during the formation of these ectopic somites. If the formation of these ectopic somites results in the activation of the “c-hairy” genes, we will use the Quail/Chick chimera model to determine which cell type(s) is (are) able to

upregulate this expression (Quail/donor/Hensen's node cells or Chick/receptor/lateral mesodermal cells). Experiments involving ectopic overexpression of Noggin or BMP4 (by using *in ovo* electroporation) will allow us to evaluate the relationship between these genes and the segmentation clock.

Mechanisms of head induction in vertebrates

Member: José António Belo

Students: Mário Rui Filipe, Ana Cristina Borges, Ana Cristina Silva and Sara Marques

Collaborators: Herbert Steinbeisser, Max-Planck-Institut für Entwicklungsbiologie, Tuebingen, Germany

Mouse *cerberus-like*, a member of the *Cerberus/Dan* gene family is a secreted factor expressed in the Anterior Visceral Endoderm (AVE) of the pre-streak mouse embryo. This region has been implicated in anterior neural specification. The neural inducing and mesoderm inhibition activities of *Cer-1* result from specific inhibition of BMP4 and Nodal molecules respectively. These activities are shared with the closely related *Xenopus cerberus* (a gene implicated in head induction in *Xenopus* experiments) and chick *Caronte*.

We have previously reported the targeted inactivation by homologous recombination in ES cells of the mouse *cer-1* gene. Currently we are studying possible genetic interactions of *cer-1* with other genes involved in head formation to look for compensatory pathways involved in this process. We have reported on the interaction of *cer-1* and *noggin* (Borges et al, 2001) and of *cer-1* and *goosegoid* (Borges et al, 2002). Currently we are pursuing these studies using mouse mutants for the genes *Otx2* and *Cripto*.

We have generated pPMcer1.EGFP transgenic mouse lines, which contain the fluorescent marker EGFP under the control of the upstream promoter sequences of *cer-1*. Expression of EGFP can be observed in the AVE. Currently we are using these lines for a differential screening approach, to isolate novel genes expressed in the Anterior Visceral Endoderm at peri-gastrulation stages and with neural inducing properties.

When this plasmid is injected in dorsal blastomeres of *Xenopus* embryos, the *cer-1* promoter was able to drive the expression of EGFP in the deep endodermal cells of gastrulating embryos, overlapping with the domain of expression of *Xcer*. Electroporation of pPMcer1.EGFP in chick embryos also resulted into a *Caronte* like type of EGFP expression.

By this we used the *cerberus-like* promoter to drive the expression of *Xwnt8*, *Xnr-1* or *XBMP4* factors in the *Xenopus* embryo anterior endoderm. We also generated and validated a *Cerberus* morpholino that efficiently abolishes transcription of *Xcerberus* mRNA. Using these two combined approaches we were able to find that by challenging endogenous *Cerberus* activity, one can conclude that it is essential for proper head formation (Ana C. Silva et al., in preparation).

Transcriptional regulation of *caronte* during embryonic development

Members: José António Belo and Ana Teresa Tavares

Students: Sara Marques, Mário Rui Filipe, Ana Cristina Borges

Xenopus Xcer, mouse *cer-1* and chick *Car* are expressed in equivalent embryonic structures such as the anterior endomesoderm, anterior visceral endoderm, and hypoblast, respectively. In mouse and chick embryos, these genes are also expressed in the anterior definitive mesendoderm. However, at later stages, *Xcer* transcripts are no longer detected, mouse *cer-1* RNA is found in the rostral domain of nascent somites, and chick *Car* is expressed in the left lateral plate and paraxial mesoderm. The general aim of this project is to dissect the transcriptional regulatory mechanisms that establish these similarities and differences in *Cerberus-like* gene expression patterns.

For this end, we cloned and mapped the genomic regulatory regions of *Car* that determine its expression pattern (genomic sequences driving the expression of the EGFP reporter gene are introduced in the chick embryo by electroporation) and identified putative transcription factor-binding sites that may act as *cis*-regulatory elements.

A series of generated deletion constructs containing *Car* regulatory regions from 2.5 kilobases (kb) to 0.36 kb (upstream the ATG) were able to drive EGFP expression specifically in derivatives of the early streak and anterior mesendoderm. Green fluorescence was first detected in two regions of the anterior mesendoderm and later co-localises with the cardiogenic mesoderm, with the lateral extraembryonic mesoderm and in the left lateral plate mesoderm. At older stages, EGFP expression can be seen in the heart tube primordia and in scattered foci of cells that appear to mark future blood islands.

Currently we are investigating the regulation of *Car* transcription by signalling molecules like Shh, FGF8 and BMP4, known to activate or repress *Car* asymmetric expression. Also, a transgenic mouse line generated using a 2.5 kb fragment of 5' genomic region of chick *caronte* driving EGFP, shows expression in the heart precursor cells and in the left lateral plate. These results suggest that the upstream regulators of *caronte* expression are present in equivalent developing regions of the chick and mouse embryos.

To investigate the extent of evolutionary conservation between the regulatory sequences of mouse, *Xenopus* and chick *cer-like* genes, intra and cross species promoter studies are being performed.

The cell biology and molecular basis of neurulation in the chick embryo

Members: António Jacinto and Ana Coutinho

Collaborator: Alfonso Martinez Arias, University of Cambridge, Cambridge, UK.

During early embryogenesis of chordates, a flat sheet of epithelial cells rolls up to form a dorsal, hollow neural tube, the rudiment of the adult central nervous system. The developmental process by which an embryo forms the neural tube is called neurulation. It is a complex morphogenetic program requiring precisely choreographed cell

proliferation, differentiation, adhesion and migration. Neural tube defects affect nearly one in every five hundred human births, reflecting the sensitivity of neurulation to even the slightest alteration in its developmental program. We are establishing methods to elucidate the cell biology of neurulation using non-invasive microscopy techniques to follow neurulation in chick embryos in real time, and we are investigating the molecular basis of neurulation by testing candidate genes known to be involved in similar morphogenetic processes, namely dorsal closure in *Drosophila melanogaster*

Functional targets for the *Hoxa2* gene

Member: Moisés Mallo

Student: Marta Carapuço

Collaborators: Nicoletta Bobola, Max-Planck Institute of Immunobiology, Freiburg, Germany; Jacques Drouin, Laboratoire de Génétique Moléculaire, Institut de Recherches Cliniques de Montréal, Montréal, Canada and Annette Neubuesser, Research Institute of Molecular Pathology, Vienna, Austria.

The gene *Hoxa2* belongs to the large family of Hox genes which play a wide variety of roles during embryonic development and are involved in the pathogenesis of several diseases. In particular, genetic analyses have shown that *Hoxa2* is essential for proper development of the ear, neck and hindbrain regions of the mouse embryo. Our current goals are focused on the identification of the molecular mechanisms that mediate the activity of the *Hoxa2* gene. Using a transcription profiling approach with Affimetrix Gene Chips, we have identified several genes that are up-regulated in the mutant *Hoxa2* embryos. The analysis of the functional relevance of the up-regulation of these genes for the activity of *Hoxa2* is currently under analysis using a combination of genetic and molecular approaches. We have already been able to show that *Hoxa2*-mediated downregulation of one of the identified target genes (*Ptx1*) is essential for proper ear development, as the double *Hoxa2*^{-/-};*Ptx1*^{-/-} animals show partial rescue of the *Hoxa2* null mutant phenotype. These studies also led to the identification of one of the major roles of *Hoxa2*: inhibition of Fgf8 signalling in mesenchymal cells. The molecular mechanisms of this inhibition are also currently under analysis in our laboratory.

The role of *Tbx1* in development of ear.

Member: Moisés Mallo

Students: Filipa Moraes and Ana Nóvoa

Collaborators: Virginia Papaioannou, Department of Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, New York, USA.

Genetic analysis in the mouse have identified the gene *Tbx1* as one of the major players in the pathogenesis of the DiGeorge syndrome. This syndrome, first described in humans, acts dominantly with incomplete penetrance and expressivity, and results from

haploinsufficiency of the *Tbx1* gene. The *Tbx1* heterozygous phenotype includes malformations in the heart outflow tract, hypo or athymia leading to immunodeficiency, thyroid deficiency and hypoparathyroidism. In the homozygous mutant state, some of these characteristics are enhanced and additional phenotypes become evident. Among them are the extreme underdevelopment of the ear region. All the ear compartments, outer, middle and inner ears, are affected in the *Tbx1* null mutant mice. We are currently performing an anatomical, cellular and molecular characterization of the ear phenotypes of the *Tbx1* null mutants in order to understand the role of this gene in the formation of these sensory structures.

The role of Bmp2 signalling in embryonic development

Member: Moisés Mallo
Student: Emília Almeida

BMPs (bone morphogenetic proteins) belong to a large family of signalling molecules that play fundamental roles in a wide variety of animals, both during embryonic and postnatal stages. We have found that one of these roles is to participate in the production of neural crest cells. These are a very important population of cells, as they are the ones making most of the peripheral and autonomic nervous systems, the skeletal structures of the face and neck, the melanocytes and are required for the formation of the heart outflow tract and pharyngeal organs like the thymus. Using transgenic and gene inactivation approaches, we have shown that *Bmp2* is the family member involved in early neural crest development in the mouse. We are now analyzing the exact role of *Bmp2* for neural crest development. We are approaching this issue by analyzing the different stages of neural crest production in *Bmp2* null mutant embryos. In addition, it has been suggested that *Bmp2* also plays additional roles at later stages of embryonic development that cannot be analyzed in these mutants because they do not develop further than E8.5. Therefore, we are using a conditional approach to investigate the possible functions of *Bmp2* at later developmental stages in processes like autonomic neuron differentiation and heart and kidney development. We are using both a classical cre/Lox approach and our newly developed reversible gene inactivation system as it could give extra experimental possibilities.

Molecular basis of medio-lateral presomitic specification

Members: Isabel Palmeirim and Leonor Saúde
Students: Catarina Freitas, Sofia Rodrigues
Collaborators: Marie-Aimée Teillet (Institut d'Embryologie Cellulaire et Moléculaire – Nogent sur marne, France)

An essential process in early embryonic development is the subdivision of the embryo into segments along its anterior-posterior axis. For example, in vertebrates, the spine,

ribcage and breastbone derive from repeated units of identical tissue that subsequently develop into unique structures and acquire specialised functions.

In vertebrates the most obvious early segmental units are the somites. Individual pairs of somites are formed progressively from the presomitic mesoderm (PSM) in a rostral to caudal manner.

Somites can be subdivided into a medial and a lateral compartment based not only on their developmental fate, but also on their origin. Quail-chick chimera analysis revealed that medial and lateral somitic compartments are already allocated in the PSM. The medial compartment will become the vertebrae, the associated epaxial muscles and the proximal ribs. The lateral compartment will give rise to the musculature of the limbs and the body wall known as the hypaxial muscles and the distal ribs.

DiI-labelling experiments performed in chick embryos have demonstrated that medial and lateral compartments of the PSM, and later of the somites, have distinct. Somite progenitors located in the lateral part of Hensen's node become restricted to the medial PSM (M-PSM). On the other hand, somite progenitors located in the anterior third of the primitive streak will become restricted to the lateral PSM (L-PSM). Whether the allocation of cells to the M-PSM or L-PSM is a consequence of their position in the primitive streak, or an outcome of their state of cellular commitment is still an open question.

Work done recently in our laboratory has shown that M-PSM is able to form somites and to express segmentation markers in the absence of L-PSM. However, L-PSM does not have the ability to form somites and loses its segmentation markers in the absence of M-PSM. These experiments show that only the medial compartment of PSM contains the molecular information for segmentation and somite formation. It is therefore likely that a signal from the M-PSM is responsible for the maintenance of segmentation genes in the L-PSM. The nature of such a signal remains unknown.

We propose to elucidate the molecular and cellular mechanisms that confer segmentation autonomy to the M-PSM cells. Our first aim is to identify chick genes differentially expressed in the M-PSM using a subtraction strategy of cDNA libraries from M-PSM versus L-PSM. Our second aim is to study the process of cell commitment acquisition of somite progenitors cells towards a medial versus a lateral fate and function using cell replacement and imaging techniques.

Molecular and temporal characterisation of the rostral-most somites in early somitic stages of the chick embryo

Member: Isabel Palmeirim

Student: Joana Santos

In the vertebrate embryo, the segmental pattern of the body is established very early in development and becomes evident by the appearance of metameric structures called somites. Somitogenesis is linked to a molecular clock that is somehow related to the Notch signalling pathway and its downstream targets, although the identity of the oscillator is still unknown. Genetic experiments involving segmentation-related mutants

in zebrafish and mice, have demonstrated that, very often, posterior somites present disruptions in their formation while the first somites are not affected. In addition, it was demonstrated that, in zebrafish, the first six somites form more rapidly than the remaining ones, suggesting that there are rostro-caudal differences in somite formation time. Other studies have also revealed that the presomitic mesoderm of chick embryos ranging from 8 to 20 somites, contains twelve prospective somites and that this number is constant throughout development. We have characterised the expression pattern of several genes related to somitogenesis during the early stages of somite formation in the chicken embryo and observed that the rostral-most somites present different expression patterns from more posterior ones. We also monitored *in vivo* the somite formation time of embryos until the 20-somite stage. We concluded that the first somites do not form at a different rate from more posterior ones and that one somite forms in average every 81 minutes, in contrast to what has been reported. Finally, we assessed for the number of prospective somites contained within the presomitic mesoderm of early somitic stage embryos, and our results suggest that this number is not constant during development and that it correlates with presomitic mesoderm elongation.

Specification of vertebrate limb bud

Members: Joaquín Rodríguez León and Juan Carlos Izpisua Belmonte

Students: Inês Ribeiro Martins

Collaborators: Yasuhiko Kawakami and Jennifer Ng (The Salk Institute for Biological Studies, CA, USA).

The T-box transcription factor family member TBX5 has been shown as a key molecule in providing forelimb identity to the anterior limb. We have shown that this transcription factor is conserved through evolution playing a key role also during pectoral fin development in zebrafish. We have also found that *Tbx5* is necessary and sufficient for forelimb induction and that it is upstream of *Fgf10*, one of the key molecules in limb initiation. Moreover, we have made it evident that an interplaying loop between them is established during the first stages of limb development.

In parallel, we are looking for new genes involved in limb identity. We are making studies of gene expression by using microarray technology. Affymetrix chips from mouse were used to hybridize two different populations of RNA, one from the forelimb and the other from hindlimb. Since hindlimb development is delayed, we have used for this hybridization forelimbs from 10,5 d.p.c. embryos and hindlimbs from 11 d.p.c. embryos. The study of the data obtained from hybridization is currently in progress.

New aspects on coordinating limb bud development

Members: Isabel Palmeirim and Joaquín Rodríguez León

Student: Susana Pascoal

Collaborator: Delphine Duprez (CNRS, UMR 7622, Biologie du développement, Université Pierre et Marie Curie, Campus de Jussieu)

The vertebrate limb originates from a dual contribution of lateral plate and somitic mesoderm (Chevallier et al., 1977). Through differential proliferation of the flank, specific regions of the lateral plate form buds at presumptive limb levels. Shortly thereafter, cells from the lateral edges of nearby somites migrate into the limb. All adult limb muscles derive from these migratory cells. The limb bud is enveloped by an overlying ectoderm, which distal tip, forms a specialized epithelial structure, the apical ectodermal ridge (AER) at the interface of dorsal and ventral territories (Saunders, 1948; Todt and Fallon, 1984). Cells directly under the AER remain undifferentiated in a region of distal mesenchyme in close proximity to the AER, denominated the “progress zone”, while condensation initiates in proximal limb regions, causing the humeral segment to form first, followed by the radius and ulna, and lastly by the digits. The first clues to how this complex shape and form is achieved came from the work of experimental embryologists who identified specific regions of the developing chick limb bud that are essential in directing growth and patterning. Of the three cardinal limb axes (D/V; A/P and P/D) the mechanisms that lead to cell fate specification along the P/D axis are the least understood. One paradigm, largely unmodified since its conception more than 20 years ago, is the progress zone model (Summerbell et al., 1973). According to this model, cell fate along the P/D axis is specified by the time spent in the progress zone. Nevertheless, until now no evidence has been provided to explain how cells “know” the time they spend in the progress zone. Recently, in a completely different system – somitogenesis - Palmeirim et al., (1997) presented evidence for the existence of a molecular clock underlying the process of chick somitogenesis by showing that presomitic (PSM) cells undergo several cycles of *c-hairy1* gene expression, with a 90 minutes periodicity, corresponding to the time required to form one segment. These *c-hairy1* mRNA oscillations of expression occur in each PSM cell until it is incorporated into a somite. These results provide the first molecular evidence of a developmental clock linked to somitogenesis (Palmeirim et al, 1997). Later, this same type of behaviour was also described for a gene coding for a closely related transcription factor, *c-hairy2* (Jouve et al., 2000). Since “*c-hairy1*” and “*c-hairy2*” are also expressed in different limb bud regions such as the progress zone, with an apparent dynamic pattern of expression (preliminary results) we wondered whether these genes are implicated on the system of time counting that allows undifferentiated progress zone cells to “know” how much time they spend in the progress zone.

In addition, we intend to study the role of these genes in ZPA, AER and muscle development, since these genes are expressed in these tissues during limb bud development.

The role of cell-fate decision genes in human hematopoietic differentiation

Member: Leonor Parreira

Students: Isabel Alcobia, Ana Sofia Quina and Hélia Neves

The choice between alternative cell-differentiation pathways is regulated by direct intercellular contacts mediated by trans-membrane proteins expressed by adjacent and apparently equivalent stem cells. Two protein families involved in this process are the Notch receptors and their ligands, the Delta and Jagged proteins. Both protein families are phylogenetically conserved and involved in several developmental scenarios including the decision processes underlying the functional divergence of CD4/CD8 T lymphocytes and the choice between $\alpha\beta$ and $\gamma\delta$ T-cell receptors in mouse thymocytes. Using a cell coculture assay we have recently observed that the Notch ligand Delta-1 completely inhibits the differentiation of human hematopoietic progenitors into the B-cell lineage while promoting the emergence of cells with a phenotype of T-cell/natural killer (NK) precursors. In contrast, Jagged-1 did not disturb either B- or T-cell/NK development. Furthermore, cells cultured in the presence of either Delta-1 or Jagged-1 can acquire a phenotype of NK cells, and Delta-1, but not Jagged-1, permits the emergence of a de novo cell population coexpressing CD4 and CD8 (*Jaleco et al, J Exp Med, 2001, 194:991-1001*).

Now, the effects of these Notch ligands on myeloid development are under investigation. To do this, a long-term culture assay is used, where bone marrow stromal cells transduced with human Delta-1 or Jagged-1 cDNAs, are co-cultured with normal human progenitors, followed by the analysis of their differentiation potential in methylcellulose clonogenic assays. Preliminary results indicate that Delta-1 and Jagged-1 differ also have differential effects in myelopoiesis, namely on the proliferation, clonogenic and differentiation properties of pluripotent myeloid precursors.

Notch signaling and lymphocyte regulation

Members: Jocelyne Demengeot and Leonor Parreira.

Students: Manuel Rebelo, Margarida Santos and Hélia Neves

Notch receptors and their ligands are evolutionarily conserved trans-membrane proteins that regulate cell fate decisions during development and postnatal life. Notch signaling is also known to differentially affect the development of lymphoid B- and T-cell throughout life. We initiated a study to assess the roles of Notch signaling in the differentiation of regulatory T cells and in the regulation of peripheral T lymphocytes numbers.

Previous analyses by Parreira, L. et al demonstrated that Delta-1, but not Jagged-1, completely inhibits the differentiation of human progenitor cells into the B-cell lineage while promoting the emergence of T and NK cells precursors. In order to obtain a more convenient and manageable system to further characterize the T cell populations induced by Delta-1, we transposed this experimental system from human to mouse. We are now in conditions to initiate the functional analyses of various lymphocyte subsets triggered by either Delta-1 or Jagged-1 ligands.

In parallel, FACS-sorted CD4 T cells from normal mice were tested for expression of Deltex, a mediator of the Notch signaling pathway. This gene is highly expressed by naïve (CD45RB^{high}) and neither by naturally (CD45RB^{low}) or *in vitro* activated cells.

However, naïve CD4 cells injected into lympho-deficient animals (Rag-2^{-/-}) expand and acquire an apparent activated phenotype (CD45RB^{low}) but keep expressing high level of Deltex. We interpret these results as an indication that naïve CD4 cells and cells under homeostatic proliferation are constrained by Notch engagement, while activated T cells are relaxed from this control. Future activities will directly test this hypothesis.

Cellular differentiation and cell fate

Members: Matthias Haury and Paula Parra Bueno

Students: Dinis Calado, Marta Vitorino, Carl Collins, Helena Nunes and Maria da Luz Alvim

Collaborators: Dan Holmberg, University of Umea, Sweden; Antonio Bandeira, Institut Pasteur, Paris, France; Paulo Vieira, Institut Pasteur, Paris, France; Alfonso Fairen, University of Alicante, Spain and Lisa Marubio, Baylor College, Houston, USA.

Our group is focussing on the problem of cellular mechanisms in the control of cell differentiation and cell-fate in two different systems:

Immunology: we are studying the effects of cytokines on the control of cellular differentiation in the immune-system, particularly in the interaction of regulatory t-cells and auto-immune effector cells:

Cytokine control during the regulation of the immune response

Using a transgenic knock-in approach we are in the process to generate tools for the in-vivo analysis of molecular interactions during the development of auto-immune and normal immune responses. The objective is to be able to dissect the local influence of various cytokines on the generation and control of immune effector cells using different models of auto-immune diseases in mice. We will hopefully gain better understanding on the mechanisms that differentiate a healthy from an auto-immune response.

Neurobiology: using a transgenic and electrophysiological approach we want to analyse the guidance and differentiation mechanisms of cajal-retzius cells during embryonic brain development:

Cell-fate of cajal-retzius during development

Cajal-Retzius (CR) cells are transient, pioneer neurons of layer I of the cortex and believed to play an essential role in corticogenesis, neuronal migration and synaptogenesis. CR cells are involved in the guidance and emplacement of neurons in the cortex as well as the hippocampus and thought to allow the correction of erroneous projections and the creation of pathways for axonal growth. Although the majority of the cells normally undergo apoptosis during the development of the central nervous system, a subset of CR cells survive and differentiate into interneurons.

Our main objective is to investigate in more detail the molecular mechanisms leading to the decision of CR cells to enter a pathway of differentiation or apoptosis. In particular we want to examine the electrophysiological differences of these two "subsets" of neurons, in order to determine if distinct ionic channels characterise two different cells populations or if there is a single population with differences in the regulation of channels that might be involved in the control of differentiation versus apoptosis.

Formation of the myotome in the mouse

Member: Sólveig Thorsteinsdóttir

Students: Fernanda Bajanca and Marta Luz

Collaborators: Margaret Buckingham and Shahragim Tajbakhsh, Pasteur Institute, Paris, France; Marilyn Duxson, University of Otago, Dunedin, New Zealand and Sara Venters, University of California San Francisco, USA

In vertebrates, the cells that will form the skeletal muscles, are primarily derived from the somites. Myocyte precursors undergo an epithelium-mesenchyme transition and organise themselves into a parallel array of mononucleated myocytes termed the myotome. With the development of the embryo, several waves of muscle cell precursors arise, which differentiate and get organised into mature muscle. Entry into the muscle differentiation programme is controlled by transcription factors of the bHLH family, namely myf5, MyoD, myogenin and MRF4. How these transcription factors control the morphogenesis of skeletal muscle formation and what downstream molecules are involved is an area under intensive investigation.

The expression pattern of several extracellular matrix molecules and their cell surface receptors in adult skeletal muscle is known and several muscle dystrophies are associated with defects in one or more components of this connection. However, much less is known about the cell-matrix interactions involved in the differentiation and morphogenesis of skeletal muscle in the embryo, particularly regarding the early aspects of muscle development.

We have performed a detailed analysis of $\alpha 4\beta 1$, $\alpha 5\beta 1$ and $\alpha 6\beta 1$ expression in the myotome of the mouse comparing it with the expression of muscle differentiation markers. Our results show that these integrins have specific temporal and spatial distribution patterns throughout the myotome development indicating a possible involvement in different phases of myotomal cells determination and differentiation. Furthermore, we have studied the formation of the muscle in myf5 null embryos, where myotome formation is initially inhibited and muscle differentiation is delayed (Tajbakhsh et al., Nature 384:266, 1996). Our results show that the absence of myf5 in muscle precursor cells results in an inhibition of basement membrane organisation. These results suggest that the absence of the basement membrane contributes to the failure of muscle cell differentiation in these embryos.

Analysis of integrin $\beta 1$ knock-in mouse embryos

Member: Sólveig Thorsteinsdóttir

Students: Ana Sofia Cachaço and Carlos Pereira

Collaborators: Christine Mummery and Susana Chuva de Sousa Lopes, Hubrecht Laboratory, Utrecht, The Netherlands

Arnoud Sonnenberg, The Netherlands Cancer Institute, The Netherlands

Integrins are extracellular matrix receptors composed of α and β subunits involved in cell adhesion, migration and signal transduction. The $\beta 1$ subunit has two isoforms, $\beta 1A$ ubiquitously expressed and $\beta 1D$ restricted to striated muscle. They are not functionally equivalent. Replacement of $\beta 1A$ by $\beta 1D$ ($\beta 1D$ knock-in) in the mouse leads to midgestation lethality on a 50% Ola/50% FVB background (Baudoin et al., Genes Dev. 12:1202-1216, 1998).

We have crossed the $\beta 1D$ knock-in line into a less penetrant genetic background. This led to an attenuation of the midgestation lethality and revealed a second period of around birth. Midgestation death was apparently not caused by failure in cell migration, but rather by abnormal placentation. The $\beta 1D$ knock-in embryos that survived midgestation developed until birth, but exhibited severely reduced skeletal muscle mass. Quantification of myotube numbers showed that substitution of $\beta 1A$ by $\beta 1D$ impairs primary myogenesis with no direct effect on secondary myogenesis. Furthermore, myotube survival is affected in $\beta 1D$ knock-in embryos. Finally, overexpression of $\beta 1D$ in C2C12 cells impaired myotube formation while overexpression of $\beta 1A$ primarily affected myotube maturation. Together these results demonstrate for the first time distinct roles for $\beta 1$ integrins in primary versus secondary myogenesis and that the $\beta 1A$ and $\beta 1D$ variants are not functionally equivalent in this process.

Extracellular matrix and somitogenesis: causes and consequences

Members: Sólveig Thorsteinsdóttir, Isabel Palmeirim and Gabriela Rodrigues
Student: Lara Carvalho

Somites are transient epithelial segments of the paraxial mesoderm that are formed in a rostral-to-caudal progression during vertebrate embryogenesis. Ectoderm is believed to be crucial for the proper epithelialisation of somites, since cultured explants of the presomitic mesoderm (PSM) only form somites in its presence, but the extracellular matrix (ECM) has also been implicated in somite epithelialisation.

In order to investigate a possible relationship between the role of the ectoderm and the ECM in somitogenesis, explants of the PSM are cultured in the presence and absence of ectoderm and assayed for the expression of ECM proteins. An analysis of the results obtained suggest that the ECM synergises with the ectoderm in promoting somite epithelialisation. We are presently using the same culture system to analyse the expression of genes known to be involved in somite epithelialisation in order to determine which of those genes are dependent on the ectoderm signal.

Epithelial dynamics and adhesion during *Drosophila* dorsal closure and wound healing

Member: António Jacinto
Students: Sérgio Simões, Beatriz Garcia and Pedro Laires

Collaborators: Alfonso Martinez Arias, University of Cambridge, Cambridge, UK; Buzz Baum, Ludwig Institute for Cancer Research, London, UK; Carl-Philipp Heisenberg, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany and Paul Martin, University College London, London, UK.

The movement and adhesion of epithelial sheets is a fundamental morphogenetic process that occurs throughout embryogenesis and whenever a tissue is wounded. In humans, defects in tissue adhesion can be the cause for many clinical conditions for example congenital malformations and cancer, for instance, during carcinoma metastasis it is required that cells lose their capacity to recognise and adhere to their normal neighbours. Dorsal closure is a morphogenetic process during *Drosophila* development that provides a genetically tractable model of epithelial dynamics and wound healing. Two opposing epithelial fronts move dorsally to form a neat seam closing over the midline in the dorsal surface of the embryo. These epithelia are segmented prior to the closure and opposing cells in corresponding segments recognise and match each other during the process. We propose to extend our previous studies, combining genetics, genomics, proteomics and advanced imaging techniques to identify and analyse novel molecular components of the dorsal closure cell-cell recognition and adhesion system in *Drosophila*. We will test the function of such molecules during wound healing taking advantage of a simple model system that we have developed using *Drosophila* embryos.

Targeted inactivation of the mouse nuclear-encoded Complex I gene *NDUFS8*.

Members: José António Belo and Vera Lucas-Teixeira

Student: Sara Marques

Collaborators: Arnaldo Videira and Margarida Duarte, IBMC/ICBAS, Porto, Portugal

One of the complex I subunits which have been associated with the human Leigh syndrome is the NADH dehydrogenase (ubiquinone) Fe-S protein 8 (NDUFS8 or TYKY). This is a highly conserved nuclear encoded subunit well documented in humans. The aim of the project was the generation of a mouse model expressing the mutation observed in humans as a first step towards studying the Leigh syndrome.

The project was divided in three parts:

- a) Since the information concerning the genomic structure of this gene in the mouse was not available by the time we initiated this project, the first task was the cloning and characterisation of this gene in the species *Mus musculus*.
- b) Then, we designed and generated the targeting allele containing one of the point mutations observed in the human Leigh syndrome at exon V. The mutation corresponds to the change of the base 448G to an A, which consequently changes the aminoacid 104 from an arginine to an histidine.
- c) Finally, we started to generate a targeted embryonic stem cell clone with the designed targeting construct.

Currently we are starting to generate quimeric mice by microinjecting selected positive ES cell clones into mouse blastocysts.

Structural and functional characterisation of the EGF/Laminin superfamily.

Members: António Jacinto and Pedro Coutinho

Student: Joana Ribeiro

Collaborator: Sarah A. Teichmann, MRC Laboratory of Molecular Biology, Cambridge, UK.

The EGF/laminin superfamily of proteins comprises all proteins with at least one EGF/Laminin domain. These domains are defined by structural homology to a sequence of about 40 amino acids that is significantly homologous to the epidermal growth factor (EGF). These families are quite rich in terms of structural architectures and molecular function and processes, furthermore they are thought to be key players in the transmission of information at the intercellular level. Although several EGF/Laminin proteins have been worked experimentally, the functional significance of these domains in proteins that do not seem to be related is unclear. However, as a general feature, they are found mainly in extra-cellular domains of membrane bound proteins or in proteins that are secreted. They are thought to be primarily involved in receptor-ligand interactions. In order to elucidate the functional roles of the EGF/Laminin proteins and their regulatory networks, we use a combination of genetics, bioinformatics, advanced imaging techniques and micro-array technology to characterise the full EGF/Laminin protein sets of some model organisms, e.g., *C.elegans*, *D.melanogaster* and *H.sapiens*. We are in the process of characterising them with respect to protein function, domain architecture, chromosomal location and relationship to other proteins within the same species or others. In addition, we will gain an insight into the protein networks that contain EGF proteins by surveying their transcriptional regulation and expression profiles.

Transactivator Yap1– crossroad of cold and oxidative stress signaling pathways in *Saccharomyces cerevisiae*.

Members: Lisete Fernandes and Helena Soares

Students: Joana Monteiro and Ana Neves Costa

Collaborators: Axelle Balguerie, IBCG, Bordeaux, France.

Eukaryotic cells respond to both stimuli, like oxidative stress (OS) and temperature fluctuations, by activating cascades which are diverse but with discrete specificity. How does the cell keep track of multiple cascades? What are the cross-talks between such cascades and how are they regulated?

The Yap family of bZIP transcriptional factors in *Saccharomyces cerevisiae* contains members which are central players in cellular responses to stress challenges as: Yap1 in oxidative and cold signals, Yap4 in response to compounds affecting cytoskeleton as well as in cold-sensitivity of yap1-deleted cells, Yap8 in arsenite response. Although it has been previously suggested that each Yap family member plays distinct biological

function, the involvement of Yap proteins in different phenomena emphasizes putative overlapping among the respective signaling cascades. From this point of view, Yap constitutes an excellent tool to address the cross-talk of signalling pathways and, in particular, it will also provide clues on the cellular mechanisms for adaptation to low temperatures.

Our major aim has been focussed in understanding the crossroad of cold and oxidative signaling pathways mediated by Yaps. The involvement of Yaps, as the target regulatory proteins, in both phenomena is being ascertained by addressing (a) the specific role of Yap1 under cold signals, (b) how specific signals propagate to impinge on Yap1, (c) the identification of genetic factors associated with role of YAP4 under cold signals.

Mechanism of activation by Yap1:-signaling through RNA polymerase II basal machinery.

Member: Lisete Fernandes

Students: Joana Monteiro and Diogo Fonseca

Yeast *Saccharomyces cerevisiae* as well as other fungi contains a set of non classical AP-1 factors, the Yaps, that are similar in structural motifs yet distinct in their amino acid sequences. Yaps are described as key proteins in cellular response to specific stress signals. Transactivators, like Yap1, stimulate gene expression by binding regulatory *cis*-elements, contacting directly or indirectly components of the RNA polymerase II basal machinery (GTFs as TFIIA, TFIIB, components of TFIID) or the Mediator, as well as by recruiting the nucleosome-remodeling complexes.

In order to understand the specific signaling downstream of Yap1, we are addressing the role of GTFs as specific targets under stress conditions. We have selected a GTF as primary target for Yap1 activity and its specific mutants under non physiological conditions are being analysed.

Molecular mechanisms of microtubule mediated signaling under oxidative stress – cell cycle dependent regulation of bim

Member: Sukalyan Chatterjee

Students: Mário Grãos, Ana Gírio and Ana Alexandra Almeida

Collaborators: Eric Lam, Imperial College, London and Maria Morone, Università Cattolica del Sacro Cuore, Rome

Bim (Bcl-2-Interacting Mediator of apoptosis), a member of the Bcl-2 family, is a BH3 domain only pro-apoptotic protein. The Bcl-2 family comprises of both anti- and pro-apoptotic members, which by transcriptional and post-translational regulation is known to tightly control the outcome of several intracellular apoptotic signals. Bim has been described to be sequestered to the microtubules (MTs) as a complex with dynein. Under certain stimuli, this complex gets released and Bim potentially interacts with Bcl-2 in the

mitochondria, leading eventually to cytochrome-c release and subsequent activation of caspases, the steps and mechanisms of which are largely unknown.

Our data shows that in NIH-3T3 cells, two isoforms of Bim (Bim-L and Bim-EL) are phosphorylated at early mitosis (M), but not in other phases of the cell-cycle, such as G1/S/G2 or G0. The cells are arrested in M-phase by using MTs perturbing agents, which prevent them from proceeding to metaphase. We further show that these agents do not induce phosphorylation of Bim *per se*. Moreover, we show that extracts from an M-phase enriched population of normally cycling cells which were never challenged with MTs poisons also show phosphorylation of the protein. Interestingly, the phosphorylation event does not correlate with apoptosis. Taken together, our data indicates that Bim-L and Bim-EL (but not Bim-S) are phosphorylated at M phase and this post-translational modification may contribute to preventing apoptosis.

We suggest that phosphorylation of Bim attenuates the pro-apoptotic potential of the protein in a context of M-phase such that it can be rapidly reactivated by dephosphorylation in case the cell decides to enter apoptosis instead of mitosis. It is attractive to speculate that this may play a role as a checkpoint, taking accounts of the cell cycle in mitosis, otherwise an irreversible process. Preliminary data supports this hypothesis, since inhibiting Cdc2 in M-phase arrested cells cause loss of phosphorylation of Bim-L/EL, with concurrent induction of massive apoptosis.

The role of the cytosolic chaperonin CCT in mature cilia and during cilia-biogenesis

Member: Helena Soares

Students: Ana Cecília Seixas

Collaborators: Luís Viseu and Pedro Brogueira, IST, Lisbon, Portugal.

The cytosolic chaperonin CCT is a hetero-oligomeric complex of about 900 kDa that mediates the folding of cytoskeletal proteins. We observed by indirect immunofluorescence that the *Tetrahymena* TpCCT α , TpCCT δ , TpCCT ϵ and TpCCT η -subunits co-localize with tubulin in cilia, basal bodies, oral apparatus and contractile vacuole pores. TpCCT-subunits localization was affected during reciliation. These findings combined with atomic force microscopy measurements in reciliating cells indicate that these proteins may play a role during cilia biogenesis related to microtubule nucleation, tubulin transport and/or axoneme assembly. The TpCCT-subunits were also found to be associated with cortex and cytoplasmic microtubules suggesting that they can act as microtubule-associated proteins. The exclusive localization of the TpCCT δ -subunit in the macronuclear envelope indicates that at least this subunit has functions outside of the 900 kDa complex. *Tetrahymena* cytoplasm contains granular/globular-structures of TpCCT-subunits in close association with microtubule arrays. Studies of reciliation and with cycloheximide suggest that these structures may be sites of translation and folding. Combined biochemical techniques revealed that reciliation affects the oligomeric state of TpCCT-subunits being tubulin preferentially associated with smaller CCT oligomeric species in early stages of reciliation. Collectively, these findings indicate that the

oligomeric state of CCT-subunits reflects the translation capacity of the cell and microtubules integrity.

Study of the *tetrahymena* tubulin complexes: an attempt to establish a functional relationship with microtubule assembly and dynamics.

Member: Helena Soares

Student: Ana Cecília Seixas

Tubulin exists in *Tetrahymena* exponentially growing cells in different protein complexes with molecular masses ranging from 90~500 kDa, as revealed by native gels. The amount of these different complexes seems to change when cells are submitted to hyperthermic stress. In order to characterize these tubulin complexes we have prepared post-mitochondrial extracts of *Tetrahymena* exponentially growing cells that were analysed in sucrose gradients from 10% to 40%. Subsequently, the tubulin enriched fractions were pooled and applied into a FPLC gel filtration column (Superdex 200HR). The analysis of the obtained elution profile showed three main regions where tubulin co-elute corresponding to: ~500 kDa, ~200kDa and ~120 kDa. The fractions corresponding to the 500 kDa region were further purified using ion-exchange chromatography (Q-Sepharose). The 500 kDa complex elutes with 380 mM NaCl, but another complex, with approximately 300 kDa, co-purifies with it. We are now trying to perform alternative schemes of purification in order to completely purify the 500 kDa complex. The band corresponding to this complex, obtained from a native PAGE analysis, was excised and analysed under denaturing conditions. This analysis revealed that the 500 kDa complex is composed of at least 12 different proteins with molecular masses ranging from 30~150 kDa. Two of these proteins were identified by immunoblot and immunoprecipitation as corresponding to the proteins CCT α and CCT ϵ . In heat-shocked cells this complex seems to have the same composition. The other unidentified proteins of the complex should be identified under sequence analysis, as soon as the complex will be isolated. Experiments are being carried to elucidate the role of this complex in Mt biogenesis and dynamics under stress conditions and their relationship with the function of the chaperonin CCT.

Study of the role and regulation of the mammalian cofactor α : implications in tubulin folding, microtubules biogenesis/dynamics and signalling pathways

Member: Helena Soares

Students: Sofia Nolasco and Andreia Feijão

In mammalian tubulin folding pathway, nascent α - and β -tubulin chains interact with prefoldin, a chaperone protein that maintains its bound polypeptide in a relatively unfolded state and transfers it to the chaperonin CCT (chaperonin-containing-TCP1). After release of tubulins from the chaperonin complex, in quasi-native conformation,

there are five proteins identified, which are involved in the folding, and association of these polypeptides *in vitro*, named cofactors. α - and β - tubulins initially follow distinct folding pathways; α - tubulin is captured by cofactor B, while β - tubulin is captured by cofactor A which are subsequently replaced by cofactors E and D, respectively. The two pathways then converge with the formation of a quaternary complex with α - and β -tubulins and cofactors D, E and C, upon GTP hydrolysis assembly-competent α -/ β -tubulin heterodimers are released. We propose that the components of tubulin folding machinery, being putative targets for regulation of microtubules (mts) dynamics by controlling tubulin synthesis, flux and transport, might be implicated in signalling pathways involving mts dynamics and stability. In this context we are investigating whether cofactor A might respond to specific events known to determine cytoskeleton rearrangements. Cofactor A is abundantly expressed in testis, where it appears to be associated to microtubular changes and with β -tubulin processing through spermatogenesis rather than meiosis itself. Therefore, we are also investigating a putative regulatory mechanism underlying tissue-specific cofactor A expression. *In vitro* experiments showed that cofactor A plays a double role: on one hand, enhancing the dimerization rate of β -tubulin, and on the other hand, serving as a reservoir of excess β -tubulin. We are currently addressing this last possibility *in vivo* using agents described to promote the microtubule depolymerization. Preliminary results point out to a distinct role of cofactor A *in vivo* compared to *in vitro* results previously described.

The role of the cytoskeleton in the activation of nf- κ b by H₂O₂

Member: Helena Soares

Student: Nuno Pedroso

Collaborator: Fernando Antunes, Faculty of Sciences, University of Lisbon, Lisbon , Portugal.

Long-term goal of the studies is to understand the mechanism of cellular adaptation to oxidative stress. The main focus of this study is to elucidate the nature of the interaction between the redox state of the cell and NF- κ B, a major transcription factor. The redox regulation of NF- κ B is very important and hydrogen peroxide (H₂O₂), an oxidant, was postulated to be the common intermediate produced by the different agents that induce NF- κ B. It is known that oxidative conditions in the cytosol induce the translocation of NF- κ B to the nucleus, whereas reducing conditions in the nucleus are essential for the binding of NF- κ B to the promoter elements in DNA. One of the main paradoxes in this regard is that the effect of the cellular redox state is dependent upon cell type. The reason why the regulation of NF- κ B by the redox state is cell type dependent remains unknown, and is indeed the subject of the proposed investigation. The overall hypothesis to be tested is that the activation of NF- κ B is critical dependent on a well-defined balance between the redox state of the cytosol and the nucleus. This hypothesis predicts that the

absence of activation of NF- κ B by H₂O₂ observed in some cell lines is explained by the fact that at the levels of H₂O₂ necessary to activate NF- κ B in the cytosol, the levels of oxidation in the nucleus are too high to allow transactivation and binding of NF- κ B to DNA. This hypothesis is not new, but until now it was not possible to test it satisfactorily due to the lack of a suitable method allowing to quantify the cytosolic and nuclear H₂O₂ concentrations, as well as, the associated changes in the thiol status achieved upon incubation with external H₂O₂ in different cell lines. Recently, Fernando Antunes developed a method that allows the determination of such H₂O₂ and thiol profiles in cellular systems *in vivo*: the cell is stimulated with steady-state concentrations of H₂O₂ and the ensuing changes in the redox state of the cell are assessed by a kinetic and thermodynamic analysis. The cellular steady-state titration was successfully applied in the study of the induction of apoptosis by H₂O₂. In this context we are also investigating the role of cytoskeleton in nf- κ b activation and migration to the cell nucleus. *In vivo* Mts are key constituents of cytoskeleton and participate in determination of cell morphology, chromosome segregation, intracellular organization, intracellular transport, cell motility and signalling. All these functions are based on the capacity of tubulin to dynamically polymerize/depolymerize. Critical to both the formation of the tubulin dimer and to the binding of tubulin to GTP are cysteine residues. Cysteine residues contain thiols that are prone to oxidation forming disulfides, and one of the main paradoxes in this area is the conflicting evidence on the role of thiol oxidation on the functionality of microtubules. Therefore is our aim to establish regulatory links between the generation of oxidants by the cell, Mts disulfides, and cytoskeleton functionality, and how this could affect NF- κ B migration to the nucleus.

Molecular and biochemical analysis of centrosome components in *Drosophila melanogaster*

Member: Álvaro Tavares

Students: Susana Godinho, Paulo Alves and Célia Domingues.

Collaborators: David Glover, Dept of Genetics, Cambridge, UK and Maria Arménia Carrondo, ITQB, Oeiras, Portugal.

We previously showed that the protein kinase polo is a centrosomal kinase, and that is required for the formation of a bipolar spindle and for the proper execution of cytokinesis. We wish to understand how the activity of the polo protein kinase is regulated and how it functions at the level of the centrosomes. We have found that polo proteins, either from *Drosophila* embryo extracts or from *Xenopus* egg extracts, bind to several proteins forming different stable complexes. We are now on the process of identifying the complexes' components in total embryo extracts and in centrosome preparations by MALDI. We want to characterize these proteins, sorting which are polo substrates and which are activators. So far we have already isolated as new polo interactors the proteins Plkk, a kinase that we have now shown to phosphorylate and activate polo, HSP70 which is required for centrosome function, actin and myosin II, both required for cytokinesis.

MOB1 is an essential gene in *S. cerevisiae*, and the Mob1p is found at the spindle pole bodies and at the bud neck during mitosis, and mutants in this gene arrest late anaphase. We have isolated *Drosophila melanogaster* cDNAs coding for 4 different Mob-like proteins, with a degree of homology with yeast Mob1 protein ranging from 25% to 45%. By immunofluorescence we were capable of detecting, in *Drosophila* embryos, an accumulation of DMob1, DMob3 and DMob4 on the centrosomes during mitosis and on the central region of the spindle after anaphase. Interestingly, time-lapse microscopy with GFP-DMob1 and GFP-DMob4 reveal a different behavior for the two proteins. GFP-Mob1 shows a strong accumulation on the spindle poles and weak accumulation on what looks the kinetochores at the beginning of mitosis. GFP-DMob4 shows the reverse pattern: weak accumulation on the centrosomes when mitosis starts but quite strong on the kinetochores until just before anaphase, a strong indication that is required for mitotic exit.

Finally we have isolated a cDNA coding for a protein with a high degree of homology with *S. cerevisiae* Mps1 protein kinase. This kinase, required in *S. cerevisiae* for spindle pole duplication and mitotic checkpoint, in *Drosophila* S2 cells has an intracellular localization similar to DMob4: by indirect immunofluorescence, we detect a clear staining of the centrosomes that progressively moves to the kinetochores during mitosis. Overexpression of DMps1 in S2 cells induces a metaphase arrest typical of spindle checkpoint components. Therefore, DMps1 and DMob seem to be part of the signal that travels from centrosomes to the kinetochores required for mitotic progression. Preliminary results indicate a requirement for these proteins during mitotic progression and centrosome duplication, as numerous cells are observed with monopolar spindles or with problems during cytokinesis when they are overexpressed.

Isolation and characterization of mitotic mutants

Member: Álvaro Tavares

Student: Mariana Faria

Collaborators: Rui Gomes, FCUL, Lisbon, Portugal and Peter Deak, Biological Research Center, Szeged, Hungary.

We have isolated two mutant *Drosophila* lines, created by insertion of a transposable P-element, resulting in a semi-lethal phenotype. One of these lines, line718/06, shows a high mitotic index in brain neuroblasts with a high proportion of cells in late anaphase with no apparent midbody formation being observed, suggesting that cells have problems exiting mitosis and executing cytokinesis. Mitotic neuroblasts show few abnormalities, such as anaphases with unfocused poles, which may indicate alterations in the centrosome integrity. Nevertheless, immunostaining of gamma-tubulin shows no alterations in the localization of this centrosomal component. The P-element was localized by in situ hybridization on polytene chromosomes at the region 95A, and it was proved to cause the phenotype since the precise excision of this transposon reverts to the wild type. A stronger phenotype is exhibited in hemizygotes for the insertion of 718/06 over Df (3R) mbc-30, a deletion with breakpoints between 95A07 and 95C10. These

individuals have about 15% poliploid cells. Usually, these poliploid cells have more than two centrosomes, which may suggest that after a failure in division at the end of mitosis, the cell continues the cycle in poliploidy. As in the mutant 718/06, these hemizygotes present a higher proportion of cells in telophase. Again, these observations suggest problems in the regulation of mitosis exit. New stronger alleles are now being generated by imprecise P-element excision. In addition, we have recently cloned the affected gene. It codes for a protein with high homology with RPN9, a regulatory subunit of the 26S proteasome. We are now on the process of characterizing biochemically how does RPN9 affect protein turnover during mitosis.

Characterization of the human Mob1-like proteins

Member: Álvaro Tavares

Students: Claudia Florindo

Collaborators: Jonathon Pines, CRC Cambridge, UK. Didier Fesquet, Centre de Recherche de Biochimie Macromoléculaire, CNRS, Montpellier, France.

Mob1 is a recently described essential gene in *S. cereveiseae*. Mutations in this gene cause an arrest in late anaphase with an elongated spindle and segregated DNA, indicating a failure in cytokinesis. The Mob1 gene is highly conserved among eucariotes. We have isolated and cloned four different genes coding for Mob-1 like proteins in human cells. These genes (HsMob1, 2, 3 and 4) have an homology with the yeast ortologue ranging from 44% to 20%. We have determined the intracellular localization of the human Mob-like proteins by indirect imunofluorescence, and found that HsMob4 is a centrosomal protein throughout the cell cycle. Interestingly, the protein seems to accumulate between the two centrioles up until telophase, when then it is associated only with the mother centriole. Western blots with synchronized HeLa cell extracts show that the levels of HsMob4 protein do not change from mitosis to interphase. The depletion of the HsMob4 by RNAi, in HeLa cells, results in a premature separation of the two centrioles and in an increased number of binucleated cells and tetranucleated, indicating a role in the execution of cytokinesis. On the other hand, over expression of human HsMob4 results also in an increased number of binucleated cells, and high levels of cellular death. These results suggest that HsMob4, like the yeast ortologue, is involved in the final steps of cell division.

The role of VEGF and its receptors in angiogenesis and tumor growth

Member: Sérgio Dias

Student: Cátia Igreja

Collaborators: Shahin Rafii, Cornell University Medical College, New York, USA and Raquel Soares, Fernando Shmitt, IPATIMUP, Porto, Portugal.

There is ample evidence that angiogenesis regulates solid tumor growth, but its importance for the growth of liquid tumors such as leukemias or lymphomas, has not been determined. Recent studies have reported increases in bone marrow vascularization and circulating VEGF levels in patients with leukemia, which suggest angiogenesis may contribute towards leukemia growth. In response to leukemia-derived VEGF, proliferating bone marrow ECs may release growth factors that promote leukemic growth. Besides VEGF, we have shown ECs produce VEGF-C, which promotes proliferation of FLT-4 positive leukemias, and protects them from chemotherapy-induced apoptosis.

We also demonstrated that some leukemic cells not only produce VEGF but also express KDR, resulting in the generation of an autocrine loop that supports leukemia proliferation and migration. We expanded these observations by showing effective delay in the growth of VEGF receptor positive leukemias requires blocking both angiogenesis-dependent and independent VEGF/receptor loops. Since most solid and liquid tumors produce VEGF and some solid tumor cells express at least one of its receptors, such autocrine angiogenic pathways may apply to solid tumors as well.

In summary, establishment and growth of tumors may involve several autocrine (endothelial independent) as well as paracrine (endothelial dependent) VEGF/VEGF receptor angiogenic loops. Therefore, the broad, long-term aim of this project is to determine the molecular and cellular contributions of VEGF/VEGF receptor loops in tumor progression and angiogenesis, using liquid and solid tumor models.

Molecular classification of breast cancer using DNA microarray hybridization

Members: Bernardo R. Peixoto and Luisa Mota-Vieira

Collaborators: Vitor Carneiro, Victor Santos and Luís Dias, Hospital of Divino Espírito Santo, Azores, Sérgio Verjovski-Almeida and Eduardo Moraes Rego Reis, Institute of Chemistry, University of São Paulo, Brazil.

Molecular classification of tumors based on microarray expression profiling promises to overcome certain limitations of existing methods of tumor staging by histopathology techniques. Indeed, recent studies have shown that it is now possible to distinguish previously unknown tumor phenotypes with heterogeneous clinical behaviour. The aim of this project is to study differential gene expression in breast cancer using cDNA-microarrays designed and built in the University of São Paulo (Brazil) specifically for cancer-related studies. We started collecting breast tumor samples, along with appropriately annotated medical history and follow-up information, since early July 2002. All samples are collected according to ethical guidelines and patient Informed Consent. The tumor bank contains 0,5 grams of tumor tissue preserved in RNALater solution, stored appropriately for future microarray analysis.

To date 14 samples have been taken to the Laboratory of Dr. Verjovski-Almeida, in the University of São Paulo, Brazil. Total RNA were extracted from all samples using TRIZol reagent (Invitrogen), after tissue crushing on mortar and pestle. Quality of the total RNA was checked by visualization of the 28S:18S ribosomal RNA ratio on a 1% agarose gel. Total RNA was also assayed using the Bioanalyzer 2100 (Agilent

Technologies). About 20µg of total RNA from patient 1 (CM1) was used in the labelling reaction to produce both Cy3 and Cy5 fluorescent probes for array hybridization. We labelled total RNA using the post-labelling kit (Amersham Biosciences), according manufacturer's recommendations. A hybridization mix, containing equal amounts of the Cy3- and Cy5-labeled cDNA probes (both generated from the tumor sample) were prepared and hybridized onto the cDNA-microarray. This microarray, constructed in Verjovski-Almeida's Laboratory, contains a selected repertoire of 3.840 cDNAs amplified from various tumor samples; in addition a set of 30 positive and negative control genes (Score Card, Amersham Pharmacia) were also spotted onto the microarray. Fluorescent intensity obtained for each channel was normalized based on the controls mentioned above, using softwares available in Dr. Verjovski-Almeida's Laboratory. The results of this first hybridization experiment demonstrate good incorporation of the fluorophores during labelling of the probes, and specific hybridization of the probes onto the spots (data not shown).

In conclusion, the results obtained during the first year (2001) indicate that the methodology used for collection, storage and transport of tumor samples preserves the quality of RNA to an optimal level for transcriptional profiling using microarrays. This validation is paramount at this stage of the project; we may confidently proceed including bigger number of clinical cases.

Studies on antigen receptor gene rearrangement mechanisms

Member: Jorge Carneiro

Students: Nuno Sepúlveda

Collaborators: Pablo Pereira. Pasteur Institute, Paris

The role of loci accessibility in the mechanisms of rearrangement of the antigen receptor genes are still defined. We are studying these issues in the case of gamma genes encoding gamma-delta T cell receptor. We postulated three mechanisms that feature respectively : (i) a fix time window for rearrangement that is synchronized in both chromosomes, (ii) a synchronized time for rearrangement that is regulated by feedback based on the first productive rearrangement, (iii) a feedback regulation but where chromosome become asynchronously accessible for rearrangement. Statistical analysis of experimental data using probabilistic and Markov chain models shows that there is evidence in favor of the two feedback model. Moreover, the asynchronous accessibility model shows an almost perfect fit to the data suggesting that it is closer to the true underlying mechanism than the remaining models.

Quantitative analyses of T cell receptor repertoire

Members: Jocelyne Demengeot, John Stewart and Shohei Hori

Collaborator: Alexis Colette, Institut Pasteur, France

We developed a new statistical method for estimating the number of events underlying a quantitative measurement and applied the method to the analyses of T cell receptors CDR3 length spectratypes (Hori et al, 2002c). We show that this approach allows the direct and accurate quantification of lymphocytes expressing any antigen receptor with a given V, J and CDR3 length inside a diverse population of cells. This application provides an empirical validation of the method, firstly in terms of internal consistency, and secondly in terms of comparison with results obtained by more onerous classical methods. Applications of our method to the analysis of repertoire fluctuations should be numerous: quantitative estimates of clonal expansion during an immune response, lymphocyte dynamics, principle of homeostatic regulation and control of tolerance versus autoimmunity.

Hypermutation in germinal centres (GC): a random or biased process? A theoretical modelling approach

Member: Jose Faro

Students: Ana Água-Doce and Lurdes Duarte

Collaborators: Isabel Gordo, IGC, Oeiras, Portugal

During the GC reaction (GCR) follicular dendritic cells continuously present antigen (Ag) to B cells, but only in limiting amounts. While non-differentiated GC B cells require for survival a continuous engagement of their Ag receptors, the hypermutation (HM) process change frequently the affinity of those antibodies (Abs). This causes a stringent selection of B cell reactivity during the GCR. To what extent mutations are random or biased toward specific nucleotide sequences within the variable genes (hot spots) is currently an unresolved issue. In the specific response to 2-phenyloxazolone (phOx) two particular point mutations increase the affinity of germline Abs, while other mutations are either neutral or decrease the initial affinity. We have developed a deterministic and a stochastic model of GC B cell dynamics and HM. We have used both models to analyze the expected frequency distribution of anti-phOx B cells in respect to: 1) the total number of mutations, 2) the different number of mutations decreasing Ab affinity, and 3) the number of key mutations increasing Ab affinity. Both models gave results consistent with each other and, in respect to point 1, they fit well experimental results corresponding to days 14 and 21 of the response. In respect to points 2 and 3, the stochastic model fit well the experimental data for day 14. However, under the same conditions the model did not fit well all classes of experimental data at day 21. This was corrected to a great extent by incorporating into the model the fact that GCs decrease steadily in size after 2 weeks of the response. But the model still is unable to fit either class 0 or class 6 of the low affinity mutations. This suggests that some other process(es) must operate towards the end of the GCR that allow the survival of of an important fraction of highly mutated low affinity cells. The present approach shows that most of the variations in experimental data can be attributed to a small sampling size. Moreover, since all simulations assumed a random mutation process, the goodness of the fitting suggests that potential intrinsic hot spots have no special impact and that one can assume the mutation mechanism is, in effective

terms, in what respect the generation of high affinity cells, random along the V genes. Our models can be further tested by experiments in which not only neutral mutations at the aa level but also at the affinity level are derived.

Genetics of lymphocyte homeostasis

Member: Carlos Penha Gonçalves

Students: Miguel Monteiro, Susana Igreja and Vera Martins.

This project aims to identify genetic factors that control the size of lymphocyte populations in non-manipulated mice. There is little knowledge on genes controlling the actual levels of individual cell populations while maintaining the homeostatic equilibrium of the immune system. In particular, the nature of the factors that control the size of a given lymphocyte population within an organ are largely unknown.

This project will use molecular genetics and cell biology techniques to identify and study genes involved in the control of lymphocyte homeostasis. The efforts will concentrate on elucidating the role of the MHC class II E molecule in the thymus homeostasis and on the search for genetic factors controlling the size of peripheral lymphocyte sub-populations. The identification of such genetic factors may be relevant for the broader biological question relating to the nature of the signals that control cell numbers in multicellular organisms.

Differential requirements for phosphatidylinositol 3-kinase and CBP reveal two distinct pathways for inducible up-regulation of MHC class II and CD86 expression by murine B lymphocytes

Member: R.M.E. Parkhouse

Collaborator: Stuart Marshall-Clarke (Department of Human Anatomy and Cell Biology, The University of Liverpool, Liverpool, UK)

Constitutive expression of MHC class II molecules (MHC II) is restricted to dendritic cells, cells of the macrophage lineage and B lymphocytes. In B lineage cells MHC II synthesis is dramatically increased on encounter with antigen, by T cell derived signals and by microbial products. We have now shown that activity of the enzyme phosphatidylinositol 3-kinase is critical for MHC II hyperexpression and induction of CD86 in response to ligation of the BCR or CD38, but not for responses to other stimuli including IL-4, LPS and CD40 ligation. BCR-induced MHC II up-regulation was also differentially inhibited by dexamethasone, suggesting a role for the co-activator, CREB binding protein (CBP), in this pathway. Thus the existence of two signaling pathways controlling expression of MHC class II molecule is therefore suggested.

The activation molecule p58, is a marker for splenic and peritoneal B1 and marginal zone B cells

Member: R.M.E. Parkhouse

Collaborator: Leopoldo Santos-Argumedo (Department of Molecular Biomedicine, Centro de Investigación y Estudios Avanzados del I.P.N., México D. F.)

In mice, follicular B cells have been extensively studied, while other two B cell subpopulations, named: marginal zone B and B1 cells are less understood. In this work we report the expression pattern of p58, a lymphocyte activation marker, recognized by the rat monoclonal antibody NIM-R7, on these B cell subpopulations. The staining with NIM-R7 showed that undisturbed marginal zone B cells, as well as, peritoneal cavity and splenic B1 cells, constitutively expressed this molecule, while follicular B cells and T lymphocytes were negative. The analysis of different compartments showed that p58 did not appear at any stage of development, in resting T or B2 lymphocytes. However, upon polyclonal stimulation, p58 become positive on both T and B2 lymphocytes. In this work we also report the ability of the Ricin A-conjugated NIM-R7 to kill BCL1 lymphoma cells. Altogether, these results point out that p58 may be a potential target for diagnosis or therapy of B1 and marginal zone B cell malignancies.

Differentially regulated expression and function of CD22 in activated B-1 and B-2 lymphocytes

Member: R.M.E. Parkhouse

Collaborator: Shozo Izui (Department of Pathology, University of Geneva, Geneva, Switzerland)

CD22 is a B cell-restricted transmembrane protein which apparently controls signal transduction thresholds initiated through the B-cell antigen receptor (BCR) in response to antigen. However, it is still poorly understood how the expression of CD22 is regulated in B cells following their activation. Here we show that the expression and function of CD22 is differentially regulated in B-1 and conventional B-2 cells. Thus the expression levels of CD22 in conventional B-2 cells are markedly down-regulated after cross-linking of BCR with anti-IgM mAb, but up-regulated after stimulation with LPS, anti-CD40 mAb or IL-4. In contrast, treatment with anti-IgM mAb barely modulated the expression levels of CD22 in CD5⁺ B-1 cells, consistent with a weak Ca²⁺ response in anti-IgM-treated CD5⁺ B-1 cells.

Type I Interferon, regulation of B cell activation and autoimmune disease

Member: Jocelyne Demengeot

Students: Deborah Braun and Iris Caramalho

This year we concluded our investigations on the immuno-regulatory functions of IFN-I. Our analyses highlight the role of the innate cytokine IFN-I on the exacerbation of inflammatory responses and demonstrate that IFN $\alpha\beta$ *per se* enhances autoimmunity in genetically predisposed individuals. We propose that the correlation between viral infection and emergence of autoimmune syndromes reported by others result from sustained production of IFN-I (Braun et al, 2002, 2003).

Regulatory T cells and regulation of autoimmunity

Member: Jorge Carneiro

Students: Kalet Leon

Collaborators: Jose Faro, Jocelyne Demengeot. Gulbenkian Institute and Agustin Lage, Rolando Peres. Center for Molecular Immunology, Havana

We have previously proposed a model for the interplay between tolerance and autoimmunity based on regulatory T cells (Leon et al. 2000. J. Theor. Biol.; Leon et al. 2001. J.Immunol.]. This model describes the population dynamics of three mutually interaction cell types: APCs, proinflammatory T cells and regulatory T cells. Activation, progression cell cycle and survival of the proinflammatory cells is dependent on cooperation with APCs and inhibited by regulatory T cells. Regulatory T cells are activated upon cooperation with APCs and they proliferation is strictly dependent proinflammatory T cells. These interactions between the three cell types were confirmed experimentally in vitro.

To better understand autoimmune pathogenesis we followed the consequences of our model for the organisation and function of the immune system. We extended our model to include the thymic and peripheral selection of TCR repertoire of proinflammatory and regulatory T cells, as well as heterogeneity in peptide presentation patterns in APCs, identifying the conditions under which reliable tolerance host antigens and immunity to invading pathogens can be achieveve (submitted).

We used our model to understand the contribution of epidemiology and genetics of autoimmune diseases. We proposed a testable solution to the otherwise paradoxical observations that infectious diseases can both promote and protect against autoimmune diseases.

Specificity requirements for selection and effector functions of CD25⁺4⁺ regulatory T cells

Member: Jocelyne Demengeot, Shohei Hori, Matthias Haury and Antonio Coutinho

Collaborator: Juan Lafaille, NYU, NY, USA

Anti-myelin basic protein (MBP) TCR transgenic mice spontaneously develop EAE when on a recombination-activating gene (RAG)-1-deficient genetic background (T/R⁻).

In contrast, RAG-1 competent transgenic animals (T/R+) remain healthy, a result of the regulatory activities of T cells expressing endogenous $\alpha\beta$ TCRs.

We first investigated the role of CD25⁺4⁺ Treg cells in controlling EAE in this transgenic system. We show that T/R+ animals contain MBP-specific anergic and suppressive CD25⁺4⁺ cells while T/R- do not. Adoptive transfer of WT or TG CD25⁺4⁺ cells into T/R⁻ mice prevented the development of EAE while non-TG cells from T/R+ conferred only a limited protection due to their restricted repertoire diversity. The development of MBP-specific CD25⁺4⁺ Treg was dependent on the co-expression of endogenous encoded TCR- α chains together with the TG-TCR. These analyses indicate that specificity to MBP is required for effector functions but not for selection of CD25⁺4⁺ regulatory T cells. (Hori et al 2002a)

We then investigated whether previously infused regulatory T cells can recruit transgenic T cells to regulatory functions. Transgenic T cells from protected animals did not transfer tolerance to secondary recipients, and elimination of donor cells in protected recipients resulted in rapid onset of disease. In addition, we show that T lymphocyte subsets containing regulatory T cells are highly enriched for proliferating cells *in vivo*. This analysis thus excludes peripheral differentiation of regulatory T cells in this particular system. It indicates however that expansion of thymically committed cells ensures the maintenance of the peripheral pool of regulatory T cells in the adult (Hori et al 2002b).

A novel assay to quantitate regulatory T cell activity

Member: Antonio Coutinho

Student: Santiago Zelenay

Development of *in vitro* model systems has facilitated the analysis of functional properties and mechanisms of action of regulatory T cells. The available assays, however, require relatively high numbers of cells, thus limiting the evaluation of regulatory activities to T cell sub-populations that can be purified in large numbers.

We have now developed an *in vitro* assay that allows monitoring regulatory activities of very small numbers of T cells. CD4⁺ CD25⁺ regulatory T cells do not produce IL-2 and suppress IL-2 production by naïve CD4⁺ T cells. In conventional assays, regulatory activity is read out as inhibition of (IL-2-dependent) proliferation of “target” cells. The novel assay instead measures IL-2 in (co-)culture supernatants, using a very sensitive biological indicator system. In short, sorted CD4⁺ CD25⁺ T cells are titrated on fixed numbers of CD4⁺ CD25⁻ “target” cells and irradiated spleen cells (as APCs), in the presence of anti-CD3 mAb for 48 hours. The IL-2 concentration in the culture supernatants is thereafter quantitated by monitoring the proliferation of an IL-2-dependent cell line (CTLL-2). This protocol permits detection of regulatory activity with at least ten fold less cells than conventional suppression assays. Typically, we could demonstrate a significant reduction of IL-2 production by as few as 100 CD4⁺ CD25⁺ T cells in cultures containing 1000 CD4⁺ CD25⁻ target T cells.

Effect of immuno-suppressors on the onset and severity of autoimmune disease

Member: Jocelyne Demengeot and Werner Haas

Student: Francisca Fontes

Immune-suppressants (IS) such as steroids (e.g. hydrocortisone, HC) and cyclophosphamide (CYP) are widely used to treat autoimmune disease. Among other effects, steroids alter the genetic control of cytokine synthesis and alkylating agents preferentially affect cycling cells. Elsewhere we have produced evidences that peripheral regulatory T cells are enriched for proliferating cells. We used the anti-myelin basic protein (MBP) TCR transgenic mice, a murine model for human Multiple Sclerosis to investigate the effect of HC and CYP treatments on regulatory T cells. Induction of EAE in otherwise healthy T/R+ mice occurred in a dose and age related manner and associate with preferential reduction in the CD4+CD25+ T cells, a population highly enriched in regulatory cells. Moreover, CD4 cells purified from treated WT animal loose their protective function when transferred to T/R- mice. The deleterious effect of HC and CYP on Treg we thus evidenced, may explain why, even though steroids and CYP are effective therapy for relapses in Multiple Sclerosis, they do not affect the progression of the disease. This study prompts us to develop a larger screen of actual and potential therapeutic drugs.

Control of inflammatory responses by regulatory T cells

Members: Jocelyne Demengeot, Matthias Haury, Alexis Perez and Antonio Coutinho

Students: Thiago Carvalho, Iris Caramalho, Santiago Zelenay, Gustavo Rosa, and Dominique Ostler

Acute inflammatory immune responses to normally innocuous microbes can be lethal and are a frequent cause of death in immuno-suppressed patients. Recent evidence demonstrated that a population of CD4+ CD25+ regulatory T cells (Treg) prevents Helicobacter-dependent inflammatory bowel disease (IBD) in immunodeficient mice. Likewise, we have shown that Treg prevent lethal inflammatory pneumonia (IPD) induced by the hyper-responsiveness of naïve CD4+ cells triggered by Pneumocystis Carinii infection in mice. Our most recent data indicate that Treg dampen the expansion of naïve CD4+ T cells and local inflammation induced by infectious agents, but also when it is sterilely induced. Using this finding, we developed and explored an in vivo short-term assay where the cellular and molecular mechanisms underlying the operation of Treg, thus far unknown, can be readily analysed. In parallel we set new ex vivo assay where the requirements for survival and functional activation of limited number of cells can be readily monitored. We could show that regulatory T cells are activated, expanded, and acquire higher effector efficiency upon inflammatory stimuli. This activation is at least in part mediated by Toll Like Receptors selectively expressed by this subpopulation of T cells. Finally we evidenced that interaction with naïve T cells is necessary for the stability of the CD25 molecule expression on regulatory T cells. Together these findings

may offer a cellular and molecular basis for several unexplained phenomenology, such as the inverse correlation between infection and autoimmune disease incidence, inefficient immuno-therapy of cancer and chronic infections that we will further investigate.

Regulation of T-cell mediated immune responses by the stress responsive gene heme oxygenase-1

Members: Miguel Soares and Jocelyne Demengeot

Student: Angelo Chora and Sofia Simões

Collaborator: Abelhadi Saoudi, Autoimmunité et Immunorégulation Inserm, Unité de Recherche U563 Centre de Physiopathologie Toulouse-Purpan Pavillion Lefèvre, Paris, France.

The overall aim of the project is to assert the role of heme oxygenase-1 (HO-1) in the regulation of T-cell mediated responses using experimental autoimmune encephalomyelitis (EAE) as a model of T cell mediated autoimmune disease. EAE is a prototypic CD4 T helper cell-mediated autoimmune disorder considered as a recapitulating model of the human demyelinating disease referred to as multiple sclerosis. It is characterized by focal areas of inflammation and demyelination throughout the central nervous system. During EAE, HO-1 expression is observed in monocyte/macrophages infiltrating in the central nervous system, as well as in resident microglia and astrocytes. HO-1 expression correlates with the remission phase of EAE and inhibition of its enzymatic activity exacerbates EAE and blocks its remission. The mechanisms by which HO-1 and/or the products resulting from HO-1 action on heme (CO, Fe²⁺ and Biliverdin/Bilirubin) modulate the development and outcome of EAE remain to be established and are the main focus of this project. We will address this through genetic manipulation of mice strains that renders them susceptible to EAE. We will then modulate the expression of HO-1 in these mice to address how the expression of this gene acts to modulate the pathogenesis of this important autoimmune disease.

Molecular mechanisms underlying the protective effect of HO-1 derived CO: *Interaction with the NF- κ B signal transduction pathway*

Member: Miguel Soares

Student: Mark Seldon and Sofia Simões

Collaborator: Josef Anrather, University of Cornell, New York City, USA

Endothelial cells (EC) form the physical barrier between blood and tissues. Activation of EC during inflammatory reactions is associated with the acquisition by these cells of a pro-inflammatory phenotype that assists in the sequestration of leukocytes into inflamed tissues. This is a critical event to combat infectious disease, as inflammatory reactions are most often associated with microbial infections. While critical to the initiation of immune responses that ultimately lead to microbial clearance, the pro-inflammatory phenotype

associated with EC activation must be tightly controlled. When this does not occur, unfettered EC activation can lead to apoptosis, which exacerbates inflammation in a manner that causes tissue injury and disease. One of the mechanisms that control the expression of pro-inflammatory genes associated with EC activation relies on the expression of a series of anti-inflammatory genes that we refer to as “protective genes”. One of such genes encodes the stress responsive enzyme heme oxygenase-1 (HO-1). Previous work from our laboratory has shown that expression of HO-1 protects EC from undergoing apoptosis and modulates the pro-inflammatory phenotype associated with EC activation (*Soares et al. submitted for publication*). Under inflammatory conditions, HO-1 becomes the rate-limiting enzyme in the catabolism of heme into biliverdin, free Fe^{2+} and CO. Biliverdin is subsequently catabolyzed into bilirubin, through a process that is dependent on the expression of biliverdin reductase. Free Fe^{2+} up-regulates the expression of the iron sequestering protein ferritin. We have found in the past year that expression of HO-1 in EC modulates the activation of nuclear factor kappa B (NF- κ B), a transcription factor intimately involved in the expression of pro-inflammatory genes associated with EC activation. This effect of HO-1 is mimicked by the iron chelator ferritin suggesting that ferritin mediates the action of HO-1 in terms of blocking NF- κ B activation. Modulation of NF- κ B activation is probably achieved in such a manner that still allows the expression of NF- κ B dependent genes, i.e. the bcl-2 family member A1, that interact functionally with HO-1-derived CO to prevent EC apoptosis. The aim of this project is to dissect further the molecular mechanisms by which HO-1, ferritin, biliverdin/bilirubin and/or CO interfere with the signal transduction pathways leading to the activation of the transcription factor NF- κ B and to the expression of NF- κ B-dependent genes associated with EC activation.

***In vivo* delivery of TAT-fusion peptides to inhibit the activation of the transcription nuclear factor NF- κ B**

Members: Miguel Soares, Moises Cavalcante and Tatiana Vassilevskaia

Student: Sofia Simões

Collaborator: Gabriela Garcia, AstraZeneca, Boston, MA, USA

Inflammation such as it occurs during microbial infections is critical to the immune response that will ultimately lead to microbial clearance. However, inflammatory reaction must be terminated as soon as microbial infections have been cleared. When this does not occur, acute or chronic inflammation develops leading to tissue injury, organ dysfunction and disease. When exposed to pro-inflammatory stimuli endothelial cells (EC) and macrophages ($\text{M}\phi$) become “activated”. As such they express a series of early responsive pro-inflammatory genes that encode adhesion molecules (i.e. E-selectin, P-selectin, ICAM-1 and VCAM-1), cytokines/chemokines (i.e. TNF- α , IFN- γ , IL-1 β , IL-6, IL-8, MCP-1 and RANTES), co-stimulatory (i.e. B7-1, -2, -h) and pro-coagulant molecules (i.e. tissue factor and plasminogen activator inhibitor). The main objective of this project is to assess the feasibility of a novel “gene delivery” technology. It employs peptides (referred to as protein transduction domains; PTD) that enter cells spontaneously with very high

efficiency in tissue culture as well as in animals (Schwarze et al., 1999, Science, 285, 1569-72). When linked to other molecules these get transported into cell as well. We are currently testing the ability of different PTD linked to peptides or proteins to modulate biological functions in EC *in vivo* and *in vitro*. We used in a first set of experiments different PTD linked to a similar peptide (Nemo binding peptide; NBP) that down-regulates the expression of a specific set of pro-inflammatory genes in activated EC by interfering with the activation of NF- κ B. Delivery of these peptides allows to determine the effectiveness of this technology in terms of mediating biological functions in cells, by measuring the expression of NF- κ B dependent pro-inflammatory genes which are expected to be down regulated after application of the recombinant fusion protein (peptide) tested. In addition we have generated a fusion protein that consists of a PTD from the TAT peptide derived from the human immunodeficiency virus fused to the heme oxygenase-1 cDNA. The resulting fusion protein has been produced and purified and shown to keep its enzymatic function as well as to be able to transduce cultured EC. We now aim to show that TAT-HO-1 can be used to mediate biologic functions attributed to HO-1 and then use it therapeutically to suppress the development of inflammatory lesions in rodents.

Identification and characterization of putative targets of HO-1 derived CO in endothelial cells

Members: Miguel Soares and Isabel Gregoire

Student: Sofia Simões

CO is synthesized *in vivo* by enzymes of the heme oxygenase (HO) family. These control the rate-limiting steps in heme catabolism, which in addition from CO also leads to the generation of biliverdin and free iron. Three mammalian HO isoforms have been identified, one of which, heme oxygenase-1 (HO-1), is stress-responsive and induced by various oxidative agents. In previous studies, we and others have demonstrated that HO-1 protects endothelial cells (EC) from undergoing apoptosis, through a mechanism that is dependent on the generation of CO. Contrary to other biological functions of CO, its ability to protect EC from undergoing apoptosis does not involve the activation of the enzyme guanylate cyclase and/or the generation of cGMP. Instead, CO acts via a signaling transduction pathway that activates p38 mitogen activated protein kinases (MAPK). The mechanism(s) by which HO-1/CO interact with this signaling transduction pathway and how this contributes to the overall anti-apoptotic effect of HO-1/CO remains to be established. The aim of this project is to identify putative intracellular targets of CO in endothelial cells. To do this we are testing different biochemical based approaches allowing to detect the several steps of the enzymatic activity of HO-1. We will then use these to isolate and characterize in endothelial cells, putative molecules to which CO binds.

Mechanism underlying the anti-proliferative effects of heme oxygenase-1 (HO-1) in smooth muscle cells: *the role of the transcription factor Yin and Yang 1 (YY1)*

Members: Miguel Soares and Isabel Gregoire

Student: Sofia Simões

Collaborator: Anny Usheva, Harvard Medical School, Boston, USA

Intimal hyperplasia arising from vascular injury, subsequent to procedures such as angioplasty, bypass surgery or organ transplantation, limits the success of these therapeutic interventions. Smooth muscle cell (SMC) migration and proliferation into the blood vessels intimal region is thought to be a central event in the the pathogenesis of intimal hyperplasia. Recently the expression of the stress responsive gene *Heme oxygenase-1* (HO-1) has been shown to prevent the development of intimal hyperplasia. We have recently shown that this effect of HO-1 is mediated via its ability to generate the gas CO, through heme catabolism (*Otterbein et. al. Nat. Med. In press*). Our working hypothesis is that CO prevents the developmemt of intimal hyperplasia by acting directly on SMC to block their proliferation. We have validated this hypothesis and found that adenovirus mediated overexpression of HO-1 in SMC or exposure of SMC to exogenous CO downregulates the expression of Yin and Yang 1 (YY1) a transcription factor that regulates cell cycle transitions in SMC. The focus of this project is to understand the molecular mechanisms by which HO-1/CO regulate the expression and activity of the transcription YY1 and how this effect contributes to suppress SMC proliferation and thus prevent intimal hyperplasia. This project is a collaborative effort between the Inflammation Laboratory, at the Intituto Gulbenkian de Ciência, Oeiras, Portugal and the laboratory of Dr. Anny Usheva at the Beth Israel Deaconess Medical Center, Department of Medicine, Harvard Medical School, Boston, MA, USA.

Molecular mechanisms underlining the anti-apoptotic effect of heme oxygenase-1 (HO-1) and of its catalytic product carbon monoxide (CO): *role of the p38 MAPK signal transduction pathway*

Members: Miguel Soares and Gabriela Silva

Student: Sofia Simões

Collaborators: Leo Otterbein, University of Pittsburgh, Boston, USA

Endothelial cell (EC) apoptosis, such as it occurs during acute or chronic inflammation, is a pro-inflammatory event that can lead to irreversible tissue injury, organ failure and disease. Understanding how EC protect themselves from undergoing apoptosis in situations of oxidative stress may be critical in the development of therapeutic strategies aimed to suppress the deleterious effects associated with acute and/or chronic inflammation. The basis of this project is to analyze the molecular mechanism by which the gene heme oxygenase-1 (HO-1) and one of its active products, the gas carbon monoxide (CO) protect endothelial cells from undergoing apoptosis. We have previously shown that one of the major cytoprotective effects of HO-1 relies on its ability to protect

EC from undergoing apoptosis. This anti-apoptotic effect is dependent on the generation by HO-1 of the gaseous molecule CO. The anti-apoptotic effects of CO act via a mechanism that is not yet clear but that is dependent on the activation of a signaling transduction pathway that activates p38 Mitogen Activated Protein Kinases (MAPK). The mechanism(s) by which HO-1 and/or CO interact with this signaling transduction pathway and how this contribute to the anti-apoptotic effect of HO-1/CO remains to be established and is the main focus of this project.

Modification of vascular endothelial cells using viral gene transfer to promote the acceptance of allogeneic vascular prostheses

Member: Miguel Soares

Student: Cornelia Doeblis and Sofia Simões

Collaborators: Prof. Hans Dieter Volk, Charite, Berlin, Germany

In order to improve the function/survival of small caliber vascular grafts, e.g. coronary bypass prostheses, autologous endothelial cells (EC) are seeded in the luminal surface of these grafts to suppress thrombosis, the main event leading to graft failure. Because of the limited availability of autologous EC for this purpose we thought of using allogeneic or xenogeneic EC for the coverage of such prostheses. In view of the known immunologic mechanisms leading to the rejection of these grafts it appears useful to modulate the immunogenic features of EC before transplantation. Therefore we set to investigate the ability of “protective genes” to prevent the cytotoxic effects inflicted to EC and leading to rejection of small caliber vascular grafts. To do so we are testing whether the *Heme oxygenase-1* (HO-1) gene, which participates in a variety of defense mechanisms that protect EC from oxidative stress, hypoxia and/or ischemia/reperfusion, can protect EC from those events leading to the rejection of these grafts. We are analyzing specifically whether lentiviral mediated HO-1 over-expression in EC can be used therapeutically to protect EC from complement-mediated lyses, a central event in the rejection of small caliber vascular grafts. The final aim of this approach being to generate an approach allowing to use HO-1 transduced EC in vitro that can be used to seed small caliber vascular prostheses and promote the survival and function of these grafts.

Pathogenesis of HIV1 and HIV2 infections

Member: Jorge Carneiro

Students: Nuno Sepúlveda and Catarina Serrano

Collaborators: Rui Victorino, Ana Espada de Sousa. Institute for Molecular Medicine, Lisbon and Zvi Grossman. Telaviv University, Israel and NIH, USA

Study of HIV-2 infection, “nature's experiment” with inherently attenuated HIV disease, provides valuable insights into the causal relationships between CD4-cell depletion, viral replication and immune-activation in HIV infection. We found that in HIV-2 and HIV-1

patients with a comparable degree of CD4 depletion the imbalance in the relative sizes of the naïve and memory T-cell populations and the upregulation of CD4 and CD8 cell-activation markers are similar, even though the viral load in the plasma of HIV-2-infected patients is two orders of magnitude lower than in HIV-1 patients and HIV-2 patients are known to have slower rates of CD4 T-cell decline and a better clinical prognosis. Moreover, we found similar increase in the frequency of cycling CD4 T-cells (measured by Ki67 expression), which was in strong correlation with the expression of activation markers. Finally, the level of T-cell anergy, as assessed by the proliferative responses to CD3 stimulation and to a panel of microbial antigens proved to be comparable in HIV-1 and HIV-2 patients with similar degree of CD4 depletion despite large differences in viral load. Our data are consistent with a direct causal relationship between immune-activation and CD4-cell depletion in HIV disease and an only indirect relation of these parameters to the virus replication rate. This hypothesis is currently being studied by mathematical modeling and computer simulation. Specifically we investigate if immune activation is the cause or the consequence of CD4-cell depletion, and if both these perturbations during HIV infection are caused by other host-pathogen interactions.

The potential and application of virus host evasion genes that modify apoptosis

Member: R.M.E. Parkhouse and Ana Crespo

Students: Sílvia Almeida, Sílvia Correia, Rute Nascimento and Vívian Oliveira

The aim is to identify and exploit viral modifiers of apoptosis and cytokine responses (particularly TNFRs) as a potential source of novel health care pharmaceuticals for manipulation of immune responses and treatment of certain diseases. Such virus genes will be identified by nucleotide sequence and functional analysis of cloned viral ORFs and cDNA libraries of three large DNA viruses (African swine fever (ASFV), Ectromelia virus (EV) and Mouse herpes virus (MHV 68)). As a direct approach towards identifying novel virus evasion genes which do not have homologies in the data base, the genes of these three viruses will be systematically screened in functional assays for their impact on apoptosis and for binding to chemokines and cytokines.

Most of the year's work has been occupied with subcloning virus genes into expression and retrovirus transfer plasmids. As a necessary prelude we have had to modify existing The construction of mice transgenic for selected virus "evasion" genes is now well underway, and will provide a novel approach to explore the mechanism and exploitation of these genes.

Molecular interactions in murine herpesvirus 68 latent infection of B-lymphocytes

Member: J Pedro Simas

Students: Patricia Madureira, Sofia Marques, Lenia Rodrigues and Claudia Istrate

Collaborators: Stacey Efstathiou, University of Cambridge, UK and Philip Stevenson, University of Cambridge, UK

Studies into the molecular basis of gammaherpesvirus latency have been hindered by the lack of amenable animal model systems and the lack of fully permissive cell lines, which are required for the genetic manipulation of these viruses.

Research on the utilisation of a gammaherpesvirus, designated murine herpesvirus 68 (MHV-68), whose pathogenesis can be readily investigated in the laboratory mouse (for recent reviews see Simas & Efstathiou, 1998; Doherty and Christensen, 2000). MHV-68 is genetically related to Epstein-Barr virus and Kaposi's sarcoma associated herpesvirus, which are important Human pathogens. Experimental infection of inbred strains of mice with MHV-68 results in acute productive infection of the lung followed by latent infection of B-lymphocytes. Comparison of the genomic organisation of MHV-68 with other gammaherpesviruses shows the presence of virus specific ORFs and a number of cellular homologues, which are predicted to determine the particular biological properties of these viruses, e.g. host range, immune evasion, latency and disease. MHV-68 has 14 unique such genes, designated M1 to M14, and several cellular homologues, including a complement control protein, a D-type cyclin and an IL8 receptor. In addition to these cellular homologues two of the CEM¹ genes, M1 and M11, show low level similarity to serpins and bcl2 cellular protein, respectively.

Our research interests are focused in trying to understand how co-ordinated expression of these cellular homologues and unique ORFs, in a developing B-lymphocyte, result in immune evasion and latent infection.

To this end, we have adopted the following strategies; (i) to determine the tropism and virus transcription pattern, during the establishment and maintenance of latent infection in B-cells and, (ii) to construct recombinant viruses with specific genes deleted and study their phenotype upon infection of mice and, (iii) to identify cellular molecular targets for virus latency-associated genes.

Antiviral peptides blocking herpes simplex virus type 1 entry into cells

Member: J Pedro Simas

Student: Lidia Fonseca

Collaborators: Massimiliano Galdiero, University of Napoli, Italy; Helena Browne, University of Cambridge, UK; William Gibbons, ARISTOS Scientific Ltd, UK and Paolo Sarmientos, PRIMM, Italy.

Human herpes simplex virus 1 (HSV-1) affects over 50% of the European Union adult population and is highly transmittable: No vaccines, despite repeated attempts, are yet available for prevention, but classical antivirals such as nucleosides are the choice chemotherapies. Nevertheless, HSV resistant strains and the failure to completely relieve symptoms (especially in immunocompromised individuals) make it imperative that antivirals that utilise new mechanisms of action are developed and marketed. Symptoms of HSV-1 infections include erythematous vesicles, at mucocutaneous sites, which can ulcerate and be quite painful. The mouth, lips and genitalia are the dominant sites of these lesions. HSV-1 however causes herpes keratitis, a recurrent infection of the eye as well as

the much feared manifestation of HSV-1 infection, herpes encephalitis, which has a 1/500,000 incidence and high mortality if not treated. Systemic infection of the newborn and the chronic lesions of immunocompromised patients (especially HIV and cancer patients) and patients undergoing organ transplantation (which necessitates use of immunosuppressive drugs) can often be complicated by HSV-1 infections.

All of the above, including the pain, loss of work and productivity, are potent reasons for possessing effective new and possibly multiple therapies against HSV-1 infections. New therapies and in particular those which act by novel antiviral mechanisms should be developed. Many arguments against the use of peptide therapeutic agents, due principally to their rapid proteolysis and short half-lives are now dissipated. An excellent example is the anti-HIV aspartyl protease which enters cells and inhibit viral assembly. A second development, the existence of virions technologies to stabilize and deliver therapeutic peptides, has permitted administration of peptide chemotherapeutics by oral, I.V., nasal and other routes.

The objective of this research program is to develop of a new class of anti-HSV-1 drugs – a peptide that acts at the cell surface blocking virus entry – when used alone or in combination with, for example, nucleoside antivirals should increase the quality of life of many in the EU who suffer pain, discomfort and sometimes morbidity. It is hoped that this work will form a basis for developing peptides moieties as anti-HSV (not part of this proposal) and provide an example of this class of antiviral that block virus entry into cells. When sold in the EU and globally it should prove economically beneficial to the EU and provide R&D training to young scientists. Because it operates via an extracellular novel mechanism these anti-HSV peptides will prove complementary not competitive with current antiviral chemotherapies; predictably combined therapy, as with anti-HIV therapies, may prove the most common use of antiviral peptides that block virus entry.

Study of immunogenic proteins of *Mycoplasma mycoides* subsp. *mycoides* small colony type

Member: J Pedro Simas

Collaborator: Jose Regalla, LNIV, Lisbon, Portugal

Mycoplasma mycoides subsp. *mycoides* (Mmm SC) is the causative agent of contagious bovine pleuropneumonia (CBPP) in domestic cattle. Due its economic impact for agriculture this disease has been included in List A by the Office International des Epizooties and has been eradicated from most European countries. Although, extensive control and eradication programs have been implemented in Portugal this disease is still endemic. Part of the difficulties of the control and eradication programs are due to the fact that the available diagnostic tests fail to detect asymptomatic carrier animals and lack sensitivity and specificity.

The proposed work is part of a formal collaboration between the Laboratório Nacional de Investigação Veterinária (LNIV) and the Instituto Gulbenkian de Ciência (IGC). Its objective is to develop a specific, sensitive and rapid serological diagnostic test by: i) identification and cloning of Mmm SC immunodominant protein encoding genes (IGC),

ii) epitope mapping of Mmm SC immunodominant proteins (IGC), iii) analysis of intraspecific variability of Mmm SC (LNIV), iv) to develop improved serological reagents to serological tests for CBPP (LNIV) and to develop improved serological tests for CBPP.

Genetics of malaria in wild mouse models

Members: Dan Holmberg, Carlos Penha Gonçalves, Sylviane Pied and Pierre André Cazenave

Student: Susana Campino

The aim of this project is to identify the genetic factors contributing to the control of susceptibility/resistance to murine malaria taking advantage of the fact that some inbred wild-derived mouse strains display unique phenotypes of resistance. Crosses of these mice with susceptible laboratory strains C57BL/6 are used for genetic mapping and identification of genes controlling resistance/susceptibility. Recently, we have studied the WLA strain, which is highly resistant to cerebral malaria. In a genome wide screening of a F2 cohort we have obtained evidence for two novel loci associated with resistance to murine cerebral malaria. Ongoing studies aim at identifying additional loci linked to resistance to hyperparasitemia observed in the WLA mouse strain. These data may have important implications for the search for genetic factors controlling cerebral malaria in humans

Host-parasite interactions during the hepatic stages of malaria infections

Member: Maria M. Mota and Pedro Coutinho

Students: Patricia Leirião, Miguel Casanova Parente and Bruno Mateus

Collaborators: Ana Rodriguez (New York University Medical Center, New York, USA), George Dimopoulos (Imperial College of Science Technology and Medicine, London, UK)

Since it is becoming evident that intracellular parasite are masters at manipulating the host cell pathways for their own benefit to create a more hospitable environment, we proposed to characterize the hepatocyte response to *Plasmodium* sporozoite infection (Aim 1). In addition, we also proposed to take a careful examination on the host apoptosis pathways altered by infection as well as to identify *Plasmodium* genes responsible for apoptosis inhibition (Aim 2). During the first year of this project we have collected samples of normal mouse hepatocytes and infected hepatocytes in order to obtain cDNA of both populations. These cDNAs were hybridized to mouse microarrays and some preliminary data has been already analyzed. Sporozoite invasion/infection causes a massive up-regulation of mitochondrial transcripts, a possible signature of apoptosis or just stress. It also up-regulates thioredoxin, peroxidase and peroxiredoxin.

This does imply an oxidative stress response. In addition, a down-regulation of genes linked to immune-related processes, such as MHC molecules, is observed.

The full sequence of the *P.falciparum* genome and sequences of other *Plasmodium* species revealed proteomes that are quite distinct from the previously known. The result is that for example, approximately 60% of the predicted proteins of *P.falciparum*, have not had a function assigned. This problem exists in all proteomes, but not at this scale. Pedro Coutinho is not only generating and testing new computational procedures to determine function of proteins as well as performing computational studies to define a set of potential regulators of apoptosis.

During this year we also attempt to collect preliminary results in an independent project but also related to hepatocyte response to *Plasmodium* infection. *Plasmodium* sporozoites migrate through several hepatocytes by breaching their plasma membranes, before infecting a final one. We hypothesize that hepatocytes wounded by sporozoites release one or more growth factors that render neighbouring hepatocytes susceptible to infection. We were able to show that wounding caused by sporozoite migration induces traversed cells to secrete hepatocyte growth factor (HGF), which alters the neighbour hepatocytes making them susceptible for infection. Hepatocyte infection is dependent on activation of HGF receptor (MET) by secreted HGF, since blocking the ligand with antagonist antibodies or interfering with MET signalling results in infection decline. Moreover, direct activation of the receptor by use of an agonist anti-MET antibody results in increased infectivity. Our data reveal a novel mechanism whereby the malaria parasite exploits the hepatic microenvironment in order to achieve infection and identifies HGF/HGF receptor targeting as a possible novel approach for malaria prevention.

Infected-erythrocyte interactions with host cells during malaria infections

Member: Maria M. Mota

Students: Daniel Carapau and Cristina Rodrigues

Collaborators: Ana Rodriguez (New York University Medical Center, New York, USA) and George Dimopoulos (Imperial College of Science Technology and Medicine, London, UK)

Plasmodium infected-erythrocytes interacts with different cells during its life cycle, namely dendritic cells and endothelial cells. We have shown recently that the capacity of interact with dendritic cells is the basis for the observed capacity to re-infect the same individual several times, without any immunological memory being established that could protect the host from secondary infections. We have recently demonstrated that the efficient CD8 response against the liver stages of *plasmodium* is abolished once the parasite evolves into blood stages. Dendritic cells (DC), as the main antigen-presenting cells (APC), are natural targets of pathogens, in order to modulate the immune response of the host. In fact, we observed that erythrocytes infected with *P. yoelii* (Py), a rodent-infective species, can drive dc into an unresponsive state to maturation stimuli such as LPS, similarly to what was found for the human-infecting *P. falciparum*. In this study, our aim is to study the global genomic response of mouse DC to Py, as well as the extent

to which the parasite impairs LPS-driven DC maturation, through cDNA microarray analysis.

When gene expression of DC incubated with infected-erythrocytes was compared to normal erythrocytes, a total of 66 genes were regulated at least 2 fold in 2 independent experiments (44 induced and 22 repressed). The majority of the induced genes were either immunity-related genes (eg. MHC class I heavy chain), transcription factors (eg. STAT1 and 2) and also interferon-induced signalling proteins. DC stimulated with LPS showed a strong induction of a large number of genes (165 more than 2 fold and 27 more than 4 fold). Of the 30 with stronger induction (for instance cyclin D2 and TNF receptor), 20 (67%) showed a much weaker induction (>40% inhibition) by LPS when DC were previously incubated with infected-erythrocytes. This was not the case with non-infected blood.

Thus, microarray analysis suggests that, when in contact with *Plasmodium*, the genetic reprogramming induced by LPS is considerably different from what is the normal maturation program. There is a considerable number of signalling proteins and transcription factors that are either induced or repressed in DC by *Plasmodium* infected-erythrocytes and not by normal erythrocytes.

Role of microglia activation in cerebral malaria associated neuropathogenesis

Member: Sukalyan Chatterjee and Teresa Faria Pais

Students: Catarina Figueiredo, M^a Hortense Matos and Rui Peixoto

Collaborators: Laura Santambrogio, Harvard Medical School Boston

Microglia, the resident macrophages of the brain, constitutes 5-20% of the cell population of the central nervous system (CNS). Although in the normal CNS microglia are in a "resting state" and have architectural and reparative functions they promptly respond to physical trauma and microbial infection. Microglia migrate to the site of injury where it can proliferate and/or get activated. Activated microglia have up-regulated surface receptors and produce cytokines which initiate a local immune response. Cerebral malaria (CM), the most serious clinical complication of infection with the parasite *Plasmodium falciparum* causes millions of deaths each year and neurological sequelae in the survivors. There is evidence that microglia are activated very early on after infection. Whether activation of microglia is a key event in causing neuronal damage culminating in CM is still a matter of debate. It is unclear whether apoptosis sets in to cause neuronal damage and what are the determinants of the cell death cascade. One hypothesis is that the parasite mediates neuronal cell death and then signals from dying neurons activate microglia. It is also likely that infection can activate microglia which causes cytotoxic damage to neurons. This project will address these issues and will also investigate the signaling mechanisms in activation of microglia. Moreover, the project will investigate the role of quinolinic acid secreted by activated microglia in the neuropathogenesis of CM. Using in vivo mouse model for CM and in vitro tissue culture systems microglial activation and neuronal death will be studied by immunocytochemistry and confocal microscopy. The signaling cascades, mechanisms initiating microglial activation and

the consequences of activation are largely unknown. Microglia in the central nervous system are both protective and cytotoxic. A controlled balance of which needs to be maintained to prevent pathogenesis. Here we will address the signaling mechanisms in activated microglia by infection (extracts of parasitized erythrocytes) and dying neurons adopting a molecular approach to identify secreted entities like cytokines, intracellular signaling cascades and mechanisms of transcriptional regulation. This may elucidate the crux of the balance between cytotoxic and protective role of microglia. The novelty of this project is in addressing, for the first time, the role of microglia as a decisive player in the pathogenesis of cerebral malaria and the results will have significant relevance in combating the disease.

The role of Toll-like Receptors in cerebral malaria

Member: Antonio Coutinho and Andrew Waters

Student: Vasco Correia

Despite intensive research, the pathogenic mechanisms of cerebral malaria – the major cause of death in *P. falciparum* infection - are not fully characterised. Known central features are the T cell-dependence of the process in murine malaria, a key role of TNF- α , and the sequestration of mature forms of parasitized erythrocytes and ring stages within the microvasculature of the major body organs, following interactions between surface molecules on parasitized red blood cells and host receptors.

Toll-like receptor 4 (TLR-4) is known to be a mediator of cellular activation and production of proinflammatory cytokines. TLR signalling operates through the release of cytoplasmic NF- κ B and its translocation to the nucleus, which we have now demonstrated occurs in endothelial cells of the brain microvasculature in animals undergoing cerebral malaria.

We have now investigated whether brain inflammation in cerebral malaria involves TLR-4 signalling, by comparing the course of *P. berghei* infection in wild-type and TLR-4 mutant mice. The results show that TLR-4 “null mutants” (C57BL/10.Sc.Cr) mice do not develop cerebral malaria when inoculated with *P. berghei*, and do not translocate NF- κ B into the nucleus of endothelial cells in the brain microvasculature. In contrast, C57BL/6 controls develop cerebral malaria at a very high frequency, and show readily detectable NF- κ B translocation in brain endothelia.

As B10.Cr mice carry a second mutation in the gene encoding one IL-12 Receptor chain, we used the C57BL/10.SN mouse strain to confirm that resistance to cerebral malaria is imparted by the TLR-4 mutation. Moreover, by producing hemopoietic bone-marrow chimeras in the B10.Cr/B6 combination, we show that the presence of TLR-4 on hematopoietic cells, but not on endothelial cells, is required for the development of cerebral malaria in this genetic background.

Interestingly, C3H/HeJ (carrying a TLR-4 point mutation that results in a complete loss of function phenotype) and C3H/HeN (wild-type) mice do not differ in susceptibility to cerebral malaria, indicating variability in the respective molecular pathogenesis. These alternative mechanisms are currently under investigation.

In conclusion, the present results support the notion that TLR-4, at least in some “backgrounds”, plays an important role in the development of cerebral malaria. Innate immunity, however, does not provide all the critical factors in the pathogenic process, as demonstrated by the complete resistance of Rag-1 $-/-$ mice. Hence, cerebral malaria represents a novel form of pathology resulting from the interaction of innate and adaptive immunity.

Immunophysiology of malaria.

Members: Sylviane Pied and Margarida Vigario

Student: Rui Rodrigues

Collaborators: Virgílio do Rosario, CMDT, UNL, IHMT, Lisbon, Portugal and Pierre-André Cazenave, IPPI, Institut Pasteur, Paris, France.

The main objective of our project is to analyse the T-cell responses involved in pathogenesis of Cerebral Malaria (CM). Immune responses triggered by the malarial parasite play a major role in controlling parasite replication (protection) but also associated with pathological changes that occur during malarial infections (pathogenesis). A proper understanding of the interaction of the parasite with the host immune system would be essential for the development of interventions to reduce malarial morbidity and mortality. We have previously shown that occurrence of the neuropathology is linked to $\alpha\beta$ T cells in mouse infected with *Plasmodium berghei* ANKA, a parasite strain able to induce cerebral malaria in susceptible mouse strain. Our activities are concentrated on the characterisation of these $\alpha\beta$ T cell subpopulations and the study of their pathogenic function. Particularly, we have observed a significant increase in the number of CD8⁺ T cells in the brain of mice developing CM infected with sporozoite or red blood stage parasites when compared with those that do not. These CD8⁺ T cells exhibit a phenotype of activated cells as demonstrated by the level of expression of CD25, CD69, CD44 and CD62L surface markers and are not undergoing on apoptosis. They secrete γ -IFN and α -TNF, two cytokines known to play a role in severe malaria. A better characterization of the CD8 population from the brain of CM positive mice has been done by studying their TCRBV repertoire using the Immunoscope approach and a new informatic tools (ISEApeaks). Data obtained showed a polyclonal CD8 T cell response and allow the identification of putative pathogenic recurrent clones expanded in CM⁺ mice but not in infected mice that do not developed CM (CM⁻).

In spite of all these indications, the exact mechanism by which these CD8⁺ T cells are involved in the pathological processes linked to CM is at present unknown. For this reason, we focus our work on two aspects: 1) To determine whether pathogenesis during malaria result from a deficient control of pathological CD8 T cell clones by looking if regulatory T cells are involved in this process. One of the approach used to establish if regulatory T cells are implicated in the pathology or in the protection against malaria, was to determine if absence (or important reduction) *in vivo* of CD4⁺CD25⁺ regulatory T cells change the outcome of an infection by *Plasmodium berghei* ANKA, a strain of malaria parasite able to induce CM in mice. In parallel, we are studying the dynamic of T

regulatory cells in infected mice. We are also determining if there is possible functional differences between different strains of mice in our model of infection. 2) To study interactions of CD8 T cells sequestered in the brain of CM mice with other local cells i.e. endothelial, microglial cells and astrocytes. To address this issue, we are using an in vitro co-culture systems composed of primary cultures of glial cells (astrocyte and microglia) derived from brain of newborn mice co-cultivated with brain endothelial cells. Preliminary data allowed us to identify different molecules involved in these interactions which could be therapeutic targets. This part of the project is done by taking advantage of the expertise in microscopic analysis offered in the IGC and using the Affymetrix GeneChip System for expression analysis.

In search of malaria mitogens

Member: Antonio Coutinho, Elsa Seixas, Christophe Gregoire and Andrew Waters
Students: Margarida Cunha, Vasco Correia and Dominique Ostler

Using three inbred mouse strains and *P. berghei* as well as *P. chabaudi chabaudi*, we have now analysed in detail the alterations in cellular composition and state of activation of lymphocytes in representative secondary lymphoid organs, bone marrow and thymus. The results confirm that malaria infection is accompanied by a marked polyclonal activation of T and B lymphocytes and serum hypergammaglobulinemia. We initiated, therefore, the search for “malaria mitogens”, following two complementary approaches: on the one hand, we screen plasmodium products for activating lymphocytes and/or dendritic cells; on the other hand, we use the information contained in the recently completed plasmodium genome sequence in order to identify “candidate” molecules with this ability.

Innate immunity in malaria infection: interactions of Dendritic Cells (DC) and other Antigen Presenting Cells with blood stages *P. chabaudi*.

Member: Antonio Coutinho and Elsa Seixas
Student: Dominique Ostler

Primary infection of mice with *P. chabaudi chabaudi* is characterized by a rapid inflammatory response where IL-12, TNF- α and IFN- γ are produced in the spleen and are transiently present in the plasma. The cells involved in this early response are unknown. Previous results (Seixas et al. 2001), however, have shown that interaction of bone marrow-derived DC (BMDC) with schizont-stage parasites leads to production of TNF- α , IL-6, and IL-12, and to up-regulation of MHC class II, CD86 and CD40, as well. Such a DC response could explain the rapid cytokine production upon infection, and the preferential activation of TH1 cells that occurs early in the primary infection with *P. chabaudi chabaudi*, but this needs to be established in more physiological conditions.

Accordingly, this work aims at investigating the “innate response” of splenic DC to blood stages of the parasite, and to establish the molecular basis of DC activation.

Accordingly, we conduct in vivo studies of splenic DC (characterized by the differential expression of surface markers and production of cytokines) during the malaria infection, and study DC responses in mutant mice carrying selective defects at each of the known TLRs. The first results show that TLR-4 is not essential for malaria innate immunity.

CD4+CD25+ T cells facilitate murine infection by *P. berghei*

Member: António Coutinho and Elsa Seixas

Student: Dominique Ostler

It has been recently demonstrated at the IGC that regulatory T cells, in addition to controlling inflammation, autoimmune diseases and allergy, reduce protective responses and the elimination of microorganisms in naturally infected animals (*see 2001 Annual Report*). We have now investigated the influence of regulatory T cells in the course of *P. berghei* infection in mice, and found that such cells facilitate infection. Thus, preferential elimination of regulatory T cells in BALB/c mice, by treatment with cyclophosphamide, results in significantly lower parasitemias that are reconstituted by the transfer of CD4+CD25+ (but not of CD4+CD25-) cells. Similar results were obtained in C57BL/6 mice. Furthermore, alymphoid (Rag-1 -/-) mice show significantly higher parasitemias if reconstituted with CD4+CD25+ cells, as compared to animals receiving CD4+CD25- T cells.

The molecular and cellular mechanisms underlying such facilitation of *P. berghei* infection by CD4+CD25+ T cells are currently under investigation. We hypothesise that they might owe to the production by the parasite of “nonspecific” mediators on “innate immunity”.

Tuberculosis transmission and control

Member: Gabriela Gomes

Student: Ana Franco (PGDB)

Collaborators: Manuel do Carmo Gomes, Faculty of Sciences, Univ. Lisbon, Portugal and Graham Medley, Department of Biological Sciences, University of Warwick, UK.

The efficacy of Bacille Calmette-Guérin (BCG) vaccination against tuberculosis (TB) has been estimated to vary between 0% and 80%, generating great controversy about its usefulness. The reason for such variability is the subject of much ongoing debate. Whether to use BCG in mass vaccination programmes is a question that public health policy makers currently face. We observe that BCG efficacy is inversely related to TB prevalence and propose a hypothesis for these trends. Our hypothesis is based on two postulates: (1) the potential for transmission varies between populations, due to differences in socioeconomic factors, for example; (2) previous exposure to mycobacteria

induces an immune response that reduces the risk of reinfection, and cannot be significantly improved by BCG vaccination. We developed mathematical models to demonstrate that these two postulates produce variable trends consistent with observations. Not only variabilities in the prevalence of TB and the outcome of vaccination programmes are retrieved, but also the efficacy of vaccination appears lower at high prevalence. The development of a vaccine more potent than natural immunity is crucial to the success of global TB control by vaccination.

To supersede the immune protection induced by natural infection is a major challenge in vaccine development, and the aim of several important research groups. Preliminary studies are promising but, before a better vaccine is available, we should do the best with existing means. Improved detection and treatment of active TB infection is the most immediate measure, and this is highly stressed by the World Health Organization (WHO). Increasing emphasis is being placed on the extension of treatment to latent TB infection. This represents a substantial shift in the approach to tuberculosis control, and so far it is restricted to persons in high risk groups. We developed mathematical models to investigate the potential impact of various implementations of treatment of latent TB. As in the case of vaccine efficacy, the models predict major variabilities for the impact of community-wide treatment of latent TB.

Modelling the efficacy of immune protection

Member: Gabriela Gomes

Collaborators: Graham Medley, Department of Biological Sciences, University of Warwick, UK; Lisa White, Department of Biological Sciences, University of Warwick, UK; Carlota Rebelo and Alessandro Margheri CMAF, Univ. Lisbon, Portugal.

We are all familiar with the image of a child with a rash or spots accompanied by a fever that lasts a few days. The child usually recovers and is then immune for life. Most of the so-called childhood infectious diseases (for example, measles, mumps and rubella - MMR) are now preventable by vaccination. Mathematical epidemiological models for the dynamics of such infectious diseases have been extensively developed and used as predictive tools to assist in the design of control programmes.

Childhood infectious diseases are unusual in generating such effective immunity. More common is the occurrence of several reinfections throughout life. In simple terms, susceptibility to reinfection is attributed to a combination of two factors: (1) immune protection may wane over time (temporary immunity); (2) immunity may not be fully protective (partial immunity). Each of these factors is determined by a complex of mechanisms that different infections combine in different degrees. Loss of protection over time may be due to decay in specific immune effectors, or due to antigenic changes in the pathogen to prevent immune recognition (human influenza A). Lack of full protection may be due to a general lack of efficacy in responding to the pathogen (tuberculosis, malaria), or due to antigenic diversity of the pathogen population (influenza B, respiratory syncytial virus, malaria). Rather than making the exact mechanisms explicit, we construct a series of simple models to investigate the

epidemiological consequences of varying the duration and degree of immune protection. The models are systematically analysed and reveal two important outcomes: the duration of immunity has a crucial impact on potential inter-epidemic periods; the degree of immune protection determines a new transmission threshold associated with a steep increase in the prevalence of infection. Furthermore, vaccination programmes are unlikely to be successful if transmissibility is above this threshold. More detailed models were developed for the particular case of two infectious diseases: tuberculosis and respiratory syncytial virus.

Viral evolution and epidemiology

Members: Gabriela Gomes, Jorge Carneiro, Francisco Dionísio, Jose Faro and Isabel Gordo.

Student: José Nuno Martins

Viruses evolved a variety of ways to circumvent immunity. Here we are particularly interested in the ability to escape recognition by changing appearance (influenza viruses, respiratory syncytial virus). A major challenge is to relate such elaborate host-pathogen interactions with observations of infection prevalence at the population level. Mathematical and computer models for the dynamics of viral mutation, infection, immunity and transmission are invaluable tools to make the correspondence. The aim of this project is to develop such tools, and use them to investigate mechanisms of viral evolution, and infer the consequences for the epidemiology and control.

Our primary focus is on influenza viruses. There are three types of influenza: A and B cause annual winter epidemics of respiratory disease, and C causes only minor illness. Many subtypes of influenza A are found in wild aquatic birds, but only few have crossed to humans. The appearance of a new subtype in humans is called antigenic shift, and is associated with a pandemic (worldwide epidemic) as human hosts have no acquired immunity. As the new subtype infects people, it begins to drift under immune selection. Influenza B viruses are structurally very similar and cause a disease like influenza A, but evolve more slowly revealing no clear antigenic drift. The pattern of variation is very erratic and extensive antigenic differences can be found within a single epidemic. The reason for such different evolutionary patterns is the puzzle that we are addressing. We sketched a computer model that will be implemented next year.

Genetics, disease and biology

Members: John Stewart and Constantin Fesl

Student: Marta Barreto

Collaborator: Florence Demenais INSERM EMI , Genopole, Evry, France

The general aim is to reactivate the original intention behind the Elston-Stewart algorithm: i.e. physiological characterisation of the effects of individual loci underlying

quantitative variation. The specific aim is the estimation of allele frequency and epistasis in multifactorial genetic diseases. Methods: In a general genetic model, the probability of disease is a sigmoid function of the number of disease alleles summed over all loci. This model has just 4 parameters: the number of loci; the population frequency of disease alleles; a threshold expressed as a proportion of disease alleles; and the slope of the sigmoid curve. Assuming 10 loci, the remaining parameters can be estimated from empirical data: population frequency of the disease, monozygotic twin concordance rates, and disease frequency in sibs of affected probands. Results: For 10 typical multifactorial diseases, the estimates of allele frequency are generally high, of the order of 20%, with strong epistatic interactions between loci. It follows that the frequencies of subphenotypes specific for a single disease locus will also be high, and only about two-fold greater in affected individuals than in normal controls. Conclusions: Because of allelic heterogeneity, purely genomic approaches are unlikely to succeed in unravelling the genetics of multifactorial diseases; this will rather require articulation with physiology and the identification of biologically meaningful subphenotypes.

Genetic structure of the Azorean population

Member: Luisa Mota-Vieira

Students: Cláudia C. Branco and Paula P. Pacheco

Collaborator: Ana Luisa Araújo, MD (Serviço de Hematologia) Hospital of Divino Espírito Santo

Isolated populations often constitute good model for genetic studies. One of the important characteristics of these populations is their reduced genetic heterogeneity. To obtain a better understanding of the genetic structure of the Azorean population we conducted a survey based on the frequencies of surnames listed in the 2001 telephone book. We tested the models first to São Miguel island and then we conducted the survey for the whole archipelago. We calculated the following parameters: isonymy (I), coefficient of inbreeding (F_{st}), abundance of surnames according to Fisher (α), Karlin-McGregor's migration rate (v) and Nei's distance. The data reveals a migration phenomenon, which occurs mainly towards the big islands. The isonymy similarity assessed by the dendrogram of Nei's distance revealed three major clusters corresponding to the geographic distribution of the nine islands. Also, the data suggest that Graciosa, the second smallest island, is genetically more isolated when compared to the other islands. Moreover, the diversity analysis of surnames for all islands demonstrates that 11% of all 57,385 subscribers are distributed by only 3 surnames. Finally, the value of F_{st} obtained for this population (0.0039) indicates little genetic differentiation (Wright's $F_{st} < 0.05$) and relative homogeneity. Ongoing work in our group is to analyze the genetic variability of the São Miguel population using amplification of polymorphic markers. For this, we are collecting DNA samples from 1000 anonymous healthy individuals with the agreement of the HDES Ethical Committee. Haplotype and phylogenetic analysis based on microsatellite and sequence data will be performed. The knowledge of the genetic

structure of the Azorean population will be very useful for mapping both genes underlying susceptibility to complex diseases and genes causing Mendelian disorders.

Genetic epidemiology of autism spectrum disorders

Member: Astrid Vicente and Constantin Fesel.

Students: Ana Margarida Coutinho, Susana Correia da Silva, Susana Antunes da Silva and Catarina Correia

Collaborators: Guiomar Oliveira, Hospital Pediátrico de Coimbra; Teresa Morgadinho, Dept. Farmacologia, FMUC, Luísa Mota Vieira, Hospital do Divino Espírito Santo, S. Miguel, Açores, Patricia Maciel, IBMC, Autism Genetics Cooperative.

Genetic Epidemiology of Autismo in Portugal: The objectives for this project have been fully achieved during 2002, with the determination of the prevalence and other epidemiological parameters for autism in Portugal and the Azores, and the recruitment of patients and their relatives for genetic research. Our present database and sample collection includes 212 nuclear families with one or more autistic patients and extensive clinical, behavioral, genealogical and biochemical information on patients and relatives. In this sample we have been analysing the association of candidate genes of the serotonergic system, and others, with autism and with associated quantitative endophenotypes, as well as exploring the hypothesis of the involvement of autoimmune mechanisms in the disease pathogenesis. We have joined an international consortium for research on the genetics of autism, the *Autism Genetics Cooperative*, funded by National Alliance for Autism Research (NAAR). This consortium proposes to conduct a genome wide association scan for autism in a sample population of unprecedented size. This collaborative effort will undoubtedly yield crucial information for the understanding of autism etiology, while opening opportunities for follow up research by the associate groups on the pathological mechanisms associated with autism symptoms and deficits.

Pharmacogenetics of risperidone therapy in autism spectrum disorders. Project developed at the IGC (PI Astride Vicente), in collaboration with the HPC. Submitted to Fundação para a Ciência e Tecnologia, funding pending. We are investigating the role of selected candidate genes in the variability of response, in efficacy/side effects, to specific medication for autism, aiming at the prediction of individual response based on specific genotypic and phenotypic information, with a major impact on therapeutic decisions in clinical settings.

Autism and Mitochondrial Disorder – microarray analysis of the expression profile of nuclear genes for mitochondrial enzymes in autism. We have found an unexpectedly high rate of mitochondrial disorder associated with autism in our sample (17% initially suspected cases, with 11/205 already confirmed and 24 under evaluation); no mtDNA mutations were detected; we have initiated the analysis of nuclear genes encoding mitochondrial-related proteins.

Clinical, epidemiological and genetic study of Rett Syndrome in Portugal. We have established a collaboration in a project developed at the Instituto Biologia Molecular e

Celular (PI Dr. P. Maciel), funded by Fundação para a Ciência e Tecnologia, to determine the prevalence of *MECP2* gene mutations in autism.

Genetics of human systemic lupus erythematosus (SLE)

Members: Astrid Vicente, Constantin Fesel, Jocelyne Demengeot, Werner Haas, John Stewart and Jorge Carneiro

Students: Francisca Fontes, Marta Barreto and Ricardo Ferreira

Collaborators: Carlos Ferreira, Associação dos Doentes com Lupus Carlos Vasconcelos, Associação dos Doentes com Lupus; Berta Martins, Instituto de Ciências Médicas Abel Salazar; Luísa Mota Vieira, Hospital do Divino Espírito Santo, S. Miguel, Açores.

SLE is a multifactorial disorder with heterogeneous presentation, in which genetic susceptibility plays a major role. The main objective of this study is the identification and characterization of genetic susceptibility factors for SLE. The strategy used is the identification of lupus-associated traits that are genetically less complex, and therefore more amenable to genetic mapping. The collection of SLE patients and family members has progressed throughout 2002, in collaboration with the Associação de Doentes com Lupus. Presently, 35 multigenerational families have already been collected, including 51 patients, 149 unaffected relatives, and 52 unrelated patients. Identification of familial cases is progressing in the Azorian islands, with collection initiation scheduled for beginning of 2003. A database has been established, gathering clinical and serological information as well as disease-associated phenotype and genetic data. Given that antinuclear antibody (ANA) production is a main characteristic of SLE, we are focusing on the analysis of autoantibody reactivities in patients and relatives. For this purpose, and given the systematic presence of ANA in patients, we have analyzed serum IgG antibody repertoires against a HEp2 nuclear extract in a sample of 16 multiplex families, including 26 patients and 72 unaffected relatives, and 46 adult healthy controls. A quantitative immunoblotting procedure and multiparametric analysis show a distinct pattern of autoantibody reactivities in patients, compared with controls ($p=0.0092$ for principal component analysis (PCA) factor 1 and $p=0.042$ for PCA factor2), with two major antigenic bands contributing for these PCA factors. Correlation coefficients for relative pairs were established for PCA factor 1 ($\rho_{M/F}=-0.419$, $\rho_{M/D}=0.348$, $\rho_{F/S}=0.428$, $\rho_{MA/N}=0.532$) and for PCA factor 2 ($\rho_{M/F}=0.127$, $\rho_{M/D}=0.128$, $\rho_{F/S}=0.653$, $\rho_{MA/N}=0.331$), indicating the presence of a genetic component for the autoantibody reactivity patterns in these SLE multiplex families. Heritability was estimated at 6% for PCA factor 1 and 21% for PCA factor 2. The present results confirm the specificity of ANA in SLE patients. Most important, the multiparametric analysis uncovered shared autoantibody reactivity patterns among relatives, indicating that this may constitute a genetic trait appropriate for mapping. Detailed segregation analysis of the patterns of autoantibody reactivities in a larger sample is required, paving the way for genetic mapping of this SLE associated phenotype. The epitope specificities of the antinuclear antibodies in patients and unaffected relatives and controls are also being analysed, and preliminary results indicate that known ANA are inherited among affected and unaffected family members.

Genetics of infections in wild mouse models

Member: Carlos Penha Gonçalves, Pierre-Andre Cazenave and Dan Holmberg

Student: Joana d'Avila

This project aims to identify genetic factors that confer resistance to clinical forms of various infections in mouse models. Unraveling the identification of such genetic factors controlling resistance will provide important contribution to the understanding of pathogenesis and will suggest therapeutic and vaccine strategies to improve resistance to disease.

The work-plan comprises (1) the genetic mapping of resistance loci using genetic crosses of mouse strains that are resistant to malaria and (2) the isolation of the genetic factors involved combining a candidate gene and a positional cloning approach.

This project takes advantage of unique wild mouse strains that, unlike most laboratory strains, show to be resistant to different infections. There are 13 wild mouse inbred strains available to the project. These mouse strains constitute a unique genetic resource, as they are likely to carry allelic variants that are not represented in the common mouse laboratory strains. Indeed, these wild mouse strains were shown to be different from the laboratory strains in respect to a number of lymphocyte properties and in the immunological response to pathogens. This included malaria infection to which at least two wild strains were demonstrated to be resistant. This project initiated the genetic analysis of these wild strains at the genomic level. A genome-wide microsatellite map, which is a requirement for the studies here proposed, was constructed and will be most useful for future genetic studies involving these mouse strains.

Genetics of type 1 diabetes

Members: Dan Holmberg and Carlos Penha Goncalves

Students: Miguel Monteiro, Marie-Louise Bergman, Nadia Duarte and Susana Campino

Type 1 diabetes is a multifactorial and polygenic disease. To date, few etiological mutations have been definitely identified as contributors to T1D pathogenesis. This project aims at identifying and genetically map subphenotypes associated with development of type 1 diabetes in humans as well as in the Non-Obese Diabetic (NOD) mouse model for type 1 diabetes. The present project builds upon previous studies in which we have been able to partly dissect the NOD-specific trait of thymocyte resistance to glucocorticoid-induced apoptosis and dysfunctional control of thymocyte proliferation. NOD mouse strains congenic over a region on Chr 6 controlling these traits have also been established and will now be used for the studies proposed here. Further analysis of the other potential NOD T1D sub-phenotypes including resistance to radiation- and chemical induction of lymphocyte apoptosis has also included the analysis of the corresponding NOD congenic mice. The continuation of this project will include the use

of the available congenic strains for sub-phenotype-based, microarray analysis of gene expression to identify candidate genes.

Control of African swine fever (ASF) through improved diagnosis

Member: R.M.E. Parkhouse

Student: Ana Luísa Reis

Collaborator: Alexandre Leitão (Laboratório de Doenças Infecciosas, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa)

African swine fever is one of the most important diseases of pigs. Its control relies solely on efficient diagnosis and application of strict sanitary measures. An effective vaccine has never been obtained. ASFV infects domestic pigs and *Ornithodoros sp.* ticks (shown as vectors in Iberian Peninsula before the disease was eradicated). The danger of ASF re-emergence and/or new introduction is a major concern for the EU, due to the lack of knowledge of mechanisms of viral persistence in pigs and ticks and because several European countries have close contacts with ASFV-endemic African countries. The rationale of this project is to improve diagnostic methods to enable rapid implementation of prophylactic measures.

Our previous work has identified the 12 principle serological determinants of ASFV and the principal aim of this project is to express these viral proteins and assess their potential value as serological diagnostic probes using sera from infected pigs previously obtained. To date, the 12 viral genes have been subcloned into the expression vector and their purification is underway.

Control of human, bovine and porcine cysticercosis through vaccination and improved diagnosis

Member: R.M.E. Parkhouse

Collaborators: Dr. T. Garate (Instituto de Salud Carlos III, Centro Nacional de Microbiología, Madrid, Spain), Dr. L. Harrison (University of Edinburgh, Department of Tropical Animal Health, Centre for Tropical Veterinary Medicine, Scotland), Dr. E. Sciutto (Universidad Nacional Autónoma de México, Instituto de Investigaciones Biomédicas, México), Dr. M. Cortez (Universidad de Carabobo, Venezuela) and Dr. H. García, Universidad Peruana Cayetano Heredia, Lima, Peru)

The zoonotic tapeworm *Taenia solium*, causal agent of life threatening human neurocysticercosis, constitutes an increasingly major health risk. The adult, or tapeworm stage, lives in the intestine of man, whilst the intermediate metacestode stage, responsible for cysticercosis, may occur both on pig and man. The related parasite, *Taenia saginata*, similarly infects man as an intestinal tapeworm but passes its metacestode stage only in cattle. Rural transmission is mediated by poor sanitation and uncontrolled pig and cow management practices, and so the prevalence of these parasites is an objective indicator

of rural poverty. Recently, population movement linked to close human/pig and cow contact in the rural-urban interface has exacerbated the problem. Control through improved sanitation is a major, long-term and expensive goal. This project focuses on the shorter-term, more cost-effective strategies of improving pig and cow management, including village pig vaccination (transmission control) and the development of sensitive and specific diagnostic assays to detect parasites and anti-parasite antibodies; the latter based on synthetic peptides, recombinant reagents and PCR, not parasite material. New diagnostic assays will improve hospital patient monitoring/treatment and man/pig screening and hence epidemiological knowledge.

To date, we have succeeded in developing the following diagnostic tests: 1) PCR tests for the differential diagnosis of cestode parasites (*Taenia solium*, *Taenia saginata* and *Echinococcus*); 2) Synthetic peptide based assays to detect antibodies to *Taenia* parasites and 3) An ELISA assay which detects secreted metacestode antigens and thus viable metacestode parasites in pigs, cattle and man. These are all now being applied in endemic areas, principally Mexico, Peru, Bolivia and Venezuela, and, on occasions, clinical material in Spain.

Finally, we have developed a potential vaccine for bovine cysticercosis, based on a recombinant oncospherical surface and secreted molecule which, interesting is functionally, an adhesion molecule, possibly facilitating tissue invasion by the parasite in the intermediate host.

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SEMINARS AT THE IGC

January

Marta de Menezes-Graça (Imperial College, London, UK)

The laboratory as an art studio - collaborations between scientists and artists.

Thomas Shenk (Dept. Molecular Biology, Princeton University, USA)

Genetic and genomic studies of human cytomegalovirus.

Louis du Pasquier (Basel Institute for Immunology, Basel, Switzerland)

Polyploidy and the immune system.

Maria Curotto de Lafaille (Skirball Institute of Biomolecular Medicine, University Medical Center, NY, USA)

Regulation of IgE production in vivo by inhibition of T helper 2 cell differentiation.

Michel Bornensn (Institut Curie, Paris, France)

What is the role of the centrosome in animal cells.

Antonio Coutinho (IGC, Oeiras, Portugal)

Polyclonal lymphocyte responses: an alternative to malaria vaccination ?

Robin Weiss (Wohl Virion Centre, Windeyer Institute of Medical Sciences, UCL, London, UK)

AIDS associated Cancer.

Stan Marée (Dep. Mathematics, The Univ. of British Columbia, Vancouver, Canada)

From pattern formation to morphogenesis: multicellular coordination in the cellular slime mould.

Miguel Soares (Lab. Associado/ IGC, Oeiras, Portugal)

Heme oxygenase-1: a stress responsive gene that regulates inflammatory reactions through the generation carbon.

Maria Mota (IGC, Oeiras, Portugal)

How can the host affect malaria infections?

Tiago Magalhães (UCSF, Berkley, USA)

Genetic control of axonal guidance.

February

Leonor Oliveira (ITQB, Oeiras, Portugal)

Post-transcriptional regulation of intracellular iron metabolism in mammalian cells: role of nitric oxide and iron regulatory proteins.

John Stewart (Univ. Compiègne/CNRS/IGC, Oeiras, Portugal)

Genetic analysis of multifactorial diseases.

Arnold Levine (President of the Rockefeller Univ., Head of the Robert and Harriet Heilbrunn Laboratory of Cancer Biology, New York, USA)

The regulation of p53 mediated apoptosis.

George Dimopoulos (Centre for Molecular Microbiology & Infection, Imperial College of Science, Technology and Medicine, London, UK)

Dissecting mosquito refractoriness to malaria on a microarray.

Leonor Parreira (CEBIP, FMUL/ IGC, Oeiras, Portugal)

The 3rd and 4th dimensions of the genome in normal and malignant cell differentiation.

Maria Mota (IGC, Oeiras, Portugal)

How Plasmodium-induced host factors are able to influence a malaria infection?

Jorge Carneiro (IGC, Oeiras, Portugal)

How does the body count its lymphocytes?

March

António Jacinto (IGC, Oeiras, Portugal)

Living on the edge of dorsal closure.

Tom Maciag (Director, Center for Molecular Medicine, Maine Medical Center Research Institute, USA)

The molecular mechanisms of angiogenesis.

David Ish-Horowicz (ICRF, London, UK)

Specificity and mechanisms of RNA transport in drosophila oogenesis and embryogenesis.

Juan Carlos Izpisua-Belmonte (The Salk Institute, La Jolla, USA/IGC, Oeiras, Portugal)

Molecular basis of left-right asymmetry.

M. Angela Nieto (Instituto Cajal, Madrid, Spain)

Ancestral and derived functions of the snail gene family: from embryonic development to tumour progression.

Antonio Simeone (MRC, Kings College, London, UK)

Regulatory control and role of Otx2 in brain morphogenesis.

Martin Raff (UCL, London, UK)

Size control in animal development.

Alfonso Martinez Arias (Dep. Genetics, Univ. Cambridge, Cambridge, UK)

Notch and signal integration during the assignation of cell.

Maya Kestermann (Quiagen, Hilden, Germany)

Quiagen innovations in molecular diagnostics & biomedical research.

Ivan Matic (INSERM, Necker Institute, Paris, France)

Horizontal transfer of mismatch repair genes and the variable speed of bacterial evolution.

Yannis Michalakis (CNRS: Centre d'Etudes sur le Polymorphisme des Microorganismes, Equipe: Evolution Theorique et Experimentale, Montpellier, France)

Biotic interactions and life-history trait evolution in mosquitoes.

Astrid Moura Vicente (IGC, Oeiras, Portugal)

Autism spectrum disorders - phenotypes and genotypes.

Marie-Aimée Teillet (Institut d'Embryologie Cellulaire et Moleculaire du CNRS et du College de France, France)

Floor plate heterogeneity, dual origin and cellular interactions. experimental study in the avian embryo.

April

Gonçalo Abecassis (Univ. Michigan, Michigan, USA)

Methods for association mapping of quantitative traits in human pedigrees.

Patricia Maciel and Teresa Temudo (IBMC, Porto, Portugal)

Rett Syndrome: clinical presentation and genotype-phenotype correlation.

Solveig Thorsteinsdottir (FCUL/IGC, Oeiras, Portugal)

From somites to muscle: role of cell-matrix communication.

Fernando Antunes (Dep. Chemistry and Biochemistry, FCUL, Lisbon)
Cellular titration of apoptosis with steady-state concentrations of H₂O₂.

Gabriela Gomes (IGC, Oeiras, Portugal)
Infection and reinfection dynamics of tuberculosis and the impact of integrated control programmes.

Silvia Giordano (Inst. Cancer Research and Treatment, University of Torino Medical School, Italy)
Scatter factor and semaphorin receptors control invasive growth.

Michael Parkhouse (IGC, Oeiras, Portugal)
From field to laboratory, from laboratory to field.

Sukalyan Chatterjee (IGC, Oeiras, Portugal)
Mechanism of cell fate decisions.

May

Alfonso Fairen (Instituto de Neurociencias, Consejo Superior de Investigaciones Cientificas, San Juan de Alicante, Spain)
The cells at the surface of the cerebral cortex and their possible developmental roles.

José Feijó (IGC, Oeiras, Portugal)
Ion dynamics and the control of cell development: pursuing arrogant simplicities?

José Eduardo Gomes (Institute of Molecular Biology, Eugene, USA)
*Orientation of the mitotic spindle, asymmetric cell division and cell fate patterning in the *C. elegans* early embryo: role of the gene *spn-4*.*

Isabelle Llano (CNRS FRE 2199, Lab. Physiologie Cérébrale, UFR Biomédicale, Univ. Paris 5, Paris, France)
Confocal studies of intracellular calcium dynamics in axons of GABA-releasing interneurons.

John Owen (Univ. Birmingham, Birmingham, UK)
The role of the thymus in the development of the immune system: unresolved issues.

Alain Marty (CNRS FRE 2199, Lab Physiologie Cérébrale, UFR Biomédicale, Université Paris 5, Paris, France)
Retrograde signaling in the cerebellar cortex.

Álvaro Tavares (IGC, Oeiras, Portugal)
So many proteins, so little time...

Yoichi Taya (National Cancer Center Research Institute, Tokyo, Japan)
Regulation of the functions of p53 and the RB protein by phosphorylation.

Abdelhadi Saoudi (INSERM U 563, Autoimmunité et Immunorégulation, Centre de Physiopathologie Toulouse-Purpan, Hôpital Purpan, Toulouse, FRANCE)
Evidence that the balance between CD45R^{high} and CD45R^{low} T cell subsets play an important role in the development of pathological immune responses in rats.

Enzo Medico (Institute for Cancer Research and Treatment and Univ. Torino, Medical School, Turin, Italy)
Genomic dissection of the invasive growth genetic program triggered by hepatocyte growth factor.

Jocelyne Demengeot (IGC, Oeiras, Portugal)
Organization of the immune system: questions on diversity and community.

June

Isabel Gordo (IGC, Oeiras, Portugal)
On the lack of sex and the death of the Y chromosome.

Maria Rescigno (Dept. Experimental Oncology, European Institute of Oncology, Milano, Italy)
Dendritic cells at the host-pathogen interface.

Peter Jordan (Centro de Genética Humana, Instituto Nacional de Saúde, Dr. Ricardo Jorge, Lisbon, Portugal)
Alternative spliced Rac1 cannot be downregulated by Rho-GDI.

Marie-Louise Bergman (IGC, Oeiras, Portugal)
A sub-phenotype approach for the dissection of murine type 1 diabetes.

Manuela Rosado (Ospedale Pediatrico Bambino Gesù, Rome, Italy)
The absence of IgM memory B cells correlates with increase susceptibility to infection by encapsulated bacteria.

Luiz Stark (Instituto de Pesquisas Energéticas e Nucleares /CNEN, São Paulo Brazil.)
The role of anti-TCR antibodies on regulation of encephalitogenic T cells.

Joaquin Rodriguez Leon (IGC, Oeiras, Portugal)
MAP kinase phosphatase 3, a modulator of MAP kinase pathway, is involved in limb development.

Ruy Ribeiro (Los Alamos National Laboratory, USA)

Modeling lymphocyte activation, proliferation, and death in HIV-1 infection.

Max Cooper (Howard Hughes Medical Institute, The University of Alabama at Birmingham, USA)

When will we understand B cell differentiation?

Sylviane Pied (Institut Pasteur, Paris, France/IGC, Oeiras, Portugal)

T-cell responses in pathogenesis of malaria: from mice to the field.

July

François Huetz (Institut Pasteur, Paris, France)

Human B cells immunoscope for VH repertoire size determination.

Ana Domingos (The Rockefeller University, New York, USA)

Drosophila larva: a new model for olfaction.

Manuela Martins-Green (Dept. Cell Biology and Neuroscience, Univ. California, USA)

Looking beyond the functions of chemokines in host defense and inflammation.

Jose Faro (IGC, Oeiras, Portugal)

Germinal centers (GC) and the GC reaction: a combined theoretical/experimental approach.

Luís Silvestre (Focus Journal)

The workings of the media.

Nuno Crato (Expresso Newspaper)

Ten recipes for science journalists.

Luisa Figueiredo (Unite Biol. des Interactions Hôte-Parasite, Institut Pasteur, Paris, France)

Biology of chromosome ends in the human malaria parasite plasmodium falciparum.

Graham Medley (Univ. Warwick, Warwick, UK)

Hepatitis B endemicity: heterogeneity, catastrophic dynamics and control.

Alcino Silva (Brain Research Institute, Univ. California, LA, USA)

Molecular and cellular mechanisms of cognitive function.

Roberto Motterlini (Vascular Biology Unit, UCL, London, UK)

Carbon monoxide for therapy.

Helena Soares (IGC, Oeiras, Portugal)

The cytosolic chaperonin CCT- how many complexes and functions ?

Lisete Fernandes (IGC, Oeiras, Portugal)

Response(s) to cold and oxidative signals in yeast.

August

Arne Akbar (Dept. Clinical Immunology, The Royal Free Hospital, London, UK)

The study of human regulatory T cells in vivo.

Gabriela Gomes (IGC, Oeiras, Portugal) Martyn Parker (Univ. Watwick, UK) and Eliana Pinho (Univ. Porto, Portugal)

Blackeye patterns: symmetries, toys and pictures.

September

Moises Mallo (IGC, Oeiras, Portugal)

Hox genes, Hox codes and morphogenesis.

H Robson MacDonald (Ludwig Institute for Cancer Research, Univ. Lausanne, Switzerland)

Role of Notch-1 in T cell development and lineage commitment.

Marcelo Jacobs-Lorena (Case Western Reserve Univ., Dep. Genetics, Ohio, USA)

Anti-malarial mosquitoes ? transgenic anopheline mosquitoes impaired in transmission of a malaria parasite.

Thomas Jenuwein (Research Institute of Molecular Pathology, Vienna, Austria)

Translating the histone code.

Epigenetic control by histone methylation.

Geneviève Almouzni (CNRS UMR 218, Institut Curie, Paris, France)

Chromatin assembly and chromatin alteration.

Jacqueline Mermoud (X Inactivation Laboratory, MRC Clinical Sciences Centre ICSM, Hammersmith Hospital, London, UK)

Mechanisms of X inactivation.

Margarete Heck (The Wellcome Trust Centre for Cell Biology, Institute of cell and Molecular Biology, Univ. Edinburgh, Edinburgh, UK)

Using the fly to dissect chromosome organization and dynamics.

What mitotic chromosomes can tell us about DNA replication and cell cycle control.

Marie-Louise Bergman (IGC, Oeiras, Portugal)

A Sub-phenotype approach to dissect the genetic control of murine Type 1.

Rui Gonalo Martinho (NYU, New York, USA)

Drosophila germ cells: quiet cells in a noisy neighborhood.

Miguel Godinho Ferreira (Cancer Research, London, UK)

Living dangerously at the edge of the chromosome.

Leonor Boavida (IGC, Oeiras, Portugal)

Bees and birds do it...Plants do it... A case of cell communication.

Manuel Gomes (FCUL, Lisbon, Portugal)

Towards the eradication of childhood diseases with multiple dose vaccination.

October

Pedro Coutinho (IGC, Oeiras, Portugal)

Snow white and the seven dwarfs: The molecular characterisation of sneezy, dopey and happy.

Richard White (School of Biological Sciences, Univ. Southampton, Southampton, UK)

Linking biodiversity databases.

Tasuku Honjo (Faculty of Medicine, Kyoto University, Kyoto, Japan)

PD-1, a negative regulator involved in peripheral tolerance.

Gabriela Gomes (IGC, Oeiras, Portugal)

Population dynamics of multi-strain pathogens.

Helder Maiato (IBMC, Porto, Portugal)

How kinetochores attach to dynamic microtubules.

Kalet Leon (IGC, Oeiras, Portugal)

A quantitative approach to dominant tolerance.

Maria Marone (IGC, Oeiras, Portugal)

TGF-beta signalling in hematopoietic progenitors.

Wagner Gouvea dos Santos (Medical College of Virginia/Virginia Commonwealth University, USA)

The use of gene therapy as an approach for the treatment of brain tumors.

Thomas Olegschlager (Marie-Curie Research Institute, Surrey, UK)
Mitotic bookmarking of active genes by promoter bound TF II D.

Patrick Varga Weiss (Marie-Curie Research Institute, Surrey, UK)
Chromatin remodelling factors + heterochromatin replication.

Agustin Lage (Centre for Molecular Immunology, Havana, Cuba)
Cancer immunotherapy and autoimmunity.

Jessica Kissinger (Center for Tropical & Emerging Global Diseases & Dep.Genetics, Univ. Georgia, USA)
A computational approach to organellar function - mining parasite genomes for nuclear-encoded apicoplast genes.

Philip Ashton-Rickardt (Gwen Knapp Center for Lupus and Immunology Research, Univ. Chicago, USA)
Molecular differentiation of memory CD8 cells.

Ken-ichi Katsube (Graduate School of Tokyo Medical and Dental Univ., Tokyo, Japan)
A novel viewpoint of notch signaling-toward understanding the mechanism of stem cells.

José António Belo (IGC, Oeiras, Portugal)
Heads, hearts, handedness... genomic approaches to the role of the cerberus-like gene family in vertebrate development.

José Mengel (Fundação Osvaldo da Cruz, Brazil)
NK T cells as helper cells.

November

Buzz Baum (Ludwig Institute for Cancer Research, London, UK)
Investigating cellular space - a functional genomic approach.

Giampietro Schiavo (NeuroPathoBiology Laboratory, Cancer Research UK London Research Institute, UK)
Fast retrograde transport in motor neurons: an essential process for neuronal survival molecular.

Carl Philipp Heisenberg (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany)
The role of Wnt signalling in cell polarization and directed cell migration during zebrafish gastrulation.

Jorge Kalil (Univ. S. Paulo, S. Paulo, Brazil)

From throat infection to rheumatic heart disease: antigenic recognition and molecular targets.

Harald Herrman (Dep. Cell Biology, German Cancer Research Center, Heidelberg, Germany)

Molecular mechanisms underlying intermediate filament assembly.

Luís Rocha (Los Alamos National Laboratory, USA/IST and IGC, Oeiras, Portugal)

Computational biology.

Eva Caamitjana-Martinez (Dep. Developmental Genetics, University of Utrecht, The Netherlands)

Control of cell division/cell differentiation in the arabidopsis root meristem.

Iain Hagan (Univ. Manchester, Manchester, UK)

An important role for localised events on the spindle pole in regulating commitment to mitosis.

Marie-France Sagot and Raquel Tavares (Logiciels et Banques de Donnees, Institut Pasteur, Paris, France)

Combinatorial algorithms and molecular biology.

Gene expression and repeated sequences: could "junk" mean "regional regulation"?

Jonathan Pines (Univ. Cambridge, Cambridge, UK)

Getting in and out of mitosis.

Carl Smythe (Univ. Sheffield, Sheffield, UK)

Intra S-phase checkpoints.

Claudio Sunkel (IBMC, Porto, Portugal)

Genetic analysis of mitotic functions in drosophila.

December

Bruce Bowerman (Institute of Molecular Biology, Univ. Oregon, USA)

Cytokinesis and cytoskeletal polarity in the early embryo.

Arnoldo Façanha (Univ. Norte Fluminense, Rio Janeiro, Brazil)

Bioenergetic aspects of the plant stress: the role of proton pumps.

Mike Jones (Chester Beatty Laboratories, Institute of Cancer Research, London, UK)

Mechanisms of patterning the xenopus gastrula.

Ana Teresa Tavares (IGC, Oeiras, Portugal)

Transcriptional regulation of caronte during embryonic development.

Francois Schweisguth (Ecole Normale Supérieure, Paris, France)

Cell polarity and asymmetric cell divisions in the drosophila PNS.

Alan Wainman (IGC, Oeiras, Portugal)

Adventures with the drosophila Mob1 homologues.

Maria Soares (Dep. Clinical Immunology, Royal Free and University College Medical School, London, UK)

The regulation of virus specific CD8⁺ T cells by apoptosis and replicative senescence.

SYMPOSIA, CONFERENCES AND MEETINGS ORGANISED BY THE IGC

January

BioCASE – First Meeting of the BioCASE Project
Instituto Gulbenkian de Ciência
25-27 January 2002

Organisers: Pedro Fernandes and Ana Brandão (*IGC, Oeiras, Portugal*)

The first meeting of the BioCASE consortium has held at the IGC in January. The meeting brought to Oeiras a total of 50 specialists in Biological Collection Databases, country representatives and project administrative staff. The meeting was used to enable contact between consortium members, define the project schedules in fine detail and establish technical strategies for reaching the milestones in time.

April

EMBO Practical Course
Gulbenkian Biology Course
Plant Development: Molecular and Cellular Basis
Instituto Gulbenkian de Ciência
3-19 April 2002

With the sequencing of several flowering plant genomes near completion, molecular and genetic tools are becoming available for integrative organism-centred approaches. The time has come for putting the organism together again and this will requires close monitoring of in vivo properties and follow-up of the extended processes that each gene or group of genes controls.

The course aims at providing the basis for post-genomic work, with a solid theoretical background and practical training on current and novel molecular tools and approaches, fostering interdisciplinary interfaces with biophysical and imaging techniques, all embodied in a strong sense of integrative biology.

Course format: 60 hours of theoretical sessions (lectures, seminars and discussions) and 60-90 hours of laboratory work, divided in 3 packs: "getting started", advanced techniques, and free-running projects. Globally, advanced training will be offered in nucleic acids extraction and characterisation, plant transformation methods, functional analysis of expression, gene cloning, cDNA library screening, database screening and primer design; Arabidopsis mutant screening, mutagenesis and gene tagging; visual probe design and imaging of GFP and GFP-related proteins; electrophysiology and DNA microchips. The course includes a workshop on imaging, with hands-on practice on ratiometric widefield, confocal and multi-photon microscopies, (with suport from the

leading imaging companies in the world) and advanced image analysis (with support from Universal Imaging Co.).

Organisers: **José Feijó** (IGC/FCUL, Portugal) and **Margarida Oliveira**, (IBET/UL, Portugal)

Speakers: **Cathie Martin** (John Innes RC, UK), **Colin Brownlee** (Mar.Biol.Lab, Plymouth, UK), **Dan Cosgrove** (U.Pennsylvania, USA), **Fernando Catarino** (Univ.Lisboa and Lisbon Botanical Garden, Portugal), **Gerd Jurgens** (Univ.Tuebingen, Germany), **Ioan Negrutiu** (ENS Lyon, France), **Jim Haseloff** (Cambridge Univ., UK), **Keith Roberts** (John Innes RC, UK), **Sheila McCormick** (U.California, Berkeley, USA), **Sydney Shaw** (U.Stanford, USA), **Ton Bisseling** (U.Wagenigen, Netherlands), **Tony Trewavas** (Univ. Edinburgh, UK), **Ueli Grossniklaus** (ETH, Zurich, Switzerland), **William Lucas** (Univ. California, Davis, USA).

May

IGC Seminars to the Scientific Advisory Board
Instituto Gulbenkian de Ciência
9 May 2002

Jan Andersson

Rearrangements at the κ L-chain locus during B cell development. Is there evidence for antigen induced editing of B cell receptor specificity?

Henrique Teotónio

Experimental evolution and the genetic mapping of adaptation.

Élio Sucena

Dissecting the mechanistic basis for morphological evolution.

Sérgio Dias

Tumor angiogenesis: cellular and molecular mechanisms.

Domingos Henrique

Cell Polarity and Cell Fate in the developing chick CNS.

Conferences of the IGC Scientific Advisory Board
Instituto Gulbenkian de Ciência
9 May 2002

Nicole Le Douarin (Academie des Sciences / College de France, France)

Interactions between Hox-negative cephalic neural crest cells and the foregut endoderm in patterning the facial skeleton in the vertebrate head

Lewis Wolpert (University College of London, London, UK)

Is science dangerous?

Sydney Brenner (The Salk Institute, USA)

What still remains to be done on the human genome

July

PROCURA Inaugural Session

Instituto Gulbenkian de Ciência

2 July 2002

Organisers: Pedro Fernandes (IGC, Oeiras, Portugal) and Deborah Penque (INSRJ, Lisbon, Portugal)

The launching of the Portuguese Proteomics Network, PROCURA, was officially held at the IGC. An open day of conferences about Proteomics was organized, that brought us four international experts and allowed for some Portuguese researchers to reveal their efforts in establishing Proteomics activities:

Stephen Pennington, Univ. Liverpool, Liverpool, UK

*Applications of Proteomics to Biomedical Sciences.***Holger Husi, Univ. Edinburgh, Edinburgh, UK**

From Neuroproteomics to a Protein-Protein Interaction Database.

Pedro Fernandes, IGC, Oeiras, Portugal

The Portuguese Proteomics database.

Deborah Penque, INSERJ, Lisboa, Portugal

ProCura - Portuguese Proteomics Network: Who we are.

Francisco Amado, Univ. Aveiro, Aveiro, Portugal

*Proteomics in Cystic Fibrosis.***Luisa Romão, UNL, Monte da Caparica, Portugal**

Non-sense Mediated Decay of Human Globin mRNA by Proteomics.

Adriano Henriques, ITQB/UNL, Oeiras, Portugal

Structural Proteomics of the Bacillus Subtilis Endospore Coat Organelle.

Samir Hanash, Univ. Chicago, Chicago, IL, USA and President of the Human Proteome Organization - HUPO.

The Human Proteome Organization.

September

Workshop - Chromatin Dynamics and Gene Expression

Instituto Gulbenkian de Ciência

12-13 September 2002

Organisers: João Ferreira (Faculdade de Medicina de Lisboa, Portugal) and Leonor Parreira (FML/IGC, Portugal)

The traditional view that the regulation of cell-type specific gene expression was mostly dependent on the correct targeting of specific combinations of transcription factors to the correct DNA sequences, within regulatory regions of specific genes, has recently been challenged. The identification of molecular mechanisms capable of establishing transient or heritable states of the chromatin which can be “open” or “closed” to transcription, together with the evidence that the 3-D organization of chromosomes and genes in the nucleus is important for gene regulation, founded a new way of thinking gene expression. In this Workshop, leading scientists in the field were brought together to present and informally discuss their data and concepts with the audience. The meeting was open to outdoors scientists, who came from several Universities and Institutes and included in the PGDB program. Ample opportunity for discussion was provided to the students during and after sessions.

Speakers:

Thomas Jenuwein, Research Institute of Molecular Pathology, Vienna, Austria; **Geneviève Almouzni**, Section de Recherche, Institut Curie, CNRS, Paris, France.; **Jacqueline Mermoud**, X Inactivation Group, MRC Clinical Sciences Centre, Imperial College School of Medicine, Hammersmith Hospital, London, U.K; **Margaret Heck**, Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology, University of Edinburgh, Edinburgh, U.K.

EMBnet AGM02

Instituto Gulbenkian de Ciência

27-28 September 2002

Organisers: **Pedro Fernandes** , **Isabel Marques** and **Ana Brandão** (*IGC, Oeiras, Portugal*)

The Bioinformatics Unit has organised the 2002 session for the General Assembly Meeting of the EMBnet.

This organisation, funded by the EU and with National nodes in 37 countries, delivers Bioinformatics services to an overall community of more than 32,000 registered users. The AGM02 took important decisions towards the future of this structure, by far the largest organized effort in making Bioinformatics available to the scientific, medical and industrial communities.

Scientific Meeting on Bioinformatics

(satellite to the EMBnet AGM02)

Instituto Gulbenkian de Ciência

26 September 2002

Organiser: **Pedro Fernandes** (*IGC, Oeiras, Portugal*)

Luciano Milanese, CNR-ITBA, Milano, Italy

Use of expressed sequences tags (ESTs) for gene mining in genomics sequences

Jörg Becker, IGC, Oeiras, Portugal

Analysis of DNA array data - user's requirement

Maria del Mar Alba, UPF, Barcelona, Spain

Virus Bioinformatics

Francisco Couto, FCUL, Lisbon, Portugal

Extracting biological information from the literature: automatic annotation of FlyBase and CAZy databases

José Leal, EBI, Hinxton, United Kingdom

Mining protein interaction data

Alfonso Valencia, CNB-CSIC, Madrid, Spain

Protein interaction networks, the Bioinformatics approach

Rita Casadio, UNIBO, Bologna, Italy

Neural networks for protein structure prediction

October

1st International Workshop on Lysosome-Related Organelles

Instituto Gulbenkian de Ciência

24-26 October 2002

Organisers: **Dan Cutler** (*University College, UK*) and **Miguel Seabra** (*Imperial College, UK and IGC, Portugal*)

The 1st International Workshop on Lysosome-Related Organelles was held at the IGC from the 24th to the 26th October 2002 and was attended by 40 investigators, all international leaders in Cell Biology research.

This Workshop was the first ever to be held on the specific topic of lysosome-related organelles, a new field within cell biology. Lysosomes are intracellular organelles recognised since the 50s that serve as the cellular digestive apparatus. Lysosome-related organelles are intracellular granules that share characteristics with lysosomes, but do not function primarily as a digestive organ. Instead lysosome-related organelles are tailored to fulfil specialised functions. Examples include platelet granules released to prevent haemorrhage, pigmented granules called melanosomes where melanin is made, and lung cell granules where surfactant is made.

The rising interest in this area is due to the large range of cellular functions regulated by these organelles. Also, recent progress indicates that certain genetic diseases that affect multiple organs and systems are due to defects in genes responsible for the biogenesis, maturation and secretion of these lysosome-related organelles. This represents another beautiful example of the complementarity of fundamental research and clinical research. In addition to the scientific interactions, the Workshop will serve as a platform for the organisation of future regular meetings in the field.

The Workshop was sponsored by PGDB (Programa Gulbenkian de Doutoramento em Biomedicina), FLAD (Fundação Luso-Americana para o Desenvolvimento, Instituto Gulbenkian de Ciência/Fundação Calouste Gulbenkian and Câmara Municipal de Oeiras.

Participants: Amy Chow, Yale University, USA, Clare Futter, University College London, UK, Colin Hopkins, Imperial College, UK, Colin Watts, University of Dundee, UK, Dan Cutler, University College, UK, Deborah Nelson, University of Chicago, USA, Elisabeth Cramer, Université de Paris, France, Esteban C. Dell'Angelica, University of California Los Angeles, USA, Gillian Griffiths, University of Oxford, UK, Glynis Scott, University of Rochester, USA, Graça Raposo, Institut Curie, France, Gudrun Stenbeck, University College London, UK, Guy Reed, Harvard University, USA, Helmut Kramer, University of Texas, USA, Isabelle Maridonneau-Parini, Université de Toulouse, France, Jacques Neefjes, Netherlands Cancer Institute, The Netherlands, Jean Gruenberg, University of Geneva, Switzerland, John Hammer, National Institutes of Health, USA, Juan Bonifacino, National Institutes of Health, USA, Judit Klumperman, Utrecht University, The Netherlands, Kalervo Väänänen, University of Turku, Finland, Luanne Peters, Jackson Laboratories, USA, Mickey Marks, University of Pennsylvania, USA, Miguel Seabra, Imperial College London, UK, Mike Horton, University College London, UK, Mike Rogers, University of Aberdeen, UK, Mitsunori Fukuda, RIKEN, Japan, Monique Kleijmeer, Utrecht University, The Netherlands, Niels Borregaard, University of Copenhagen, Denmark, Norma Andrews, Yale University, USA, Paul Luzio, University of Cambridge, UK, Peter Peters, W.C.I. Amsterdam, The Netherlands, Pierre Cosson, University of Geneva, Switzerland, Richard Swank, Roswellpark Cancer Institute, USA, Ronit Sagi-Eisenberg, Tel Aviv University, Israel, Rosangela Da Silva, University of Oxford, UK, Sandra Haberichter, University of Wisconsin, USA, Tim Weaver, University of Cincinnati, USA, Vincent Hearing, National Institutes of Health, USA, Willem Stoorvogel, Utrecht University, The Netherlands.

SLE Meeting

Convento da Arrábida

26-28 October 2002

Organisers: The Associação de Doentes com Lupus and the IGC organised a meeting to discuss the projects on Systemic Lupus Erythematosus (SLE).

Participants: Rita Andreia, Associação de Doentes com Lupus, Portugal, Maria João Antunes, Associação de Doentes com Lupus, Portugal, Jorge Carneiro, IGC, Portugal, Antonio Coutinho, IGC, Portugal, Jocelyne Demengeot, IGC, Portugal, Constantin Fesel, IGC, Portugal, Carlos Ferreira, Hospital Sta. Maria, Portugal, Werner Haas, IGC, Portugal, Carlos Penha Gonçalves, IGC, Portugal, José Mengel, Univ. São Paulo, Brazil, Isabel Reis, Associação de Doentes com Lupus, Portugal, Eugenia Santos, Hospital Egas Moniz, Portugal, Ana Tam, Associação de Doentes com Lupus, Portugal,

Carlos Vasconcelos, Hospital Sto. António, Portugal, **Maria de Belem**, Porto, Portugal, **Astride Vicente**, IGC, Portugal, **Marta Barreto**, IGC, Portugal, **Iris Caramalho**, IGC, Portugal, **Francisca Fontes**, IGC, Portugal, **Santiago Zelenay**, IGC, Portugal.

November

EMBO Sectoral Meeting on Immunology: Crossroads of Immunology

Instituto Gulbenkian de Ciencia

22-24 November 2002

EMBO sporadically organises "sectoral meetings", inviting all EMBO members in a given research area to discuss the current state and perspectives in their field. This year, EMBO is organising a Sectoral Meeting in Immunology from November 22-24 at the IGC.

Organisers: **Jan Taplick**, Germany, **António Coutinho**, Portugal

Participants: **Adriano Aguzzi**, Institute of Neuropathology, Switzerland, **Ruth Arnon**, Weizmann Institute of Science, Israel, **Brigitte Askonas**, Imperial College, UK, **Jeroen van Bergen**, Leiden University Medical Center, The Netherlands, **Philippe Brachet**, INSERM, France, **Frank Gannon**, EMBO, Germany, **Jose Lopez de Castro**, Centro de Biologia Molecular Severo Ochoa, Spain, **Franco Celada**, Italy, **Antonio Coutinho**, IGC, Portugal, **Sandor Damjanovich**, University Medical School of Debrecen, Hungary, **Daniel Davies**, Imperial College of Science, Technology and Medicine, U.K., **Antonella Folgori**, IRBM, Italy, **Alexander von Gabain**, Intercell AG, Austria, **Nicolas Glaichenhaus**, Institut de Pharmacologie Moléculaire et Cellulaire, France, **Julian Gordon**, Abbott Labs, USA, **Sirpa Jalkanen**, University of Turku, Finland, **Günther Hämmerling**, Institute of Immunology & Genetics, Germany, **Jonathan Howard**, Institut für Genetik der Universität, Germany, **Raymond Kaempfer**, Faculty of Medicine/Hebrew University, Israel, **Dimitris Kioussis**, NIMR, UK, **George Kollias**, Institute of Immunology, Greece, **Antonio Lanzavecchia**, Institute of Research in Biomedicine, Switzerland, **Bernard Malissen**, Centre de Immunologie INSERM, France, **Alberto Mantovani**, Istituto di Ricerche Farmacologiche Mario Negri, Italy, **Carlos Martinez-A.**, Centro Nacional de Biotecnología, UAM, Spain, **Fritz Melchers**, Germany, **Michael Neuberger**, MRC, UK, **Israel Pecht**, Weizmann Institute of Science, Israel, **Philippe Pierre**, CIML-CNRS-INSERM, France, **Ulf Rapp**, Julius-Maximilian University, Germany, **Michael Reth**, Abt. Molekulare Immunologie, Germany, **Paola Ricciardi-Castagnoli**, University of Milano-Bicocca, Italy, **Claude-Agnes Reynaud**, Faculté de Médecine Necker-Enfants Malades, France, **Angela Santoni**, Policlinico Umberto I, Italy, **Michael Sela**, Weizmann Institute of Science, Israel, **Roberto Sitia**, DIBIT-HSR, Italy, **Maria de Sousa**, ICBAS, Portugal, **Jack Strominger**, Harvard University, USA, **Socrates Tzartos**, Hellenic Pasteur Institute, Greece, **Rolf Zinkernagel**, Institute of Experimental Immunology, Switzerland.

December

Drosophila Meeting

Instituto Gulbenkian de Ciência

20 December 2002

Drosophila has been, for about a 100 years now, the paradigm model organism for genetic studies in high eukaryotes. The advantages presented for using this biological model, were extend with the completion of the sequencing of the *Drosophila* genome, and modern molecular and genetic tools now allow an integrative organism-centred approach.

The exchange of information and material among researchers using *Drosophila* was always one major factor that contributed to the advancement of the field. There are a few international *Drosophila* conferences taking place annually, and many small local “*Drosophila*” gatherings.

The purpose of this Portuguese *Drosophilists* Meeting is to bring together the maximum of researchers that use the fly as biological material. This will allow not only the discussion of the latest technology and the discussion of ideas, but, more importantly, will allow the portuguese scientific community to open up, and establish connections with the “outside” and the “inside” of the country. The meeting it deliberately to follow an informal model, to stimulate discussion and in particular to encourage the participation of graduate and pos-graduate students. It is intended to turn this meeting into the annual *Drosophila* meeting, with the second event planned to take place December 19th, 2003.

Organiser: Álvaro Tavares (IST, Lisbon, Portugal and IGC, Oeiras, Portugal)

Participants: Sofia Araujo (London, UK); Victor Barbosa (New York, USA); Claudia Barros (Cambridge, UK); Mónica Bettencourt (Cambridge, UK); Fernando Casares (IBMC, Porto); Paula Coelho (IBMC, Porto); Mariana Faria (IGC, Oeiras, Portugal) Rui Gomes (FCT, Lisbon, Portugal) António Jacinto (IGC, Oeiras, Portugal); Helder Maiato (IBMC, Porto, Portugal and Edinburgh, UK); Rita Teodoro (UCSF, USA); Rui Martinho (New York, USA) Alexandra Moreira (IBMC, Porto, Portugal); Paulo Pereira (IBMC, Porto, Portugal); Paula Sampaio (IBMC, Porto, Portugal) Sheila Vidal (Gif-sur-Yvette, France); Álvaro Tavares (IGC, Oeiras, Portugal); Alan Wainman (Cambridge, UK).

TEACHING

POST-GRADUATE EDUCATION

Post-graduate education has always been a strong valence of the IGC, and this tradition has been maintained through the establishment of the Gulbenkian Programme in Biology and Medicine which ended in 1999 and was followed by the Gulbenkian Programme on Biomedicine.

GULBENKIAN PhD PROGRAMME IN BIOMEDICINE

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Joana Duarte Antunes Ramos dos Santos
Rita Graça da Silva
Juliette Elisabeth Savin
László Tokaji

Gulbenkian PhD programme in Biomedicine for 2002

Programme for 2002/2003 conducted at the IGC in 2002:

16 September: Introduction day IGC

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

16-18 September: Recombinant DNA Techniques

Lisete Fernandes (IGC, Oeiras, Portugal)

24-27 September: Biochemistry I – Techniques

Miguel Teixeira (ITQB, Oeiras, Portugal)

Eurico de Melo (ITQB, Oeiras, Portugal)

Ricardo Pires (ITQB, Oeiras, Portugal)

Patrícia Madureira (IGC, Oeiras, Portugal)

José Feijó (IGC, Oeiras, Portugal)

Sérgio Gulbenkian (IGC, Oeiras, Portugal)

30 September-7 October: Advanced Protein Chemistry: Folds, Forms and Function

Sukalyan Chatterjee (IGC, Oeiras, Portugal), Miguel Teixeira (ITQB, Oeiras, Portugal)

Claudio Gomes (ITQB, Oeiras, Portugal)

Eurico de Melo (ITQB, Oeiras, Portugal)

Teresa Catarino (ITQB, Oeiras, Portugal)

Avihai Danon (Weizmann Institute, Rehovot, Israel)

Joerg Hoehfeld (Institute for Cell Biology, Bonn, Germany)

Manuel Prieto (IST-UTL, Lisboa, Portugal)

8-11 October: Genetics: Drosophila, Mouse & Human; Mendelian & Non-Mendelian Genetics

John Stewart (Université de Technologie de Compiègne, France; IGC, Oeiras, Portugal)

Dan Holmberg (Umeå Univ., Sweden; IGC, Oeiras, Portugal)

Moises Mallo (IGC, Oeiras, Portugal)

António Jacinto (IGC, Oeiras, Portugal)

Pedro Coutinho (IGC, Oeiras, Portugal)

Carlos Penha-Gonçalves (IGC, Oeiras, Portugal)

14-25 October: Molecular Genetics: Transcription, Post-Transcriptional Processing Translation, Recombination, Replication

Sukalyan Chatterjee (IGC, Oeiras, Portugal)

Maria Marone (Catholic University, Rome, Italy)

Valeria Poli (Università di Torino, Torino, Italy)

Thomas Oelgeschlager (Marie Curie Research Institute, Oxford, UK)

Miguel Godinho Ferreira (Telomere Biology Laboratory, Cancer Research UK, London, UK)

Eric Lam (Ludwig Institute for Cancer Research, London, UK)
Karim Labib (Paterson Institute for Cancer Research, Manchester, UK)
João Ferreira (IHEFM-UL, Lisboa, Portugal)
Margarida Gama-Carvalho (IHEFM-UL, Lisboa, Portugal)
Luisa Figueiredo (Institut Pasteur, Paris, France)
Patrick Varga-Weisz (Marie Curie Research Institute, Oxted, UK)

25-31 October: Signal Transduction

Marcus Thelen (Institute for Research in Biomedicine, Bellinzona, Switzerland)
Mattias Peter (Institut Suisse de Recherche Experimentale sur le Cancer, Epalinges, Switzerland)

4-8 November: Apoptosis

Yuri Lazebnik (Cold Spring Harbor Laboratory, Cold Spring Harbor, USA)
Michael Hengartner (IMB, University of Zurich, Switzerland)
David Vaux (The Walter and Eliza Hall Institute, Victoria, Australia)
Scott Kaufmann (Mayo Clinic, Rochester, USA)

11-15 November: Membrane Traffic

Dan Cutler (MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, UCL, London, UK)
Graça Raposo (Institut Curie, CNRS UMR 144, Paris, France)
Adam Linstedt (Carnegie Mellon University, Pittsburg, USA)
GiamPietro Schiavo (Cancer Research UK, London Research Institute, London, UK)

18-22 November: Cytoskeleton

Helena Soares (IGC, Oeiras, Portugal)
Victor Small (Institute of Molecular Biology, Salzburg, Austria)
Harald Herrmann-Lerdon (German Cancer Research Center, Heidelberg, Germany)
Jesus Ávila (Universidad Autonoma de Madrid, Madrid, Spain)
Peter Jordan (INS Dr. Ricardo Jorge, Lisboa, Portugal)
Lisete Fernandes (IGC, Oeiras, Portugal)

25-29 November: Cell Cycle

Álvaro Tavares (IST-UTL, Lisboa & IGC, Oeiras, Portugal)
Carl Smythe (University of Sheffield, Sheffield, UK)
Jonathon Pines (University of Cambridge, Cambridge, UK)
Iain Hagan (University of Manchester, Manchester, UK)
Claudio Sunkel (IBMC, Universidade do Porto, Porto, Portugal)

2-13 December: Developmental Biology

António Jacinto (IGC, Oeiras, Portugal)
Domingos Henrique (Instituto Histologia e Embriologia, FML, Lisboa, Portugal)
Acaimo Gonzalez-Reyes (IPB - CSIC, Granada, Spain)

Lola Martin Bermudo (IPB "López-Neyra", Granada, Spain)
Bruce Bowerman (University of Oregon, Eugene, USA)
Martin Cohn (University of Reading, Reading, UK)
Pierre Gönczy (Swiss Institute for Experimental Cancer Research, Epalinges/Lausanne, Switzerland)
Miguel Constância (The Babraham Institute, Cambridge, UK)
Mike Bate (University of Cambridge, Cambridge, UK)
François Schweisguth (CNRS UMR 8542, Paris, France)
Mike Jones (ICR, London, UK)
Derek Stemple (NIMR, London, UK)
Miguel Manzanares (CSIC-Universidad Autonoma de Madrid, Spain)
Moises Mallo (IGC, Oeiras, Portugal)

16 December: Biology of the Tetrahymena

Helena Soares (IGC, Oeiras, Portugal)

17-20 December: Oncogenesis & Metastasis

Sérgio Dias (IPO, Lisboa & IGC, Oeiras, Portugal)

Carla Mouta (Maine Medical Center Research Institute, Scarborough, USA)

**Annual Meeting of PGDBM/PGDB in Curia
19-23 September 2002**

According to the tradition, Curia hosted the Annual Meeting of the PGDBM/PGDB from 19th-23rd September. The scientific sessions included a poster session presented by the PGDB-II students as part of their requirement for passing the first year of the graduate programme. The posters were kept for the whole duration of the meeting, enabling frequent daily discussions around the posters. In addition there were oral presentations from 47 students, which stimulated lots of discussions. A newly-appointed research review committee constituted by recently graduated ex-students of PGDBM (Isabel Palmeirim, António Jacinto, Miguel Castelo-Branco, Miguel Godinho Ferreira, Vasco Barreto and Francisco Dionísio) followed closely all the presentations and provided feedback to the PhD programme Executive.

There were also two presentations from ex-students, Bruno Santos and Nuno Arantes de Oliveira, who spoke about their work and recent experience, and three keynote conferences by invited speakers: Professor Michael Morgan of the Wellcome Trust, UK, "The Biotechnology Revolution: Can Europe Compete Globally, Professor Alfonso Martinez-Arias of the University of Cambridge, UK, "Are organisms important for the study of developmental biology?" and Professor Antonio Garcia-Bellido, Universidad Autonoma de Madrid, Spain, "My experiences in developmental genetics".

Many important visitors kindly participated in the Annual Meeting. On the opening day, we were visited by Professor Diogo de Lucena (FCG board member) and Dr. Idalina Salgueiro (FLAD), and by the Minister for Science and Higher Education, Dr. Pedro

Lynce, and the Secretary of State for Science and Technology, Dr. Manuel Fernandes Thomaz the following day.

The new format, of the meeting starting on a Thursday and finishing on a Monday, provided for a more condense and intense meeting. Nevertheless, the official ceremonies were held on the Saturday, as is now tradition. The official ceremonies included an open session of discussion about science policy in Portugal, a round table composed of Professor Ramôa Ribeiro, Director of FCT, Professor Marçal Grilo, Board member of FCG, and Professor António Coutinho, Director of the IGC. The audience included a number of important guests including Professor João Caraça, Director of Science at FCG, and influential personalities in science, education and government.

THE GULBENKIAN TRAINING PROGRAMME IN BIOINFORMATICS (GTPB)

The Bioinformatics Unit of the IGC, which maintains the Portuguese Node of the European Molecular Biology Network (EMBnet), develops a specific educational programme to train scientists in the proper use of Bioinformatics tools and techniques. The courses take place in a specifically set-up training room where practical knowledge is easily acquired. A total of 110 attendees (21 from the IGC, 87 with other Portuguese affiliation and 7 with international affiliation) have received more than 240 hours of tuition. Extensive documentation has been handed in CDROM form.

Local Organizers: Fernandes P.L. and Marques I.

June

BSA02 - Biological Sequence Analysis

Faculty: David Judge, University of Cambridge, UK, Lisa Mullan, HGMP-RC, Hinxton, Cambridge, UK

September

BMBCD02 - Building and Managing Biological Collections Databases

Faculty: Richard White, School of Biological Sciences, Univ. Southampton, UK, Eduardo Dalcin, Univ. Federal Pernambuco - UFPE, Recife, Brazil

October

PDA02 - Phylogenetic Data Analysis

Faculty: James McInerney, National University of Ireland, Maynooth, IE

AMB02 - Applied Malaria Bioinformatics

Local Organizers: Fernandes P.L., Marques I., Mota M.

Faculty: Pedro Fernandes, IGC, PT, Chuong Huynh, NIH/NLM/NCBI, USA, Jessica Kissinger, Univ. Georgia, USA

November

PGGM02 - Population Genetics and the use of Genetic Markers

Faculty: Mark Beaumont, University of Reading, UK, Lounes Chikhi, Université Paul Sabatier, Toulouse, FR

(Note: Co-sponsored by the EMBnet)

December

BC02 - Basic Course

Faculty: David Judge, University of Cambridge, UK, Lisa Mullan, HGMP-RC, Hinxton, Cambridge, UK

SCIENCE AND SOCIETY

CIÊNCIA VIVA

“Ciência Viva” is a programme of the Ministry of Science and Higher Education for the promotion of science in society. The IGC participates in several of its initiatives.

Project “Scientific occupation for teenagers during holidays”

The programme “scientific occupation for teenagers during holidays”, started in 1997 and has provided secondary education students with the opportunity of being in close contact with the reality of scientific research work, through their participation in training sessions in public and private laboratories, research centres and entities for the promotion of science.

Within the context of this programme the IGC, in 2002, participated with the following training sessions:

Estudo da Selecção Natural na Evolução de Bactérias

IGC Member: Francisco Dionísio

22-27 July 2002

Manutenção dos Animais de Laboratório do Biotério do IGC

IGC Member: Bruce Lenhart

22-31 August 2002

Biologia Molecular e Morfógenese

IGC Member: António Jacinto

16-20 September 2002

National week for the disclosure of scientific activities:

23-30 November 2002

IGC Open Day

28 November 2002

With reference to the “National week for the disclosure of Scientific Activities” during the period 23 – 30 November, the IGC had an open day on the 28th November. The IGC was visited by:

Escola Secundária de Fonseca Benevides, Lisbon – 12 students.

SCHOOL VISITS AT THE IGC

Esc. Secundária Júlio Dantas (Lagos)	35 students
Esc. Secundária Bocage (Setúbal)	45 students
Esc. Salesiana Manique (Alcabideche)	40 students
Esc. Secundária Bocage (Setúbal)	45 students
Esc. Sec. Alves Martins (Viseu)	45 students
Esc.Sec.Morgado Mateus (Vila Real)	25 students
Esc. Sec. Sebastião da Gama (Setúbal)	64 students
Colégio Sagrado Coração Maria (Lisbon)	27 students
Esc. Sec. Viriato (Viseu)	40 students
Colégio Sagrado Coração Maria (Lisbon)	28 students
Esc. Salesiana Manique (Alcabideche)	40 students
Esc.Sec.Padrão da Légua (Matosinhos)	16 students
Esc.Salesiana Manique (Alcabideche)	25 students
Saint Dominic's (Carcavelos)	27 students
TOTAL 14 Schools	502 students

SCIENCE AND SOCIETY INITIATIVES AT THE IGC

One of the remits of the Instituto Gulbenkian de Ciência (IGC) is to promote the dialogue between scientists and society, thus contributing the cultural, social and economic well-being of the Portuguese society. In this context, several initiatives have been undertaken aimed at improving the communication links between scientists, the media and the public.

Informal meetings between scientific researchers and science journalists

The main objectives of these meetings are to discuss and clarify the background and most recent findings in biomedical research, as well as their applications and implications for society as a whole. A further aim is to establish a network of journalists and scientists, thus improving communication between the two groups.

The meetings are held in a very informal setting, where both journalists and scientists are free to discuss all aspects of biomedical sciences, from basic research to implications for public health. On average, the meetings have had the participation of five science journalists, from the press (daily newspapers, weekly magazines) and television. The meetings are held once every three weeks, and each lasts approximately 2 hours.

The first meeting took place in June 2002, and a total of 6 have been held to date (December 2002). The following scientific fields/themes have been covered: Cloning and Ageing, Genetically Modified Organisms, Immunology (two sessions), Cell Proliferation – from Biology to Cancer, Inflammation. In each meeting, two or three researchers, from both the IGC and other portuguese research institutions, have taken part, answering questions and discussing scientific findings and reports.

Live Radio Broadcast on Science supported by the Gulbenkian Foundation

On 21st November 2002, the IGC hosted the daily morning programme of the national radio broadcaster, Antena 1. The programme, a live broadcast, focused on how the Calouste Gulbenkian Foundation (FCG) has supported science in Portugal, over the last 40 years. A follow-up to the live programme, focused on Gulbenkian Foundation-supported research by young biomedical and social scientists, was broadcast on 23rd November 2002.

The broadcasts were organised in close collaboration between Dr. Jorge Wemans, head of the “Serviço de Comunicação” of the FCG, and the IGC, through Dra. Ana Paula Coutinho.

A total of 21 researchers and students took part in the broadcasts, including the Science Administrator of the Gulbenkian Foundation, Prof. Diogo de Lucena, the Head of the “Serviço de Ciência” of the FCG, Prof. João Caraça and the Director of the IGC, Prof. António Coutinho. Representing the Oeiras Campus were Prof. António Xavier, former Director of ITQB and Prof. Manuel Carrondo, Director of IBET.

In respect to the efforts of the FCG in promoting scientific research and in studying the impact of science on society, the broadcast counted on two young students of Mathematics, winners of the “Novos Talentos da Matemática” prize, awarded by the FCG’s Serviço de Ciência, and Prof. Firmino da Costa, author of a FCG-supported book entitled “Os Públicos da Ciência”. Also present was António Contador, a sociologist who undertook a study on multiculturalism in schools, supported by the FCG.

Nuno Arantes de Oliveira, Margarida Trindade (from Toulouse) and Cristina Costa contributed as former research scientists (all with PhDs), now pursuing careers in biotechnology enterprises, non-governmental organisations and practising medicine, respectively.

Several graduate students, post-doctoral researchers and young team leaders of the IGC took part in both the live broadcast and in the edited programme. They were: Cristina Pina, Manuel Rebelo, Ana Paula Coutinho, Maria Mota, Astrid Vicente, António Jacinto, Sérgio Dias and Matthias Haury.

THESES

The following Theses were prepared in part at the IGC and were presented in 2002:

PhD Theses

Ricardo Pimenta-Araujo “Allo transplantation of embryonic tissues: the thymic epithelium paradigm”, University of Paris VI, Paris, France, February 2002.

Marie-Louise Bergman “A sub-phenotype approach to dissect the genetic control of murine type 1 diabetes” Umeå University, Umeå, Sweden, June 2002.

Kalet Leon Monzon “A quantitative approach to dominant tolerance”, Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Lisbon, Portugal, October 2002.

BsC Theses

Lara Carvalho “The role of fibronectin in chick somitogenesis” FCUL, Lisbon, Portugal, October 2002.

Inês Conceição “O Papel dos Plasmídeos na Evolução Bacteriana”, FCUL, Lisbon, Portugal, October 2002.

Ana Roxo Leão Neves Costa, “Interacções genéticas associadas à função do YAP4”, FCUL, Lisbon, Portugal, October 2002.

Andreia Lino “Caracterização do mecanismo molecular da activação dos linfócitos T”. FCUL, Lisbon, Portugal, July 2002.

Marta Luz “Myotome colonisation and early muscle differentiation in normal versus myf5 null mouse embryos: possible role of cell-extracellular matrix interactions” FCUL, Lisbon, Portugal, October 2002.

Sara Rute Lamas de Oliveira Marques “Pesquisa de genes envolvidos na organogénese de Gallus gallus: o papel de Plutão na diferenciação da mesoderme pré-somítica”. FCUE, Évora, October 2002.

Gustavo Rosa “Interacções recíprocas entre linfócitos T e as respostas inflamatórias”, FCUL, Lisbon, Portugal, July 2002.

Joana Santos “Molecular and temporal characterisation of the rostral-most somites in the early somitic stages of the chick embryo”, FCUL, Lisbon, Portugal, July 2002.

PARTICIPATION IN ACADEMIC COMMITTEES

Jorge Carneiro

Member of Jury of the Ph.D Thesis of Cristina Girão, University of Porto, Porto, Portugal.

Member of the Jury of the Ph.D Thesis of Kalet Leon, University of Porto, Porto, Portugal.

Member of the Jury of Ph.D Thesis of Alexis Collette, University of Paris VI, Paris, France.

António Coutinho

Member of the Jury of the PhD Thesis of Ricardo Pimenta-Araújo, University of Paris VI, Paris, France.

Jocelyne Demengeot

Member of the Jury of the PhD Thesis of Elodie Mohr, University de la Mediterranée, Marseille, France.

Francisco Dionisio

Member of the Jury of the Bs.C. Thesis of Inês Conceição, FCUL, Lisbon, Portugal.

Lisete Fernandes

Member of the Jury of the Bs.C Thesis of Ana Roxo Leão Neves Costa, FCUL, Lisbon, Portugal.

Isabel Palmeirim

Member of the Jury of the undergraduate theses of Joana Santos and Lara Carvalho - FCUL, Lisbon, Portugal.

Member of the Jury of the Ph.D Thesis of Arantza Barrios, University of London (UCL), Department of Anatomy and Developmental Biology, London, United Kingdom.

Michael Parkhouse

Member of the Jury of the M.Sc. Thesis of Nataly Manjarrez-Orduño, at Centro Nacional de Estudios Avanzados, Mexico, D.F., Mexico.

Sylviane Pied

Member of the Jury of the Ph.D Thesis of Padma Das, Sambalpur University, Orissa, India.

J. Pedro Simas

Member of the Jury of the Ph.D Thesis of Rui Tato Marinho, FMUL, Lisbon, Portugal.

Miguel Soares

Member of the Jury of the Ph.D Thesis of Delphine Bouchet, University of Paris VII, Paris, France.

Álvaro Tavares

Member of the Jury of the PhD Thesis of Elsa Logarinho, ICBAS, Porto, Portugal.

Member of the Jury of the MSc. Thesis of Marta Agostinho, ISTUTL, Lisbon, Portugal.

Member of the Jury of the MSc. Thesis of Cristina Fragoso, Univ. Aveiro, Aveiro, Portugal.

Sólveig Thorsteinsdóttir

Member of the Jury of the undergraduate theses of Inês Baptista, Joana Santos, Gonçalo Neto, Lara Carvalho, Marta Luz, Ana Catarina Martins and Sandra Nobre, FCUL, Lisbon, Portugal.

PARTICIPATION OF IGC PERSONNEL IN CONFERENCES, SEMINARS AND SCIENTIFIC MEETINGS

January

Coutinho A.

From innate to adaptive immunity.

Advanced Course in Immunology, DEA de l'Institut Pasteur, Institut Pasteur, Paris, France.

February

Belo J.A.

Mechanisms of head induction: evolutionary conservation of the transcriptional regulation of cerberus-like genes.

Instituto de Neurociencias, Universidad Miguel Hernandez, Alicante, Spain.

Coutinho A.

TCR specificity and central commitment of MBP-related regulatory T cells.

Collège de France/Institut Pasteur Workshop on Autoimmune Diseases, Institut Pasteur, Paris, France.

Coutinho A.

Tolerance, regulatory T cells and autoimmunity

3rd International Congress on Autoimmunity, Geneva, Switzerland.

Feijó J.A.

Two-photon microscopy application in plant cells.

New trends on femtosecond laser spectroscopy in chemistry, physics and neurobiology.

Fundacion Ramon Areces, Madrid, Spain.

Feijó J.A.

Plant cell growth and morphogenesis regulation: the pollen tube paradigm.

CSIC/ Univ. Salamanca, Salamanca, Spain.

Vicente A.M.

Genética do Autismo.

Seminars Cycle: Investigação aplicada em Saude, Escola Superior de Tecnologias da Saúde de Coimbra. Coimbra, Portugal.

March

Belo J.A.

Manipulação genética e clonagem: impacto e perspectivas na sociedade.

Jornadas Engenharia Biotecnológica 2002, UALG, Algarve, Portugal.

Belo J.A.

Manipulação genética – legitimidade e implicações.

VI ENEB Lisboa 2002, FCUL, Lisbon, Portugal.

Coutinho A.

TCR specificity and thymic commitment of MBP-related regulatory T cells.

Colloque en l'honneur de Philippe Druet, INSERM/CHU Purpan, Toulouse, France.

Tavares A.T., Filipe M. and Belo J.A.

Comparative study of the transcriptional regulation of cerberus-like genes during embryonic development.

Poster. Meeting on Evolution of Developmental Mechanisms, University of York, York, UK.

April

Carneiro J.

Modelação do sistema imunitário.

Bioinformática 2002. University of Aveiro. Aveiro, Portugal.

Coutinho A

A model for developmentally-acquired self-tolerance.

The 3rd International Workshop of the Kyoto T cell Conference (KTCC), Kyoto University, Kyoto, Japan.

Coutinho A.

Progresso em Imunologia: doenças autoimunes e alergias.

Sessão Clínica Faculdade/Hospital, FMUL, Hospital de Santa Maria, Lisbon, Portugal

Fernandes P.L.

Bioinformática (Genoma Humano).

II Jornadas de Engenharia Biotecnológica “Genómica e Proteómica, que Futuro?”, Escola Superior Agrária de Bragança, Bragança, Portugal.

Fernandes P.L.

Perspectivas para o Futuro da Bioinformática.

Bioinformática 200, Univ. Aveiro, Aveiro, Portugal.

Fernandes P.L.

Bioinformática.

Conferência em Biotecnologia, Univ. Lusófona de Humanidades e Tecnologias, Lisbon, Portugal.

Homberg D.

Genetic control of immune dysfunction in type 1 diabetes

Karolinska Institute, Stockholm, Sweden

Marques I.

Introdução à Bioinformática

Perspectivas em Química e Bioquímica, FCUL, Lisbon, Portugal

Rodriguez-Leon, J. and Izpisua Belmonte J.C.

Expression and regulation of colloid and ventroptin during limb development.

Poster. Workshop in Limb development. Instituto Juan March de Estudios e Investigaciones, Madrid, Spain.

Sobrinho L.G., Simões M. , Barbosa L., Raposo J., Pratas S., Fernandes P.L. and Correia I.

Respostas Endocrinológicas a Emoções Provocadas sob um Estado Modificado de Consciência.

Poster. 4º Symposium of the Fundação Bial, Porto, Portugal.

May

Alves P., Godinho S., Faria M. and Tavares A.

Cloning and characterisation of DPLKK, the Drosophila Polo kinase kinase.

Poster. The Cell Cycle, Cold Spring Harbour, USA.

Coutinho A.

Carreira de Investigação.

VII Seminar on “ Saídas Profissionais”, Associação de Estudantes da FMUL, Lisbon , Portugal

Coutinho A.

Contemporary topics in immunology

Chairman of the Session on MHC at the Marcus Wallenberg Symposium, Nobel Forum, Karolinska Institutet, Stockholm, Sweden

Feijó J.A.

Plant cell growth and morphogenesis regulation: the pollen tube paradigm.

CNC/ Coimbra Univ., Coimbra, Portugal.

Haury M.
ISAC International World Congress in Cytometry.
San Diego, CA, USA.

Marques I.
O papel da Bioinformática no estudo do Genoma Humano.
Genoma Humano Século XXI – Impacto nas Ciências Farmacêuticas, FFUL, Lisbon, Portugal

Marques I.
Introdução à Bioinformática.
Workshop de Biologia Molecular, IHMT, Lisbon, Portugal

Palmeirim I.
The molecular clock is working, at least, in two embryonic dimensions.
Seminar at the National Institute for Medical Research (NIMR), Mill Hill, UK.

Rodriguez-Leon, J. and Izpisua Belmonte J.C.
Expression and regulation of colloid and ventroptin during limb development.
EuroConference on Tissue Specification and Patterning during Development. Euresco Conferences, Granada, Spain.

June

Bajanca F.
Cloning genes at the computer screen: possibilities and problems.
Practical course, Instituto Nacional de Saúde Dr. Ricardo Jorge, Centro de Genética Humana, Lisbon, Portugal

Carvalho T.
Functions and dynamics of CD4+CD25+ T cells in LPS induced inflammation.
University of Alabama, Birmingham, USA

Carvalho T.
Regulatory T cells and Inflammation
Princeton University, Princeton, USA

Coutinho A.
Identidade e Imunologia.
Mestrado em Psicossomática “A identidade e o somático”, ISPA, Lisbon, Portugal

Demengeot J.
Kirin idea shop on regulation of autoimmunity.
FOCI satellite meeting, San Francisco, USA.

Feijó J.A.

Advanced microscopy application in plant cells.

Leica course on confocal and digital microscopy. Viana do Castelo, Portugal.

Jacinto A.

Life in the edge of dorsal closure.

ELSO 2002, 2nd ELSO conference, Acropolis Conference Center, Nice, France.

Melo L. V., Casalou C., Nolasco S., Seixas A.C., Brogueira P. and Soares H.

Study of the regeneration of cilia in ciliate Tetrahymena by atomic force microscopy.

Spring Meeting, 2002 European Materials Research Society (E-MRS), Strasbourg, France.

Parkhouse M.

Protection and diagnosis in human, porcine and bovine cysticercosis.

University of Oxford, Oxford, U.K.

Sobrinho L.G., Simões M., Barbosa L., Raposo J.F., Pratas S., Fernandes P.L. and Santos M.A.

Hormonal responses to emotions elicited during a hypnoidal state.

24th European Conference on Psychosomatic Research, Lisbon, Portugal.

July

Belo J.A.

Heads, hearts, handedness...evolutionary conservation and functional genomics with the cerberus-like gene family.

Montagskolloquium Seminars, Max-Planck Institute for Developmental Biology, Tuebingen, Germany.

Carneiro J.

Models of the immune system: homeostasis in lymphocyte populations and etiology of autoimmune diseases.

Summer School on Mathematical Biology. Complexo Interdisciplinar da Universidade de Lisboa. Lisbon, Portugal.

Coutinho A.

Diversidade, cooperatividade e tolerância.

Ciclo de colóquios “ A Medicina e a Cidade”, Conversas do Campo de Sant’ana, FCMUNL, Lisbon, Portugal

Gomes M.G.M.

Pattern formation in populations of antigenically diverse pathogens.

Poster. 6th Meeting on Molecular Epidemiology and Evolutionary Genetics of Infectious Diseases (MEEGID VI), Institut Pasteur, Paris, France.

Gomes M.G.M.

Population dynamics of multi-strain pathogens.

Courses on Summer School on Mathematical Biology. CIM-Coimbra /CMAF-Lisbon, Portugal.

Leon K.

Mathematical Modeling & Computing in Biology and Medicine.

5th European Conference of the ESMTB, Milano, Italy.

Parkhouse M.

Protection and diagnosis in human, porcine and bovine cysticercosis.

Instituto Biomed, University de Carabobo, San Carlos, Venezuela.

August

Andersson J.

Natural Tolerance

Regulatory T cells

Mini-Curso de Inmunologia, Fundación Ciencia para La Vida, Santiago, Chile.

Casalou C., Seixas A.C., Melo L.V., Nolasco S., Brogueira P. and Soares H.

The oligomeric state of the Tetrahymena cytosolic chaperonin CCT and in vivo association of CCT α , CCT δ , CCT ϵ and CCT η -subunits with specialized microtubule structures are perturbed by cilia biogenesis.

17th European Cytoskeleton Forum, Nyon-Geneva, Switzerland.

Coutinho A.

Testar princípios: Alguns anos de experiência no Instituto Gulbenkian de Ciência

Sessão Científica FIOCRUZ-BA, Fundação Oswaldo Cruz, Centro de Pesquisa Gonçalo Moniz, Salvador, Brazil.

Coutinho A.

Natural Tolerance

Regulatory T cells

Mini-Curso de Inmunologia, Fundación Ciencia para La Vida, Santiago, Chile.

Filipe M., Silva A., Tavares A.T., Marques S., Kuerner K-M., Steinbeisser H. and. Belo J.A.

Genomic approaches to the study of the mechanisms of vertebrate head induction.

Poster. Santa Cruz Conference on Developmental Biology", UCSC, Santa Cruz, USA.

Jacinto A.

Cytoskeleton dynamics during Drosophila dorsal closure and wound repair.

17th European Cytoskeleton Forum, Nyon, Switzerland.

Parkhouse M.

Protection and diagnosis in human, porcine and bovine cysticercosis.

Centro Nacional de Estudios Avanzados, Mexico.

September

Bajanca F. and Thorsteinsdóttir S.

Expression of integrins during myotome formation in the mouse.

Poster. EMBO Workshop on the Molecular Genetics of Myogenesis and Muscle Diseases, Cambridge, UK.

Cachaço A.S., Chuva de Sousa Lopes S.M., Kuikman I., Bajanca F., Abe K., Baudoin C., Sonnenberg A., Mummery C.L. and Thorsteinsdóttir S.

Knock-in of integrin beta1D affects primary but not secondary myogenesis in mice.

Poster. EMBO Workshop on the Molecular Genetics of Myogenesis and Muscle Diseases. Cambridge, UK.

Caramalho I.

Regulatory T cells selectively express toll like receptors and are activated by lipopolysaccharide.

Poster. FEBS International Summer School on Immunology. The Ionian Village, Greece.

Carvalho T.

CD4+CD25+ T cells control CD4+CD25- cells during LPS induced inflammation.

Poster. FEBS International Summer School on Immunology. The Ionian Village, Greece.

Coutinho A.

Induction of tolerance to self by the endodermal component of the thymus

Colloque on Tolerance and autoimmune diseases.

Fondation des Treilles, Provence, France.

Fernandes P.L.

Databases and Tools for Metabolic Engineering and Research.

FEBS Lecture Course: Advanced Technologies for Metabolic Engineering in Biotechnology and Medicine, Carcavelos, Portugal.

Faria M., Alves P., Godinho S. and Tavares A.

Mps1 kinase, a novel checkpoint kinase in Drosophila melanogaster.

Poster. Le cycle cellulaire et ses mécanismes de surveillance, Jacques Monod Conferences, Roscoff, France.

Gomes M.G.M.

Infection and reinfection dynamics of tuberculosis and the impact of control programmes.
Poster. Advanced School on The Immune System in the Protection and Susceptibility to Tuberculosis, Ruggero Ceppellini Advanced School of Immunology, Naples, Italy.

Gomes M.G.M.

Applied Research in Spatial Epidemiology (ARISE).
IRD, Montpellier, France.

Haury M.

Moflo User Meeting.
Freiburg, Germany.

Jacinto A.

Epithelial dynamics during Drosophila dorsal closure.
Morphogenesis, Growth and Death, London Drosophila Meeting (Genetics Society), University College London, London, UK.

Jacinto A.

Epithelial movement during Drosophila dorsal closure.
Epithelia in Development and Disease, First Symposium of SFB 590, Heinrich-Heine-Universität, Düsseldorf, Germany.

Mallo M.

Hox genes, Hox codes and morphogenesis.
EMBO Practical Course, Zagreb, Croatia.

Parkhouse M.

Protection and diagnosis in human, porcine and bovine cysticercosis.
International Meeting “Molecular and cellular biology of Helminth parasites”, Hydra, Greece.

Rebelo M.

Age-dependent variation of the Notch signaling pathway in lymphoid organs.
Poster. 5th EFIS Immunology Conference, Slovakia.

Sepúlveda N.

Análise estatística da recombinação dos genes gamma do receptor dos linfócitos T gama-delta.
X Congresso Nacional da Sociedade Portuguesa de Estatística. Porto, Portugal.

Sobrinho L.G., Simões M., Barbosa L., Raposo J.F., Pratas S., Fernandes P.L. and Santos M.A.

Cortisol, Prolactin, growth hormone and neurovegetative responses to emotions elicited during a hypnoidal state.

Poster. 10th Meeting of the European Neuroendocrine Association, Munich, Germany.

Tavares A., Wainman A, Domingues C, Alves P, Faria M and Glover D.

Mob1-like proteins in Drosophila melanogaster.

Centrosomes and Spindle Pole bodies, EMBO/EMBL Conference, Heidelberg, Germany.

Vigario A.M. and Pied S.

Murine models of cerebral malaria.

Workshop on “Host pathogens interactions”. Third European congress on tropical medicine and international health. Lisbon, Portugal.

October

Barreto M., Fesel C., Fontes F., Andreia R., Viana J.F., Crespo F., Vasconcelos C., Vicente A.M. and Ferreira C.

Genetics of autoantibody repertoires in Systemic Lupus Erythematosus (SLE).

52nd Annual Meeting of The American Society of Human Genetics, Baltimore, USA. October 2002.

Belo J.A.

Culturas celulares e manipulação genética dirigida.

ISTUTL, Lisbon, Portugal.

Coutinho A.

Thymus and regulatory cells.

XIII Congresso Associazione Italiana di Neuroimmunologia, Moltrasio, Italy

Coutinho A.

Imunologia e Cancro.

Curso “Avanços em Oncologia”, IPOFG, Lisbon, Portugal.

Coutinho A.M., Silva S., Fesel C., Morgadinho, Macedo T.R., Bento C., Marques C., Ataíde A., Miguel T., Oliveira G. and Vicente A.M.

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Demengeot J.
Tolerance and autoimmune diseases.
Workshop, Les Treilles, Nice, France.

Holmberg D.
Hunting for disease susceptibility genes in mice and man.
Malmo General Hospital, Malmo, Sweden

Lenhart B.
Utilização de Animais em investigação/experimentação.
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Parkhouse M.
Protection and diagnosis in human, porcine and bovine cysticercosis.
Centro de Investigaciones en Enfermedades Tropicales, Universidad de Carabobo, San Carlos, Venezuela.

Silva S., Fesel C., Coutinho A.M., Barreto M., Marques C., Miguel T., Ataíde A., Bento C., Oliveira G. and Vicente A.M.
Autoantibody repertoires against brain tissue in autism nuclear families.
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November

Caramalho I.
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Carneiro J.
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Carvalho T.
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Centro de Pesquisas Gonçalo Muniz- Fundação Oswaldo Cruz, Salvador Brazil.

Coutinho A.
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III Congresso de Investigação em Medicina
Univ. Coimbra, Coimbra, Portugal

Coutinho A.
How evolution of development tinkered the emergence of complex behaviours in the immune system.
IISREEC, International interdisciplinary seminar on new robotics, evolution and embodied cognition, FCG, Lisbon, Portugal.

Coutinho A.
Chairman of the Plenary Session: Tolerance
XXVIII Reunião Annual da Sociedade Portuguesa de Imunologia
“Transplantation, Tolerance and Inflammation”
Coimbra, Portugal.

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Faro J., Agua-Doce A. and Gordo I.
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Poster. XXVIII Annual Meeting of the Portuguese Society for Immunology Universidade de Coimbra, Coimbra, Portugal.

Feijó J.A.
Two-Photon Excitation Imaging of Plant Cells.
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Fontes. F
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Poster. XXVIII annual meeting of the Portuguese society of Immunology, Coimbra. Portugal.

Gomes M.G.M.
Epidemiologia da tuberculose.
Conferences “Matemática, Ciência e Arte”. Mathematics Dep., Faculty of Sciences, University of Lisbon, Lisbon, Portugal.

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XXVIII Annual Meeting of the Portuguese Society for Immunology Universidade de Coimbra, Coimbra, Portugal.

Parkhouse M.

Protection and diagnosis in human, porcine and bovine cysticercosis.

Colegio de Veterinarios, Santa Cruz de la Sierra, Bolivia.

Rebelo M.

Role of Notch in the peripheral immune system.

Poster. XXVIII annual meeting of the Portuguese society of Immunology, Coimbra, Portugal.

Rosa G.

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Poster. XXVIII annual meeting of the Portuguese society of Immunology, Coimbra, Portugal.

Santos M.

The role of delta-1 and jagged-1 in lymphoid differentiation.

Poster. XXVIII annual meeting of the Portuguese society of Immunology, Coimbra, Portugal.

Tavares A.

O ser humano em ATGCs.

Clonagem – O genoma humano – Abordagem Biopsicológica, ISTUTL, Lisbon, Portugal.

Vicente A.M.

Autismo – Avanços Genéticos.

Síndrome de Rett e Perturbações do Espectro Autista. Simpósio, IBMC, Porto, Portugal.

Zelenay S.

A novel in vitro assay to measure regulatory T cell activity in small numbers of lymphocytes

Poster. XXVIII annual meeting of the Portuguese society of Immunology, Coimbra, Portugal.

December

Alcobia I., Quina A.S., Neves H., Clode N., Parreira L.
The spatial organization of heterochromatic compartments in human peripheral blood cells is established during hematopoietic differentiation.
American Society of Hematology Annual Meeting, Philadelphia, Pennsylvania USA.

Alves P., Faria M., Godinho S. and Tavares A.
Initial characterisation of the Drosophila MPS1 kinase.
Poster. XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Carvalho L.; Rodrigues G.; Palmeirim I. and Thorsteinsdóttir S.
The role of ectoderm and the extracellular matrix during somitogenesis in the chick embryo.
Poster. XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Carvalho T.
Immunological Tolerance and Inflammation: Regulatory T cell responses to bacterial lipolissacharide.
Department of Genetics, Institute of Biology, State University of Sao Paulo, Campinas (UNICAMP), Brazil.

Coutinho A.
Immunology is not doing well, or is it ?
EMBO Sectoral Meeting Crossroads of Immunology, IGC, Oeiras, Portugal

Coutinho A.
Round Table on *Perspectives NeuroImmunoPsychology*
Curso de Imunologia Clínica , Hospital Geral Santo António, Porto, Portugal

Domingues C., Wainman A., Glover D. and Tavares A.
Mob1-like proteins in Drosophila melanogaster
Poster. XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Faria M., Parelho V., Deak P., Glover D., Gomes R. and Tavares A.
Nobody, a regulatory subunit of the 26S proteasome important for mitotic exit.
Poster. XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Florindo C., Perdigão J. and Tavares A.
Human Mob1-like proteins.
Poster. XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Godinho S., Alves P., Parelho V. and Tavares A.
Cloning and characterisation of the Drosophila Polo kinase kinase, DPLKK.
XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Godinho S., Alves P., Parelho V. and Tavares A.
DPLKK, the Drosophila Polo kinase kinase.
Poster. 42nd Annual Meeting of the ASCB, San Francisco, USA.

Gomes M.G.M.
Daan Mulder Memorial Symposium.
London School of Hygiene and Tropical Medicine, UK

Luz M.; Bajanca F. and Thorsteinsdóttir, S.
Myotome colonisation and early muscle differentiation in normal versus myf5 null mouse embryos: possible role of extracellular matrix.
XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

Neves H., Lúcio P, Alcobia I., Clode N., Parreira L.
Differential role of NOTCH ligands DELTA-1 and JAGGED-1 in human hematopoietic progenitor cell differentiation.
American Society of Hematology Annual Meeting, Philadelphia, Pennsylvania USA.

Wainman A, Domingues C, Alves P, Faria M, Glover, D and Tavares A.
Mps1 kinase and Mob1-like proteins.
XIII Congresso Nacional de Bioquímica, Lisbon, Portugal.

OTHER ACTIVITIES OF THE IGC PERSONNEL

Coutinho A.

1st Meeting of the “Consejo Asessor Externo del Instituto Nacional de Ciências Médicas y Nutricion Salvador Zubiran”, Mexico City, Mexico. (April 2002)

FOCIS 2003 European Planning Committee Meeting, Federation at Clinical Immunology Societies (FOCIS) , Paris, France. (May 2002)

EMBO Council Meeting, Heidelberg, Germany. (June 2002)

Homenagem da Sociedade Brasileira de Imunologia aos Cursos Yakult
Universidade de S. Paulo, S. Paulo, Brazil. (August 2002)

EMBC/EMBO Working Party; EMBO Council Meeting, Heidelberg, Germany.
(September 2002)

Evaluation Committee for the Institut Claude de Preval (IFR30-FR23)
Hôpital Purpan, Toulouse, France. (October 2002)

Juri Prémios Pfizer 2002. (October 2002)

Haury M.

Scientific Expert, Chairman, Mid-term Review EU Programm QoL 1998-2002 Project
QLK2CT 2000-00795, Paris, France. (May 2002)

Mallo M.

Evaluation Panel for FCT's MsC, PhD and Post-doc fellowships. (June 2002)

Parkhouse M.

External expert for appraisal of exotic disease proposals submitted to the Department for
Environmental Food and Rural Affairs (DEFRA), U.K. (December 2002)

Soares H.

President of the Jury to recruit an Assistant Professor to the scientific area of Dietetics of
the Escola Superior de Tecnologia da Saúde de Lisboa. (November 2002)

Abbreviations

BAI	Bolsa de Assistente de Investigação
BIC	Bolsa de Iniciação à Investigação Científica
BTI	Bolsa de Técnico de Investigação
BI	Bolsa de Investigação
CEBIP	Centro de Biologia e Patologia Molecular
CIM	Centro de Inmunología Molecular, Cuba
CNRS	Centre National Recherche Scientifique, France
DGV	Direcção Geral de Veterinária
EAN	Estação Agronómica Nacional
ESTSL	Escola Superior de Tecnologias da Saúde de Lisboa
FCG	Fundação Calouste Gulbenkian
FCUE	Faculdade de Ciências da Universidade de Évora
FCUL	Faculdade de Ciências da Universidade de Lisboa
FCUP	Faculdade de Ciências da Universidade do Porto
FMUL	Faculdade de Medicina da Universidade de Lisboa
FCMUNL	Faculdade de Ciências Médicas da Universidade Nova de Lisboa
FCT	Fundação para a Ciência e Tecnologia
FCTUNL	Faculdade de Ciência e Tecnologia da Universidade Nova de Lisboa
FERNUA	Faculdade de Engenharia de Recursos Naturais da Univ. do Algarve
FMVUTL	Faculdade de Medicina Veterinária da Universidade Técnica de Lisboa
FFUL	Faculdade de Farmácia da Universidade de Lisboa
HDES	Hospital do Divino Espírito Santo, Açores
HUSM	Hospital Universitário de Santa Maria, Lisboa
IBMC	Instituto de Biologia Molecular e Celular da Universidade do Porto
ICBAS	Instituto de Ciências Biomédicas Abel Salazar
IEFP	Instituto do Emprego e Formação Profissional
IHMT	Instituto de Higiene e Medicina Tropical
INSA	Instituto Nacional de Saúde Dr. Ricardo Jorge
INSERM	Institut National de la Santé et de la Recherche Médicale
IPOFG	Instituto Português de Oncologia de Francisco Gentil, Lisboa
ISA	Instituto Superior de Agronomia da Universidade Técnica de Lisboa
ISCE	Instituto Superior de Ciências Educativas
IST	Instituto Superior Técnico da Universidade Técnica de Lisboa
ITQB	Instituto de Tecnologia Química e Biológica
LNIVL	Laboratório Nacional de Investigação Veterinária de Lisboa
PDEE	Programa de Doutoramento no País com Estágio no Exterior, Brasil
PRODEP	Programa de Desenvolvimento Educativo para Portugal
UALG	Universidade do Algarve
UCSC	Università Cattolica del Sacro Cuore, Rome, Italy
UE	União Europeia
UNL	Universidade Nova de Lisboa