

THE ESACT NEWSLETTER

Published by the
**European Society for Animal
Cell Technology**



April 2003

CONTENTS

1. *Editorial - looking forward to Granada!*
2. *Introduction to the Committee Members & Newsletter Co-Editor*
3. *A Word from the Chairman*
4. *General Assembly at Granada*
5. *ESACT in Granada update*
6. *19th ESACT meeting in Harrogate, UK*
7. *Stem Cell Bioreactors*
8. *Cell Factory - European 5th Framework Programme*
9. *15th JAACT meeting*
10. *10th ESGT meeting*
11. *Future meetings*
12. *The Enhanced doctorate at UCL*
13. *New Members and Joke corner*
14. *ESACT Secretariat*

Editorial – Looking forward to Granada!

Dear Readers,

Hola and Buenos Dias! (just practising my Spanish for next month.) In this issue, we have decided to put together some photos of the committee members and their brief bio so that you can recognise and approach us at the next meeting. I'd also like to introduce our new Co-editor, Merlin, who will be helping me make this newsletter more colourful. Both Merlin and I hope to meet with many of you and look forward to getting contributions from members for the next issues. We look upon you all as 'family' and every 2 years we have an opportunity for reunion dinners and long chats, as the Chinese traditionally do, so do write to us.

I am happy to report that a previous contributor, Dr. Ferruccio Messi has received new interest in serum free media for animal cells after his contribution to the April '02 newsletter. This newsletter is and will continue to be your means of communication with each other; we being the facilitators.

A word from our Chairman, Otto, follows. Alain, our Secretary will present matters for discussion at the General Assembly which we hope many members will attend. Quico, who has been most busy with Granada's meeting, writes a short note.

Next, there are 2 articles from Merlin: Stem Cell Bioreactors and Cell Factory. Otto reports on the exciting JAACT and ESGT meetings. Finally we end with announcements of future meetings, an advertisement about the enhanced doctorate at UCL, new members, the joke corner and ESACT Secretariat. Adios.

Chief Editor, Steve Oh

Introduction to the Committee Members & Newsletter Co-Editor

For the benefit of all ESACT members we would like to introduce the committee members so that you will be able to look for and talk with them at the Granada meeting on any matters of interest. Please also welcome Merlin Goldman who has enthusiastically volunteered to be our new Co-editor.

Alain R. Bernard – Secretary



Alain Bernard is currently Director of Manufacturing Process Development for Serono. His main responsibility is the development of production processes for therapeutic proteins derived from recombinant mammalian cell culture technologies. He joined Serono in 1998 and before occupying his current position he was responsible for R&D in the biotechnology department. In that position, he was in charge of generating recombinant proteins for research use, developing screens for organic small molecule discovery and running these screens in high-throughput, automated laboratories. Prior to that move, he worked for 10 years at the Glaxo-Wellcome Institute of Molecular Biology in Geneva, Switzerland. He holds a PhD in biochemical engineering and worked both in the US and Europe on process development and reactor design for a variety of biotechnological processes.

Elisabeth Lindner-Olsson – Treasurer



Elisabeth Lindner holds an MSc in Chemical Engineering (1981) and an MBA (1996). She has worked in the pharmaceutical industry since 1981. Her first 5 years were as a bioprocess scientist with Fermenta AB, Pharmacia Fine Chemicals (now Amersham BioSciences) and Kabi Pharmacia BioScience Centre, on eukaryotic and prokaryotic process development and manufacturing. She has held

senior management positions in Pharmacia. From 1996-2000 she was Director, Process R&D and Senior Director New Product Introduction and member of the Global Supply Europe management team.

Since 2000: Director of Manufacturing at Metcon Medicin AB which is a venture cap company in diabetes research and development. Responsible for sourcing of product from a major site in the UK along with business development and licensing questions.

She is the Founder and Managing Director of BioSource Europe AB, a private consultancy which currently has two business areas: Pharmaceuticals and Recruitment. BioSource Recruitment sells services in Life Science recruitment. BioSource Pharmaceutical sells services in CMC development and product sourcing for biotech and pharma.

Manuel Carrondo – Committee member



Manuel Carrondo is the former Chairman of ESACT and Director of Instituto de Biologica et Experimental & Technologica in Portugal.

Stefanos Grammatikos – Committee member



Stefanos Grammatikos is 36 years old and was, born in Corfu in Greece. He is currently a dual citizen of Greece and Germany. Married and father of two daughters. He studied Chemical Engineering at UC Berkeley (USA) and Biochemical/Cell Culture Engineering at Northwestern University (USA) with Bill Miller and Terry Papoutsakis. After a 1.5-year post-doc at the GBF in Braunschweig (Germany) with Roland Wagner, entered the industrial world (Boehringer Ingelheim Pharma in Biberach, Germany) via a post-doc position in Biopharmaceutical Process Development Cell Culture Technology (back then headed by Wolfgang Noé). In charge of the laboratory of

Cell Biology at BI Pharma (Cell Line Development, cell characterization and cell banking) for 2.5 years and since summer 2001 Head of Upstream Operations for G104, the new biopharmaceutical manufacturing facility of BIP in Biberach (6 x 12,000l bioreactors). The spiritual father of JIN, and very interested and active (within and outside of ESACT) in the area of training young academics for industry and in providing career and job orientation for students and university graduates.

Martin Fussenegger – Committee member



Martin Fussenegger is a group leader in Mammalian Cell Engineering at the Swiss Federal Institute of Technology (the ETH Zürich). He graduated in molecular biology and genetics with Werner Arber in the Division of Microbiology of the Biocenter in Basel (University of Basel, Switzerland; 1992). From 1993-1994 Martin Fussenegger joined the Max Planck Institute of Biology in Tübingen (Germany) where he studied host-pathogen interactions of human pathogenic bacteria and received his Ph.D. with Thomas F. Meyer and Volkmar Braun. These studies were continued in 1995 as a postdoctoral fellow at the Max Planck Institute of Infection Biology in Berlin (Germany) before joining the research unit of Professor James E. Bailey at the ETH Institute of Biotechnology beginning of 1996. Martin Fussenegger is co inventor of several patents and cofounder of the biotechnology start-up company Cistronics Cell Technology GmbH.

Other committee members are:-

Otto Merten (Chairman), Francesc Godia, and Florian Wurm. Christophe Losberger is our ESACT website manager.

Merlin Goldman, our new Co-editor



Merlin Goldman recently completed an MBA full-time at the University of Edinburgh in Scotland where he has lived for

the last 5 years. Previously, he worked as a Fermentation Development Scientist at Serologicals Ltd. – a large scale producer of monoclonal antibodies for diagnostic kits. This involved process design, production and supervision duties. Prior to that he worked for PPL Therapeutics as a Research Scientist characterising Factor IX, a human clotting protein, expressed in transgenic sheep. While he worked there he was Editor of the company newsletter. Prior to working in industry, his PhD at UCL focussed on the influence of process conditions on the quality of a human recombinant protein expressed in CHO cells.

Steve Oh – Your Chief Editor



Steve Oh gained his Ph.D. at Birmingham University, UK with Prof. Al-Rubeai in 1991 and spearheaded the Cell Culture Lab at the Bioprocessing Technology Unit in Singapore, when research was still in its infancy. After 3.5 years and 1 patent which has successfully been commercialised, he joined Pall Corporation as Technical Manager of biomedical and biopharmaceutical products for the Asia Pacific and Australasia regions. Returning to the now much larger Bioprocessing Technology Centre (BTC) <http://www.eng.nus.edu.sg/btc/> in Sept. 2001; Steve is teaching the next generation of M.Sc. and Ph.D. Biochemical Engineers and forging new research territories in the Stem Cell and Fermentation Groups. He is married to Lilian and has a 5 year old son Emmanuel.

A Word from the Chairman:

Dear ESACT-Member,

We are now approaching the next ESACT Meeting and only two months are separating us from this event in Granada/E. I will not talk too much on this event because Francesc Godia, the organizer from the UAB in Barcelona, will give the latest news. However, I am confident and very pleased with the organizational advances of the Granada Meeting. Each meeting is an important event in the existence of ESACT in particular, since

we have changed the frequency of the ESACT Meetings. We meet each other only every second year. These meetings are not only the most important meetings on animal cell culture, biology, and technology in the world, but they are also a very important gathering of (almost) everybody active in this field. The satellite meeting, the five sessions, one workshop, and the posters will be an excellent mixture of science and technology and will present domains such as animal cell culture, optimisation, gene therapy aspects of animal cell technology, and cell based therapies, only to mention some of the topics. A very important happening will be the **General Assembly** which is the only direct and personal way to get a discussion between the members and the Executive Committee. Because of this importance every ESACT member should come and take part in this assembly.

The agenda will deal with usual points, such as the election of the new Executive Committee for the next two years. In addition, the General Assembly has to decide on the modification of the constitution of ESACT. Just to remind you, during the last General Assembly held in Tylösand almost two years ago there was a short discussion on the question of whether ESACT should transform associated membership status (held by all non-European members of ESACT) into ordinary membership status (held by all European members who live and work in Europe). From thereon the Committee has started to discuss the matter of modifying the membership status of the associated members, because it seems outdated and also unfair to maintain two different membership stati, one for Europeans, and a second one for Non-Europeans. Therefore a proposition for modifying the membership status of the associated members will be put forth for the next General Assembly. The modified/amended version of the constitution has been sent together with the ballot forms to all ordinary members in order to vote for this amendment. The vote can be done by post or personally in Granada before the General Assembly. For more immediate information, please take a look at the ESACT Newsletters edition of September 2002 (or our

website: www.esact.org) as well as at the article by Alain Bernard.

Regular readers of these Newsletters are aware of the sad fact that Lisa Hunt, the co-editor of these Newsletters, passed away last year, and ESACT will honour her by nominating a poster session with her name. Meanwhile our Newsletter editor Steve searched and found a new co-editor: Dr. Merlin Goldman. He has a biotech background (he worked in the lab of Mohamed Al-Rubeai in Birmingham) and is working on a publishing venture. Please, take a closer look at the introduction by Steve.

Finally, some news concerning the training network (in the frame of the Marie Curie Programme) to be organized with ACTIP. As the time is short, this project will be organized in the next months and will be submitted in November 2003. For the moment we got interest from several ACTIP members and the main focus of this training network will be established in the next months, before the next ESACT Meeting will take place. During this meeting the final group of participants should be established and the final programme will be finished during the following months.

If there are urgent points to discuss, I would be happy if you contact me; if not, we will meet at the ESACT Meeting in the nice city of Granada.

Otto-W. Merten Crespières, 14 March, 2003

GENERAL ASSEMBLY OF ESACT - Granada, 13th May 2003

The next General Assembly of ESACT will take place, as usual, during the 19th ESACT Meeting in Granada, Spain from May 11th to 14th. This bi-annual General Assembly of ESACT will take place during lunch time (1300 - 1430) on the 13th of May 2003.

The provisional agenda is as follows:

- Chairman's Report (Otto-Wilhelm Merten)
- Treasurer's Report (Elisabeth Lindner-Olsson)

- Secretary's Report (Alain Bernard)
- Elections of the new committee
- Proposal for modification of the constitution - status of "associate" members
- Vote on the new constitution
- Proposition and vote for new honorary members: Prof. Bryan Griffiths and Prof. Caroline MacDonald
- First feedback on the 18th ESACT-Meeting , Granada, Spain (Francesc Gòdia)
- Presentation of the 19th ESACT-Meeting , Harrogate, UK (Rodney Smith)
- Any other business

The nominated candidates for the 4 open positions of ordinary member of the Executive Committee are (by alphabetical order):-

S. Clarke (Lorantis, UK)
J. Coco-Martin (DSM Biologics, NL)
Q. Godia (Barcelona University, E)
S.Grammatikos (BI Pharma, D)
H. Hauser (GBF, D)
R. Wagner (GBF, D)
F.Wurm (EPFL, CH)

Every ESACT member is invited to participate in this assembly, however, in accordance with the rules only ordinary members can vote. The voting form has been mailed to each member in a separate, earlier mailing. The election is your opportunity to influence the composition of the Executive Committee and you should not miss it.

You are also asked to vote for or against the nomination of Bryan Griffiths and Caroline McDonald to the status of honorary members of ESACT. You will find below short biographies of these two outstanding personalities which have clearly contributed a great deal to the advancement of the ESACT society in recent years. To qualify for election, a nominee must be supported by two thirds of the votes. In addition, this specific General Assembly will include a vote to modify the ESACT Constitution which eliminates the status of Associate Member. All members, including those who cannot attend the Granada meeting are entitled to vote by post. **Please**

use the nomination forms that were sent out earlier in April for voting.

Remember that we need your feedback on the composition of the Executive Committee and the proposals above and I encourage every single member to use this unique opportunity to express themselves and contribute to the ESACT's life!

Alain BERNARD, ESACT Secretary

Nomination for honorary membership: B. Griffiths and C. MacDonald

Bryan Griffiths



After gaining a first degree in Microbiology from Brunel University, Bryan obtained a Ph.D. from Queen Elizabeth College, London University working in nutrition and regulation control of Animal Cells. Subsequent work in cryobiology led to his involvement in initiating the European Collection of Animal Cell Cultures, ECACC. Bryan became Deputy Director of the Vaccine Research & Production Laboratory at the Centre for Applied Microbiology Research (CAMR) where, through successive promotions, he reached the top as Director of Research. He has also been associated with the School of Biological Sciences of the University of Surrey where he was a Visiting Professor for over 15 years.

Bryan is the co-editor of two of the bibles in the area, the 6 volume edition of "Animal Cell Biotechnology" and "Cell and Tissue Culture – Laboratory Procedures"; overall, Bryan authored over 130 research papers, co-edited 25 books and is co-inventor of five patents. He was the Founder and Managing Editor of Cytotechnology until 1998. Bryan has strongly contributed to ESACT, where he has been involved since before its existence as a Founder Member (1976), Committee Member (1976-2001), Meeting Secretary (1979,

Oxford), Secretary/Treasurer (1979-1985), Past Chairman (1985-1988) and, currently, responsible for its Secretariat.

Caroline MacDonald



At the University of Glasgow, Caroline attained a first degree in Molecular Biology and a Ph.D. in Biochemistry; continuing to work in Animal Cell Biochemistry and Genetics as a Post-doc in the same University, she isolated the first mammalian arginine transport mutant cell line and developed numerous hybridoma cell lines. Caroline has been one of the pioneers in the generation of novel cell lines by introducing immortalizing oncogenes into differentiated primary cells. Caroline joined the University of Strathclyde as a Lecturer where later she became Senior Lecturer and Visiting Professor to the Bioengineering Unit. She is currently Professor and Head of the Department of Biological Sciences at the University of Paisley and is Assistant Principal responsible for Research and Commercialisation.

She has published over one hundred refereed research papers, co-authored three books and was Editor of The Genetic Engineer and Biotechnologist. Her involvement with ESACT has been long, extensive and varied: she held a position in the Executive Committee for fourteen years (1988–2001), was the Meeting Secretary for Brighton (1991), Secretary Treasurer in 1991-1994 and Chairman from 1994 to 1997.

ESACT in Granada update

The scientific programme is completed and it is **NOW** available on the web <http://www.esact.org/esact2003/science/index.htm>. All authors should have received their notifications, as well as the bursaries. Presently, we are working on the final details of the proceedings book, social programme, and trade exhibition. The

programme will have 5 Keynote lectures, 6 invited oral presentations and 31 oral presentations selected from the abstracts. There will also be 205 posters, half of them belonging to session 5, which has received a lot of attention from the delegates. As in previous meetings, we will organise a poster prize, which this time is provided by Hoffman La Roche.

The total number of delegates is 600. We have received generous support from a lot of companies, and the trade exhibition will be very well attended, with around 60 booths. So, we look forward to have an **exciting** meeting!!

Francesc Godia aka Quico, Barcelona, Spain.

The 19th ESACT Meeting

The UK is pleased and honoured to be the chosen venue for the 19th ESACT meeting. After extensive investigation and visits to many suitable sites in the UK the Organising Committee have chosen Harrogate, North Yorkshire as the venue and in particular the International Conference Centre (www.harrogateinternationalcentre.co.uk) as the most appropriate site for the ESACT meeting. Although it is a long way off please mark up your diaries with the following dates:-

June 5th – 9th 2005
19th ESACT Meeting
Harrogate, UK

Harrogate is situated in the heart of North Yorkshire surrounded by stunning countryside. It has a history as an old spa town dating back to 1571 and still has many Victorian buildings that confirm the history. The town is renowned for its beautiful floral displays and its Valley Gardens. Harrogate is also known as a thriving conference town. The surrounding

area has various sites of interest ranging from medieval city such as Ripon to the Jorvik Viking Centre at York. We are sure that there will be something of interest for everyone's tastes. The Harrogate International Centre will accommodate the ESACT meeting with a warmth that ensures our needs will be met more than adequately. The town is well equipped with numerous hotels and access from Leeds/Bradford International airport. The organising committee would welcome any suggestions from members as to how the meeting could meet your needs further. Come and speak to us in Granada. Please forward all ideas and suggestions to rod.smith@tesco.net. We look forward to seeing you all in Harrogate 2005.

The composition of the organising Committee for the 19th ESACT meeting is as below:

Meeting Secretary

Rod Smith, UK, Dr.Rodders@tesco.net

Scientific Committee

Prof. Jürgen Lehman, University of Bielefeld, Bielefeld, Gr.

Prof. Mohamed Al-Rubeai, University of Birmingham, Birmingham, UK

Dave Venables, Q One-Biotech, Glasgow, UK

Rob Arathoon, Genentech, San Francisco, USA

Glyn Stacey, NIBSC, South Mimms UK

Martin Fussenegger, Inst. Biotechnology, ETH Zürich, CH

Trade Committee

Fiona Godsman, Q One-Biotech, Glasgow, UK

John Bonham-Carter, Adaptive Biosystems, Luton, UK

Organising Committee

Tracey Zecchini, Astex Technology, Cambridge, UK

Glenda Bland, Global Meeting Planning, Cardiff, UK

Stem Cell Bioreactors

Stem cells have received a great amount of publicity lately, due both to recent achievements in their isolation and cultivation and their potential widespread therapeutic uses. Most tissues contain a variety of functional cell types which cannot divide to form new cells. Stem cells are relatively undifferentiated and are able to form these specialised cells in the body. However, they occur in small numbers in adults and have proved difficult to cultivate. If large numbers of cells could be cultivated then they could be used in animal models for testing of drugs and in research and development toward cures for disease.

Neural stem cells were discovered in 1992 and the Pharmaceutical Production Research Facility (<http://ench.ucalgary.ca/~pprf>) at the University of Calgary has done much to advance their culture in bioreactors. Kalos and colleagues have determined optimal growth conditions and culture of these cells in suspension culture for 35 days while retaining normal karyotype and their ability to differentiate into all the major CNS cell types. They grew the cells in aggregates which they believe will be an asset in a perfusion bioreactor system, allowing the cells to be easily retained. Serial passaging of a mixture of aggregate sizes resulted in high viable cell densities, and good control of aggregate diameter. Single cells shed from the surface of these aggregates retained the capacity to generate new aggregates and were able to differentiate into all CNS primary cell phenotypes. In addition, aggregate size could be controlled by monitoring kinematic viscosity of the medium and the power input per unit mass of medium. In sufficient numbers, neural stem cells could be used to treat neurodegenerative disorders such as multiple sclerosis, Huntington's and Parkinson's disease.

The culture of haematopoietic stem cells offers potential applications in bone-marrow transplantation, immunotherapy, gene therapy and the production of blood products. Cell products produced in bioreactors have already reached phase 1 clinical trials. All blood cells are derived from a haematopoietic stem cell. Nielsen's (1999) review of the state-of-the-art of the culture of these cell types concludes that there are still many unanswered questions. For instance, do haematopoietic stem cells age during culture? Haematopoietic stem cells are non anchorage dependent and have been successfully cultured in both serum and serum-free stirred culture systems. A number of bioreactors configurations have been tried: perfusion, stirred micro carrier, hollow fiber,

collagen sphere and airlift packed bed. Dang et al. (2002) compared four standard embryonic stem cell differentiation culture systems which suggest routes for the development of efficient, scalable bioprocesses. Professor Harvey Lodish of the Whitehead Institute, MIT (<http://www.wi.mit.edu>), has recently revealed success in expansion of foetal liver stem cells in culture by incubating them with certain populations of non-stem cells. Stem cell bioreactor expertise is still its infancy but these successes suggest exciting developments ahead.

References

- Nielsen, LK. Annu. Rev. Biomed. Eng., Volume 1, 1999.
- Noll T, Jelinek N, Schmid S, Biselli M, Wandrey C. Adv Biochem Eng Biotechnol 2002;74:111-28
- Dang SM, Kyba M, Perlingeiro R, Daley GQ, Zandstra PW. Biotechnol Bioeng 2002 May 20; 78(4):442-53

Cell Factory - European 5th Framework Programme

In March 2003 activities funded by the European 5th Framework Programme, Cell Factory, were announced. The aim of the funding was to encourage collaborations between both academia and industry in different countries to bring research results more quickly to use, benefiting health, environment, agriculture or industrial produce and in the longer term employment, prosperity and quality of life in general. The average funding was between 1 and 2 million Euro and the average number of partners was 8. From a total of 1296 proposals 78 research and demonstration projects were selected for funding. The Cell Factory has three priority areas

- Improving the diagnostic and therapeutic arsenal for health care.
- Improving environmental sustainability.
- New biological and biotechnological products and processes for agro-industry, agri-food and high value added chemicals.

The activity of partners is shown below:

Activity Type of Partners	%
Higher education	46
Industry	18
Research Institutes	27
Other	9

The applications were not exclusively open to EU parties.

	Participants	%
EU Countries	8289	87.1
NAS Countries	536	5.7
Other associated countries	597	6.3
3 rd countries	89	0.9
Total	9511	100

The Accompanying Measures scheme which supplemented the activities of the Cell Factory were intended to support a wide spectrum of initiatives, from meetings on individual scientific matters to studies of a wider societal interest. The Cell Factory supported a total of 53 Accompanying Measures throughout the programme. The budget contribution by the EC amounted to over 3.5 million Euros. One of these was a special session within the tenth European Congress on Biotechnology, which was organised by the European Federation of Biotechnology and held in Madrid in July 2001 (<http://www.efbweb.org/topics/ecb10.htm>). A second initiative was BioBiz, a series of seminars/workshops, which have been held in several cities within the European Union and associated states (<http://europa.eu.int/comm/research/biotech/biocour1.html>). They have attracted over 200 scientists who are interested in entrepreneurship. They state that 25 companies have been started-up or developed partially as a result of the attendance of scientists to those workshops.

In addition, Marie Curie Fellowships were created to support the training and mobility of researchers throughout Europe. This scheme is particularly focused on the provision of post-doctoral level research training. Over 150 have been awarded. These can be of two types: Industry Host and Training Site Fellowships. The first, Industry Host, is a legal entity whose principle activity is of an industrial or commercial nature. The second, Training Site, is a clearly identifiable part of an institution, such as a research group e.g. in a university or interrelated research groups within the same institution. The UK, France, Spain and Denmark have captured the majority of host and training sites. Further details can be found at http://europa.eu.int/comm/research/quality-of-life/cell-factory/volume2/index_en.html.

Information about Framework 6 is can be found at <http://fp6.cordis.lu/fp6/home.cfm>.

Merlin Goldman, Co-editor

merlin@magnetical.com

Report on the 15th JAACT-Meeting

The 15th JAACT Meeting was held in Fuchu (Tokyo, Japan) on 11-15 November 2002. More than 200 delegates from 17 countries attended and about 100 lectures and posters were presented. The meeting had plenary lectures, symposia, oral sessions, seminars, technical workshops and ESACT special lectures.

Recent Advances in the Materials of Reconstitutive Therapy and Tissue Engineering:

J. Myllyharju (University of Oulu, Finland) presented a new method of producing recombinant human collagen from *Pichia pastoris* and SF9. The optimisation of the expression plasmids, enzyme coexpression and culture conditions improved yields from 15mg/l to 2g/l in 2 l bioreactors using *Pichia pastoris*. The SF9/baculovirus expression system achieved 50 mg/l. Pepsin was used to harvest the collagen because it is not secreted although a pepsin-free process is planned. Matrigel is currently derived from the Engelbreth-Holm-Swarm (EHS) Tumor and used as a basement membrane for adherent cells. A recombinant form would be beneficial and Y. Kitagawa (Japan) described their work to develop human laminin, the major component of Matrigel, in yeast. Laminin consists of 3 different types of chains and presently 45 different combinations are known. Currently, the yeast system suffers from transport problems to the ER but the *Drosophila* hemocyte cell line K-147 may be more suitable because it is able to produce large quantities of laminins and collagens.

The group of Y. Hayashi (Aichi Medical University, Japan) expressed recombinant laminin-8 and 10 in 293 cells and were able to obtain about 1mg from 10 l of culture following purification. However, this represents a hundredth of the quantity possible using EHS cells. This is probably due to the fact that EHS cells contain high levels of chaperones and proteins involved in the secretion machinery. He suggested that parietal endodermal cells would be the best host for the production of laminins since they produce naturally high quantities of laminins.

M. Nomizu's group (Hokkaido University, Japan) has systematically screened oligopeptides (12AA, 4AA overlap) from laminin for biological activity. They have identified 3 peptides with different biological functions: cell binding-activity: AG73 (RKRLQVQLSIRT, proteoglycan-mediated cell

attachment) and RGD-A99 (AGTFALRGDGNPQG, integrin-interaction).

S. Enosawa (National Research Institute for Child Health and Development, Japan) presented a new artificial liver model. A hamster GS cell line expressing HepG2 was cultivated in a circulatory flow bioreactor (CYGNUS). It demonstrated ammonia removal activity of 15% in comparison to porcine hepatocytes. The cell line could be cultured for more than 80 days achieving a titre of 4×10^9 cells. The expression of other genes was possible e.g. cytochrome 340 which were able to clear lidocaine. The activity was about 1.7x higher (430pmol/min/mg-protein) than that observed in human hepatocytes. In order to use these cells, a dual phase reactor will be necessary with the hepatic side (recombinant GS-cells) + bloodside (blood flow).

Production of biologicals, functional cell lines, immunologicals, monoclonal antibodies and vaccines and gene therapy

M. Carrondo (Portugal) presented work on the production of biopharmaceuticals using genetic and chemical synchronisation approaches. D. Jayme (USA) presented evidence that for certain cell lines a fed-batch system using low glucose and glutamine concentrations achieved better cell growth and higher product titres. Some cell lines preferred an equilibrated sugar composition (glucose/fructose/mannose) for optimal growth and production. This did not seem to be necessary for CHO cells, which grew in 0.1g/l of glucose. The glycogenic amino acids were the most heavily utilised and it was found that the most successful strategy was to feed Asn, Gln, and Cys which were metabolised to NH_4^+ .

T. Fletcher (USA) indicated that the presence of glutamine in SF-Media with and without plant hydrolysates increased the sialylation of expressed recombinant proteins. G. Schmid (Switzerland) reported on their large scale non-GMP production of recombinant proteins. Using 10-100 l reactors and 50-100 mg of plasmid they produce 10-100 mg of recombinant protein every 1-2 weeks. Their optimal transfection cell number is $5-6 \times 10^5$ c/ml. D. Barnes (USA) presented work on establishing fish cell lines (*Fugu* or *Danio*). In contrast to mammalian primary cells which undergo senescence, when not transformed, many fish cells do not show senescence and keep a normal karyotype. In the case that they do not proliferate continuously, immortalisation or transformation via transfection methods is possible. Fish cells show a high and stable TERT expression.

T. Banu (Japan) described research on human articular chondrocytes which show only differentiation and high collagen II expression when cultured on poly-glycolic acid (PGA). The collagen expression is weaker when grown on other polymers. Aggrecans are also highly expressed, when chondrocytes are grown on PGA. H. Kallel (Tunisia) presented process developments concerning the production of a veterinary rabies vaccine using BHK-21 cells. Three g/l of Cytodex III carriers were used allowing the production of 3×10^6 cells/ml. The cultures were run in perfusion mode in order to maintain the residual lactate concentration at relatively low levels (~ 15 mM). The process was scaled up to 20 l providing 2×10^8 FFU/ml or 40000 doses per run.

F. Wurm (Switzerland) gave a talk on large scale (100 l) transient gene expression. They use 293 cells cultivated in BioWhittaker's 293G medium and obtain single cell cultures. The transfection efficiency (using Ca^{++} - Phosphate) is up to 100%, and 1 mg of plasmid DNA is used per litre. Protein yields ranged between 1 and 40 mg/l. S. Takuma (Japan) studied the effects of glucose and CO_2 concentrations on CHO cell physiology in high density perfusion culture. They used the Sonosep-System for cell retention and an YSI-8500 probe to determine CO_2 concentration. Under high glucose conditions (14.1 ± 0.1 mM), the glucose was mainly converted to lactate, and the lactate yield from glucose was not affected by CO_2 concentration up to 30%. The growth rate declined (from 0.021 to 0.009 hr^{-1}) as the CO_2 concentration increased to 30%, and the cell specific antibody productivity decreased slightly (by 6%, $p=0.05$).

M. Takagi (Japan) studied the effect of static pressure on the production of hGM-CSF by CHO cells. Cultivating the CHO cells in IMDM + 10% FCS, the authors have tested a pressure range of 0.1-0.9 MPa by keeping the $p\text{O}_2$ (0.005 MPa) and the $p\text{CO}_2$ (0.021 MPa) constant. Although the specific growth rate of CHO DR1000L4N decreased slightly as the static pressure increased, the specific production rate of hGM-CFS increased from 1.23 to 1.62×10^{-13} mg/cell/h in proportion to be the pressure increase. The expression levels of hGM-CSF mRNA in CHO cells at 0.1 and 0.9 MPa were 0.33 ± 0.06 ($n=8$), 0.4 ± 0.07 ($n=8$) in arbitrary units, respectively, which were significantly different ($p < 0.05$). In conclusion a high static pressure stimulates the expression of hGM-CSF mRNA in CHO cells, which increases the production of hGM-CSF. These data suggest that the expression of hGM-CSF mRNA is performed through the PKC pathway in CHO cells. However,

hGM-CSF production in response to a high pressure may be PKC-independent.

G.M. Lee (Korea) developed an apoptosis-resistant DHFR CHO cell line. They cloned the anti-apoptotic gene (bcl-2) into the cells and could efficiently use Na-butyrate for increasing the productivity of a MAb without inducing apoptosis at the same time. In the presence of Na-butyrate, these cells showed prolonged growth duration, 5-fold better product accumulation and an increased productivity in comparison to the bcl-2-negative control cells.

O.-W. Merten (France) presented the advantages of using fructose instead of glucose as a sugar source for cell growth and retroviral vector production by different producer cell lines. In fixed bed reactor cultures, it could be shown that TEFLY as well as PG13 cells produced 4 to 8 times higher cumulative infectious vector titres than in glucose supplemented cultures. Whereas the TEFLY cells showed a slightly increased cell growth in the fructose supplemented media, the PG13 cells grew 3 to 4 times slower in the fructose cultures. In spite of this, the vector accumulation was increased by a factor of 8 due to the increase in the specific vector production by a factor of about 35. J. N. Warnock (UK) showed work to optimise the production of retroviral vectors using TEFLYRD-lacZ cells. They observed an optimal vector production at a serum concentration of 2.5% and cell growth was only negatively affected when the FCS concentration was reduced below 1%. With respect to vector decay rates, the vector was somewhat more stable in 10% serum ($k=0.114/\text{h}$) than in 1% serum ($k=0.15/\text{h}$). In semi-continuous cultures they observed a 42% increase in the vector production at a serum level of 2.5% in comparison to 10%. The virus production was positively affected by an increase in the cell growth.

Cell Regulatory Functions of Food Factors and Nutritional Resources with special reference to health promotion and disease prevention

J. Hayashi (Japan) reported on the isolation of a melanin synthesis inhibitor from a marine red alga (*Laurencia nipponica*) which might be of interest in whitening cosmetics for reduction/prevention of skin pigmentation. There were several lectures on the use of electrolysed reduced water (ERW) for different applications in cell culture but also for potential future uses for the treatment of diseases. T. Hamasaki (Japan) found that ERW produced a reduction in lipid peroxidation (60% at 180 h in comparison to control experiments) compared to vitamin E (100-10% lipid peroxidation at

concentration increasing from 0 to 300 μM over 150h). The reducing activity seems to be due to platinum colloids originated from the production of the reduced water. T. Komatsu (Japan) compared M-acetylcysteine (MAC) and ERW with respect to cell growth and differentiation. Both induced differentiation in K-562 human leukemia cells. T. Kashiwagi (Japan) presented results concerning use of ERW to reduce cell death in neuronal cells (PC-12) induced by glutamate. ERW inhibited the deadly effect of H_2O_2 at concentrations of 100 and 200 μM . J. Ye (Japan) presented the positive effects of ERW on the down-regulation of MMP-2; in addition, ERW reduced Matrigel invasion by HT1080 cells in the presence or absence of H_2O_2 .

Glycoengineering in animal cell technology

K. Ajisaka (Japan) presented chemoenzymatic approaches for the preparation of oligosaccharides and glycopeptides in vitro as a first step in the synthesis of glycoproteins. The procedure required three steps: (1) step by step elongation of sugar chains of the peptide using β -Galactosidase from *Bacillus circulans* expressed in *E. coli*, recombinant sialyltransferase. (2) One step transfer of the N-linked sugar chain using Endo M which hydrolyses the linkage between GlcNAc and GlcNAc. (3) One step transfer of O-linked sugar chains, using endo N-acetyl- α -D-galactosaminidase which hydrolyses the linkage between GlcNAc and Ser in O-linked glycopeptides and can transfer this sugar chain on the OH-group of a Ser-residue of other peptides. S. Koizumi (Japan) presented the biosynthetic production of oligosaccharides by coupling of engineered bacteria. By expressing glycotransferase from other bacteria in *E. coli* and by providing the indispensable sugar nucleotides, large scale bioprocesses can be used to produce, for instance CDP-choline.

Y. Jigami (Japan) presented work on protein glycosylation in yeast cells. The disruption of OCH1, MNN1 and MNN4 genes of the yeast, which are responsible for the mannan structure of yeast derived glycoproteins (leading to an absolute incompatibility for a human use), leads to the human type of high mannose sugar chains (M8), which then allows the application of such proteins for human treatment. M. Asanagi (Japan) reported on the remodelling of sugar residue structures of recombinant proteins expressed in CHO cells. Intracellular competition between β -1, 4-galactosyltransferase with N-acetylglucosaminyltransferase IV and V was controlled allowing the expression of highly branched structures. T. Sato (Japan) presented work on the biosynthesis of oligosaccharides using

saccharide primers. Cultivating several cell lines in SF media supplemented with lactose or N-acetylglucosamine-bearing saccharide primers he was able to establish an oligosaccharide library. For instance, with COS 7 cells it was possible to produce GM3, GM2, GM1 and GD1 α (glycosphingolipids), when Lac-Cer (best chain lengths C-12) was used as a primer.

The **Murakami Memorial Lecture** on the Manzanar Project was given by Gordon Sato (Lifetime Achievement Award in Animal Cell Technology) one of the fathers of SF-Media and who did a lot of early work to establish the needs of animal cells with respect to growth factors, hormones, etc. This project involves the reforestation of the seaside of Eritrea with mangrove trees.

Posters

The study by A. Okamura (Japan) demonstrated that the addition of ascorbic acid-2 phosphate, EGF and HGF stimulated ammonium metabolism and albumin secretion and furthermore enhanced the proliferation of porcine hepatocytes. S.O. Hwang (Korea) showed that the specific production rate of thrombopoietin by recombinant CHO cells could be increased by elevating the expression level of the chaperone Erp57. M.S. Kim (Korea) used a biphasic culture strategy for optimising MAb production by recombinant CHO cells. Hyperosmolarity was good for increasing the specific production of vectors, but reduced cell growth. By separating the culture into two osmotic phases (294 mOsm/kg and 522 mOsm/kg) the specific production rate was increased from 2.1 to 11.1 μg of MAb/ 10^6 cells per day. S.H Kim (Korea) observed an increased specific production rate of EPO by recombinant CHO cells when the culture temperature was reduced from 37°C to 33°C (the optimal temperature for getting the best titre) and to 30°C (highest production rate, but low titres due to growth arrest). T.M.P Keijzer (Holland) presented a 1000 l ultrasonic retention system, which is composed of 5x200 l systems. This system was tested using a 1000 l yeast perfusion culture. The highest allowable perfusion rates were 1500 l/day for a biomass concentration of 10g/l and 1200 l/day for 20g/l. T. Sakai (Japan) presented a small scale fixed bed reactor system based on the use of hydroxyapatite modules. Cell densities of 10^8 cells/ml sheet were obtained.

O. -W. Merten

Report on the 10th ESGT-Meeting, Antibes/F, 13th to 16th of October 2002

The 10th ESGT meeting was organised in Antibes/F at the Côte Azur and the weather situation was quite good, allowing a swim in the Mediterranean. The meeting was organised by D. Klatzmann and took place in the Congress Center of Antibes. About 650 people participated and 23 exhibitors took part. The exhibition was organised together with the poster exhibition and the coffee breaks and wine and cheese tasting in the evening in the same place in a marquee outside the congress center.

The scientific part had a duration of three days (Sunday afternoon – Wednesday noon), and was separated into at most two parallel sessions and two poster sessions (there were approximately 360 posters). In contrast to previous meetings, the topics of the sessions were application/disease oriented and not vector oriented.

Session on cancer 1 – Immunotherapies

P. Lowenstein and M.C. Castro (USA) presented the stimulation of immune responses against tumors located in privileged sites (brain glioblastoma). This cancer constitutes a very serious disease and the life expectancy is very low, because it leads to death in more than 95% of the patients within 6-12 months. The immune response is not very efficient because of the lack of afferent antigen presenting dendritic cells. The actual gene therapy protocol making use of the expression of HSV1-TK and gancyclovir is not sufficient in animal models because no dendritic cells are recruited (brain = immuno-privileged site). In order to increase the efficiency, a combined treatment was developed, making use of the HSV1-TK/gancyclovir approach for killing the cancer cells as well as for exposing the tumor antigens to dendritic cells which have been recruited by the concomitant expression of cytokines. This approach works in animal models leading to a novel development of gene therapies for brain glioblastomas. Gut-less adenoviral vectors were much more efficient than normal adenoviral vectors.

D. Godelaine (Ludwig Inst. for Cancer Research, Brussels/B) presented a talk on the CTL responses of melanoma patients vaccinated with the MAGE-3 antigen which is one of the cancer germ line genes located on the X-chromosome. This antigen is expressed in many tumours (e.g. 56% of melanomas), but not in normal tissue (except in testis). In vaccinated patients, showing tumor regression and/or clinical responses, a CTL

response was detected in the blood of the patients. This response was monoclonal (for patients immunised with the MAGE-3A1 peptide and recombinant ALVAC) or polyclonal (for patients vaccinated with dendritic cells pulsed with MAGE-3A1 peptide).

L. Sanz (Hosp. Puerta de Hierro, Madrid/E) presented the use of single chain antibodies for blocking functionally active sites of extracellular matrix. Such antibody fragments binding to laminin block the morphogenesis of endothelial cells into capillary-like structures, thus blocking angiogenesis. The direct delivery of plasmid DNA encoding this antibody gene significantly delayed the growth rate of established murine tumors. Stable transformation of human fibrosarcoma cells with the gene significantly inhibited their implantation and growth.

Plenary session on Basic Science Perspectives: Issues for Cell and Gene Therapies of Tomorrow

G. Cossu (Stem Cell Res. Inst., DIBIT, Milano/I) presented the actual state of stem cell research and the problems associated with their use.

N. Taylor (Inst. Genet. Moleculaire, Montpellier/F) presented a view on gene transfer to T-cells and the fact (problem?) that there seems to be some sort of homeostasis signifying that the total number of T-cells stays constant within the T-cell compartment. However, this means also, that after the establishment of memory T-cells, the number of T-cells equivalent to that of memory T-cells has to die/is eliminated.

ESACT-ESGT session on Regulatory Affairs, Safety issues, and Cell Technology

The ESACT-ESGT session, which took place in parallel to the satellite session organised by Prime Biotech, was well visited, indicating that questions concerning regulatory affairs, safety issues, and cell technology became more important to the participants of ESGT Meetings.

L. Tsang (MCA, London/U.K.) presented an update on European Guidelines on gene transfer medicinal products, dealing with definitions on qualitative and quantitative composition of viral gene transfer products, virus shedding, lentiviral vectors, germ line integration, etc. A common European Position is in preparation for agreement in view of the International Conference on Harmonisation in Washington/DC.

H. Merget-Millitzer (MainGen Biotechnology GmbH, Frankfurt/D) presented the retroviral transduction of peripheral blood stem cells in a

GMP compliant setting for a phase I gene therapy trial. In order to reduce adventitious contaminations during production and transduction of peripheral blood stem cells, MainGen has developed and validated a closed bag system. The essential difference between the gene therapy trial of Prof. Fischer Hôp. Necker Paris/F) is the fact that in Germany the transduced cells have to be frozen and are only allowed to be injected after all safety tests have been successfully passed. By using a vector containing the coding sequence of gp91^{phox} - one essential sub-unit of phagocyte NADPH oxidase - they obtained on average 50% genetically modified CD34⁺ stem and progenitor cells. After having performed pre-clinical investigations using the NOD/SCID xenotransplantation model, a gene therapy trial of X-CGD has been authorised by the regulatory authorities in Germany and started in June 2002. For the first patient (> 18 years) the transduction efficiency was 50% and 44% of the stem cell population was gene marked.

Y. Takeuchi (UCL, London/U.K.) presented a lecture on the identification of human receptors for pig endogenous retrovirus, which is of high relevance in view of the xenotransplantation of pig-derived tissues and organs. His group has identified two related human receptors for PERV-A encoded on chromosomes 8 and 17. Homologous equally functional virus receptors were identified on baboon and porcine cells, however, the murine homologue was inactive. Expression of the human PERV-A receptors is widely spread in human tissues, including peripheral blood mononuclear cells. Whereas PERV-C can only be transmitted to porcine cells, PERV-A and PERV-B can also be transmitted to 293 human cells.

Session on Dermatology, Systemic Diseases and Endocrine System

G. Meneguzzi (INSERM U385, Nice/F) presented the pre-clinical and clinical development of gene therapy in epidermolysis bullosa (EB). There are different forms of genodermatoses depending on the gene defect involved. The dystrophic form of EB (DEB) is associated with mutations in the collagen VII gene. Pre-clinical tests using human DEB cells expressing recombinant collagen VII secreted a correctly folded and biologically active protein that is deposited at the basement membrane of artificial skin reconstructed in vitro. With respect to the deficiency in laminin-5, it could be shown in in-vitro tests, that keratinocytes defective in this protein, could be genetically corrected showing a fully restored cell adhesion machinery. At present the first phase I/II clinical trial of ex vivo gene

therapy is performed in selected EB patients in Italy.

T. Magnaldo (CNRS, Villejuif/F) presented advances towards a cutaneous therapy of the cancer prone/DNA repair deficient xeroderma pigmentosum (XP). UV induced DNA damages are not repaired and leads sooner or later to the development of skin cancers. The transduction of XP-C keratinocytes with retroviral vectors transferring a functional XPC gene, lead to a fully reversal of the non functional gene, i.e. DNA repair deficiency, UV-cell mortality. These repaired cells show a completely normal behaviour as known for normal keratinocytes (normal lasting of p53 expression after UV-exposure, normal level of β 1-integrins). These data allow a better understanding of the disease, and opens a realistic perspective for gene therapy.

F. Bosch (UAB, Bellaterra/E) and N. Giannoukakis (University of Pittsburgh/USA) described approaches for the treatment of diabetes 1, which is due autoimmune destruction of the insulin producing β -cells. The group of F. Bosch works on different approaches. The first one consists in inducing the regeneration of β -cells via growth factors (IGF-I), which can prevent autoimmune destruction of new islets. This approach was tested by expressing IGF-I in β -cells of streptozotocin treated transgenic mice, which counteracts cytotoxicity and insulinitis. The mice showed gradually a normalisation of all metabolic parameters. The second approach is the genetic manipulation of skeletal muscle (replacement therapy) and pancreas (regeneration therapy). The main objectives are: i) to study the skeletal muscle capacity to decrease diabetic hyperglycemia by producing mature insulin and increasing glucose uptake, and ii) to establish a new therapeutic approach to type 1 diabetes by endocrine pancreas regeneration. The group of Giannoukakis used an immunological approach to prevent destruction of the β -cells. The treatment of non-obese diabetic (NOD) dendritic cells (DC) with antisense oligonucleotides targeting the CD40, CD80, and CD86 primary transcript, achieved significant suppression of CD40, CD80, and CD86 expression in response to LPS in culture. Engineered DC promoted the suppression of NOD T-cell proliferation and a significant decrease in TH1-type cytokines in the presence of NOD islet antigen. A single injection of engineered NOD DC achieved significant long-term prevention of diabetes onset in NOD recipients and promoted a significant increase in CD4 CD25 T-cells. The protection was transferable to NOD-SCID mice.

Session on vaccination

P. Pumpens (Biomedical Research and Study Center, Riga/Latvia) presented the construction of VLPs (virus-like particles) as molecular carriers for vaccination purposes. The strategy is to get an ordered structure and the addition of epitopes for exposing to the immunessystem. VLPs are non-infectious, they do not contain a genome, thus they cannot replicate (empty particles). They can be produced in *E. coli* (= preferable because easier and cheaper), yeast and other eukaryotic cells. The most popular example is the Hepatitis B virus core (HBc) having an icosahedral form. In contrast to HBsAg, HBcAg can be expressed in all existing expression systems. The HBcAg is a dimer and they form spikes out of 4 α -helices. It consists of 183 amino acids, the amino acids 144-183 are necessary. The tips of the spikes (amino acids 74-90) which are particularly immunogenic can be exchanged against other amino acid sequences (maximal length of foreign sequence: 200 amino acids). Uniform and mosaic structures are possible, as well as multi-epitope presentations. There is no need for adjuvants. So, HBcAg particles were used to display immunodominant epitopes of HepB, HepC, HepE, human rhino, papilloma, hanta, and influenza virus, and many other viruses. As other functions can be integrated, it is thinkable that artificial vectors capable of packaging of RNA or DNA might be possible. The introduction of the RGD-sequence will present a possibility to target special cells. For more information, it is advised to contact the author: paul@biomed.lu.lv.

P. Liljeström (Karolinska Inst. Stockholm/S) presented DNA/RNA replicon vectors for vaccination, and in particular, the Semliki Forest Virus system. This system can be used for the vaccination against viruses but also against tumors.

F. Tangy (Institut Pasteur, Paris/F) presented the use of measles vaccine as vector for preventive vaccination of children against AIDS. Measles virus is an enveloped RNA virus and is used as a live attenuated virus for vaccination. Measles virus can be stably produced at 37°C on CEF cells, whereby no mutations are observed. Even in the case of pre-existing immunity, recombinant vaccines are working because the virus infects cells, replicates and leads thus to an immune response.

A HIV vaccine based on this technology should work at least with respect to an antibody response. In order to achieve this, different SIV and HIV genes were introduced into the measles virus: gag, pol, env, tat, rev, and nef. Using a helper cell based rescue system, the recombinant viruses were stably

produced over several passages. Immunisation of animals with these viruses led to long lasting humoral and cellular immune responses against measles and the products of the transgenes. This vaccine seems to be a good candidate for paediatric vaccination against HIV.

E. Cancellotti (Univ. Padua/I) presented the use of non-viral gene transfer to deliver DNA into epithelial cells. This vector is based on the use of a fusion protein between the *E. coli* heat labile enterotoxin and a 27-poly-lysine peptide and binds to and complexes DNA. Such complexed DNA reaches the cytoplasm within 2 hours and the nucleus within 6 hours. Furthermore after 2 hours of incubation 10-15% of the cells were transfected. Using such a complex with DNA encoding the glycoprotein D2 of herpes simplex type 2 for vaccination via mucosal administration, a strong systemic and mucosal immune response was observed in vaccinated animals. The immune system was much more triggered towards a Th1 response than when a naked plasmid vaccine was administered.

B. Bellier (CNRS-UMR7087, Paris/F) presented the use of recombinant retroviruses for antigen display in view of the development of a new vaccine. They have modified the env protein of the recombinant replication incompetent retrovirus. They have inserted a 9 amino acid sequence (GP³³⁻⁴¹) of a cytotoxic T-lymphocyte epitope derived from lymphocytic choriomeningitis virus glycoprotein at the amino terminus of the MLV 4070A-env protein. Three envelopes expressing GP³³⁻⁴¹ flanked by 0, 3, or 5 amino acids were generated and used to pseudotype replication-defective retroviral particles transducing GFP. The +3 type was not correctly processed and incorporated into the virions. The other constructions led to equal or even better (+5) infectivity. It could be shown that these modified env proteins were correctly processed and presented in the context of major histocompatibility complex class I (H-SD^b), because cells transiently expressing the +0 or +5 4070A- GP³³⁻⁴¹ envelopes could be lysed by autologous GP³³⁻⁴¹-specific cytotoxic T-lymphocytes (strong CTL response). It could be shown that the immune response induced against GP³³⁻⁴¹-expressing tumor cells (B16F10) by using recombinant retroviral vectors was extremely efficient in absence of any adjuvant.

Session on Clinical perspectives: learning from experiences in relevant clinical fields

D. Houssin (Etablissement français des Greffes, Paris/F) gave an interesting comparison of the development and final application of organ transplantation with respect to the actual developmental state of gene therapy.

J. Bell (London/U.K.) gave an overview on the use of imaging techniques (MRI and PET), mainly in a clinical environment.

M. Cavazzana-Calvo (INSERM/ U 429 Hopital Necker, Paris/F) gave an update on the gene therapy trial for γ c chain X-SCID. She presented the actual situation of patient 4 who shows a lymphoproliferative syndrome after 30 months. The patient was treated (chemotherapy) against these proliferative T-cells at the end of August 2002 and is doing well. Altogether the transduced T-cells showed 1 copy/cell and 40 different integration sites were observed. Professor Cavazzana-Calvo presented all molecular biological tests and biological tests performed on the patient in order to figure out the reasons for this proliferative disease. Presently, the other patients are screened. Due to this problem which arrived during the γ c chain X-SCID trial, all MLV-based gene therapy trials concerning the transduction of lymphocytes or their precursors are suspended for the moment.

Posters

R. Sakuma (Inst.Med.Sci., Univ. of Tokyo/Jp) presented cautions against the general use of central polypurine tract (CPPT) and central termination sequence (CTS) in HIV type 1 vectors: recently cPPT/CTS was found to enhance nuclear import of viral complementary DNA and transduction efficiency when introduced in HIV-1 vectors. The aim was to examine if HIV-1 vector cPPT/CTS itself express transgene arrays more efficiently than those without CPPT/CTS. The insertion of cPPT/CTS (282 nt) increased the production of vector particles in terms of p24 and viral RNA, but vector titre per unit p24 decreased in the vectors carrying cPPT/CTS (282 nt).

cPPT/CTS did not always enhance gene transduction efficiency. The insertion of cPPT/CTS increased luciferase activity independent of the orientation, so cPPT/CTS might have transcriptional enhancer activity on heterologous promoters, but when LTR presented just upstream of cPPT/CTS, it decreased luciferase activity. cPPT/CTS inhibit transcription in context of HIV-1 vectors. Upstream HIV-1 LTR was involved in this inhibition even in the absence of Tat. The enhancing effect of cPPT/CTS would require i) optimal promoter/gene partners, ii) optimal length.

The 173 nt increases transduction efficiency. The 282 nt decreases transduction efficiency. An enhancing/inhibitory effect on the insertion of cPPT/CTS should be examined for each vector.

F. Salvatori (MolMed, Milano/I) presented a QRT/PCR method for quantifying the relative frequency of the spliced form and therefore non-functional form of HIV-TK in biological samples.

J. Stadler (Amersham Biosciences Europe, Freiburg/D) presented a new purification process of supercoiled plasmid DNA, which can be easily transferred to GMP-conditions. This process consists of 3 chromatographic steps: RNA-removal and buffer exchange on Sepharose 6 Fast Flow (prep-size exclusion chromatography); capture of supercoiled plasmid DNA on Plasmid Select (thiophilic aromatic chromatography); polishing and concentration on Source 30Q (anion exchange chromatography). The final product characteristics in a non-GMP environment are: ccc plasmid DNA: > 97%; plasmid (μ g/ml): up to 850; endotoxins (E.U./mg plasmid DNA): < 10; RNA (μ g/mg plasmid DNA): < 0,2; genomic DNA (μ g/mg plasmid DNA): <2; proteins (μ g/mg plasmid DNA): < 3.

J. Urthaler (Boehringer Ingelheim Austria, Wien/A) presented a second protocol for the purification of plasmid DNA for therapeutic use, after alkaline lysis of bacteria, HIC was used to separate the majority of the impurities (open circular DNA, RNA, proteins) from the supercoiled plasmid DNA. The second step is an anion exchange chromatography (CIM short monolithic column) and further separates and concentrates suspended pDNA. The third step (size exclusion chromatography) guarantees the removal of residual low molecular weight impurities and salts. The final bulk is obtained after adjustment of the desired concentration (> 10mg pDNA/ml possible) and filtration (0.2 μ g). The purity is >99% and the homogeneity is >98%. No RNase is used in this protocol.

P. Cruz (IBET, Oeiras, Portugal) presented a study on degradation kinetics and physico-chemical properties of MLV-vectors. It is known that a given retroviral vector produced in different cell lines or at different temperatures exhibits different properties, including its temperature stability. They envisaged the use of Electron Paramagnetic Resonance (EPR) to evaluate the physical-chemical properties of the membrane of retroviral vectors with amphotropic and GALV envelopes produced in TE Fly cells at 32 and 37°C. The use of EPR

provided data that clearly showed that the viral membrane changed both with the producer cell line and the production conditions, encouraging future research on the improvement of the characteristics of retroviral vectors.

X. Lu (VIRxSYS Co., Gaithersburg MD/USA) reported on a large-scale transfection method (multitray: 10 layers of 293 cells) for the production of lentiviral vector for the treatment of AIDS. He used a safe 2-plasmid system, consisting of the gene transfer vector and the packaging construct. The helper construct leads to the expression of the structural and envelope protein for HIV vector production; however, in order to improve the system, they deleted HIV accessory genes and separated the ORFI of the structural and env genes by 2-poly-A-sites and a transcriptional pause site. In addition, some regions of the construct were degenerated to decrease the amount of homology with the vector construct. Final vector titres of more than 10^9 TU/ml and the yields of about 10^{11} TU per lot were obtained. No RCL were detected, primary CD4 T cells could be efficiently transduced (>90%). Challenge tests with wild type HIV showed that the transduced cells were able to inhibit viral replication by more than 2 log units of p24.

U. Bantel-Schaal (German Cancer Research Center, Heidelberg/D) could show that AAV-5 is differently routed depending on the target cells. Whereas, in HeLa cells, AAV-5 is routed to the Golgi area, large amounts of AAV-5 are found in the lysosomes of fibroblast cells. In both cells, AAV5 is endocytosed via clathrin-coated pits and vesicles and by non-coated structures.

K. Wikström (Vectura AB, Novum, Huddinge/S) reported on the GMP production of MLV vector using a stable production system (293 cells in CellCube). The growth was done in SVF containing medium, which was replaced (without a washing step for cell adherence reasons) to a serum free medium. Production batches of 10-20 L were produced, the average vector concentration was 2×10^6 IU/ml. They have observed a half-life of 18 months, when the vector was stored at -80°C (other half-lives were; 8h at 37°C ; 40h at 20°C ; 14 days at 4°C).

By the way, other GMP vectors producers in EU are **EUFETS**, **AMT** and **Henogen** (non-exhaustive list).

O.-W. Merten

Future Meetings

Biotechnology 2003 V International Specialized Exhibition

June 16-19, 2003, Petersburg Sport and Concert Complex Saint-Petersburg

Tel: +7-812-3246416

email: biotech@sivel.spb.ru

www.sivel.spb.ru

11th European Congress on Biotechnology, 25th Anniversary of EFB: building bridges between biosciences and bioengineering

24-29 August, 2003, Basel, Switzerland

Organisation: ECB11, Tel + 41 61 686 28 28, Fax: +41 61 686 21 85; email: info@ecb11.ch; web:

<http://www.ecb11.ch>

6th Conference on Protein Expression in Animal Cells

7-11 September, 2003, Quebec, Canada

Organisation: www.bri.nrc.ca/6thPEACE

11th Annual Meeting of the European Society of Gene Therapy

14-17 November, 2003, Edinburgh, UK

Organisation: Congrex Sweden AB, P.O. Box 5619, SE-114 86 Stockholm, Sweden, Tel + 46 8 459

6600, Fax: + 46 8 661 9125, email:

esgt@congrex.se

Annual Meeting of American Institute of Chemical Engineers: Advances in Cell Culture Processing

16-21 November, 2003, San Francisco, USA

Organisation: American Institute of Chemical Engineers, 3 Park Ave, New York, 10016-5991, USA, Tel + 1 212 591-7338, Fax: + 1 212 591-8894, email: meetmail@aiche.org

The Enhanced Doctorate with UCL Biochemical Engineering Department

DOES YOUR COMPANY NEED NEW INNOVATIVE IDEAS OR NEED HELP TO DEVELOP THEM?

The Enhanced Doctorate with UCL Biochemical Engineering Department can help you.

What is the Enhanced Doctorate?

The programme combines the scientific rigour of a PhD with industrial relevance and business perspective to give your company a fully costed and

investigated project, which could be an area of future research strategy.

The programme is heavily subsidised by the UK Engineering and Physical Sciences Research Council (EPSRC pay 70% of all running costs) and provides a dedicated research engineer working full time on your project needs. Not only that, for this minimum investment, access is also gained to the services of the UCL_{BE} academic staff. The department is world-renowned in the field of bioprocess engineering -its research underpins the process development of biopharmaceuticals.

What has it achieved?

So far, over the four years of the programme, UK and International companies have sponsored over 30 projects. Sponsors have included both large (e.g. GSK, Pfizer and Eli Lilly) and small companies (Astex, Beocarta, Biopharm Services).

Project successes include:

- * A new application of a chromatographic resin using project results on expanded bed chromatography (with Amersham Biosciences)
- * Development of commercial software from research on fermentation modeling (with Adaptive Biosystems)
- * A new manufacturing strategy based on a scale-down and modeling study (with BioProducts Laboratory)

What are the benefits?

We believe gains for the company include

- * Attraction of the best graduates using the special EPSRC stipends
- * Cost effective research
- * Access to the resource of an international centre
- * Opportunity to screen potential leadership candidates for your company

How do I find out more?

If you would like to discuss about potential research collaboration please contact myself, Dr Dipankar Dey, at the following address

Dr Dipankar Dey
The Advanced Centre for Biochemical Engineering
Department of Biochemical Engineering
University College London
Torrington Place
London, UK
WC1E 7JE
Tel: + 44 (0)20 7679 4414
Fax: + 44 (0)20 7916 3943
www.biochemeng.ucl.ac.uk
Email d.dey@ucl.ac.uk

New Members

ESACT would like to welcome the following new members;

Dana Anderson (Genentech Inc.); Heiner Bottinger (Universitat Stuttgart); Monika Burg (Cardion AG); Christophe Giese (ProBioGen AG); Yun Jiang (Biovitrum AB); Ingo Jordan (ProBioGen AG); Susann Koch (ProBioGen AG); Karlheinz Landauer (Igeneon Forschungs-und Entwicklungs GesmbH); Alexander Loa (Cell Culture Service GmbH); Marco Reidal (ProBioGen AG); Volker Sandig (ProBioGen AG); Claudia Schulz (ProBioGen AG); Ann Smith (Lorantis Ltd.); Catherine Sonderegger (Biochemie GmbH); Angelika Viviani (Hochschule Weadenswil); Karsten Winkler (ProBioGen AG); Catherine Yandell (GroPep)

Joke Corner

Children speaking the 'truth'...

"HOW DO YOU DECIDE WHO TO MARRY?"

You got to find somebody who likes the same stuff. Like, if you like sports, she should like it that you like sports, and she should keep the chips and dip coming. Alan, age 10

No person really decides before they grow up who they're going to marry. God decides it all way before, and you get to find out later who you're stuck with. Kirsten, age 10

"WHAT IS THE RIGHT AGE TO GET MARRIED?"

Twenty-three is the best age because you know the person FOREVER by then. Camille, age 10

No age is good to get married at. You got to be a fool to get married. Freddie, age 6

"HOW CAN A STRANGER TELL IF TWO PEOPLE ARE MARRIED?"

You might have to guess, based on whether they seem to be yelling at the same kids. Derrick, age 8

"WHAT DO YOU THINK YOUR MOM AND DAD HAVE IN COMMON?"

Both don't want any more kids. Lori, age 8

“WHAT DO MOST PEOPLE DO ON A DATE?”

Dates are for having fun, and people should use them to get to know each other. Even boys have something to say if you listen long enough.
Lynnette, age 8

“IS IT BETTER TO BE SINGLE OR MARRIED?”

I don't know which is better, but I'll tell you one thing. I'm never going to have sex with my wife. I don't want to be all grossed out. Theodore, age 8

It's better for girls to be single but not for boys. Boys need someone to clean up after them. Anita, age 9

CRAZY English Language

Why the English language is so hard to learn:-

1. There is neither egg in eggplant nor ham in hamburger; neither apple nor pine in pineapple.
2. English muffins weren't invented in England or French fries in France.
3. Sweetmeats are candies while sweetbreads, which aren't sweet, are meat.
4. We take English for granted. But paradoxically quicksand work slowly, boxing rings are square and a guinea pig is neither from Guinea nor is it a pig.
5. And why is it that writers write but fingers don't fing, grocers don't groce and hammers don't ham?
6. If the plural of tooth is teeth, why isn't the plural of booth beeth?
7. One goose, 2 geese. So one moose, 2 meese? One index, 2 indices?
8. In what language do people recite at a play and play at a recital? Ship by truck and send cargo by ship?
9. Have noses that run and feet that smell?
10. How can a slim chance and a fat chance be the same, while a wise man and a wise guy are opposites?

You have to marvel at the unique lunacy of a language in which your house can burn up as it burns down, in which you fill in a form by filling it out and in which, an alarm goes off by going on. English was invented by people, not computers, and it reflects the creativity of the human race, which, of course, is not a race at all. That is why, when the stars are out, they are visible, but when the lights are out, they are invisible.

ESACT Secretariat

A reminder that 2003 subscription fees are now due;

Members working in Europe (Full) 20 Euros (= £13)
Members working outside Europe (Associate) 30 Euros (= £18)

Please remember when paying by credit card to include the **security number** (3 digit on the reverse on the card usually on signature strip; 4 digits for Amex) and the **post/zip code** of your billing address.

Bryan Griffiths

**Please note the ESACT OFFICE email address is;
esact@griff.evesham.net**

Also, as courier services etc. do not recognise PO Box addresses the following ESACT Office address should be used; PO BOX 1723, 5 Bourne Gardens, Porton, Salisbury, Wilts., SP4 0PL, UK

ESACT INFORMATION

COMMITTEE MEMBERS

Chairman:

Otto-Wilhelm MERTEN
Généthon III
Gene Therapy Programme Tel: (+33) 1 6947 2590
1 rue de l'Internationale, BP 60 Fax: (+33)1 6947 2838
F-91002 Evry Cedex 2
France omerten@genethon.fr

Secretary:

Alain BERNARD
Serono Biotech Center
Route de FENIL Z1B Tel.: (+41) 21 923 23 57
1804 CORSIER-sur-VEVEY Fax: (+41) 21 923 20 13
Switzerland Alain.Bernard@Serono.com

Meeting Secretary:

Francesc GODIA
Universitat Autònoma de Barcelona,
Dept. d'Enginyeria Quimica, Tel: (+34) 93 5812692
Edifici C, Fax: (+34) 93 5812013
E-08193 Bellaterra (Barcelona) francesc.godia@uab.es
Spain

Treasurer:

Elisabeth LINDNER-OLSSON
Metcon Medicin AB
Dalenum 17, Tel: (+46) 8 695 9186
SE-18170 LINDINGO Fax (+46) 8 695 4099
Sweden elisabeth.lindner-olsson@swipnet.se

Committee Members:

Manuel CARRONDO
IBET/CTQB
Apartado 12 Tel.: (+351) 21 442 7787
P-2781-801 Oeiras Fax: (+351) 21 442 1161
Portugal mjtc@itqb.unl.pt

Martin FUSSENEGGER
ETH Honnerberg
HPT Tel.: (+41) 1633 3448
CH8093 Zurich Fax: (+41) 1633 1051
Switzerland
fussenegger@biotech.biol.ethz.ch

Stefanos GRAMMATIKOS
Boehringer Ingelheim Pharm. Tel.: (+49) 7351 544022
84 397 Biberach an der Riss Fax: (+49) 7351 844022
Germany
stefanos.grammatikos@bc.boehringer-ingelheim.com

Florian WURM

EPFL,
Dept. de Chimie, Tel: (+41) 21 693 6141
Centre de Biotechnologie, Fax: (+41) 21 693 6140
CH-1015 Lausanne Florian.Wurm@epfl.ch
Switzerland

ESACT Office:

PO Box 1723
5 Bourne Gardens Tel.: (+44) 1 980 610 405
Porton, Salisbury Fax: (+44) 1 980 610 405
Wilts SP4 0PL, UK esact@griff.evesham.net

Web Site Manager

Christophe LOSBERGER
Serono Pharmaceutical
Research Institute Tel: (+41) 22 70 69637
14 Chemin des Aulx Fax: (+41) 22 79 46965
CH-1228 Plan-des-Ouates
Geneva, Switzerland
christophe.losberger@serono.com

Newsletter Editor:

Steve Oh
Fax: +65 67754933
btcohs@btc.a-star.edu.sg

Membership Subscriptions:

We propose the following alternative payment methods:

Master, Visa, Access, Delta Eurocard, Amex or

1. 20 Euro per year paid by cheque drawn on a UK bank*.
2. USD 25.00 per year if drawn on any other bank*.

Note:

- We recommend you consider payment of more than one year's subscription at a time so as to minimise local bank charges to your account.

Newsletter Correspondence/Contributions:

Correspondence for publication in the ESACT Newsletter, meeting reports and comments should be sent direct to the Newsletter Editor.

PLEASE NOTIFY ESACT OFFICE OF ANY ADDRESS CHANGES/TEL/FAX/email etc.