# Plenary Lectures

Lecture

NIH funding of the 3Rs (reduce, refine, replace)

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The National Institutes of Health (NIH) is charged with pursuing fundamental scientific knowledge about the nature and behaviour of living systems and with applying that knowledge to extend healthy life and reduce the burdens of illness and disability. The use of animal and other models of disease has been an essential component of NIH's efforts and successes in fulfilling its mission. The NIH has played an active role in contributing to a better understanding and utilisation of animal models of disease, including lower phylogenetic species, and in supporting science that has led to refinements in techniques and practices that have reduced pain and distress in the laboratory animal. As NIH looks to the future, the NIH Roadmap and other scientific initiatives are creating an exciting world of opportunities that will allow NIH to continue its commitment to the 3Rs.
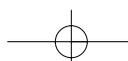
Lecture

Systems Biology and the 3Rs

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Pancreatic beta-cells, the only physiological source of insulin production, die by apoptosis in early type 1 diabetes mellitus (T1DM). Apoptosis is an active, gene directed process, and recent observations by our group suggest that beta-cell fate following exposure to immune mediators is a complex and highly regulated process, depending on the duration and severity of perturbation of key interacting gene networks. This departs from the traditional view of phenomena, based on the study of signaling pathways by intuitive inferences based on the study of individual pathway components. Identification of complex and interacting gene/protein patterns poses a formidable challenge, but the sequencing of the human genome, and of the genome of several other species, makes it possible to address it by the use of new high throughput technologies, such as microarray analysis and proteomics. To fully use these data we will need a global multivariate strategy, as proposed by the systems biology approach. This approach seeks to devise models based on the comprehensive, qualitative and quantitative analysis of all constitutive parts of a cell or tissue with the ultimate aim of explaining biological phenomena through the interaction of all its cellular and molecular components.

Against this background, we are utilising microarray analysis, detailed promoter studies and *in silico* analysis to clarify the pattern and regulation of gene expression in primary rat beta-cells and in human islets exposed for different time points to the pro-apoptotic cytokines IL-1β + IFN-γ. The data obtained are deposited at the "Beta Cell Gene Expression Bank", which is already accessible at http://t1dbase.org/cgi-bin/enter_bcgb.cgi. The ultimate goal of this open access resource is to identify and annotate all genes expressed in rat, mouse and human beta-cells.

By allowing us to obtain massive and integrated information on limited amounts of tissue, and by increasing the predictive power of different biological and *in silico* models, this novel approach may lead to a decrease in the number of animal experiments. This potential impact of the systems biology approach on the "3Rs" will be discussed at the lecture.

Lecture

ECVAM´s progress in implementing the 3Rs in Europe

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Starting with the animal welfare Directive from 1986 and continuing until most recent chemicals and cosmetics legislation, Europe has laid the ground for the implementation of alternative methods. In order to meet these political expectations, a couple of technical and strategic developments became necessary:
- An analysis of current *in vivo* test performance to set benchmarks for alternatives
- An analysis of the frequency (prevalence) of toxic health effects in different areas of test application
- An inventory and database of the alternative methods available
- A coached development of lacking tests also making use of novel technologies

- An acceleration and international harmonisation of the validation process and regulatory implementation
- A development of quality assurance systems for *in vitro* methods such as Good Laboratory Practice and Good Cell Culture Practice
- A transition from single tests as stand-alone replacements to the composition of test strategies and their validation.

The European Centre for the Validation of Alternative Methods (ECVAM) has played a proactive role in all these processes co-ordinating many stakeholder activities. A review of the state of these developments shall be given, in order to show how a new type of evidence-based toxicology is emerging which is based on validated and quality controlled test strategies.

Lecture

Education in alternatives to animal experimentation. Who shall be educated?

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The contribution of animal experimentation to human welfare can not be ignored. Although the value of animal experimentation is recognised, one's sentiment to love animals must be remembered even in scientific communities. The well-educated liberalists have established a new idea for laboratory animal welfare, 3Rs that is a key of alternative studies.

Because the basement of 3Rs is sentiment and ethical mind of human beings, the early education is essential. The primary school pupils, high school students shall be educated. The university students in particular medical, dental, veterinary and biology schools shall be educated. These students are the future science or biology teachers for children and students. The post-

graduate students in biomedical sciences shall be educated before they are planning animal experimentation for their thesis. The tutors, lecturers and professors to look after biomedical postgraduate students shall be educated because they are teachers for future researchers and scholars and also they are researchers who are making experimental protocols. All of members in education at not only academic institutions but also research institutions shall be educated.

Finally but most importantly, the citizens shall be educated to understand the alternatives to animal experimentation as tax payers who are patrons of any scientific activities including animal experimentation.

Lecture

Willi Halle's registry of cytotoxicity

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In toxicology, we are forced to test hazard potential in animals as alternates to men. Inter-species sensitivity differences are then bridged by applying safety factors to NOAEL's observed in animals. However, in general, data from acute systemic toxicity tests are not used to derive NOAEL's for risk assessment procedures, so that valuable information derived from these studies is often reduced to the crude information needed for classification and labelling: A rough estimate of the LD_{50}, or an estimate of a toxicity class. Cell biologists have therefore since long investigated whether the loss of cellular functions *in vitro* can be used to predict lethal doses *in vivo*, in particular the pioneers of the principle of basal cytotoxicity, Björn Eckwall and Willi Halle.

In the late 60ies of the last century, Willi Halle started in former East Germany to collect published IC_{50} values from

cytotoxicity studies provided these had met his defined acceptance criteria. Once LD_{50} values of these chemicals became available, the data were entered into a data base, the Registry of Cytotoxicity (RC). However, Willi Halle did not receive the necessary support for his pioneering work before the reunification of Germany, and only after the Berlin Wall fell, his work received the attention and support it deserved. With support of the German Ministry of Education and Research (BMBF) and continuous support by ZEBET, the BfR is currently in the lucky position to hold the RC as an electronic database – with 537 chemicals the largest collection of *in vitro* IC_{50} and related LD_{50} values. Willi Halle's model for prediction of acute oral LD_{50} shows particular strength in the prediction of the absence of oral toxicity, which holds for the majority of industrial chemicals.