Annual Report 2004

Institut für Pharmakologie (PKI)
der Universität Bern

Department of Pharmacology
of the University of Bern

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1. Introduction

1.1. Vorwort

Dies ist der vierte umfassende Jahresbericht des Instituts für Pharmakologie (PKI) der Universität Bern. Das PKI hat sich auch im Jahr 2004 bemüht, seine Aufgaben in Lehre und Forschung innerhalb der Medizinischen Fakultät vorbildlich zu erfüllen. Die Pharmakologie besitzt eine Brückenfunktion zwischen biologischer Grundlagen- und klinischer Forschung. Das PKI arbeitet deshalb eng mit den verschiedensten Kliniken des Inselspitals und mit anderen Forschungseinrichtungen der Universität Bern zusammen. Damit wollen wir helfen, die klinische Forschung sowie die Aus-, Weiter- und Fortbildung an der Medizinischen Fakultät zu stärken. Zum anderen sind wir an der Zusammenarbeit mit Firmen interessiert, wie die weiter hinten aufgeführten gegenwärtigen Kontakte der einzelnen Forschungsgruppen zeigen. Auch im Jahr 2004 trugen wir dazu bei, die Kommunikation zwischen Wissenschaftlern und Öffentlichkeit zu fördern. Dazu dienten u.a. die Vernissagen, die viele Gäste in das PKI lockten.

Das Jahr 2004 war für die in der Lehre tätigen Mitglieder unseres Instituts vor allem mit der weiteren Umsetzung der Studienreform im 3. Studienjahr Medizin (Problem-based Learning) verknüpft. Insbesondere ging es darum, die Qualität der Lehre weiter zu verbessern. Dies ist auch gelungen, wie Resultate aus internen und externen Befragungen bestätigen. In der Kerngruppe zur Planung und Umsetzung des PBL-Systems arbeitet Prof. Porzig mit, als Pharmakologie-Fachvertreter in den einzelnen Themenblöcken sind die Proff. Honegger, Porzig, Sigel und Simon vertreten. Umfangreiche Lehrverpflichtungen wurden auch im Rahmen der Ausbildung für Zahnmediziner und Pharmazeuten wahrgenommen.

Eine weitere wichtige Aktivität im Rahmen der Lehre stellt unsere Arbeit innerhalb des Programms für die interfakultäre Ausbildung des Forschungsnachwuchses der Universität Bern (PIAF) dar. Prof. Sigel ist Mitglied der PIAF-Kommission. Dazu kommen zusätzliche Bildungsangebote in Form eines Praktischen Kurses (Prof. Sigel) und einer Summer School (Prof. Simon), die beide weitgehend aus eigenen finanziellen Mitteln bzw. Sponsorengeldern bestritten werden. Im Institut arbeiten gegenwärtig 15 DoktorandInnen (10 PhD, 5 MD), und 2 haben im Berichtsjahr ihre Arbeit erfolgreich abgeschlossen.

Die Mitarbeiter und Mitarbeiterinnen des Pharmakologischen Instituts publizierten in 2004 insgesamt 31 Originalarbeiten sowie 10 Übersichtsartikel in internationalen Fachzeitschriften (Summe der „impact factors“ ca. 168). MitarbeiterInnen des Pharmakologischen Instituts wurden zu insgesamt 55 Vorträgen bzw. Seminaren eingeladen. Dr. Frank Altznauer und Dr. Stephan von Gunten wurden mit Forschungspreisen der Universität Bern ausgezeichnet. Gegenwärtig werden 5 Mitarbeiter mit namhaften Beiträgen des Schweizerischen Nationalfonds unterstützt. Zahlreiche Persönlichkeiten besuchten das Institut und hielten Forschungsseminare. Der Berner Immunologie-Club erfreut sich, auch durch die aktive Hilfe aus dem PKI, einer grossen Beliebtheit. Ein gemeinsam mit Prof. Dr. Th. Brunner (Institut für Pathologie) organisierter Apoptose-Kongress zog über 200 Teilnehmer aus dem In- und Ausland an. Im Jahr 2005 sind wir Gastgeber der 4. Konferenz der Internationalen Eosinophilen-Gesellschaft (http://www.pharmacology.unibe.ch/eos2005). Diese Aufzählung belegt den hohen Stellenwert, den die Forschung in unserem Institut besitzt.

Ein grosses Problem ist die zu geringe finanzielle Unterstützung unseres Instituts zur Aufrechterhaltung der Infrastruktur. Wir haben deshalb die grosse Hoffnung, dass mit der Unterzeichnung des Leistungsvertrages zwischen der Medizinischen Fakultät und dem PKI eine Besserung eintritt.

Das PKI nimmt auch ausserhalb der Universität seine Verantwortung für die Weiterentwicklung des Fachs Pharmakologie wahr. Wir bemühen uns um enge Kontakte zu anderen universitären Instituten für Pharmakologie vor allem innerhalb der Schweiz und Deutschlands. Innerhalb der Schweizerischen Gesellschaft für Pharmakologie und Toxikologie (SGPT) wurden von uns zahlreiche Initiativen gestartet, z.B. bei der Organisation von wissenschaftlichen Veranstaltungen. Prof. Simon dient gegenwärtig als Präsident der SGPT.

Ich danke allen Mitarbeitern und Mitarbeiterinnen für ihren Einsatz, welcher auch im Jahr 2004 zu einer Bilanz beitrug, die internationalen Massstäben gerecht wird. Ebenso danke ich allen Sponsoren und Freunden des Instituts.

Prof. Dr. med. Hans-Uwe Simon
Direktor

Bern, Februar 2005

1.2. Foreword

This is the fourth comprehensive report of the Department of Pharmacology of the University of Bern. Clearly, our department has worked hard to fulfil its tasks in teaching and research within the Medical Faculty in 2004 in high quality. Pharmacology fulfils functions in both basic biological science and clinical research. The Department of Pharmacology wants to succeed in both areas and, therefore, maintains intense contacts with several clinics of the University Hospital (Inselspital) as well as with the different research institutes of the University of Bern. This way, we hope to strengthen both research and teaching at the Medical Faculty. On the other hand, we are very much interested in collaborating with the industry on new developments. Current activities are listed in this report. Also in 2004, we tried to promote communication between scientists and the public. The organization of art exhibitions within our institute is an example for these efforts.

The year 2004 was, at least for the teaching staff of our institute, associated with the further implementation of the new “Problem-based Learning (PBL)”-system for medical students in their third study year. In particular, our goal was to further improve the quality of the whole teaching process. This goal was largely achieved, as demonstrated by the results of several internal and external evaluations. Prof. Porzig is a member of the core group that oversees teaching in the third study year. As specialists for Pharmacology, the professors Honegger, Porzig, Sigel, and Simon contribute to all of the thematic teaching blocks. The institute was also greatly involved in teaching dentistry students and students of Pharmacy.

Another important teaching activity is required within the graduation program for MD/PhD students of the University of Bern (PIAF). Additional teaching offers, such as a practical course (Prof. Sigel) and a summer school (Prof. Simon), were provided. Importantly, both events were organized using mainly our own financial resources and/or with the help of external sponsors. Currently, 10 PhD students and 5 MD students work at the PKI and two students successfully finished their graduate study in 2004.

In 2004, the scientific staff of the Department of Pharmacology published 31 original and 10 review articles in international peer-reviewed journals (the sum of the “impact

factors" is approximately 168). Co-workers of the institute were invited to 55 lectures or seminars. Dr. Frank Altznauer and Dr. Stephan von Gunten received research prizes of the University of Bern. Five co-workers are currently supported by grants of the Swiss National Science Foundation. Several prominent researchers visited the institute and presented seminars. An international congress on Apoptosis with more than 200 participants was organized together with Prof. Dr. Th. Brunner (Dept. of Pathology, University of Bern). In 2005, we will be host of the 4th Congress of the International Eosinophil Society (http://www.pharmacology.unibe.ch/eos2005). In summary, research plays an important role at the PKI and is performed at a high level.

One major problem is the financial support of the institute within the ordinary budget of the Medical Faculty that is not sufficient to keep the infrastructure in shape and running. We very much hope that the planned contract between the Medical Faculty and the PKI will improve our situation in the near future.

The PKI also takes responsibility to develop the field "Pharmacology" outside of the university. We have close contacts to other institutes of Pharmacology, in particular in Switzerland and Germany. We are actively working within the Swiss Society of Pharmacology and Toxicology (SSPT), for instance in the organization of scientific events. Prof. Simon currently serves as the president of SSPT.

I thank all co-workers for their hard work that contributed to the success of the PKI in 2004. I also thank all the sponsors and friends of the institute for their support.

Prof. Hans-Uwe Simon, MD, PhD
Director

Bern, February 2005

2. Staff 2004

Director

Prof. Dr. Hans-Uwe Simon, MD, PhD

Deputy Director

Prof. Dr. Hartmut Porzig, MD

Permanent Members

Prof. Dr. Ulrich E. Honegger, PhD
Prof. Dr. Hartmut Porzig, MD
Prof. em. Dr. Harald Reuter, MD
Prof. Dr. Erwin Sigel, PhD
Prof. Dr. Hans-Uwe Simon, MD, PhD
Prof. Dr. Jörg W. Stucki, PhD

Scientific Staff

PD Dr. Kurt Baltensperger, PhD (until February 2004)
Roland Baur, head technician
Dmytro Berezhnoy, PhD student (until July 2004)
Nathalie Boulineau, PhD student*
Dr. Andreina Bruno, PhD (since April 2004)
Dr. Sibylle Bürgi, PhD
Dr. Sébastien Conus, PhD
Arezoo Daryadel, PhD student*
Gian Marco De Marchis, MD student* (until February 2004)
Niculina Gebhardt, MD student* (since August 2004)
Remo Filippo Grifone, MD student*
Roberto Hess, MD student*
Reto Kaderli, MD student* (since August 2004)
Ganna Kostylina, PhD student*
Ivana Kotevic, PhD student*
Evelyne Kozlowski, technician
Sibylla Martinelli, PhD student*
Dr. Frédéric Minier, PhD*
Susanne Probst, technician
PD Dr. Claes Ruedeberg, PhD, consultant*
Inès Schmid, head technician
Kelly Tan, PhD student* (since September 2004)
PD Dr. Robert Urbanczik, PhD
Ekatherina Vassina, PhD student
Anton Vichalkovski, PhD student*
Dr. Clemens Wagner, PhD
Adrian Wirz, PhD student
Dr. Shida Yousefi, PhD
Dr. Stephan von Gunten, MD, PhD student*

External University Teachers

PD Dr. Armand Cachelin, MD, PhD*
Prof. Dr. Peter Hoffmann, MD*
Prof. Dr. Francesca Levi-Schaffer, PhD* (Visiting Professor of the University of Bern)
PD Dr. Uwe Zangemeister-Wittke, PhD*

Guest scientists

Dr. Dagmar Simon*
PD Dr. Alex Straumann*

External Computer Support

Faton Shala*
Dominik Wyss*

Office

Erika Fritsche, head secretary
Peggy Shala, secretary
Franziska Marti*, secretary to Prof. Reuter

Workshop

Hans Andres

House Keeping

Mariter Vieites
Esther Weber

*at least partially paid from external sources, mostly research grants

3. Teaching Activities
3.1. Lectures

Lectures for medical students

Date	Lecturer	Titel of the lecture
Jan 05, 2004	Prof. Hartmut Porzig	Antiarrhytmika
Jan 14, 2004	Prof. Hartmut Porzig	Beeinflussung der kontraktilen Herzfunktion durch Pharmaka
Jan 28, 2004	Prof. Hartmut Porzig	Diuretika
April 07, 2004	Prof. Erwin Sigel	Pharmakokinetik III
April 16, 2004	Prof. Erwin Sigel	Störungen des Zuckerstoffwechsels
April 16, 2004	Prof. Erwin Sigel	Störungen des Fettstoffwechsels
April 16, 2004	Prof. Erwin Sigel	Gicht, Syndrom X, Gewichtskontrolle
April 26, 2004	Prof. Ulrich Honegger	Antiepileptika
April 28, 2004	Prof. Hartmut Porzig	Lokalanästhetika
May 17, 2004	Prof. Erwin Sigel	Nebenniere
May 19, 2004	Prof. Erwin Sigel	Schilddrüse / Kropf
May 12, 2004	Prof. Ulrich Honegger	Pharmakotherapie von Demenz-erkrankungen (M. Parkinson, M. Alzheimer)
June 07, 2004	Prof. Ulrich Honegger	Angriffspunkte von Psychopharmaka
June 07, 2004	Prof. Ulrich Honegger	Neuroleptika
June 09, 2004	Prof. Ulrich Honegger	Anxiolytika
June 09, 2004	Prof. Ulrich Honegger	Antidepressiva
June 16, 2004	Prof. Ulrich Honegger	Pharmakokinetik im Alter
June 21, 2004	Prof. Ulrich Honegger	Schmerztherapie
June 21, 2004	Prof. Ulrich Honegger	Anästhesiologie I
June 21, 2004	Prof. Ulrich Honegger	Anästhesiologie II
July 07, 2004	Prof. Hans-Uwe Simon	Immunmodulation

Oct 19, 2004	Prof. Hans-Uwe Simon	Pharmakodynamik 1
Oct 25, 2004	Prof. Hartmut Porzig	Pharmakodynamik 2
Oct 27, 2004	Prof. Ulrich Honegger	Pharmakokinetik 1
Oct 27, 2004	Prof. Ulrich Honegger	Pharmakokinetik 2
Oct 27, 2004	Prof. Peter Hoffmann	Einführung in die Toxikologie
Nov 03, 2004	Prof. Hans-Uwe Simon	Entzündungshemmung
Nov 09, 2004	Prof. Hartmut Porzig	Das autonome Nervensystem (2. study year)
Nov 17, 2004	Prof. Ulrich Honegger	Polypharmazie im Alter (4. study year)
Nov 24, 2004	Prof. Hans-Uwe Simon	Pharmakotherapie bei Lungenkrankheiten
Dec 01, 2004	Prof. Hartmut Porzig	Antithrombotische Therapie II und Antikogulantien
Dec 08, 2004	Prof. Hartmut Porzig	Pharmakologie des sympathischen Nervensystems
Dec 08, 2004	Prof. Hartmut Porzig	Wirkprinzipien der Antihypertonika
Dec 15, 2004	Prof. Hartmut Porzig	Vasoaktive und antianginöse Substanzen

Lectures for dental students (Coordinator: Prof. Dr. J. W. Stucki)

Date	**Lecturer**	**Title of the lecture**
March 22, 2004	Prof. Hartmut Porzig	Einführung, Rezeptoren
March 25, 2004	Prof. Hartmut Porzig	Dosis-Wirkungskurven
March 29, 2004	Prof. Jörg Stucki	Antagonisten
April 01, 2004	Prof. Jörg Stucki	Absorption, Verteilung
April 05, 2004	Prof. Jörg Stucki	Bioavailability, Ausscheidung
April 08, 2004	Prof. Jörg Stucki	Arzneimittelmetabolismus

April 15, 2004	Prof. Jörg Stucki	Gesamtkinetik, Dosierung
April 19, 2004	Prof. Hartmut Porzig	Sympathikus
April 22, 2004	PD Dr. Armand Cachelin	Starke Analgetika
April 26, 2004	Prof. Erwin Sigel	Schwache Analgetika I
April 29, 2004	Prof. Erwin Sigel	Schwache Analgetika II
May 03, 2004	PD Dr. Armand Cachelin	Immunsuppressiva
May 06, 2004	Dr. Sibylle Bürgi	Lokalanästhetika
May 10, 2004	Dr. Kurt Baltensperger	Kreislaufpräparate
May 15, 2004	Dr. Sibylle Bürgi	Insulin, Orale Antidiabetika
May 24, 2004	Prof. Ulrich Honegger	Magensäurehemmer
May 27, 2004	Prof. Ulrich Honegger	Psychopharmaka
June 07, 2004	Prof. Erwin Sigel	Anxiolytika
June 10, 2004	Dr. Sibylle Bürgi	Antibiotika I
June 14, 2004	Dr. Sibylle Bürgi	Antibiotika II
June 17, 2004	Prof. Jörg Stucki	Examensvorbereitung

Lectures for Pharmacy students

Date	**Lecturer**	**Title of the lecture**
March 26, 2004	Prof. Ulrich Honegger	Einführungsvorlesung
April 02, 2004	Prof. Ulrich Honegger	Logistik bis zur Apotheke (mit Exkursion)
April 16, 2004	Prof. Ulrich Honegger	Industriepharmazie (mit Exkursion)
May 14, 2004	Prof. Ulrich Honegger	Zukunftsaussichten
May 14, 2004	Prof. Ulrich Honegger	Pharmazeuten in Lehre und Forschung
June 25, 2004	Prof. Ulrich Honegger	Führung durch das Departement Biowissenschaften der ETH Zürich

Lectures for students of the Natural Sciences Faculty

Date	Lecturer	Title of the lecture
April 14, 2004	Prof. Hans-Uwe Simon	Regulation der Granulozyten-Apoptose
Jan-March, 2004	Dr. Clemens Wagner	Uebungen zu Physik I
March-June, 2004	Dr. Clemens Wagner	Uebungen zu Physik II
March-June, 2004	Dr. Clemens Wagner	Lineare Algebra II
Sept-Dec, 2004	Dr. Clemens Wagner	Moderne Ansätze der System-biologie: die Netzwerk Analyse

3.2. Coordination PBL Medical Students, 3. year (2004/2005)

Core group:
Prof. Hartmut Porzig

Representatives of Pharmacology in teaching blocks:
Prof. Ulrich E. Honegger (blocks V and VI)
Prof. Hartmut Porzig (blocks II and III)
Prof. Erwin Sigel (block IV)
Prof. Hans-Uwe Simon (blocks I and VII)

3.3. Tutorials (study year 2004/2005)

Medical students 3. year:
Dr. Sébastien Conus
Dr. Stephan von Gunten
Prof. Dr. Ulrich Honegger
Prof. Dr. Hans-Uwe Simon
Prof. Dr. Hartmut Porzig
Dr. Shida Yousefi
PD Dr. Uwe Zangemeister-Wittke

3.4. Seminars of Invited Speakers

Date	Teacher	Title of the seminar
Feb 04, 2004	PD Dr. K. Baltensperger University of Bern	Image acquisition and advanced image analysis in microscopy (part 1 of 2)
Feb 11, 2004	Prof. Dr. A.-C. Andres University of Bern	Protein tyrosine kinases in mammary gland development and carcino-genesis: from mice to humans to mice
Feb 18, 2004	PD Dr. K. Baltensperger University of Bern	Image acquisition and advanced image analysis in microscopy (part 2 of 2)
Feb 25, 2004	Prof. Dr. S. Schuster University of Jena, Germany	Detecting routes in metabolism - fundamentals and applications
July 14, 2004	Dr. M. Wüthrich University of Wisconsin-Madison, USA	The good, the BAD and the compen-satory: Host responses to attenuated and virulent strains of Blastomyces dermatitidis
Aug 09, 2004	Dr. R. Braun University of Wisconsin-Madison, USA	T cell subpopulations in pulmonary fibrosis
Oct 06, 2004	Dr. K. D. Philipson UCLA, USA	Genetic manipulation of cardiac sodium-calcium exchange
Oct 13, 2004	Prof. Dr. S. Krähenbühl University of Basel	Rhabdomyolysis associated with statins: Epidemiology, risk factors and mechanisms
Nov 03, 2004	Dr. D. Burger University of Geneva	Chronic inflammation and cellular contact: Induction and modulation of Cytokine production
Nov 10, 2004	Prof. Dr. U. Rudolph University of Zurich	Pharmacological and physiological functions of $GABA_A$ receptor subtypes
Nov 17, 2004	Dr. D. Stroka University of Bern	Responses to hypoxia in the liver
Dec 01, 2004	Dr. A. Ziegler University of Zurich	Plasma DNA analysis: Prognostic applications in lung cancer

In addition, the scientific staff of the institute meets to discuss ongoing research projects and recently published work each Tuesday at 5 pm.

3.5. Bern Immunology Club

Date	Teacher	Title of the seminar
Jan 21, 2004		Clinical Immunology Conference on the hypereosinophilic syndrome:
	Prof. Dr. J. Ring	Clinical aspects of the syndrome
	Prof. Dr. H.-U. Simon	Molecular mechanisms causing the syndrome
	Prof. Dr. S. G. Plötz	Anti-IL-5 antibody treatment
Feb 05, 2004	Prof. Dr. D. Dobbelaere	Leukocyte signalling pathways, used and abused by the transforming parasite Theileria
March 24, 2004	PD Dr. F. Seibold	Immune reactivity to mannans in inflammatory bowel disease
April 28, 2004	Prof. Dr. C. Müller	Methods and Tools: Workshop
May 26, 2004	Prof. Dr. B. Engelhardt	Molecular mechanisms involved in lymphocyte homing to the central nervous system
June 30, 2004	PD Dr. P. Mohacsi	How to influence the immune system during and after transplantation
Sept 29, 2004	PD Dr. J. Greeve	Die APOBEC-1 Genfamilie: Cytidin Deaminierungen als universelles genetisches Prinzip in der angeborenen und erworbenen Immunität
Oct 27, 2004	Prof. Dr. A. Wendel	The use of GM-CSF to reconstitute the immune response in transplantation
Nov 24, 2004	Dr. D. Simon	Immunomodulation in atopic dermatitis

3.6. Academic Degrees

Kurt Baltensperger, PD Dr. sc. nat.
Habilitation, University of Bern, October 2004

Gian Marco Gabriele Giorgio De Marchis, Dr. med.
Thesis: Vitamin E as a mean to elucidate the antidepressant mode of action of Hypericum extracts.
University of Bern, February 2004

Dmytro Berezhnoy, Dr. phil. nat.
Thesis: Relative orientation of benzodiazepines in their binding pocket on $GABA_A$ receptors.
University of Bern, June 2004

4. Research Activities

4.1. Research Projects and Publications

Group Prof. Ulrich E. Honegger

Group members: Dr. Sibylle Bürgi, PhD
Adrian Wirz, PhD student
Roberto Hess, MD student
Susanne Probst, technician
PD Dr. Claes Ruedeberg, PhD, consultant

Interested in antidepressant drugs, we focus on the elucidation of their modes of action. For many years we have concentrated on classical, well established synthetic compounds but have recently expanded our research efforts to the antidepressant activity and properties of plant extracts. Studies are performed in *in vitro*-systems including cultured cells and brain slices of rats. Cell culture models are also used for the investigation of the kinetic behaviour of lipophilic, persistent polyhalogenated compounds. As a research group, known within the Department of Clinical Research of the Medical Faculty (DKF), for its expertise in receptor ligand binding we have collaborated with different research groups of our university. In particular Dr. S. Bürgi has helped to establish and to perform binding studies as part of ongoing projects with the team of Prof. Draeger, Dept. of Anatomy, with Prof. Mullis' group, University Children's Hospital, Inselspital, with the group of Prof. Blum from the Veterinary Faculty and others.

A closer look at the antidepressant-induced mechanisms of β-adrenoceptor down-regulation

S. Bürgi, K. Baltensperger, U. Kämpfer*, J. Schaller*, U. E. Honegger
(Collaboration with the *Department of Chemistry and Biochemistry, University of Bern)

Long-term use of most antidepressants leads to a decrease in the number of functional β1-adrenoceptors in central postsynaptic membranes and in cultured cells. Due to the coincidence this reduction in receptor density is thought to play a critical role in mediating the therapeutic effect of antidepressants. The exact mechanisms for β1-adrenoceptor down-regulation are not completely understood. Recent studies in our laboratory using cultured rat astrocytoma C6 cells have demonstrated that antidepressant-induced reduction of receptor surface expression may be caused by an impairment of β1-adrenoceptor recycling. In chronically antidepressant-treated cells, β1-adreno-ceptors underwent normal agonist-induced internalization, but instead of recycling back to the cell surface, they were retained in intracellular compartments. It has been shown that changes in the phosphorylation pattern of the distal cytoplasmic tail of G-protein coupled receptors can lead to a strong intracellular retention of endocytosed receptors. Metabolic labeling with ^{32}P-orthophosphate

followed by SDS-PAGE and phosphor-imager analysis of receptor bands indicated that levels of β1-adrenoceptor phosphorylation were altered by chronic antidepressant-treatment. We currently analyse drug-induced changes in β1-adrenoceptor phosphorylation using electro-spray ionisation mass spectrometry. The aim of the MS studies is to identify the phosphorylation sites in the carboxy-terminal region of the β1-adrenoceptor and to determine site specific changes in receptor phosphorylation following chronic antidepressant treatment.

Chronic antidepressant-induced changes in recycling of serotonin receptor subtypes

A. Wirz, S. Bürgi, U. E. Honegger

Our studies on antidepressant-induced alterations in the recycling of β1-adrenoceptors have demonstrated that this phenomenon is specific for individual receptors and not a general consequence of altered membrane trafficking. I*n vivo*-studies investigating antidepressant effects on the serotonin system have shown that the 5-HT2A receptor subtype is down-regulated following chronic drug treatment, while number and characteristics of the 5-HT1A receptor subtype are not affected by the antidepressant treatment. We transfected COS cells transiently with a 5-HT2A receptor-GFP construct and HEK cells permanently with either the 5-HT2A receptor-GFP construct or with the 5-HT1A receptor. This should allow us to study functionality and trafficking of these receptor subtypes in control and antidepressant exposed cells. The aim will be to distinguish the differences in recycling behavior of these receptor subtypes in response to antidepressants and to correlate it with differences in posttranslational modifications such as receptor phosphorylation or glycosylation.

Antidepressant effects on the β-adrenergic signal pathway. A comparison between hypericum perforatum (St. John's wort) extracts, TCA- and SSRI-antidepressants.

A. Wirz, S. Bürgi, U. E. Honegger

β1-adrenoceptor density as well as isoproterenol- and forskolin-stimulated cAMP responses were studied in rat astrocytoma cells chronically exposed to St. John's wort extract, to the tricyclic antidepressant desipramine or to the SSRI antidepressant fluoxetine. It was of interest to see that all treatments were comparably efficient in inducing receptor down-regulation, while the inhibitory effectiveness of the synthetic compounds on cAMP-formation was more pronounced than that of the plant extracts.

Pharmacology of hypericum perforatum (St.John's wort) extracts, and fractions of the plant extract containing different amounts of hyperforin.

S. Probst, C. Ruedeberg, R. Hess, U. E. Honegger

St. John's wort extracts are widely and successfully used in the treatment of mild and moderate forms of depression. *In vitro*-test systems are routinely used in our laboratory to investigate antidepressant effectiveness. A model to simulate the acute effects of antidepressant compounds on neurotransmitter reuptake is the use of freshly prepared rat brain slices. The constantly oxygenated slices are incubated with radioactively labelled ^{3}H-norepinephrine or ^{3}H-serotonin in the presence and in the absence of hypericum extract and extract-fractions, varying in their contents of hypericine or hyperforin. 10 μM imipramine is used as a measure for 100% inhibition of uptake. The extracts showed a dose-dependent inhibition of neurotransmitter uptake. Fractions of the whole extract were the more potent the more apolar the contents were. Very similar results were seen when down-regulation of β-

adrenoceptors was investigated in extract-exposed astrocytoma cells. The plant extracts and their fractions showed corresponding efficacies on receptor down-regulation as on neurotransmitter uptake inhibition.
In collaboration with Prof. Hamburger from the Friedrich-Schiller University in Jena, Germany we investigated fractions of an alcoholic St. John's wort extract for antidepressant effectiveness in our *in vitro*-systems. Fractions are either highly enriched with or depleted of hyperforin. Hyperforin, a major constituent of St. John's wort is known for its high potency to induce metabolic enzymes and thus for its interactions with co-administered drugs The antidepressant activity of this constituent, however, is still a matter of controversy. We could demonstrate that hyperforin-free fractions were still effective and that hyperforin is by no mean the only effective antidepressant principle in the extract although the efficacy was higher with increased contents of hyperforin.

Cellular kinetics of persistent compounds with endocrine effectiveness
S. Probst, S. Mühlebach, U. E. Honegger
(Collaboration with S. Mühlebach, Kantonsspital Aarau and G. Karlaganis, BUWAL, Bern)
Persistent lipophilic compounds are relevant representatives of environmental contaminants. They have entered the biosphere partly unintended from waste deposits, through leakage from closed systems or as residues of incineration due to resistance to high temperature. They show chemical blockage by chloro- or bromo-substitution of metabolically vulnerable positions in the molecule, e.g. of lipophilic aromatic ring systems normally degradable by cytochrome P_{450} enzymes as shown in PCB or DDT derivatives. The global distribution and marked bioaccumulation of such compounds through the food chain is a consequence of their extreme lipophilicity and high level of metabolic resistance leading to persistence in fat deposits eventually in man. There is little knowledge on mechanisms of fat storage and release of such compounds nor is a simple test method available to screen new chemical entities for their potential of bioaccumulation. To study more thoroughly ecotoxicological aspects of such compounds their kinetic behaviour has to be characterised in defined test models such as cell culture systems using well-defined and reproducible conditions. Apart from methodological studies to establish useful screening or test systems with representative cell lines to imitate important uptake and storage organs like fat, brain or skin, such cell culture systems allow to study toxicokinetics of selected model compounds using varying experimental conditions. Specific interactions with defined receptors may be investigated which may have relevance for acute or long-term effects. An ultimate goal will be to establish structure-effect correlations for a better ecotoxicological risk assessment of new chemical compounds developed and released into defined technical application fields. The aim of this study was to define methodological and experimental conditions in single and multiple (sector) cell culture systems (lit) using fibroblasts, adipocytes (differentiated 3T3 cells) and astrocytoma C6 cells. Selected model compounds with different molecular size and degree of halogenization were investigated. From our present results we can conclude that *in vitro*-cell culture systems are useful tools for the pharmacokinetic screening of highly persistent lipophilics. A correct and stable solution of these compounds in the culture media can best be achieved after incorporation into liposomes. The use of different cell types with distinct properties allows to detect differences in cell-specific kinetics and storage of lipophilics. The combination of up to four sectors covered with monolayers of different cell types in one plate represents a simple *in vitro*-system to analyze competitive cellular uptake of persistent lipophilic contaminants. Extents of

uptake and accumulation were drug- and cell-specific. Rates of uptake were fast and reached equilibrium within 15 minutes.

Effects of extracts of Valeriana plants on GABA uptake into rat brain slices.
C. Ruedeberg, U. E. Honegger
(Collaboration with Prof. W. Schaffner, Institute of Pharmaceutical Biology, University of Basel, Switzerland)
Valeriana plants of genetically mutated species or extracts prepared by different solvents and individual, isolated constituents were compared for their potency to modulate radiolabelled GABA uptake into freshly prepared rat brain slices. Specific GABA uptake was characterized in the presence of nonradiolabelled GABA or with selective GABA uptake inhibitors. It was of interest to find that plant extracts inhibited ^{3}H-GABA uptake dose-dependently, while e.g. valerenic acid was without inhibitory effectiveness.

The NK1 receptor localizes to the plasma membrane microdomains and its activation is dependent on lipid raft integrity
(Collaboration with Katia Monastyrskaya*, Andrea Hostettler*, Annette Dräger*
from the Institute of Anatomy and * Department of Cell Biology, University of Bern)
Neurokinin 1 receptor (NK1R) is expressed in central and peripheral nervous system as well as in endothelial and smooth muscle cells. It is involved in mediation of pain, inflammation, exocrine secretion and smooth muscle contraction. The group of Prof. Dräger demonstrated that the NK1R is localized in lipid rafts. Cholesterol depletion of NK1R-expressing HEK cells with methyl-b-cyclodextrin (MBCD) abolished receptor signalling. The NK1R raft localization and the NK1 receptor-mediated signalling were rescued by cholesterol replenishment. To rule out the possibility that cholesterol extraction diminished substance P/NK1R-mediated signalling by depleting the pool of NK1R on the cell surface, we performed radioligand binding studies in untreated and cholesterol-depleted NK1R-expressing cells. These binding studies revealed that cholesterol extraction did not affect the expression of NK1R on the cell surface. Our results support their findings that NK1R signalling is dependent on lipid raft integrity.

Short stature caused by a biologically inactive mutant growth hormone (GH-C53S)
(Collaboration with Amélie Besson*, Souzan Salemi*, Johnny Deladoey*, Jean-Marc Vuissoz*, Andrée Eblé*, Martin Bidlingmaier**, Christa Flück*, Primus E. Mullis*
* University Children's Hospital, Pediatric Endocrinology and Metabolism, Inselspital, Bern, ** University's Hospital, Clinical Medicine, Munich)
Short stature can be associated with bio-inactive growth hormone. Prof Mullis and his group have found one homozygous missense mutation, C53S, in the growth hormone molecule of one Serbian patient with growth retardation showing all the clinical characteristics of a bio-inactive growth hormone. Using radioligand binding assays we were able to show that the bio-inactivity of the growth hormone missense variant C53S is due to lower affinity of this mutant for the growth hormone receptor.

Original publications

1. K. Monastyrskaya, A. Hostettler, S. Bürgi, A. Dräger:
The NK1 receptor localises to the plasma membrane microdomains and its activation is dependent on lipid raft integrity.
J. Biol. Chem., in press (published online Dec. 8, 2004).

2. A. Besson, S. Salemi, J. Deladoey, J. –M. Vuissoz, A. Eblé, M.Bidlingmaier, S. Bürgi, U.E. Honegger, C. Flück, P.E. Mullis:
Short stature caused by a biologically inactive mutant growth hormone (GH-C53S).
J. Clin. Endocrinol. Metab., in press.

3. S.F. Muehlebach, G. Karlaganis, U.E. Honegger:
Kinetic assessment of persistent halogenated xenobiotics in cell culture models. Comparison of mono-and poly-halogenated compounds.
Chemosphere, in press.

Review article

U.E. Honegger:
Pharmakologie der Antiepileptika im Alter.
Geriatrie Praxis 3 (2004), 26-29.

Group Prof. Hartmut Porzig

Group members: Dr. Kurt Baltensperger, PhD[1] (until February 2004)
Ivana Kotevic, PhD student
Anton Vichalkovski, PhD student
Niculina Gebhardt, MD student (since August 2004)
Reto Kaderli, MD student (since August 2004)

[1]In addition, independent research work with own Swiss National Science Foundation projects.

The research interests of our group center on mechanisms regulating proliferation and differentiation of human hematopoietic, in particular erythroid progenitor cells. In principle, during blood cell formation there are three major problems that have to be solved: (1) maintain a constant pool of undifferentiated stem cells, (2) regulate proliferation and lineage commitment according to the overall needs of the body, (3) maintain a constant number of terminally differentiated blood cells. To reach these objectives, a host of humoral signals participate in determining the fate of hemato-poietic progenitor cells. Best known among these is the cytokine family of peptide growth factors acting via stimulating cellular tyrosine kinases. In recent years it became increasingly clear that the effects of cytokines are modulated by signals that act via G protein-linked receptors. This latter group includes, among others, chemokines, thrombin, purine nucleotides and lipids and constitutes the focus of our most recent research projects. The cross-talk between cytokine - and G protein-coupled receptor – linked signal transduction pathways is independently investigated by Dr. K. Baltensperger. Of particular interest in this respect is the development of new strategies for the treatment of malignant diseases, such as BCR-Abl positive leukemias.

A second line of ongoing research deals with functional aspects of the sodium/ calcium exchanger protein in cardiac cells and with the expression pattern of its three major subtypes in primary neuronal cell cultures and in brain tissue. This membrane transport system is an important element in maintaining cellular Ca^{2+} homeostasis in excitable cells.

A second line of ongoing research deals with the effects of BCR-Abl activity on G protein-linked cellular Ca^{2+} signaling in leukemic cells. Several model systems allowing to switch between inactive and highly active BCR-Abl are used to dissect the role of this tyrosine kinase in cellular Ca^{2+} handling and hence, on the chemotactic behavior of leukemic cells.

Modulation of cytokine signaling by thrombin and SDF-1 during growth and differentiation of hematopoietic progenitor cells

A. Vichalkovski, H. Porzig

The effects of two G protein-coupled receptor agonists, the chemokine CXCL12 (SDF-1) and thrombin, on growth and survival have been analyzed in multipotent and erythroid human hematopoietic progenitor cells. While the receptors for the two agonists, CXCR4 and PAR-1, respectively, are expressed in both cell populations, DNA synthesis was enhanced by CXCL12 only in multipotent cells and was inhibited by thrombin only in erythroid cells. We show that CXCL12 on its own promotes cell survival and provides an additive proliferative effect together with cytokines. This effect required the activation of the RhoA-Rho kinase pathway and the stimulation of Ca^{2+}-dependent kinases. The activation of RhoA relied upon a G_i-mediated stimulation of tyrosine kinases and was blocked both by inhibitors of Src kinases and of Jak kinases. In erythroid, but not in multipotent cells, Epo and the PKC activator PMA stimulated the Src kinase Lyn and enhanced cell growth. Thrombin reduced the stimulating effect of Epo, and tyrosine kinase inhibitors antagonized this effect. Hence, thrombin appeared to block erythroid DNA synthesis by mediating a tyrosine kinase-dependent inhibition of Epo-stimulated PKC subtypes. Detailed analysis of PKC subtype activity under these conditions revealed PKCβ_2 as a specific target for the inhibitory action of Src and Abl tyrosine kinases. We conclude from these data that erythroid commitment of multipotent hematopoietic progenitors is associated with pronounced changes in PKC-tyrosine kinase interactions through establishing a negative feedback loop between PKCβ and tyrosine kinases. These developmental stage-dependent changes may determine which signaling pathways are recruited and whether growth and survival of hematopoietic cells are promoted or inhibited by G protein-coupled receptor agonists.

See original publication No. 1

'Functional half-life' of the Na^+-Ca^{2+} exchanger in neonatal rat cardiac myocytes revealed by an antisense oligodeoxynucleotide approach

M. Egger, H. Porzig, E. Niggli, B. Schwaller

Cooperation with the Institute of Physiology, Bern and the Department of Histology, University of Fribourg

The Na^+-Ca^{2+} exchanger is essential for the Ca^{2+} homeostasis in many cell types but a specific pharmacological blocker is still lacking. We used an antisense oligodeoxynucleotide (AS-ODN) directed against the rat cardiac Na^+-Ca^{2+} exchanger (NCX1) to suppress the *de novo* synthesis of the protein. The specificity of this approach was examined in neonatal rat cardiac myocytes and, as a new strategy, in baculovirus-infected Sf9 insect cells transiently expressing NCX1. Suppression of NCX1 synthesis by AS-ODNs was assessed with immunohistochemistry and a quantitative binding assay using the radiolabeled monoclonal antibody R3F1. The reduction of NCX1 activity by AS-ODN treatment was evaluated by recording Na^+-Ca^{2+} exchange currents with the voltage-clamp technique and by examining Ca^{2+} transport via NCX1using laser-scanning confocal imaging of transient Ca^{2+} signals. In cultured neonatal cardiac myocytes the total amount of NCX1 epitopes recognized by the antibody was unaltered after 48 h AS-ODN treatment, while the functionally active fraction of NCX1 in the sarcolemma virtually disappeared. In contrast, in NCX1-baculovirus-infected Sf9 cells devoid of endogenous NCX1, the exposure to AS-ODN resulted in a significant reduction of the synthesis and function of NCX1. Taken together, these results indicate that the functional half-life of the NCX1 protein in the plasma membrane of neonatal cardiac myocytes is surprisingly brief and, in fact,

shorter than reported half-lifes of about 30h for other membrane proteins. On the other hand, the epitope recognized by the R3F1 antibody remains much longer inside the cells, resulting in a significant difference in the half life measured with exchange activity compared to the one determined by epitope binding.
See original publication No. 2

Regulation of G protein-dependent Ca^{2+} signaling in BCR-Abl expressing chronic myeloid leukemia (MEL) cell lines
N. Gebhardt, R. Kaderli, A. Vichalkovski, H. Porzig
Previous work in our laboratory had shown that cellular Ca^{2+} transients in primary hematopoietic progenitors, induced by G protein-coupled receptor (GPCR) agonists and involved in chemotaxis, are under the control of PKC. An inhibitory feedback mechanism links PKC activity with tyrosine kinase activity of Src and Abl kinases (see 1). Deregulation of tyrosine kinase (TK) activity by expression of the Bcr-Abl kinase is a hallmark of chronic myeloid leukemia (CML). Therefore, we used the human CML cell lines EM-2 and K562 to assess possible changes in TK-PKC crosstalk under native conditions (Bcr-Abl active), after blocking of Bcr-Abl kinase activity with the specific inhibitor imatinib and in sublines made imatinib-resistant. These experiments were complemented with studies in a recombinant mouse cell line (TonB210) expressing Bcr-Abl under the control of an inducible promoter to test acute effects of TK over-expression. Cellular Ca^{2+} transients induced by GPCR agonists (involved in chemotaxis) are highly sensitive to suppression by PKC in normal and leukemic hematopoietic cells and can thus be used as functional readout system to follow TK-mediated effects on PKC activity. Under control conditions, magnitude and kinetics of Ca^{2+} transients and of store-operated Ca^{2+} influx (SOC) in Bcr-Abl expressing cells remained unchanged or became smaller after kinase inhibition with imatinib. However, inhibition of PKC with bisindolylmaleimide revealed a highly significant increase in GPCR agonist-activated SOC in imatinib-treated cells. Ca^{2+} release from cellular stores was much less affected. From our, still preliminary, data we conclude that Bcr-Abl reduces SOC by inhibiting the activity of several PKC subtypes. Since SOC forms a prominent part of total Ca influx after GPCR activation, the TK-mediated loss of SOC may contribute to the known loss of chemotactic homing response in leukemic cells.

G protein-dependent signal transduction and induction of differentiation in hematopoietic cells
I. Kotevic, K. Baltensperger
Hematopoietic cells express a unique heterotrimeric G protein α-subunit, $G_{\alpha 16}$, a member of the $G_{\alpha q}$ family. It is involved in Ca^{2+}-signaling and is capable of inducing cellular differentiation when overexpressed in a leukemia cell line [(Ghose, S., et al., J. Biol. Chem. 274, 12848-12854 (1999)]. Earlier experiments indicated that UTP-stimulated $P2Y_2$ nucleotide receptor ($P2Y_2R$) signalling critically depends on the presence of $G_{\alpha 16}$.
In the context of a thesis project we tested whether $P2Y_2$ receptors could physically interact with $G_{\alpha 16}$. In order to detect molecular proximity we used fluorescence resonance energy transfer (FRET) analysis in combination with an acceptor photobleaching technique and confocal fluorescence microscopy. Receptor and G protein were fused to cyan (CFP) and yellow (YFP) variants of the green fluorescent protein to provide the fluorescence donor and acceptor, respectively. When expressed in K562 leukemia cells, the fusion proteins were capable of triggering a Ca^{2+}-signal upon receptor stimulation, demonstrating their functional integrity. In

FRET measurements a strong signal from the plasma membrane region of fixed, resting cells was detected when the receptor was co-expressed with the G protein as the FRET acceptor, as well as when the CFP-tagged receptor was co-expressed with receptor fused to YFP. We conclude that, under resting conditions, i.e. in the absence of agonist, $G_{\alpha 16}$ and $P2Y_2$ receptors form constitutive complexes, and the $P2Y_2$ receptor is present as an oligomer. The data provide a possible explanation for the basal activity of G proteins in resting cells.
See original publication No. 3

Original publications

1. A. Vichalkovski, K. Baltensperger, D. Thomann, H. Porzig:
Two different pathways link G-protein-coupled receptors with tyrosine kinases for the modulation of growth and survival in human erythropoietic progenitor cells
Cell Signal 17 (2005), 447-459.

2. M. Egger, H. Porzig, E. Niggli and B. Schwaller:
Rapid turnover of the 'functional' Na+-Ca2+ exchanger in cardiac myocytes revealed by an antisense oligodeoxynucleotide approach
Cell Calcium 37 (2005), 233-243.

3. I. Kotevic, K. M. Kirschner, H. Porzig and K. Baltensperger:
Constitutive interaction of the P2Y2 receptor with the hematopoietic cell-specific G protein $G_{\alpha 16}$ and evidence for receptor oligomers
Cell Signal 17 (2005), in press (published online Dec. 21, 2004).

Book Chapters

1. H. Porzig, S. Engelhardt:
Pharmaka mit Wirkung auf das vegetative Nervensystem. In: Pharmakologie und Toxikologie, Hrsg. C.-J. Estler und H. Schmidt, Schattauer Verlag, 6. Aufl. (in press).

2. H. Russ, H. Porzig:
Antiparkinsonmittel. In: Pharmakologie und Toxikologie, Hrsg. C.-J. Estler und H. Schmidt, Schattauer Verlag, 6. Aufl. (in press).

3. H. Porzig, F. Grimminger:
Pharmaka zur Behandlung der arteriellen Hypertonie und des Schocks. In: Pharmakologie und Toxikologie, Hrsg. C.-J. Estler und H. Schmidt, Schattauer Verlag, 6. Aufl. (in press).

4. J. Allendörfer, M. Kaps, H. Porzig:
Pharmaka zur Behandlung des Schlaganfalls. In: Pharmakologie und Toxikologie, Hrsg. C.-J. Estler und H. Schmidt, Schattauer Verlag, 6. Aufl. (in press).

5. J. Allendörfer, H. Porzig, M. Kaps:
Pharmaka zur Behandlung der Migräne. In: Pharmakologie und Toxikologie, Hrsg. C.-J. Estler und H. Schmidt, Schattauer Verlag, 6. Aufl. (in press).

Group Prof. Erwin Sigel

Group members: Roland Baur, head technician
Dmytro Berezhnoy, PhD student (until July)
Nathalie Boulineau, PhD student
Dr. Frédéric Minier, PhD
Kelly Tan, PhD student (since September)

The $GABA_A$ receptors are the major inhibitory neurotransmitter receptors in the mammalian nervous system. They are integral membrane proteins consisting of five pseudo-symmetrically arranged subunits surrounding a central chloride ion selective channel. Modulation of their function influences our state of vigilance, anxiety and muscle tension. They represent the molecular targets of the frequently used tranquilizers of the benzodiazepine type (Valium®). We are interested in finding novel isoform-specific modulators of the receptor, in the receptor architecture and in the agonist and drug binding sites. For this purpose, we use expression of recombinant proteins in HEK-293 cells and primary neuronal cells (transient transfection) and Xenopus oocytes (mRNA microinjection), pharmacological (radioactive ligand binding studies), electrophysiological (2-electrode-voltage clamp, patch-clamp), biochemical, and molecular biology techniques.

Architecture of the benzodiazepine binding site on $GABA_A$ receptors
D. Berezhnoy, R. Baur, E. Sigel (Collaboration with Dr. M. Goeldner, University of Strasbourg, France).
Benzodiazepines exert their effects through a specific high affinity binding site on the $GABA_A$ receptor channel, where they act as positive allosteric modulators. To start to elucidate the relative positioning of benzodiazepine binding site ligands in their binding pocket, $GABA_A$ receptor residues thought to reside in the site were individually mutated to cysteine, and combined with benzodiazepine analogs carrying substituents reactive to cysteine. Direct apposition of such reactive partners is expected to lead to an irreversible site-directed reaction. We found a covalent interaction of α_1H101C with a reactive group attached to the C-7 position of diazepam. This interaction was studied at the level of ligand binding and at the functional level using electrophysiological methods. Covalent reaction stabilizes the receptor in its allosterically stimulated conformation. Covalent modification is not observed in wild type receptors or using mutated α_1H101C containing receptors in combination if the reactive ligand is pre-reacted and modification rate is reduced by the ligand Ro15-1788.
See original publication No. 1

Conformational changes in $GABA_A$ receptors
D. Berezhnoy, E. Sigel (Collaboration with Dr. M. Goeldner, University of Strasbourg, France).
The rate of covalent reaction between a benzodiazepine carrying a cysteine reactive moiety with mutated receptor having a cysteine residue in the benzodiazepine

binding pocket, α_1H101C$\beta_2\gamma_2$, was used as a sensor of its conformation. By using concatenated subunits we demonstrated that the covalent reaction occurs either exclusively at the α/γ subunit interface, or if it occurs in both α_1 subunits, exclusively reaction at the α/γ subunit interface can modulate the receptor. We found evidence for an increased rate of reaction of activated receptors, whereas reaction rate with the desensitized state is slowed down. The benzodiazepine antagonist Ro15-1788 efficiently inhibited the covalent reaction in the presence of 100 μM GABA but only partially in its absence or in the presence of 10 μM GABA. It is concluded that Ro15-1788 efficiently protects activated and desensitized states, but not the resting state.
See original publication No. 6

Defined subunit isoforms of $GABA_A$ receptors
F. Minier, E. Sigel
Subunit concatention allows a forced assembly of receptors with either two different α or β subunit isoforms in defined positions. Thus, receptors carrying α_1 and α_6 were prepared ($\beta_2\alpha_6\gamma_2/\beta_2\alpha_1$, $\beta_2\alpha_1\gamma_2/\beta_2\alpha_6$, $\beta_2\alpha_1\gamma_2/\beta_2\alpha_1$ and $\beta_2\alpha_6\gamma_2/\beta_2\alpha_6$). These four receptor types were expressed in Xenopus oocytes and functionally characterized for their activation by GABA and the partial agonist P4S, their inhibition by furosemide and their modulation by diazepam (Table 1). It turns out that this set of functional properties can be used as a diagnostic tool for cerebellar granule cells which express the α6 subunit. This will also open the way for the screening of potentially useful substances at defined $GABA_A$ receptor subtypes. α_6 subunits also have been reported to confer a specific subcellular localization. It will be investigated how ($\beta_2\alpha_6\gamma_2/\beta_2\alpha_1$, $\beta_2\alpha_1\gamma_2/\beta_2\alpha_6$, $\beta_2\alpha_1\gamma_2/\beta_2\alpha_1$ and $\beta_2\alpha_6\gamma_2/\beta_2\alpha_6$ receptors localize after transfection into primary neurons.
See original publication No. 3

Table 1

	GABA	Diazepam	Furosemide	P4S
	EC_{50} (μM)	stim (%)	IC_{50} (μM)	E_{max} (%)
γ-β-α_1/β-α_1	150–200	370	>5000	5
γ-β-α_6/β-α_6	1	0	30	44
γ-β-α_1/β-α_6	100	0	30	15
γ-β-α_6/β-α_1	40	300	30-40	11

Relative positioning of β subunit isoforms in $GABA_A$ receptors
N. Boulineau, E. Sigel
$\alpha_1\beta_1\gamma_2$ receptors have properties different from $\alpha_1\beta_2\gamma_2$ receptors. In contrast to $\alpha_1\beta_1\gamma_2$ receptors, $\alpha_1\beta_2\gamma_2$ receptors are strongly stimulated by the anticonvulsant loreclezole and by the anesthetic etomidate. β_2 subunits also confer a specific subcellular location while β_1 subunits do not. Concatenated subunits $\alpha_1\beta_1\alpha_1$, $\beta_1\gamma_2$, $\alpha_1\beta_2\alpha_1$ and $\beta_2\gamma_2$ have been prepared. $\alpha_1\beta_1\alpha_1/\beta_1\gamma_2$, $\alpha_1\beta_2\alpha_1/\beta_2\gamma_2$, $\alpha_1\beta_1\alpha_1/\beta_2\gamma_2$ and $\alpha_1\beta_2\alpha_1/\beta_1\gamma_2$ receptors were expressed in Xenopus oocytes and their pharmacological properties analyzed. The presence of only one β_1 subunit is nearly sufficient to confer the low

sensitivity typical for $\alpha_1\beta_1\alpha_1$ receptors for gating by anesthetic etomidate. $\alpha_1\beta_1\alpha_1/\beta_2\gamma_2$ and $\alpha_1\beta_2\alpha_1/\beta_1\gamma_2$ receptors display a degree of stimulation by loreclezole and etomidate intermediate between $\alpha_1\beta_1\gamma_2$ and $\alpha_1\beta_1\gamma_2$ receptors. No positional effect of β_1 and β_2 was observed. Transient transfection into primary neurons in combination with immunocytolocalization experiments will reveal the subcellular localization of the four types of receptor.

The flavone Hispidulin isolated from Sage stimulates $GABA_A$ receptors
R. Baur, E. Sigel
(Collaboration with D. Kavvadias, Drs. Ph. Sand and P. Schreier, Dept. of Food Chemistry and Clinical Neurochemistry, University of Würzburg)
Hispidulin was investigated on recombinant $GABA_A$ receptors expressed by *Xenopus laevis* oocytes. Hispidulin strongly stimulated the GABA-induced chloride currents at all receptor subtypes ($\alpha_{1\text{-}3,5,6}\beta_2\gamma_2$) indicating a positive allosteric profile. In contrast to diazepam, hispidulin also enhanced the GABA-activated current by binding to the $\alpha_6\beta_2\gamma_2$-$GABA_A$ subtype receptor.
See original publication No. 5

An isolated plant compound allosterically stimulates $GABA_A$ receptors independently of the benzodiazepine site with a unique subunit specificity
R. Baur, E. Sigel
(Collaboration with M. Senn, Drs. U. Séquin and U. Simmen, Dept. of Chemistry and Pharmacy, University of Basel)
Patenting and manuscript in preparation.

Functional characterization of a human mutation in a muscle chloride channel leading to Myotonia Congenita
M.T. Schaerer, E. Sigel
(Collaboration with Drs. L. Chen, D. Lang, J. Fritschi, L. Kappeler and J. Burgunder, Laboratory of Neuromorphology, Departments of Neurology and Clinical Research; and Drs. F. Joncourt and S. Gallati, Laboratory of Molecular Genetics, Children's Hospital; and Prof. Dr. J. Weis, Division of Neuropathology, Institute of Pathology; all University of Bern)
See original publication No. 2

Zolpidem insensitive mice
E. Sigel
(in collaboration with the groups of Profs. P. Somogyi, W. Sieghart, W. Wisden and E.R. Korpi)
We have shown earlier that the point mutation in the γ2 subunit F77I leads to zolpidem insensitive $\alpha1\beta2\gamma2$ $GABA_A$ receptors that still respond normally to diazepam. Transgenic mice expressing in place of normal γ2 this mutated subunit were shown to be insensitive to zolpidem but not to diazepam. These mice may be useful to dissect the differential contribution of the γ2 subunit in the brain by restoring normal γ2 subunit expression in selected brain regions.
See original publication No. 4

Original publications

1. D. Berezhnoy, Y. Nyfeler, A. Gonthier, H. Schwob, M. Goeldner, E. Sigel:
Towards a relative orientation of benzodiazepines in their binding pocket on $GABA_A$ receptors.
J. Biol. Chem. 279 (2004), 3160-3168.

2. L. Chen, M. T. Schaerer, Z. H. Lu, D. Lang, F. Joncourt, J. Weis, J. Fritschi, L. Kappeler, S. Gallati, E. Sigel, J. M. Burgunder:
Exon 17 skipping in CLCN1 leads to recessive myotonia congenita.
Muscle Nerve 29 (2004), 670-676.

3. F. Minier, E. Sigel:
Positioning of α subunit isoforms confers a functional signature to $GABA_A$ receptors.
Proc. Natl. Acad. Sci. USA 101 (2004), 7769-7774.

4. D. W. Cope, P. Wulff, A. Oberto, M. I. Aller, M. Capogna, F. Ferraguti, C. Halbsguth, H. Hoeger, H. E. Jolin, A. Jones, A. N. J. Mckenzie, W. Ogris, A. Poeltl, S. Sinkkonen, O. Vekovischeva, E. R. Korpi, W. Sieghart, E. Sigel, P. Somogyi, W. Wisden:
Abolition of zolpidem sensitivity in mice with a point mutation in the $GABA_A$ receptor γ_2 subunit.
Neuropharmacology 47 (2004), 17-34.

5. D. Kavvadias, Ph. Sand, K. A. Youdim, C. Rice-Evans, R. Baur, E. Sigel, D. Rausch, P. Riederer, P. Schreier:
The flavone hispidulin, a benzodiazepine receptor ligand with positive allosteric properties, is able to cross the blood brain barrier and exerts anti-convulsive effects.
Br. J. Pharmacol. 142 (2004), 811-820.

6. D. Berezhnoy, R. Baur, A. Gonthier, B. Foucaud, M. Goeldner, E. Sigel:
Conformational changes at benzodiazepine binding sites of $GABA_A$ receptors detected with a novel technique
J. Neurochem., in press.

Review articles

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Ligand-Operated Membrane Channels: GABA. In: Encyclopedia of Biological Chemistry (W.J. Lennarz and M.D. Lane, eds.), Elsevier, Oxford, Vol. 2 (2004), p. 562-566.

2. E. Sigel:
The benzodiazepine recognition site on $GABA_A$ receptors.
Medicinal Chemistry Reviews (2004) - Online, in press.

3. F. Minier, E. Sigel:
Use of concatenated subunits for the study of ligand-gated ion channels.

Trends in Pharmacol. Sci. 25 (2004), 499-503.

4. E. Sigel, F. Minier:
Educational paper: The Xenopus oocyte: System for the study of functional expression and modulation of proteins
Molecular Nutrition and Food Research, in press.

Group Prof. Hans-Uwe Simon

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Dr. Shida Yousefi, PhD[1]

[1]In addition, independent research work with own Swiss National Science Foundation and Bern Krebsliga projects.

We are interested in the precise features of chronic inflammatory responses. Several diseases serve as models to study such processes. In particular, we investigate pathogenic mechanisms of the following diseases: Atopic dermatitis, idiopathic eosinophilia, eosinophilic esophagitis, cystic fibrosis, sepsis, rheumatoid arthritis, chronic obstructive pulmonary disease, and cancer. Our research goal is the identification of new drug targets for future therapeutic approaches in these diseases. Besides the pathogenic aspects of our research, we have developed several in vitro and in vivo test systems to determine potential effects of a given drug on the immune system. Moreover, we are involved in several clinical drug studies, some of them were finished and published in 2004. Our research requires a network of physician-scientists from many different clinics. Most of the participating groups are located at the Medical Faculty of the University of Bern. Results of these collaborative interactions are seen in the following abstracts, which briefly describe our research activities in 2004.

Calpain-1 regulates Bax and subsequent Smac-dependent caspase-3 activation in neutrophil apoptosis

F. Altznauer, S. Conus, A. Cavalli, G. Folkers, H.-U. Simon

(Collaboration with the Department of Pharmaceutical Sciences, University of Bologna, Bologna, Italy, and the Department of Pharmaceutical Sciences, University of Zurich, Zurich, Switzerland)

In the absence and in the resolution of inflammatory responses, neutrophils rapidly undergo spontaneous apoptosis. Here we report about a new apoptosis pathway in these cells that requires calpain-1 activation and is essential for the enzymatic activation of the critical effector caspase-3. Decreased levels of calpastatin, a highly specific intrinsic inhibitor of calpain, resulted in activation of calpain-1, but not

calpain-2, in neutrophils undergoing apoptosis, a process, which was blocked by a specific calpain-1 inhibitor or by intracellular delivery of a calpastatin peptide. Further support for the importance of the calpastatin-calpain system was obtained by analyzing neutrophils from patients with cystic fibrosis that exhibited delayed apoptosis associated with markedly increased calpastatin and decreased calpain-1 protein levels compared to neutrophils from control individuals. Additional studies were designed to place calpain-1 into the hierarchy of biochemical events leading to neutrophil apoptosis. Pharmacological calpain inhibition during spontaneous and Fas receptor-induced neutrophil apoptosis prevented cleavage of Bax into an 18-kDa fragment unable to interact with Bcl-x_L. Moreover, calpain blocking prevented the mitochondrial release of cytochrome c and Smac, which was indispensable for caspase-3 processing and enzymatic activation, both in the presence and absence of agonistic anti-Fas receptor antibodies. Taken together, calpastatin and calpain-1 represent critical proximal elements in a cascade of pro-apoptotic events leading to Bax, mitochondria, and caspase-3 activation, and their altered expression appears to influence the life span of neutrophils under pathologic conditions.

See original publication No. 1

Inflammation-associated cell-cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils

F. Altznauer*, S. Martinelli*, S. Yousefi, C. Thürig, I. Schmid, E. Kozlowski, E. M. Conway, M. H. Schöni, P. Vogt, C. Müller, M. F. Fey, U. Zangemeister-Wittke, H.-U. Simon

(Collaboration with the Departments of Oncology, Pediatrics, and Pathology, University of Bern, the Departments of Medical Oncology and Clinical Pathology, University of Zurich, Switzerland, and the Center for Transgene Technology and Gene Therapy, University of Leuven, Belgium)

Survivin has received great attention due to its expression in many human tumors and its potential as a therapeutic target in cancer. Survivin expression has been described to be cell-cycle-dependent and restricted to the G_2-M checkpoint, where it inhibits apoptosis in proliferating cells. In agreement with this current view, we found that survivin expression was high in immature neutrophils, which proliferate during differentiation. In contrast to immature cells, mature neutrophils contained only little or no survivin protein. Strikingly, these cells re-expressed survivin upon GM-CSF or G-CSF stimulation *in vitro* and under inflammatory conditions *in vivo*. Moreover, survivin-deficient mature neutrophils were unable to increase their life span following survival factor exposure. Taken together, our findings demonstrate that: (i) Overexpression of survivin occurs in primary, even terminally differentiated cells and is not restricted to proliferating cells; and (ii) Survivin acts as an inhibitor of apoptosis protein (IAP) in a cell-cycle-independent manner. Hence, survivin plays distinct and independent roles in the maintenance of the G_2-M checkpoint and in apoptosis control, and its overexpression is not restricted to proliferating cells. These data provide new insights into the regulation and function of survivin, and have important implications for the pathogenesis, diagnosis, and treatment of inflammatory diseases and cancer.

*Shared First-Authorship

See original publication No. 2

Induction of genes mediating interferon-dependent extracellular traps formation during neutrophil differentiation

S. Martinelli*, M. Urosevic*, P. A. Oberholzer, A. Daryadel, C. Baumann, M. F. Fey, R. Dummer, H.-U. Simon, S. Yousefi
(Collaboration with the Department of Oncology, University of Bern, and the Department of Dermatology, University of Zurich, Switzerland)

Interferons (IFNs) are cytokines that possess potent anti-viral and immunoregulatory activities. In contrast, their potential role(s) in anti-bacterial defense and neutrophil activation mechanisms is less well explored. By comparing gene expression patterns between immature and mature human neutrophils, we obtained evidence that intracellular proteases and other anti-bacterial proteins are produced at earlier stages of maturation whereas the genes for receptors and signaling molecules required for the release of these effector molecules are preferentially induced during terminal differentiation. For instance, mature neutrophils strongly expressed genes that increase their responses to type I and type II IFNs. Interestingly, granulocyte/macrophage – colony-stimulating factor (GM-CSF) was identified as a repressor of IFN signaling components and consequently of IFN-responsive genes. Both IFN-α and IFN-γ induced strong tyrosine phosphorylation of STAT1 in mature but not in immature neutrophils. Functional in vitro studies suggested that IFNs act as priming factors on mature neutrophils allowing the formation of extracellular traps upon subsequent stimulation with complement factor 5a (C5a). In contrast, both IFN-α and IFN-γ had only little capacity to prime immature cells in this system. Moreover, both IFNs did not have significant anti-proliferative effects on immature neutrophils. These data contribute to our understanding regarding changes of gene expression during neutrophil differentiation and IFN-mediated anti-bacterial defense mechanisms.

*Shared First-Authorship

See original publication No. 3

Functional expression of CD134 by neutrophils

R. Baumann, S. Yousefi, D. Simon, S. Russmann, C. Müller, H.-U. Simon
(Collaboration with the Departments of Dermatology, Clinical Pharmacology, and Pathology, University of Bern, Switzerland)

CD134 (OX40) is a member of the tumor necrosis factor (TNF) receptor superfamily expressed on activated T-cells. Here, we show that human peripheral blood neutrophils express CD134. Activation of CD134 by soluble CD134 ligand (OX40 ligand/gp34) resulted in delayed caspase-3 activation and consequently in delayed neutrophil apoptosis *in vitro*. Moreover, CD134 ligand, like G-CSF, maintained anti-apoptotic Mcl-1 levels and inhibited cleavage of the pro-apoptotic Bcl-2 family members Bid and Bax in these cells, suggesting that CD134-mediated signals block apoptosis pathways proximal to mitochondria activation. In conclusion, CD134 regulates neutrophil survival, suggesting that this molecule does not only contribute to adaptive but also to innate immune responses.

See original publication No. 4

Reduced dermal infiltration of cytokine-producing inflammatory cells in atopic dermatitis following short-term topical tacrolimus treatment

D. Simon, E. Vassina, S. Yousefi, L. R. Braathen, H.-U. Simon
(Collaboration with the Department of Dermatology, University of Bern, Switzerland)

In several clinical studies, topical immunomodulators have been shown to be effective in the treatment of atopic dermatitis (AD). As calcineurin inhibitors, they

target signaling pathways that control gene expression, in particular the expression of cytokines. We examined the cellular infiltrate of skin lesions of ten AD patients and characterized the cytokine pattern expressed by the infiltrating cells before and after short-term topical therapy with tacrolimus 1% ointment. Skin biopsies were examined for histological alterations (HE staining), composition of the inflammatory infiltrate (immunofluorescence) and cytokine expression (ELISA, immunofluorescence) one and three weeks after initiation of tacrolismus therapy. Systemic immunological effects were assessed by analyzing peripheral blood leukocytes (immunofluorescence) as well as in vitro stimulated pan-T cell cytokine production and proliferation (ELISA, lymphocyte proliferation test). All patients showed a significant improvement of their skin lesions associated with a marked regression of spongiosis, acanthosis, and of the density of the inflammatory infiltrate in the dermis. The latter was due to reduced infiltration of T cells, B cells, and eosinophils. In contrast, the numbers of mast cells did not change. Moreover, the expression of the T helper (Th) 2 cytokines interleukin (IL)-5, IL-10, and IL-13 in CD4+ T cells was reduced after therapy. Interestingly, tacrolimus therapy was also associated with a reduction of CD8+ T cells expressing the Th1 cytokine interferon-γ. Furthermore, the numbers of epidermal CD1a+ dendritic cells increased following treatment. In the peripheral blood, a decrease of granulocytes (eosinophils and neutrophils), but no changes in the distribution of lymphocyte subpopulations were noticed. In conclusion, topical tacrolimus treatment has anti-inflammatory effects on AD skin as indicated by reduced infiltration of cytokine expressing inflammatory cells.
See original publication No. 5

CINCA syndrome: a defect in peripheral tolerance mechanisms?
T. Bihl, E. Vassina, M. K. Boettger, R. Goldbach-Mansky, M. Seitz, P. M. Villiger, H.-U. Simon
(Collaboration with the Departments of Rheumatology/Clinical Immunology/ Allergology, University of Bern, Switzerland, and National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH, Bethesda, MD, USA)
Chronic infantile neurological cutaneous and articular (CINCA) syndrome is a severe chronic inflammatory disease, in which the pathogenesis is unknown. We examined the capacity of CINCA peripheral blood mononuclear cells (PBMC), which carried the T348M mutated form of cryopyrin, to release cytokines upon primary stimulation of monocytes (LPS) and lymphocytes (PHA), respectively. In contrast to normal PBMC and independent from the inflammatory activity of the patient, T348M cryopyrin expressing PBMC did not generate significant amounts of interleukin (IL)-10 upon LPS stimulation, but produced normal levels of this cytokine upon PHA stimulation. The data reported here provide evidence for a defective tolerance mechanism towards microbial antigens in a so-called autoinflammatory disease.
See original publication No. 6

Siglec-9 transduces apoptotic and non-apoptotic death signals into neutrophils depending on the pro-inflammatory cytokine environment
S. von Gunten, S. Yousefi, M. Seitz, S. M. Jakob, T. Schaffner, R. Seger, J. Takala, P. M. Villiger, H.-U. Simon
(Collaboration with the Departments of Rheumatology/Clinical Immunology/ Allergology, Intensive Care Medicine, and Pathology, University of Bern, as well as with University Children's Hospital, Zurich, Switzerland)
We report about new apoptotic and non-apoptotic death pathways in neutrophils that are initiated via the surface molecule sialic acid binding immunoglobulin-like lectin

(Siglec)-9. In normal neutrophils, Siglec-9 activation induced apoptosis. Inflammatory neutrophils obtained from blood of patients suffering from acute septic shock or from joint fluids of rheumatoid arthritis patients demonstrated increased Siglec-9- but normal Fas receptor-mediated cytotoxic effects when compared with normal blood neutrophils. The increased Siglec-9-mediated death was mimicked *in vitro* by short-term pre-incubation of normal neutrophils with pro-inflammatory cytokines, such as granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α, and IFN-γ, and was demonstrated to be caspase-independent. Experiments using scavengers of reactive oxygen species (ROS) and neutrophils unable to generate ROS indicated that the Siglec-9-mediated caspase-independent neutrophil death in the presence of GM-CSF depends on ROS. Interestingly, this form of neutrophil death was characterized by cytoplasmic vacuolization and several other non-apoptotic morphological features, which were also seen in neutrophils present in joint fluids from rheumatoid arthritis patients. Taken together, these data suggest that apoptotic (caspase-dependent) and non-apoptotic (ROS-dependent) death pathways are initiated in neutrophils *via* Siglec-9. The new insights have important implications for the pathogenesis, diagnosis, and treatment of inflammatory diseases such as sepsis and rheumatoid arthritis.

Increased expression and a potential anti-inflammatory role of TRAIL in atopic dermatitis

E. Vassina, M. Leverkus, L. R. Braathen, H.-U. Simon, D. Simon

(Collaboration with the Department of Dermatology, University of Bern, Switzerland, and the Department of Dermatology, University of Magdeburg, Germany)

TRAIL, the tumor necrosis factor-related apoptosis-inducing ligand induces apoptosis of many transformed but also of non-transformed cells. In addition, TRAIL receptor activation has been reported to activate non-apoptotic signaling pathways. Here, we report an increased expression of TRAIL in peripheral blood T cells and monocytes from patients with atopic dermatitis compared to control individuals. High TRAIL expression was also observed in skin-infiltrating T cells of atopic dermatitis patients. Topical tacrolimus treatment reduced the total number of T cells in the skin, but the relative proportion of TRAIL positive cells within both CD4+ and CD8+ cell populations did not change. TRAIL was demonstrated to induce the expression of interleukin-1 receptor antagonist in keratinocytes in a caspase-independent manner in vitro. Moreover, increased expression of interleukin-1 receptor antagonist was observed in keratinocytes of atopic dermatitis lesional skin. These data suggest that TRAIL expressing inflammatory skin cells may contribute to the epidermal activation of the interleukin-1 receptor antagonist gene in atopic dermatitis.

Apoptotic pathways are inhibited by leptin receptor activation in neutrophils

A. Bruno, S. Conus, I. Schmid, H.-U. Simon

Leptin regulates food intake as well as metabolic, endocrine, and immune functions. It exerts proliferative and anti-apoptotic activities in a variety of cell types, including T cells. Leptin also stimulates macrophages and neutrophils, and its production is increased during inflammation. In this study, we demonstrate that human neutrophils express leptin surface receptors under in vitro and in vivo conditions and that leptin delays apoptosis of mature neutrophils in vitro. The anti-apoptotic effects of leptin were concentration-dependent and blocked by an anti-leptin receptor monoclonal antibody. The efficacy of leptin to block neutrophil apoptosis was similar to granulocyte colony-stimulating factor. Using pharmacological inhibitors, we obtained evidence that leptin initiates a signaling cascade involving phosphatidylinositol-3-OH

kinase and mitogen activated protein kinase – dependent pathways in neutrophils. Moreover, leptin delayed the cleavage of Bid and Bax, the mitochondrial release of cytochrome *c* and Smac, as well as the activation of both caspase-8 and caspase-3 in these cells. Taken together, leptin is a survival cytokine for human neutrophils, a finding with potential pathologic relevance in inflammatory diseases.

Eosinophils form extracellular traps in bacterial and parasitic infectious diseases

S. Yousefi, R. F. Grifone, A. Straumann, G. J. Gleich, H.-U. Simon
(Collaboration with the Department of Dermatology, University of Utah, Salt Lake City, USA, and Department of Gastroenterology, Kantonsspital Olten, Olten, Switzerland)

Eosinophils are considered as proinflammatory effector cells, which may be helpful in the defense against bacteria and parasites. Here, we demonstrate that activated eosinophils release granule and nuclear proteins, which form extracellular fibers. Similar structures have previously been described following activation of neutrophils and called neutrophil extracellular traps (NETs). We further show that eosinophil extracellular traps (EETs) bind and kill bacteria. EETs were identified in vivo in the course of bacterial and parasitic infections, respectively. Thus, eosinophils contribute to innate immune responses in tissues by inhibition of pathogen spreading and, subsequently, by pathogen killing.

Evidence for distinct cytokine expression patterns among eosinophils of the gastrointestinal tract

A. Straumann, J. Kristl, S. Conus, E. Vassina, H.-P. Spichtin, C. Beglinger, H.-U. Simon
(Collaboration with the Department of Gastroenterology, Kantonsspital Olten; Department of Gastroenterology, University of Basel; and Department of Clinical Pathology, Basel, Switzerland)

In eosinophilic esophagitis, the esophagus is infiltrated with activated eosinophils, often causing tissue damage. Since the intestine of these patients is unaffected, we hypothesized that in this disease different tissue-dwelling eosinophilic populations may co-exist: activated eosinophils infiltrating the esophagus and resting eosinophils residing in unaffected intestine. Using immunofluorescence and immunoassays, we investigated the expression of CD25 and the T_H2 cytokines IL-4, IL-5, IL-10, and IL-13 in esophageal, intestinal and blood eosinophils in controls and eosinophilic esophagitis patients. In controls, a small but significant proportion of intestinal, but no blood, eosinophils expressed CD25 and IL-13. On the other hand, eosinophils infiltrating the inflamed esophageal mucosa of eosinophilic esophagitis patients demonstrated strong evidence for activation, since the majority expressed CD25, IL-4, and IL-13. Moreover, IL-13 positive intestinal eosinophils were increased in patients compared to the normal intestinal mucosa. Taken together, there are distinct cytokine expression patterns among eosinophils under non-inflammatory and inflammatory conditions.

Anticancer drugs target survivin via a PI3K-dependent but Akt-independent signaling pathway in immature neutrophils

S. Martinelli, S. Yousefi, S. Conus, V. Niggli, C. Baumann, M. F. Fey, H.-U. Simon
(Collaboration with the Departments of Pathology and Medical Oncology, University of Bern, Switzerland)

Myelosuppression is the most common unwanted side effect associated with the administration of anticancer drugs and infections remain a common cause of death in

chemotherapy treated patients. Several mechanisms of the cytotoxicity of these drugs have been proposed and may synergistically operate in a given cell. Survivin expression has been associated with cancer, but recent reports suggest that this molecule is also expressed in several immature and mature hematopoietic cells. Here we demonstrate that specific survivin depletion increases caspase-3 activity and that survivin overexpression blocks anticancer drug - induced death in immature human neutrophils. Moreover, treatment of these cells with anticancer drugs reduced endogenous survivin levels causing apoptosis. The anticancer drugs did not directly target survivin, instead they blocked the activity of phosphatidylinositol-3-OH kinase (PI3K), which regulated survivin expression and apoptosis in immature neutrophils. Strikingly, and in contrast to other cells, this pathway did not involve the serine/threonine kinases Akt or the mammalian target of rapamycin (mTOR). These data suggest that drugs, which block either Akt or mTOR, may preferentially induce apoptosis of cancer cells since they exhibit no cytotoxicity for immature neutrophils.

Original publications

1. A. Altznauer, S. Conus, A. Cavalli, G. Folkers, H.-U. Simon:
Calpain-1 regulates Bax and subsequent Smac-dependent caspase-3 activation in neutrophil apoptosis.
J. Biol. Chem. 279 (2004), 5947-5957.

2. F. Altznauer, S. Martinelli, S. Yousefi, C. Thürig, I. Schmid, E. M. Conway, M. H. Schöni, P. Vogt, C. Mueller, M. F. Fey, U. Zangemeister-Wittke, H.-U. Simon:
Inflammation-associated cell-cycle-independent block of apoptosis by survivin in terminally differentiated neutrophils.
J. Exp. Med. 199 (2004), 1343-1354.

3. S. Martinelli, M. Urosevic, A. Daryadel, P. A. Oberholzer, C. Baumann, M. F. Fey, R. Dummer, H.-U. Simon, S. Yousefi:
Induction of genes mediating interferon-dependent extracellular trap formation during neutrophil differentiation.
J. Biol. Chem. 279 (2004), 44123-44132.

4. R. Baumann, S. Yousefi, D. Simon, S. Russmann, C. Mueller, H.-U. Simon:
Functional expression of CD134 by neutrophils.
Eur. J. Immunol. 34 (2004), 2268-2275.

5. D. Simon, E. Vassina, S. Yousefi, E. Kozlowski, L. R. Braathen, H.-U. Simon:
Reduced dermal infiltration of cytokine-expressing inflammatory cells in atopic dermatitis after short-term topical tacrolimus treatment.
J. Allergy Clin. Immunol. 114 (2004), 887-895.

6. T. Bihl, E. Vassina, M. K. Boettger, R. Goldbach-Mansky, M. Seitz, P.M. Villiger, H.-U. Simon:
The T348M mutated form of cryopyrin is associated with defective LPS-induced IL-10 production in CINCA syndrome.
Ann. Rheum. Dis., in press.

7. A. Straumann, H.-P. Spichtin, K. A. Bucher, P. Heer, H.-U. Simon:
Eosinophilic esophagitis: Red on microscopy, white on endoscopy.
Digestion 70 (2004), 109-116.

8. D. Simon, C. Boudny, H. Nievergelt, H.-U. Simon, L. R. Braathen:
Successful treatment of pityriasis lichenoides with topical tacrolimus.
Br. J. Dermatol. 150 (2004), 1033-1035.

9. A. Munitz, I. Bachelet, S. Fraenkel, G. Katz, O. Mandelboim, H.-U. Simon, L. Moretta, M. Colonna, F. Levi-Schaffer:
2B4 (CD244) is expressed and functional on human eosinophils.
J. Immunol. 174 (2005), 110-118.

10. A. Straumann, H.-U. Simon:
Eosinophilic esophagitis – escalating epidemiology?
J. Allergy Clin. Immunol., in press.

11. D. Simon, E. Vassina, S. Yousefi, L. R. Braathen, H.-U. Simon:
Inflammatory cell numbers and cytokine expression in atopic dermatitis after topical pimecrolimus treatment.
Allergy, in press.

12. J. Stucki, H.-U. Simon:
Mathematical modeling of the regulation of caspase-3 activation and degradation.
J. Theor. Biol. 234 (2005), 123-131.

13. D. Simon, H.-P. Marti, P. Heer, H.-U. Simon, L. R. Braathen, A. Straumann:
Eosinophilic esophagitis is frequently associated with IgE-mediated allergic airway diseases.
J. Allergy Clin. Immunol., in press.

14. A. Kerstan, C. Rose, D. Simon, H.-U. Simon, E.-B. Bröcker, A. Trautmann, M. Leverkus:
Bullous delayed pressure urticaria: Pathogenic role for eosinophilic granulocytes?
Br. J. Dermatol., in press.

Review articles

1. A. Straumann, H.-U. Simon:
The physiological and pathological roles of eosinophils in the gastrointestinal tract.
Allergy 59 (2004), 15-25.

2. D. Simon, L. R. Braathen, H.-U. Simon:
Eosinophils and atopic dermatitis.
Allergy 59 (2004), 561-570.

3. U. Zangemeister-Wittke, H.-U. Simon:
An IAP in action: The multiple roles of survivin in differentiation, immunity and malignancy.
Cell Cycle 3 (2004), 1121-1123.

4. A. Straumann, D. Simon, H.-U. Simon:
Eosinophilic esophagitis: new pathogenic insights.
Curr. Immunol. Rev., in press.

Book chapters

1. H.-U. Simon, H. Reuter, M. H. Bickel:
Zur Geschichte des Pharmakologischen Instituts der Universität Bern. In: Geschichte der pharmakologischen, klinisch-pharmakologischen und toxikologischen Institute im deutschsprachigen Raum (Ed. A. Philippou);
Behrenkamp Buch- und Kunstverlag, Innsbruck, 2004, p. 124-131.

2. H.-U. Simon, S. Plötz, D. Simon, R. Dummer, F. Levi-Schaffer:
IL-5 induces functional IL-2 receptors on eosinophils. In: Allergy Frontier and Futures (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, 2004, p. 161-163.

3. A. Straumann, P. Schmid-Grendelmeier, H.-P. Spichtin, H.-U. Simon:
Primary eosinophilic esophagitis: Inflammatory cell pattern and immunopathogenic mechanisms. In: Allergy Frontier and Futures (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, 2004, p. 172-176.

4. D. Simon, S. von Gunten, L. R. Braathen, H.-U. Simon:
Role of costimulatory signals for cytokine production of T-cells in atopic dermatitis. In: Allergy Frontier and Futures (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, 2004, p. 262-265.

5. H.-U. Simon, U. Zangemeister-Wittke:
Apoptosis: Regulation and clinical implications. In: K. Ruckpaul, D. Ganten: Encyclopedic Reference of Genomics and Proteonomics in Molecular Medicine.
Springer-Verlag, Heidelberg, in press.

6. A. Straumann, H.-U. Simon:
Eosinophilic esophagitis: new clinical and pathophysiological insights. In: From genes to phenotypes: the basis of future allergy management. (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, in press.

7. S. Yousefi, S. Martinelli, F. Altznauer, E. Vassina, H.-U. Simon:
Survivin – a key regulator of neutrophil and eosinophil survival. In: From genes to phenotypes: the basis of future allergy management. (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, in press.

8. D. Simon, E. Vassina, S. Yousefi, E. Kozlowski, L. R. Braathen, H.-U. Simon:
Reduced numbers of cytokine-expressing inflammatory cells in atopic dermatitis after topical tacrolimus treatment. In: From genes to phenotypes: the basis of future allergy management. (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, in press.

9. J. Ring, S. G. Plötz, U. Darsow, J. Huss-Marp, M. Braun-Falco, H.-U. Simon, H. Behrendt:
Anti-Interleukin-5 in the treatment of hypereosinophilic skin diseases. In: From genes to phenotypes: the basis of future allergy management. (Eds. J. Bienenstock, J. Ring, A.G. Togias);
Hogrefe & Huber Publishers, Cambridge, Göttingen, in press.

10. H.-U. Simon:
Physiological and pathophysiological roles of eosinophils in innate immunity. In: Allergy and Asthma in the Modern Society: The Scientific Approach. (Ed. R. Crameri);
S. Karger AG, Basel, in press.

Group Prof. Jörg W. Stucki

Group member: Dr. Clemens Wagner, PhD[1]
PD Dr. Robert Urbanczik, PhD

[1]In addition, independent research work with own Swiss National Science Foundation projects.

Prof. Dr. Jörg W. Stucki:

Some properties of the Willamowski-Rössler model were studies by numerical simulations. From the original equation a minimal version was derived which also exhibited the characteristic properties of the original model. This minimal model suggests that the Willamowski-Rössler model contains the Volterra-Lotka model as a core component. Akin to the original model, the minimal model has two steady states, a saddle point responsible for chaos and a fixed point which dictates its dynamic behaviour. The chaotic attractor is located close to the surface of the basin of attraction of the saddle node. Surprisingly, it was found that the mean values of the variables corresponded to the steady states during oscillations even under chaos. This allowed comparing the entropy production of the Willamowski-Rössler oscillator with the entropy production of the steady states. It was found that oscillations always lowered the entropy production of the system. Since under these circumstances less energy is dissipated to produce the same output, the oscillating system is more efficient than the non-oscillatory one.

Original publications

1. J. W. Stucki:
Chromokinetics of metabolic pathways.
Eur. J. Biochem. 271 (2004), 2745-2754.

2. J. W. Stucki, H.-U. Simon:
Mathematical modeling of the regulation of caspase-3 activation and degradation
J. Theor. Biol. 234 (2005), 123-131.

Dr. Clemens Wagner:

Due to the progress in genomics the reconstruction of whole cell metabolic networks has become feasible. The mode of operation of these systems can be approximated by the steady state. As a result of the thermodynamic constraints all possible states of the network describe a convex geometric object, which is called the flux cone.

From a mathematical point of view this cone has two different representations. Either it is given by a set of hyper-planes and a set of inequalities (derived from the irreversibility conditions on flows) or it is represented by the edges of the cone. Each edge forms an equivalence class, which is characterized by an elementary flux vector. This latter description is called VR-representation (Vertex: elementary flux vector; Ray: edge) whereas the former is named H-representation (Hyper-plane). The link to biochemistry is the following: each row of the stoichiometry matrix defines a hyper-plane in the space of flows and the elementary flux vectors represent minimal sets of enzymes, which operate independently. Considering a metabolic network as a graph the elementary flux vectors are non-redundant sub-graphs. This property is exploited in many different applications (see Invited Articles 1).

The null-space algorithm
R. Urbanczik, C. Wagner
The complexity of whole cell metabolic networks requires efficient algorithms to compute the elementary flux vectors from the stoichiometry matrix. We have recently devised a new method to calculate these operating modes (see Original Publication 1). Compared to previous suggested algorithms this new method has the advantage that it determines the elementary flux vectors in a space of reduced dimension. This was achieved by first satisfying the null space condition (steady state condition) and second fulfilling the irreversibility conditions instead vice versa. We have further improved the algorithm and put it on solid mathematical ground (see Original Publication 2). As a result, the computation time is considerably shortened. When we apply the new method to the central carbon metabolism of *E. Coli* the time required to compute the set of elementary flux vectors was more than 20 fold reduced.

The minimal generating set
R. Urbanczik, C. Wagner
In a unidirectional representation of the network each elementary flux mode corresponds an edge of the flux cone. If we allow for reversible reactions some of these edges become internal rays. As a consequence, the set of elementary modes, which describes the flux cone is reduced. This new subset was then called the minimal generating set. As the name suggests it is not possible to find a smaller set, which describes the flux cone. It also has an interesting link to another metabolic network analysis tool, the flux balance analysis (FBA). This approach is based on Linear Programming where the state of the network can be resolved using the thermodynamic constraints and a target function, which e.g. optimizes growth. FBA can be used to determine the phenotype phase plane. Thereby, the state of the system with optimal biomass production is determined using different input conditions. We showed that the phenotype phase plane analysis corresponds to the projection of the minimal generating set to the input/output space (see Original Publication 3).

Information processing in fractally coupled networks
C. Wagner, R. Stoop
There is experimental evidence that sensory information in the primary visual cortex is partly processed by synchronization. Furthermore, it has been proposed that neural networks in the brain develop by minimizing the total length of connections. We study synchronization behavior and information transfer in models of neural networks with different architectures. Our analysis revealed that fractally coupled networks with a bi-power law distribution of connections perform best under these constraints (see Refereed Proceedings 1).

Original Publications

1. C. Wagner:
Nullspace approach to determine the elementary modes of chemical reaction systems.
J. Phys Chem B 108 (2004), 2425 – 2431.

2. R. Urbanczik, C. Wagner:
An improved algorithm for stoichiometric network analysis: Theory and application.
Bioinformatics (2004), in press.

3. C. Wagner, R. Urbanczik:
The geometry of the flux cone of a metabolic network.
Biophys. J., in press.

Invited Article

1. C. Wagner:
Mit Systembiologie gegen Parasiten.
Bioworld 1 (2004), 2 – 5.

Refereed Proceeding

1. C. Wagner, R. Stoop:
Information processing in fractally coupled networks.
In: Conference Proceedings Nolta 2004 (2004), Fukuoka, Japan

4.2. Congress Invitations

Prof. Ulrich E. Honegger

Fortschritte in der Pharmakologie. ZNS. Bern (CH), January 29, 2004;
Fortschritte in der Anxiolytika-Forschung. Konsequenzen für die Praxis.

Schweizerischen Gesellschaft für Klinische Neurophysiologie (SGKN-SVEPTA), Kantonsspital BL Bruderholz Basel (CH), April 22-24, 2004;
Umgang mit Antiepileptika.

European Conference on Epilepsy, Workshops on basic research, Vienna (Austria), Roundtable, May 29 – June 3, 2004;
Mode of actions of antiepileptics.

Deutsche, Österreichische und Schweizerische Gesellschaften für Biologische Psychiatrie, 6. Dreiländertreffen, Psychiatr. Universitätsklinik Waldau, Bern(CH), Oct. 21, 2004;
Aripiprazole: Partieller Dopaminagonismus/-Antagonismus als therapeutisches Prinzip von Antipsychotika.

Dr. Sibylle Bürgi and Prof. Ulrich E. Honegger

Collegium Internationale Neuro-Pharmacologicum (CINP) Breaking Scientific News Session, Paris (F), June 21 –26, 2004;
Modification of cellular recycling and trafficking of receptors as a mechanism of antidepressant drug action.

Prof. Harald Reuter

Tschira Symposium on Neurobiology, Heidelberg (D), March 4–6, 2004.

National Academy of Sciences und IPSO Council Meeting, Washington (USA), April 18-23, 2004.

Synaptic transmission:From ion channels to neuronal network function. Symposium zum 60. Geburtstag von Prof. Erwin Neher, Göttingen (D), June 4-6, 2004.

Abschiedssymposium für Prof. Hasso Scholz, Hamburg (D), June 9-11, 2004.

Calcium in Health and Disease, Rovaniemi (FIN), July 5-9, 2004.

Abschiedsymposium für Prof. Beat Gähwiler, Monte Verità (Ascona) (CH), Sept. 19-23, 2004.

Paris UNESCO: IPSO Council Meeting, Paris (F), Nov. 13-15, 2004.

Prof. Hans-Uwe Simon

European Academy of Allergology and Clinical Immunology (EAACI) – Section Immunology meeting: Immune Mechanisms of Allergy, Davos (CH), Jan. 09, 2004;
Understanding neutrophil differentiation and function – implications for the therapy of inflammatory disorders.

United Airways: Entzündungsmechanismen in den Atemwegen, Fortbildung Inselspital, University of Bern, Bern (CH), March 18, 2004;
Entzündungsmechanismen der oberen Atemwege.

60th Annual Meeting of the American Academy of Allergy Asthma and Immunology (AAAAI), San Francisco (USA), March 19-23, 2004;
Eosinophil – T-cell –interactions.

60th Annual Meeting of the American Academy of Allergy Asthma and Immunology (AAAAI), San Francisco (USA), March 19-23, 2004;
Eosinophilic esophagitis: Clinical spectrum and evaluation in adults.

Annual Meeting of the Swiss Society for Allergology and Clinical Immunology (SSAI), Geneva (CH), April 15-17, 2004;
Novel aspects in the treatment of allergic diseases.

Workshop: Eosinophile im Jahr 2004, Universitätsspital Zürich, Zürich (CH),
May 06, 2004; Hypereosinophilie-Syndrom.

XXIII. Congress of the European Academy of Allergology and Clinical Immunology (EAACI), Amsterdam (NL), June 12-16, 2004;
Pharmacogenomics.

Cell Death Differ. and Apogenix Biotechnology AG, Heidelberg – Workshop on “New insights into apoptosis: from basic mechanism to therapeutic application”, Loveno di Menaggio (I), June 28 – July 01, 2004;
Neutrophil differentiation and apoptosis regulation.

British Society for Allergy & Clinical Immunology (BSACI), Annual Meeting 2004, Loughborough (UK), July 12-14, 2004;
Anti-IL-5 and eosinophil diseases.

25th Symposium of the Collegium Internationale Allergologicum (CIA), Bornholm (DK), August 24-30, 2004;
Inflammation-associated block of apoptosis by survivin in neutrophils.

69. Jahresversammlung der Schweizerischen Gesellschaft für Gastroenterologie und Hepatologie, Montreux (CH), Sept. 9-11, 2004;
Apoptosis: Basic principles and clinical implications.

4th International Conference on Cysteine Proteinases and their Inhibitors, Portoroz (SL), Sept. 11-15, 2004;
Role of cathepsins and calpains in neutrophil death pathways.

3rd Swiss Apoptosis Meeting, Bern (CH), Sept. 16-17, 2004;
The role of survivin in terminally differentiated mature neutrophils.

10th Scientific Symposium, Austrian Pharmacological Society (APHAR), Vienna (A), Sept. 23-26, 2004;
The role of eosinophils in inflammation: Lessons from anti – IL-5 antibody studies.

Innovation in der Phytotherapie: Petasites hybridus L. als Antiallergikum, Zürich (CH), Sept. 29, 2004;
Immunologische Grundlagen von Allergien und das Potential der Pestwurz auf Basis bisheriger pharmakologischer Erkenntnisse.

Ekatherina Vassina

12th Euroconference on Apoptosis, Chania (Greece), Sept. 17 – 20, 2004;
Role of inhibitor of apoptosis proteins (IAPs) in eosinophil apoptosis.

Dr. Shida Yousefi

Bern Immunology Club, Bern (CH), April 28, 2004;
Introduction into confocal microscopy and image analysis.

International Summer School, Villars-sur-Ollon (CH), August 14-16, 2004;
Global gene expression analysis during neutrophil differentiation.

4th Balkan Immunology Meeting, Istanbul (Turkey), Sept. 5-8, 2004;
Induction of genes mediating interferon-dependent extracellular traps formation during neutrophil differentiation.

10th Annual Meeting of the Austrian Pharmacological Society (APHAR), Vienna (A), Sept. 23-26, 2004;
Induction of genes mediating interferon-dependent extracellular traps formation during neutrophil differentiation.

4.3. Seminar Invitations

Prof. Ulrich E. Honegger

Ärzte und Hebammen Fortbildung, Rund ums Stillen, Inselspital, University of Bern, Bern (CH); October 10, 2004;
Medikamente und Stillen.

6th Continuation weekend for neurologists, Sils-Maria (CH); October 22-24, 2004;
Interaction of central acting drugs.

Galenicare Fortbildungstag, Bern (CH); October 21, 2004;
Medikamentinteraktionen, Ursachen und Folgen.

AGFAM Fortbildungstage Zürich (CH); November 3, 11, 24, and 30, 2004;
Psychopharmaka und Rezeptvalidierung.

Gerichtskreise VII,VIII,IX Kanton Bern, Weiterbildungstagung, Psychiatrische Universitätsklinik Waldau, Bern (CH); November 26, 2004;
Psychopharmaka, Wirkungsmechanismen und Auswirkungen.

Prof. Hans-Uwe Simon

Kinderklinik, Inselspital, University of Bern, Bern (CH); Febr. 16, 2004; guest of Prof. R. Kraemer:
Therapie allergischer Erkrankungen mit Petasites hybridus (Ze339, Pollivita).

Institute of Cell Biology and Morphology, University of Lausanne, Lausanne (CH); March 4, 2004: guest of Prof. C. Widmann:
New insights into the regulation of neutrophil apoptosis.

American Academy of Allergy Asthma & Immunology, 60th Annual Meeting, San Francisco (USA); March 21, 2004;
Effector functions of eosinophils in allergic inflammation.

Hämatologisches Zentrallabor, Inselspital, University of Bern, Bern (CH); March 25, 2004; guest of Prof. B. Lämmle:
Pathophysiologische Aspekte der Eosinophilie.

Dept. of Pharmaceutical Sciences, University of Bologna, Bologna (I); April 22, 2004; guest of Prof. A. Cavalli:
Neutrophil apoptosis in health and disease.

Dept. of Pharmaceutical Sciences, Pharmazentrum, University of Basel, Basel (CH); May 05, 2004; guest of Prof. K. Hofbauer:
Survivin – a new anti-inflammatory drug target?

Dept. of Internal Medicine (Respiratory Medicine), Philipps-University Marburg, Marburg (D); July 05, 2004; guest of Prof. H. Fehrenbach:
Von Interferonen und MIF zu Smac und Survivin: Regulation der Differenzierung, Aktivierung und Apoptose von Neutrophilen.

Dept. of Hematology, Inselspital, University of Bern, Bern (CH); August 17, 2004; guest of Prof. A. Tobler:
Understanding neutrophil differentiation and functions in inflammation and cancer – role of interferons.

Faculty of Pharmacy, University of Ljubljana, Ljubljana (SL); Sept. 13, 2004; guest of Doz. Dr. Irena Mlinaric:
Cooperation of caspases and non-caspase proteases in neutrophil death pathways.

Prof. Erwin Sigel

Hoffmann-La Roche, Basel; March 8, 2004; guest of Drs. G. Trube and F. Knoflach:
The $GABA_A$/Benzodiazepine Receptor: Architecture and Modulation

Ecole Polytechnique Fédéral de Lausanne (EPFL), SB/ISB/LCPPM (Laboratoire de chimie physique des polymères et membranes) Lausanne; November 25, 2004; guest of Prof. H. Vogel:
Use of subunit concatenation for the study of the functional architecture of $GABA_A$ receptors

Ecole Polytechnique Fédéral de Lausanne (EPFL), SB/ISB/LCPPM (Laboratoire de chimie physique des polymères et membranes) Lausanne; November 25, 2004; 3h lecture and tutorial with students; guest of Dr. R. Hovius:
Expression of membrane proteins in Xenopus oocytes

Dr. Stephan von Gunten

Dept. of Medical Oncology, University of Zurich, Colloquium in Applied Cancer Research; June 02, 2004; guest of PD Dr. U. Zangemeister-Wittke:
Role of Siglec-9 in the regulation of neutrophil apoptosis in inflammatory disease.

Dr. Clemens Wagner

Dept. of Endocrinology, University of Bern; “Biological Rhythms: Growth Hormone Oscillations in the Rat” (2004)

Inst. of Biochemistry, University of Bern; “Redundancy and Robustness in Complex Metabolic Networks” (2004)

Center for Nonlinear Dynamics, Montreal (Canada); “Taming Redundancy in Metabolic Networks” (2004)

International Symposium on Nonlinear Theory and its Application, Fukuoka (Japan); “Information Processing in Fractally Coupled Networks” (2004)

Department of Chemie and Biochemie, University of Bern; Redundancy and robustness in complex metabolic networks (2004).

4.4. Organization of Meetings and Courses

PD Dr. Kurt Baltensperger

Image Acquisition and Advances Image Analysis. Feb. 04, 2004, and Feb. 18, 2004

Prof. Erwin Sigel

5th Practical Course: “Functional Analysis of Living Cells”, March 1 – March 5, 2004

Prof. Hans-Uwe Simon

Clinical Immunology Conference: The hypereosinophilic syndrome; Bern (CH), Jan. 21, 2004

Symposium of the Swiss Society of Pharmacology and Toxicology: Tyrosine kinase inhibition as a therapeutic principle; USGEB meeting; Fribourg (CH), Febr. 26, 2004

Cell Death Differ. and Apogenix Biotechnology AG, Heidelberg – Workshop on “New insights into apoptosis: from basic mechanism to therapeutic application” (together with E. Candy, Italy, and H. Walczak, Germany); Loveno di Menaggio (I), June 28 –July 01, 2004

3rd III-Bern International Summer School; Villars-sur-Ollon (CH), August 14 - 16, 2004

3rd Swiss Apoptosis Meeting (together with Prof. Dr. T. Brunner, Bern); Bern (CH), Sept. 16-17, 2004

Bern Immunology Club (together with the other founder members); Dept. of Pharmacology, University of Bern, one meeting in each month of 2004

Dr. Shida Yousefi

DKF-course in immunofluorescent staining, confocal microscopy, and image analysis. Bern (CH), Nov. 24-26, 2004

4.5. Invited Chairperson at Congresses

Prof. Hans-Uwe Simon

European Academy of Allergology and Clinical Immunology (EAACI) – Section Immunology meeting: Immune Mechanisms of Allergy, Session 2 “Innate Immune Response”; Davos (CH), Jan. 09, 2004

16. Mainzer Allergie-Workshop, Deutsche Gesellschaft für Allergologie und Klinische Immunologie; Sitzung “Therapie/Perspektiven”; Mainz (D), March 12-13, 2004

XXIII. Congress of the European Academy of Allergology and Clinical Immunology (EAACI); Main Symposium 9 “IgE-associated AEDS versus non-IgE-associated AEDS”; Amsterdam (NL), June 12-16, 2004

5th International Cell Death Symposium, International Cell Death Society; Session IV “Agents of destruction (caspases, cathepsins, granzymes)”; Maynooth (Ireland), June 25-28, 2004

69. Jahresversammlung der Schweizerischen Gesellschaft für Gastroenterologie und Hepatologie; Session “Beyond the endoscope: Apoptosis in the GI tract“; Montreux (CH), Sept. 9-11, 2004

4th International Conference on Cysteine Proteinases and their Inhibitors; Session “Apoptotic cysteine proteases”; Portoroz (SL), Sept. 11-15, 2004

3rd Swiss Apoptosis Meeting; Session “Apoptosis Pathways I”; Bern (CH), Sept. 16-17, 2004

4.6. Referee Work for Peer-Reviewed Journals

Prof. Ulrich E. Honegger

Biochemical Pharmacology
Life Sciences
Swiss Medical Weekly
Planta Medica

Prof. Hartmut Porzig

N-S Arch Pharmacol
J Cell Biochem

Prof. Erwin Sigel

Biochim Biophys Acta
BioTechniques
Brain Res
Cell+Tissue Research
Eur J Neurosci
FEBS Lett
J Biol Chem
J Membrane Biol
J Neurochem
J Neurosci
J Pharmacol Exp Ther
J Physiol (London)
Mol Brain Res
Mol Pharmacol
Neurochem Int
Neuropharmacology
Pflügers Arch
Proc. Roy Soc B
Trends Pharmacol Sci (TIPS)

Prof. Hans-Uwe Simon

Allergy
Am J Pathol
Am J Physiol - Cell Physiol
Apoptosis
Biochem Pharmacol
Blood
Cell Death Differ
Clin Exp Allergy
Clin Exp Immunol
Cytokine
DMW
Environm Toxicol
Eur J Immunol
Eur Respir J
Exp Dermatol
FEBS letters
Hepatology
Int Arch Allergy Immunol
Int J Hyg Environ Health
J Allergy Clin Immunol
J Biochem Biophys Meth
J Exp. Med.
J Hepatol
J Immunol
J Invest Dermatol
J Leukocyte Biol
J Pharm Pharmacol
Life Science
Oncogene
Proc Natl Acad Sci USA
Planta Medica
Swiss Med Wkly
Thorax

Dr. Shida Yousefi

Cancer Detection and Prevention

4.7. Referee Work for Grant Bodies

Prof. Erwin Sigel

Swiss National Science Foundation (SNF)
The Wellcome Trust, London, UK
Medical Research Council (MRC)
Austrian Foundation for the Advancement of Science
Deutsche Forschungsgemeinschaft (DFG)
Schweizerische Stiftung für Alkoholforschung

Prof. Hans-Uwe Simon

Swiss National Science Foundation (SNF)
Deutsche Forschungsgemeinschaft (DFG)
Medizinische Fakultät der Universität Tübingen, *fortüne*-Programm
Landesstiftung Baden-Württemberg, Stuttgart
Philip Morris External Research Program, Linthicum Heights, Maryland, USA
The Wellcome Trust, London, UK
Medical Research Council (MRC)
Interdisziplinäres Zentrum für Klinische Forschung, Univ. Tübingen

Dr. Clemens Wagner

Canadian Institutes of Health Research (CHIR), International Opportunity Program, together with Prof. G.S. Tannenbaum Prof. M. Mackey, McGill University, Montreal, Canada and S.R. Caplan, Weizman Institute of Science, Rohovot, Israel

4.8. Awards

Dr. Frank Altznauer

Bürgi-Prize 2004
(University of Bern)

Dr. Stephan von Gunten

Prize for the best work in preclinical research in 2004
(Dept. of Clinical Research, University of Bern)

5. Administrative, Advisory, and Honorary Posts

PD Dr. Kurt Baltensperger

Information Technology Coordinator at the PKI (until February 2004)

Roland Baur

Coordinator for radioactive work at the PKI

Prof. Ulrich E. Honegger

Verantwortlicher für das Pharmaziestudium an der Universität Bern

Ortspräsident Pharmazie des BAG, Prüfungssitz Bern

Präsident der Kommission für Fakultätsexamen in Pharmazie der Medizinischen Fakultät der Universität Bern

Mitglied der Subkommission Pharmazie des Leitenden Ausschusses des BAG. Verantwortlich für die Universitäten Bern und Fribourg

Mitglied der Arzneimittelkommission des Schweizerischen Apothekerverbandes

Wissenschaftlicher Beirat des Apothekervereins des Kantons Bern

Member of the GlaxoSmithKline Advisory Board for Epilepsy

Member of the GlaxoSmithKline Advisory Board for Biopolar Disorders

Member of the Zeller Medical Advisory Board

Prof. Hartmut Porzig

President of the Faculty Commission for habilitation and academic promotion (until August 2004)

Member of the steering committee for the curriculum reform of 3rd year medical studies

Member of the working group: “Interfakultäre Graduate School” and Curriculum “Medizinische Biologie”

Member of the Editorial Board of Naunyn-Schmiedebergs Archives of Pharmacology

Prof. Harald Reuter

Elected Member of the International Council of Israeli-Palestinian Science Organization (IPSO)

Chairman of the “Committee on Human Rights“ of the Council of Swiss Academies

Member of the “International Human Rights Network of Academies and Scholarly Societies“

President of the “Schweizerische Stiftung für medizinisch-biologische Stipendien“ (Swiss foundation for medical-biological stipends)

Obmann (chairman) and Senator for the “Section Physiology and Pharmacology/Toxicology“ of the “Deutsche Akademie der Naturforscher Leopoldina“

Member of the Scientific Advisory Board of the Friedrich-Miescher-Institute (Basel)

Prof. Erwin Sigel

Biosafety Coordinator for the PKI

Information Technology Coordinator at the PKI (since March 2004)

Member of the committee supervising the “Programm für die Interfakultäre Ausbildung des Forschungsnachwuchses der Universität Bern (PIAF) (Interfaculty Doctorate and PhD of the Medical Faculty)

Prof. Hans-Uwe Simon

Director of the curriculum “Pharmacology” within the program for interfaculty education of graduate students at the University of Bern

Treasurer, European Cell Death Society (ECDO), since 2000

Member of the Ethics Committee, European Academy of Allergology and Clinical Immunology (EAACI), 2003-2005

Member of the Board of the Immunology Section of the European Academy of Allergology and Clinical Immunology (EAACI), since June 2003

Fellow of the American Academy of Allergy, Asthma and Immunology (AAAAI)

Member of the Annual Meeting Planning Committee (Workshops) of the American Academy of Allergy, Asthma and Immunology (AAAAI), 2002-2005

Member of the Immunomodulation Committee (Workshops) of the American Academy of Allergy, Asthma and Immunology (AAAAI), 2004-2006

Member of the Board, Swiss Academy for Medical Ethics

Member of the Central Committee of the Union of the Swiss Societies for Experimental Biology (USGEB/USSBE), since 2001

Member of the Council of the Swiss Society of Pharmacology and Toxicology (SSPT), since 2002

President of the Swiss Society of Pharmacology and Toxicology (SSPT), since 2004

Member of the Scientific Advisory Board, Society in Science: The Branco Weiss Fellowship, since 2003

Member of the Executive Committee, International Eosinophil Society (IES), since 2003

Representative of the Medical Faculty within the Foundation for the Promotion of Scientific Research at the University of Bern, since Nov. 2004

Associate Editor, Allergy

Section Editor, Apoptosis

Member of the Editorial Board, International Archives of Allergy and Immunology

Member of the Scientific Board, Allergologie

Member of the Editorial Board, Clinical and Experimental Allergy

Member of the Editorial Board, Int. Journal of Hygiene and Environmental Health

Member of the Advisory Board, Allergo-Journal

Member of the Advisory Editorial Board, Planta Medica

Dr. Clemens Wagner

Webmaster of the PKI

Dr. Shida Yousefi

Operator of the Confocal Microscopy Facility of the Dept. of Clinical Research (located at the PKI)

Operator of the Image Analysis Facility of the Dept. of Clinical Research (located at the PKI)

Coordinator for PC work at the PKI

6. Services

6.1. Confocal Microscopy

The facility hosts a Zeiss laser scanning microscope (LSM410), which may be used by members of the Medical Faculty at a small charge (CHF 10 per h). As a major expansion of the facility a workstation for quantitative image analysis and 3-D representation of microscopic data was purchased in 2002. During the past year the confocal microscope has been used by a total of 46 different users with 14 different affiliations. Together with the Image analysis station, it was in operation for approximately 450 hours. The facility for confocal microscopy and image analysis was operated by Dr. S. Yousefi. Under the supervision of the coordinator, a whole team of qualified individuals provides training for new users, as well as technical and scientific support. The operator's time spent for the facility amounted to over 500 hours.

6.2. Flow Cytometry

A service is provided for analyzing potential pathogenic mechanisms of eosinophilic disorders, sepsis, and other inflammatory diseases. Monitoring of patients under immunomodulatory therapy is also included. The costs are currently covered by research grants of the coordinator (Prof. H.-U. Simon), who can also be consulted for scientific support. Usage of the flow cytometer by non-members of the institute within collaborative projects is also possible.

7. Public Work

7.1. Art Exhibitions

Marianne Dahinden, Bern, Switzerland
Vernissage: June 03, 2004

Adrian Ciurea, Zurich, Switzerland
Vernissage: Oct. 21, 2004

8. Sponsors

8.1. Research Grants

PD Dr. Kurt Baltensperger
Swiss National Science Foundation (grant No. 31-059124.99) (until March 2004)
Novartis Stiftung für Biologisch-Medizinische Forschung

Prof. Ulrich E. Honegger

GlaxoSmithKline
Zeller Medical AG, Romanshorn (CH)

Prof. Hartmut Porzig

Schweizerische Krebsliga (together with K. Baltensperger, grants No. SKL 778-2-1999, OCS-01404-08-2003)
Sandoz Foundation, Basel (CH)
Jubiläumsstiftung der Schweizerischen Mobiliar Genossenschaft
Bonizzi-Theler Foundation

Prof. Erwin Sigel

Swiss National Science Foundation (grant No. 3100A0-105372/1)

Prof. Hans-Uwe Simon

Swiss National Science Foundation (grant No. 31-58916.99)
OPO-Foundation, Zurich (CH)
Bernische Krebsliga (together with S. Yousefi)
Stiftung zur Förderung der wissenschaftlichen Forschung an der Universität Bern

Dr. Clemens Wager

Swiss National Science Foundation (grant No. 3100A0-102269/1)

Dr. Shida Yousefi

Swiss National Science Foundation (grant No. 31-068449.02)
Bernische Krebsliga (together with H.-U. Simon)

8.2. Meetings

3rd III-International Summer School, August 14-16, 2004

Alexis Corporation, Lausen
Allergomed, Therwil
Becton Dickinson Biosciences, Allschwil
COST action 844, EU
Essex Chemie AG, Luzern
GlaxoSmithKline AG, Münchenbuchsee
LabForce AG, Nunningen
Miltenyi Biotec GmbH, Bergisch-Gladbach (D)
PerkinElmer Life Sciences, Schwerzenbach
Pharmacia Diagnostics AG, Dübendorf
Union of the Swiss Societies of Experimental Biology (USGEB)
ZLB Bioplasma AG, Bern

3rd Swiss Apoptosis Meeting, September 16-17, 2004
Alexis Corporation, Lausen
Catalys AG, Wallisellen
Cell Signaling Technologies, Allschwil
Fluka Chemie GmbH, Buchs
Juro Supply GmbH, Lucerne
LabForce AG, Nunningen
VWR International AG, Lucerne
Institute of Pathology, University of Bern
EU COST Action 844 - Switzerland

8.3. Travel Support

Hans-Sigrist-Stiftung
Support of Prof. Dr. Francesca Levi-Schaffer,
3rd III-International Summer School, August 14-16, 2004

8.4. Other Support

Bürgi fund
Seminar series of the institute