From Bench to Bedside
Translational Medicine at the GSF
At the GSF we work on the foundations of a medicine and health care future as well as on ecosystems with significant impact on human health. A better understanding of environmental influences on health and diseases helps us to derive effective measures for prevention in terms of causal therapies. Therefore, environmental diseases are the focus of our research. We are committed to ensure that patients benefit quickly from the results.

Translational research – the transfer of knowledge from basic research to clinical applications and vice versa – is our key to success.

Our objective task is not classical pharmaceutical research. Our approach is based on a very interactive network of expertise and capabilities from basic research on biological mechanisms through to phase III studies. We have the expertise and the resources to conduct research from target to phase III studies, i.e. from the search of the relevant biological mechanism all the way to the application on the patient. With this new approach we distinguish ourselves from classical pharmaceutical research, e.g. in the pharmaceutical industry.

Quite within the spirit of the central theme of the Helmholtz-Gemeinschaft Deutscher Forschungszentren (Helmholtz Association of German Research Centers), of which our research center is a member, we want to contribute to preserving and improving the basis for human life by embarking on new ways to individualized diagnosis, prevention and causal therapy also in the future.

This brochure was prepared to give you an understanding of the diversity of our translational efforts in to research. In the first part you will become acquainted with our Clinical Cooperation Groups, which are most essential instruments of translational research at the GSF. In the second part you will be given an insight into our Clinical Research Platforms, where GSF scientists find the latest methods and technology for their joint work with external clinical partners under one roof with excellent scientific support. In the last part of this brochure you will be able to take a closer look at four highlights from our current translational research program.

Prof. Dr. Günther Wess
President and CEO

A Word of Introduction
Monday morning, 9:00, Hämatologikum in Munich Großhadern: Scientists from the GSF Institutes of Molecular Immunology as well as Clinical Molecular Biology and Tumor Genetics are meeting with physicians from Klinikum Großhadern, the university hospital of Ludwig-Maximilians-University, for their „joufixe.“ They are discussing intermediate results of the ongoing clinical study on immunotherapy for kidney cancer patients. Both sides, scientists in basic research and clinicians, highly appreciate these meetings, from which they take new suggestions and ideas back to their everyday work in the laboratory and hospital, respectively.

Building the bridge from the laboratory to the hospital – summed up by experts in the field as „Translational Research“ – has long been
a fact at the GSF-Research Center. In this context the day on which the first Clinical Cooperation Group (KKG) for Aerosol Medicine was established in 1995 was a memorable date. Scientists from the GSF Institute of Inhalation Biology and representatives of what is now the Asklepios-Fachkliniken in Gauting established a project group for the purpose of linking clinically relevant questions from clinical everyday life with experimental research. In the long term new diagnostic and therapeutic strategies were supposed to enter clinical practice in the form of clinical studies.

The concept was successful and was soon to find the approval of decision-making bodies involved in science policy. Thirteen such translational working groups have been established to date.

**Pillars of the Concept of Success**

“The Clinical Cooperation Groups were, however, just one of the pillars we used to establish and further extend our concept on translational research,” says Prof. Günther Wess, the President of the GSF. The central pillar for a translational medicine of tomorrow is the excellent basic research in the life sciences. Last but not least it is the experimental facilities, such as the German Mouse Clinic or the Genome Analysis Center, as well as research platforms close to the clinic, such as the Immune Monitoring Platform, which are used by scientists all over the world, that form important components of translational research. Another pillar is the unique close link between biomedicine and environmental research in the research program of the GSF. “And finally it is the long-standing and close network with numerous external clinical partners which allows us to take completely new individualized approaches to diagnosis and prevention as well as causal therapy.” Wess explains.

Concepts are not designed to be kept in the drawer, they must be implemented in practice. At the GSF a variety of milestones witness the fruits of translational research. They build the bridge from the exploration of basic biological mechanisms to the direct benefit for the patient. This includes, e.g., the first successful bone marrow transplant, which resulted in a breakthrough in the treatment of leukemias under Prof. Hans-Jochem Kolb at the GSF Institute of Molecular Immunology in cooperation with clinics. It also includes the regional deep hyperthermia developed by Prof. Rolf Issels from the GSF Institute of Molecular Immunology, which is currently applied to tumor treatment in a model project.

**Ideas for Tomorrow**

But the GSF does not want to rest on its laurels. In its new research program the next steps for the further extension of the focus of “Translational Research” have already been determined.

The range of subjects of the 13 Clinical Cooperation Groups will be extended to include new issues. In addition to the short-term projects initiated so far, there will be long-term studies of a multidisciplinary character.

In view of the increasing socio-economic significance of lung diseases, the GSF is planning to establish an interdisciplinary translation center for lung diseases together with Ludwig-Maximilians-University Munich. This center is expected to give new impulses to lung research in Germany.

The start-up of another service facility for scientists and clinical partners, a GMP cleanroom system for the production of cell preparations is also underway.

So the GSF will continue to make its contribution to the development of translational research on an international level in the future.
Elevated body temperature causes stress to cells including tumor cells: if they are heated to 40 to 44 °C by electromagnetic waves, collective cell death sets in at a temperature of 42 °C. Temperatures of more than 40° make tumor cells more vulnerable to natural defense processes and radiation or chemotherapy. “Due to the dissipation of heat from sites with good blood circulation we cannot heat the tumor uniformly,” explains Professor Rolf Issels, head of the Clinical Cooperation Group Hyperthermia. “The areas with good circulation, however, are reached particularly well by cytostatics.”

Prof. Rolf Issels, head of the Clinical Cooperation Group “Hyperthermia” at the GSF, started his work for the benefit of regional deep hyperthermia (RHT) for cancer therapy back in the mid-eighties. Since 1993 RHT has been applied as a therapy in a model project by the statutory health insurances. In 1999 the KKG was founded with research ranging from clinical research to deep hyperthermia and biological research in the field of immunology and cell biology.
Quite in the spirit of the translational approach to research of the GSF it is not only the purely clinical studies, but also associated biological research aspects which have higher priority in the Clinical Cooperation Group “Hyperthermia.” Various groups focus mainly on two areas: the influence of heat shock proteins on the immune system, and liposome research.

**Heat Shock Proteins – Activators of the Immune System**

In biological terms treatment with hypothermia means that heat shock proteins (HSPs), also called stress proteins, are induced in the tumor. They are of interest to cancer research, because they interfere with the body’s own immune system in different ways. Among other things they mark tumor cells and make them visible to the killer cells of the immune system. Therefore, cells producing HSP can be destroyed more effectively by killer cells and the immune system can fight the tumor more effectively. In their studies Issels and his colleagues found out that the induction of protein HSP 70 intensifies the immune response against the tumor in two ways. On the one hand HSP 70 acts as a danger signal for natural killer cells and for dendritic cells, which increases their proliferation and cytotoxic activity. In addition to this cytokine function an antigen-specific T-cell response could be achieved with HSP 70 from human melanoma cells: if HSP complexes are isolated from the melanoma cells and put on dendritic cells, they will mature to become antigen-presenting cells (APCs) which process antigens and present them on their cell surface, so that they can be recognized by the T-cells. The studies on the dendritic cells were conducted in close cooperation with Dr. Elfriede Nössner from the GSF Institute of Molecular Immunology.

**Liposomes as Ferries**

The second important supporting pillar of biological research in the KKG “Hyperthermia” are liposomes. These artificial globules of phospholipids into which active substances can be introduced, are “highly interesting”, Issels says with great enthusiasm. In cooperation with the MPI for Biophysical Chemistry in Göttingen his colleague Dr. Lars Lindner could produce thermo-susceptible liposomes, which open at certain temperatures (41 to 42°C) and release their contents. This opens up undreamt-of possibilities for the therapy with hyperthermia: highly toxical cytostatics could be transported to the tumor with the liposomes and released there specifically by heating. This is being investigated for the KKG’s own liposomes using the model of the amelanotic melanoma on the Syrian hamster. On the same model another possibility for the use of liposomes is also being investigated of late: temperature-sensitive liposomes filled with a contrast medium are supposed to make temperature control during hyperthermia treatment easier. If the contrast medium is released at defined temperatures and becomes visible in the NMR, invasive temperature control using probes might become superfluous. This would mean considerable relief for the patient, because “it would allow proper non-invasive temperature measurement, not only the monitoring of hot spots,” Issels says taking a look into the future. Lindner’s working group was recently awarded a prize at the Munich Business Plan Competition.

**Clinic and Laboratory Benefit from Each Other**

“With the clinical studies and the simultaneous connection with fundamental research, the KKG is an ideal instrument of translational research,” Issels concludes. “I think that the establishment of this particular KKG was most decisive for the progress of a new treatment technique with all facets of research.”

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**Symbiosis of Biological Research and Clinic**

*Graph: Treatment with regional and part body hyperthermia 1986 to 2005*

- **RHT = regional hyperthermia**
- **PBH = part body hyperthermia**
Issels first started his work for the benefit of regional deep hyperthermia (RHT) for cancer therapy in 1986 as one of the pioneers in this field. From the start Issels used soft tissue and bone tumors as model tumors, which start in the connective and supporting tissues and are called sarcomas. In the clinical area Issels and his colleagues were particularly interested in when a particular soft tissue sarcoma can be treated better by the combination of hyperthermia with other therapies.

A phase III study was started with high-risk soft tissue sarcoma patients, which should show whether the combination of hyperthermia and chemotherapy improves the chances for recovery compared to chemotherapy only for these deep-seated, large tumors. A previous phase II study showed encouraging results: it was shown that patients responding to hyperthermia treatment have a significantly greater chance to live without any tumors after a period of five years.

Issels has meanwhile transferred the knowledge gained for sarcomas to colon and rectal cancer as well as very recently to the locoregionally advanced stage of pancreatic cancer. It is being investigated whether the combination of chemotherapy and/or radiochemotherapy with hyperthermia improves the success of the therapy. For these tumors in the abdomen or pelvis the clinical cooperation group (KKG) has a novel hybrid system consisting of a hyperthermia device and an NMR tomograph. Using this system the complete area from the pelvis to below the lungs can be heated in one go. This NMR tomograph also prevents healthy tissue from being damaged due to excessive temperatures in so-called “hot spots”. “In Germany our KKGs at Klinikum Großhadern and Charité in Berlin are the only centers doing part body hyperthermia in model projects,” Issels reports proudly. In 1991 Issels was awarded the German Cancer Award for his work on clinical hyperthermia. Under the guidance of the GSF a “Virtual Institute of Excellence” has been established between the two centers through the Initiative and Networking Fund of the Helmholtz-Gemeinschaft for an initial period of three years.

In the SIGMA EYE MR applicator, a hybrid system consisting of a hyperthermia device and an NMR tomograph, tumor patients receive part body treatment. Apart from Charité in Berlin the GSF is the only institution offering this extended method.
In Gauting near Munich the GSF has established the Clinical Cooperation Group (KKG) “Inflammatory Lung Diseases” on the campus of the Asklepios-Fachkliniken. This KKG was the first of its kind to transfer insight gained at the laboratory to clinical practice and vice versa to include clinical results in its further fundamental research. It is affiliated to the GSF Institute of Inhalation Biology and closely cooperates with the doctors of Fachkliniken (specialized hospitals) for pulmonology and thoracic surgery. This means that the scientists can, for example, obtain samples from lung patients of the hospital.

Inflammatory Lung Diseases

Chronic bronchitis and other respiratory diseases may be caused by inhaled particles. The Clinical Cooperation Group “Inflammatory Lung Diseases” of the GSF analyzes the mechanisms of the pathogenesis in cooperation with the Asklepios-Fachkliniken. With this work the Group opens up new avenues for the diagnosis and therapy of inflammatory lung diseases.
The Gauting KKG “Inflammatory Lung Diseases” focuses on environmental chronic obstructive pulmonary disease (COPD). There are overlaps with other clinical pictures, such as chronic bronchitis, asthma and emphysema. The orange areas are assigned to COPD.

Fatal Consequences of Fine Particles

The KKG focuses on environmental chronic obstructive pulmonary disease (COPD). This includes chronic obstructive bronchitis and pulmonary emphysema. In emphysema alveolar tissue is destroyed, which results in a reduction of the internal surface of the lungs, which is important for gas exchange. COPD is one of the most frequent diseases worldwide and was the fourth most common cause of death in Germany in 2001. What makes this disease so fatal is that current therapies only mitigate the symptoms, they cannot halt the course of the disease altogether. COPD is also difficult to diagnose. Most patients with COPD show all three symptoms: chronic bronchitis, emphysema and excessive secretion of mucus.

In the bronchial secretion of COPD patients the Clinical Cooperation Group showed a macrophage population of cells which are smaller than the macrophages known and are, therefore, called small sputum macrophages. The portion of this population is normally only approx. ten per cent of all macrophages, but may rise to up to 90 per cent in COPD patients. The cells seen on the screen are isolated from the sputum samples and applied to a slide. The cellular structures can be made visible by Pappenheim staining, so that a morphological differentiation is possible.
Small Sputum Macrophages refer to COPD

The KKG investigates the influence of particles on the mechanisms of the development of COPD and wants to develop new diagnostic and therapeutic methods. For this purpose, Dr. Marion Frankenberger, head of the Cooperation Group, and her team analyzed lung cells of COPD patients and discovered something very interesting: in the sputum of COPD patients, the scientists identified a new macrophage population with cells smaller in size than the known macrophages. Therefore, they were called small sputum macrophages. In healthy organisms macrophages are the main population of all white blood cells in this compartment. Their portion in the samples from the patients is quite different: their population is only 15 per cent, approx. 80 per cent of the cells are neutrophilic granulocytes. The portion of the small sputum macrophages, which normally account for only approx. ten per cent of all macrophages, can rise to up to 90 per cent in COPD patients. “This is also a way to distinguish asthma from COPD, since the concentration of the small sputum macrophages is only slightly elevated in asthma patients,” Marion Frankenberger explains.

Inflammatory Genes Activated

Macrophages are of central significance in the respiratory tract and in the periphery of the lungs, the alveoli: they take up exogenic bacteria, viruses and also aerosol particles. The small sputum macrophages, which are found in greater quantities in COPD patients, produce large quantities of the tumor necrosis factor (TNF). This cytokine stimulates inflammatory reactions and thus contributes to the development of chronic obstructive bronchitis and helps sustain it. “We suspected that air-borne particles activate particular genes of these macrophages,” says Frankenberger. The analysis of the gene expression confirmed this assumption: diesel exhaust particles and carbon particles have the effect that in the macrophage-like cell line (Mono Mac 6) higher amounts of the gene COX-2 are expressed. Cyclooxigenase-2, which is the full name of the enzyme which is synthesized according to the design of the COX-2 gene, is involved in the oxidation processes in the lungs. If many COX-2 enzymes are active, a large number of oxidatively reactive substances is produced, which initially intensify the inflammatory reaction in the lungs. Following this, further messenger substances, such as leukotriene $B_4$ (LTB4) and prostaglandin $E_2$ (PGE2) are activated, which also influence the inflammation. LTB4 has a stimulating effect, while PGE2 contributes more to bringing the inflammatory process to a halt. Whether this actually works depends on the distribution of these biological signal substances and their receiver molecules, the receptors. “In an inflammatory reaction there is an interaction of many different steps,” says Frankenberger. “We do not know yet what the exact structure of this network is. It is, however, certain that at some point in this chain the cytokine TNF is also activated, which in turn maintains the inflammation status in the cell.” So in the investigated cell line Mono Mac 6 ultrafine particles support the gene expression of pro-inflammatory and anti-inflammatory mediators of the lungs.
Whether the small particles also activate genes in cells of COPD patients and in healthy test subjects is an issue Frankenberger’s group wants to investigate in the future. At some point the scientists hope to be able to use this knowledge to shut down inflammatory genes or to intensify anti-inflammatory processes.

**Vitamin A against Tissue Degradation**

To follow the course of inflammatory diseases in the lungs, doctors and scientists have to control inflammation markers regularly. Invasive methods and the taking of sputum have always caused patients with highly advanced COPD great discomfort. But this is now over: the Cooperation Group in Gauting uses markers in the exhaled air – the air flowing out of the lungs during exhalation – to control whether, for example, the patient responds to a cortisone therapy. If cytokines and lipid mediators, such as LTB\(_4\) and PGE\(_2\), decrease during the therapy, the inflammation of the lungs has also receded.

Using markers in the exhaled air scientists from the KKG also want to check in the future whether vitamin A can stop tissue damage causing emphysema in the alveoli. First the scientists transferred vitamin A packed in little fat droplets, the liposomes, specifically to the macrophages in cell culture tests. Their enzymes are known to decompose lung tissue. In healthy organisms the protease MMP9 (matrix metalloproteinase 9) and its inhibitor TIMP1 (tissue inhibitor of matrix metalloproteinase) are in the macrophages in equal concentrations. This ensures that exactly as much lung tissue is decomposed as new tissue is produced. In COPD patients, however, there is an imbalance: the decomposing enzyme MMP9 is present in much higher concentrations than its antagonist TIMP1. “It is clear that in this case the lungs continue to be digested,” says Frankenberger. In this process vitamin A could come to the rescue: it reduces the protease MMP9 in cell lines, while it also activates the inhibitor TIMP1. So vitamin A supports the protection of the tissue in two ways. “We are currently planning phase II studies on this issue and hope that the results we have found so far will be confirmed,” says Frankenberger. This would really help COPD patients a great deal: specific vitamin A therapy would turn down mechanisms decomposing tissue in the lungs and inhaled particles could cause less damage.

On 8 March 2006 a European patent for an “Agent for Treating Illnesses of the Tracheobronchial Tract, Especially Chronic Obstructive Pulmonary Disease (COPD)” was granted for the invention.
Particularly it is due to the close interaction of laboratory and clinic that scientists in basic research and physicians of the GSF could become pioneers of bone marrow transplantation: in 1975 Prof. Hans-Jochem Kolb, now head of the Clinical Cooperation Group “Hematopoietic Cell Transplantation,” together with a colleague from the Municipal Munich Schwabing hospital saved the life of a youth with bone marrow failure by transferring healthy bone marrow. It was the first successful transplantation of this kind in Germany.

If left untreated, leukemia, a blood cell production (hematopoiesis) disorder in the bone marrow, inevitably causes the patient’s death. Therefore, attempts to destroy the patient’s diseased bone marrow and to replace it with healthy bone marrow from a suitable donor were made very early on. In 1975 Prof. Hans-Jochem Kolb together with colleagues from the Hospital of Schwabing of the City of Munich succeeded in saving the life of a youth with bone marrow failure (aplastic anaemia) by transferring healthy bone marrow.
**T-Cells Put out of Action**

The spectacular treatment had been preceded by years of experimental work at what was then the GSF Institute of Immunology, headed by Prof. Stefan Thierfelder. First suitable radiation methods had to be developed in animal experiments. They could destroy the degenerate blood cells in the bodies of leukemia patients and at the same time make space for the healthy cells of the transplant. But this was not all. Because the donated bone marrow does not only contain the vital blood-cell-forming stem cells, but also so-called T-cells, which consider the recipient as foreign and attack his/her organs and tissues. “Prof. Thierfelder had shown in experiments that the treatment of the donor with antiserum against T-cells can prevent this dangerous immune reaction of the donor against the recipient,” Prof. Kolb remembers: “But the human donor cannot really be treated with antiserum in order to prevent the reaction of the patient. Prof. Thierfelder had the idea to simply remove the T-cells from the...
bone marrow before the transfusion is made.” For the first time ever the GSF physicians at Haunersches Kinderspital (pediatric clinic) treated a girl suffering from leukemia with T-cell-depleted donor bone marrow in 1978; today “T-cell depletion” has become an established method in bone marrow transplantation.

Adoptive Immunotherapy Helps

Just a year later Prof. Thierfelder introduced another innovation: the removal of leukemia cells from the bone marrow. This means that the patient’s own bone marrow can be used for the transplant. It is taken during a quiescent phase of the leukemia and treated with an anti-leukemia serum which eradicates any remaining leukemia cells. The prepared bone marrow is returned to the patient after whole body irradiation. This method does show success, but it cannot eliminate all remaining leukemia cells. Looking for a better solution to this problem, the doctors benefited from a special feature which distinguishes the bone marrow from all other organs: it is only combated by the recipient’s immune system as a foreign body at the beginning and then tolerated only a few months after the transfer. This “tolerance” of the patient to the donor bone marrow was used by Prof. Kolb and his working group to follow a new approach to leukemia treatment: adoptive immunotherapy. The donor’s T-cells which had been removed from the bone marrow before the transplant are reintroduced into the patient who has now become “tolerant” in a second step, so that they specifically destroy his remaining leukemia cells. “We first showed that in a patient who has had a transplant and who has had recurrent leukemia this leukemia can be eliminated by the administration of T-cells of the donor – without chemotherapy or radiation,” Prof. Kolb stresses. Although the classical weapons against cancer, chemicals and radiation, are still indispensable for the preparation of any bone marrow transplant, subsequent adoptive immunotherapy can considerably reduce the dosage of the preceding chemotherapy and irradiation – and thus the stress the patient is exposed to.

Apart from the treatment of leukemia, the method developed by Kolb may be able to help solve yet another problem in the future: transplanting organs which do not match as yet. For as opposed to the classical bone marrow transplant, where maximum correspondence between the donor and the recipients is required, Kolb’s method also allows the transfer of bone marrow which does not match. Patients whose body has accepted such a transplant could also have another organ of the bone marrow donor transplanted without rejection as a foreign organ – a new chance for transplant medicine.
A s has long been known – pollen is one of the most important allergenic substances in outside air. So far the doctrine has been that the allergic inflammatory reaction is triggered, when proteins released from the pollen, the allergens, enter the human body through the skin or mucous membrane and cause a specific allergic immune reaction there. But there is little knowledge as to why these proteins cause an “abnormal” immune reaction with the production of immunoglobulin E antibodies. Scientists from the Clinical Cooperation Group “Environmental Dermatology and Allergology” (KKG UDA) at the Center for Allergies and the Environment at the Clinic for Dermatology and Allergology of the Technical University of Munich, headed by Prof. Heidrun Behrendt, made an important discovery which might mean a big step forward for allergology: in their studies on allergen release Dr. Claudia Traidl-Hoffmann and her working group discovered a completely new biological property of pollen: apart from proteins it also releases a number of unsaturated fatty acids. “Continuing our studies we were surprised to find,” says Dr. Claudia Traidl-Hoffmann, head of the working group of the KKG UDA, “that these fatty acids have a direct immunostimulatory and immuno-
modulatory effect on the human organism.” The substances which are therefore called pollen-associated lipid mediators, or PALMs, are capable of directly attracting and activating human inflammatory cells, such as neutrophilic and eosinophilic granulocytes. Apart from that they modulate dendritic cells – central cells of the human immune system – so that they stimulate an allergic immune response. This means that pollen is more than just an allergen carrier. It can pave its own way for the development of an allergic reaction by releasing PALMs.

**PALMs – Key to Many Questions?**

But this was not to be the only surprise for the members of the Clinical Cooperation Group: “The effects observed were not only found in allergic patients, but also in people with no allergies,” says Traidl-Hoffmann. This opens up completely new approaches for the scientists. In the past the focus was on sensitized patients with the question why an allergy develops. In the future the question will also be investigated which mechanisms prevent an allergy from developing in people who have no allergies. Thus, PALMs might provide answers to the question why at times of high pollen concentrations non-allergic reactions of the upper respiratory tract also occur more frequently.

Yet another question might be answered in the near future by the discovery of the PALMs: epidemiological studies have shown that in areas with greater pollution more people suffer from allergies. PALMs might also have something to do with this – since: the Clinical Cooperation Group could also show that pollen grains release more PALMs from pollutant exposed trees compared to rural trees.

Newly discovered bioactive substances: apart from their specific effect, the pollen-associated lipid mediators (PALMs) released by pollen on the mucous membrane cause an unspecific activation and modulation of the immune system, thereby paving their way for the development of an allergic reaction. (DC = dendritic cell, Th1/Th2 = T-helper cells of the immune system, IgE = immunoglobulin E antibodies)
A Cough Is Not a Cough – First Discovery of Immunospecific Markers

The Clinical Cooperation Group “Immunoregulation in Childhood” has set out to look for immunospecific markers, which will help distinguish between the diagnosis of an allergic cough and that of a cough with other causes, so that more specific therapeutic approaches will be possible than in the past. And some initial success has been seen.

Coughing is a widespread symptom, mainly in children and infants. The causes for coughing may be manifold: thus, a cough may be the only symptom of allergic asthma. A clear diagnostic differentiation has been difficult so far. But it would be important, since a cough with allergic asthma is treated differently from a cough, e.g., associated with viral infections, as they are frequent in children.

The Clinical Cooperation Group “Immunoregulation in Childhood” has set out to look for immunospecific markers, which will help dis-
Distinguish between the diagnosis of an allergic cough and that of a cough with other causes, which will make more specific therapeutic approaches possible than in the past. And it has had its first success: “A key might be in the Th1-/Th2-related pulmonary chemokines and their receptors,” Dr. Susanne Krauss-Etschman, head of the KKG, explains. In a clinical study, which has just been completed, her group examined 12 children with allergic asthma, 15 children with chronic cough without an atopic background as well as 10 children without any diseases of the respiratory tract. Among other things the concentration of the pulmonary chemokines and of the corresponding receptor-carrying lymphocytes was determined in the bronchoalveolar lavage of the children. The result: in the children with allergic asthma the content of two kinds of pulmonary chemokines as well as of specific lymphocytes was clearly elevated. On the other hand in the other children without an asthmatic background higher concentrations of other chemokines as well as of specific lymphocytes were found.

The analysis of these newly identified markers does not only open up prospects for an essential contribution to the differentiated diagnosis of allergic and non-allergic respiratory diseases. As a next step the Clinical Cooperation Group also wants to continue the search for the immune markers in sputum samples, so that in the future the invasive method of bronchoalveolar lavage will become superfluous. Furthermore, the results of a larger case number of children should be checked prospectively, before they can be applied in everyday clinical practice.

In children with allergic asthma the level of the pulmonary chemokines TARC and MDC as well as of CCR4+CD4+ lymphocytes is clearly elevated. On the other hand in the other children with no asthmatic background higher concentrations of ITAC and IFN-γ as well as CXCR3+CD8+ cells are found. The new markers identified by the Clinical Cooperation Group “Immunoregulation in Childhood” make an essential contribution to the development of a differentiated diagnosis of allergic as opposed to non-allergic respiratory diseases.
It is estimated that viruses are involved as cofactors in approx. 15 per cent of all human tumors. Thus, the Epstein-Barr virus (EBV), a herpes virus discovered in 1964, was of significance to various cancers including EBV-associated lymphomas of immunosuppressed patients. It has long been known that EBV remains in the infected individual for life, and that the immune system is of decisive significance for overcoming the active infection. The contribution that T-helper cells make to controlling the viral infection had

Groundbreaking Strategy for the Development of Cancer Vaccines

In order to be able to develop specific vaccines for malignant diseases, suitable target antigens must be identified. Thanks to a new method the Clinical Cooperation Group “Pediatric Tumor Immunology” recently achieved a strategic breakthrough in the search for T-helper cell antigens of cancer cells. Some first antigens which were identified by this method provided very promising input for the immunotherapy of virus-associated tumors. The group expects this method to make a decisive contribution to the immunotherapy of cancer in adults and children.
hardly been studied at all up to this day. With her Clinical Cooperation Group “Pediatric Tumor Immunology” its head, Dr. Uta Behrends, took a closer look at the EBV immune detection by T-helper cells. Her goal was to identify antigens of the EBV, which are detected by the T-helper cells. Behrends and her colleagues could show that T-helper cells detect EBV-infected cells, before the virus reproduces in these cells. The T-helper cells recognize proteins of the viral envelope and lyse cells presenting these coat antigens. These results point at a central significance of the T-helper cells both to the control of the primary virus spread and to the elimination of cells in which the active viral infection flares up again at a later point in time. Apart from healthy B-lymphocytes, these include the EBV-positive tumor cells. Thus, T-helper cells with a specificity for EBV coat proteins provide important new starting points for the immunotherapy of life-threatening EBV-associated diseases. Meanwhile Uta Behrends’s Clinical Cooperation Group has been able to develop a method to very quickly and reliably identify T-helper cell antigens not only for EBV, but basically also for other viruses, bacteria and, e.g., tumor cells. A patent application for this so-called DANI method, which has been recognized as groundbreaking for an application in medicine, was filed recently. Uta Behrends and her working group hope that they will be able to identify various target antigens for vaccines against infectious pathogens and tumors with this method in the near future and to contribute to better treatment of the patients concerned.
There are many prejudices about depressed persons, which equate depression with a weak character or personal failure, resulting in the idea of having attracted an untreatable destiny. But what are depressions from a medical point of view? The clinical term depression includes all affective disorders that can be characterized by a pronounced, continuously irritated, and depressed mood or the loss of interest and pleasure in usual activities. It is assumed that depression is a multifactorial disorder caused by a genetic predisposition as well as lifestyle and the environment.

The World Health Organization (WHO) assumes that by the year 2020 depressive illnesses – along with cardiovascular diseases – will represent the largest group of diseases in industrialized countries. In the Clinical Cooperation Group (KKG) “Molecular Neurogenetics” scientists from the GSF and the Max-Planck-Institute of Psychiatry join forces to elucidate the molecular mechanisms and causes of depression and anxiety-related disorders, and to find new approaches for suitable therapies. In a clinical study the scientists showed that there is a clear disturbance of the hormonal balance in patients with these psychiatric disorders, indicating that pharmaceuticals acting on a hormonal level may be applied as an alternative to classical antidepressants in the near future.

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At this point the work of the Clinical Cooperation Group “Molecular Neurogenetics” sets in, which was established by the GSF Institute of Developmental Genetics headed by Prof. Wolfgang Wurst, and the Max-Planck-Institute of Psychiatry headed by Prof. Florian Holsboer. “Our aim is to elucidate the molecular mechanisms and causes of depression and anxiety-related disorders and thus to find novel therapeutic strategies for the treatment of depressive illnesses”, says Wolfgang Wurst. For this purpose, the scientists from the GSF and their colleagues from the Max Planck Institute of Psychiatry have developed special mouse models, which emulate the clinical symptoms of human depression. Only recently a major breakthrough was achieved in clinical research. The observation that patients with psychiatric diseases have elevated levels of CRH in the cerebrospinal fluid, a reduced density of CRH receptors in the frontal cortex, and elevated stress hormone (cortisol) levels in the blood indicates that CRH plays a central role in the development and course of depression and anxiety-related disorders. The scientists found changes in the complex interactions between corticotropin-releasing hormone (CRH) and its receptors. CRH plays a key role in the physiological response of the organism to stress by regulating the hypothalamic-pituitary-adrenal (HPA) axis. In addition, CRH is released in the brain and may thus directly influence central behavioral patterns, which is an important factor when dealing with stress-related situations.

Wolfgang Wurst and his colleagues have created a mouse model that allows the dissection of these two functions. By genetically ablating the CRH receptor type 1 (CRH-R1) in the mouse brain, they could show that CRH regulates anxiety-related behavior in a particular area of the brain, the limbic system – irrespective of its role in the HPA axis. Apart from this, the KKG could also show that the function of CRH-R1 plays an important role in stress-induced alcohol consumption. These results corroborate the significance of CRH-R1 in the development of psychiatric disorders. “We now take this knowledge back to the clinic”, says Dr. Jan Deussing from the Max-Planck-Institute of Psychiatry. “In some first clinical trials we could confirm that specific CRH-R1 antagonists have an antidepressive effect.” This paves the way for an establishment of CRH-R1 antagonists as a possible alternative to classical antidepressants in the near future.

Elevated levels of corticotropin-releasing hormone (CRH) are found in the brain of depressed patients, similar to stressful situations in which the endogenous hypothalamic-pituitary-adrenal axis is activated. In animal models, it was shown that the increase in CRH levels in the brain causes changes in behavior, which can also be observed in depression.
A part from important institutions with an experimental orientation, such as the German Mouse Clinic or the Genome Analysis Center, the GSF also operates three research platforms near clinics. They each work on special interdisciplinary questions and consistently implement their findings for the prevention, diagnosis and therapy of diseases together. These are the Immune Monitoring Platform, the Antibody Platform as well as the KORA research platform.

Headed by Prof. Dr. Dolores Schendel, the scientists from the Immune Monitoring Platform utilize clinical studies to develop, standardize and validate new methods for the best possible monitoring of ongoing immune responses. It is only with a variety of methods that the effect of the therapies individually adapted to the patient can be optimally evaluated. Clinical partners benefit from the latest technologies of the platform without having to establish the complex processes themselves.

The idea is by no means new: scientists from different disciplines run a joint research unit in which they use the same resources and the same communication system and design joint studies. What is, however, rather new is the establishment of research platforms at the interface between laboratory and bedside. The GSF already operates three of these – instruments of translational research par excellence.
The growing knowledge of the role of the immune system in the development of malignant and infectious diseases has resulted in new approaches to their treatment. Thus, it is hoped today that immunotherapies will soon be able to mobilize the endogenous defense system specifically against viruses and tumor cells. More than half of the Clinical Cooperation Groups established by the GSF focus on this field of research. Their common aim is to develop new immunotherapies and to implement them in clinical applications as well as to identify and quantify immune reactions in patients in clinical studies.

Since 1995 Dr. Elisabeth Kremmer and her working group have been producing large quantities of high-quality antibodies in the research platform Monoclonal Antibodies. In close contact with partners in research they develop the antibodies best suited for each requirement. In 2000 the physician and her colleagues were awarded the Erwin Schrödinger Prize for the special quality of the antibodies.

The health platform KORA of the GSF is also situated near a clinic. The scientists keep a database which they have collected over a period of more than 20 years together with their local partner, the Zentralklinikum Augsburg. Thanks to the detailed examination of and interviews with 18,000 participants in the study many different questions on the risk factors of all cardiovascular diseases can be investigated. Numerous external partners from other research institutions, clinics and universities participate in the studies. They benefit from the excellent data management and the enormous expertise of the scientists on the KORA platform.

All three platforms cooperate with research partners in a worldwide network and continually introduce new strategies into clinical application.
For the exact monitoring of these immune responses the GSF created its own immune monitoring platform near the clinics in 2004. Not only do colleagues from the clinic get access to the latest and most powerful technologies. They also always have highly specialized experts by their side, who develop with them tailor-made monitoring processes for their clinical studies.

Cell sorting at the Immune Monitoring Platform of the GSF: Cells which were previously marked with fluorescent dyes are sorted in the cell sorter (photo above) using a breaking jet of liquid. The sorted cells are now available for further experiments or measurements.

**Stations in cell sorting**

1. Cell suspension
2. Staining
3. Cell sorting
4. Cell culture, analysis, etc.
Method of Choice for Any Requirement

Normally one single diagnostic method is not sufficient to cover the manifold consequences of different therapeutic modulations of the immune system. For what is so special about these treatments is that they are individually adapted to each patient. “We actually need a whole armory of methods from which we choose the methods suitable for each individual case,” Prof. Dolores Schendel, Director of the GSF Institute of Molecular Immunology and head of the Immune Monitoring Platform, explains.

On the one hand, established methods are available, on the other hand these methods are continuously developed further and improved. “If, for example, we do not know the antigens in a therapy study, we use T-cell tests, cytokine measurements in the micromilieu and PCR analyses to monitor the course of the immune response,” Schendel explains. If, however, the immunogenic antigens have already been defined, but the individual MHC restriction elements are unknown, other procedures are required. And finally: if the specific epitopes and MHC restriction elements are already known, fluorescence-marked MHC peptide complexes are used as markers for monitoring.

External Clinical Partners Also Benefit

The group around Schendel is an important part of the whole platform technology for translational medicine: a core unit of qualified scientists and technical staff standardizes and validates many different immune monitoring tests, thereby ensuring that the clinical partners can use the latest technologies without having to establish many complicated methods themselves. Of course, this applies not only to the GSF’s own Clinical Cooperation Groups, but also to external cooperation partners: the Institute of Medical Microbiology, Immunology and Hygiene of the Technical University of Munich as well as the Laboratory of Tumor Immunology of the Urological Clinic of the Klinikum Großhadern of the Ludwig-Maximilians-University Munich have long been on board.

Apart from providing support for clinical studies, the scientists at the Immune Monitoring Platform also conduct fundamental research, which makes valuable contributions to the basic understanding of the cellular and molecular regulation of the human immune response.

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Monitoring method on the GSF platform

- Sterile cell sorting
- T-cell receptor analyses
- Multiparameter cytometry
- Analysis of the T-cell receptor reservoir
- Test for special cell populations by antibody staining
- Typification of cytokines and human leukocyte antigens
- ELISPOT quantification of the immune response of specific T lymphocytes using their cytokine production
- Imaging for live cells

Immune Monitoring

Using ELISPOT and their cytokine production the immune response of specific T-lymphocytes is quantified: a color reaction makes the activated lymphocytes (spots) visible (picture on the right), their number is a measure for the reactivity of the immune system and, thus, allows a standardized assessment of the immune reaction during the course of the therapy.

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Contact

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It was back in the early eighties that Dr. Manfred Eulitz, then a scientist at the GSF Institute of Immunology, who established a group producing monoclonal antibodies. The basic idea of this group was that any GSF scientist interested could acquire the knowledge required to be able to develop the monoclonal antibodies he/she wanted. But it soon turned out that the logistic requirements were too much for most laboratories. This is why the group was converted to a service facility. Today Dr. Elisabeth Kremmer heads the Research Platform Monoclonal Antibodies at the GSF Institute of Molecular Immunology.

Established by the immune system for millions of years, antibodies have long become an indispensable tool in research and therapy. Making good antibodies, however, is a task reserved for specialists – and not all institutions have the excellent possibilities of the GSF. With its Research Platform Monoclonal Antibodies it is an important interface in the network of health and environmental research.
Large-Scale Production

“Today our working group can continuously produce the antibodies exactly matching the requirements of the requesting scientists,” Kremmer explains. “Under the new service concept we have been producing high-quality monoclonal antibodies ready-to-measure very quickly since 1995, approx. 300 different antigens per year, with a rising tendency.” At the same time Kremmer’s group is extremely flexible: together with the client they discuss which antigen is best suited for the production of the requested antigens. “Some proteins cause no or only a very weak immune response, they are not immunogenic,” Kremmer explains. “No antibodies against them can be made. Together with the partners we then look for more suitable antigens.”

When an immunogenic substance has been found, an immune response against it is produced in the animal model. The B cells of the bodily defense activated in this process and directed against the antigen will be extracted and fused with a tumor cell line, a so-called myeloma cell line. “When this happens, the desired property of the B cell to produce a specific antibody is transferred to the myeloma cell and a so-called hybridoma is produced,” Kremmer explains. This will now be reproduced in the cell culture, the antibodies secreted into the medium will be withdrawn and added to the antigen. “If the antibody binds specifically and strongly to the antigen introduced, it has the desired high affinity and specificity. The antibodies are sent to the partners for further characterization,” says Kremmer.

Producing good antibodies is a task reserved for specialists – and the service unit of the GSF has them: with their experienced eyes the scientists see which cells have grown so well that they are worth testing. “We examine all growth media and reagents for their suitability for the production of hybridomas,” says Kremmer. “This saves time.” It is not so much the laboratory equipment as it is this skill, diligence and the many years of experience together with a subtle feeling for whether, e.g., a culture needs an additional change of medium, which guarantee the high quality standard of the service unit.

High Efficiency Without High-Tech

“We are particularly efficient, although or maybe just because we work without any special devices, such as a pipetting robots, freezing machines and bioreactors,” says Kremmer. “With just four people we produce approx. 30 different hybridomas per week, which we propagate in culture bottles.” It would be much too complicated to charge a bioreactor for the small quantities of antibodies requested. Apart from that, if a culture bottle is accidentally contaminated with bacteria, this one culture can quickly be disposed of, while all others can continue to grow. “A bioreactor would have to be cleaned completely in this case, and all cells in it at this point in time would be useless at once,” says Kremmer.

Together with her colleagues Dr. Martin Lipp and Dr. Reinhold Forster from the Max-Delbrück-Center of Molecular Medicine as well as Dr. Eckhard Wolf from the Gene Center of the University of Munich the physician was awarded the Erwin Schrödinger Prize 2000 for the exceptionally high quality of the antibodies. The fact that, apart from GSF scientists, scientists from many universities and research institutions throughout the world have their antibodies made by the GSF platform is also owed to the special support after the production. Scientists from all over the world have their antibodies made by the GSF Antibody Platform. With just four people Dr. Elisabeth Kremmer, head of the Platform, produces approx. 300 highly specific antibodies a year. Apart from the high quality standards the clients particularly appreciate the service platform for the intensive support provided even long after the production.
Antibodies are complicated protein structures which can react with chemical structures of many different kinds due to the variation of amino acids in certain sections of the protein chains. The ability of the organism to react to noxae which have entered by forming antibodies developed over many million years. Predecessor structures of the antibodies have been found in cartilaginous fish. The fact that they can bind many different chemical structures with high specificity makes the antibodies, together with immunological detection methods, such as radio and enzyme immunoassays “RIA” and “ELISA”, unique detectives in research.

The normal antibody response of the body after contact with an antigen, however, has one disadvantage: it is inhomogeneous, because it consists of a mixture of specifically and less specifically binding antibodies. It takes a lot of time and effort to isolate high-purity molecules from this, and this is often not even crowned with success. Thanks to Georges Köhler’s and Cesar Milstein’s work those antibodies can be selected from the multitude of possible antibodies today, which bind the desired antigen with high specificity. For this hybridoma technology published in 1975 the two scientists were awarded the Nobel Prize for Medicine in 1984. Those cells are selected as the end product of this process, which produce only one antibody of the required specificity.

To Clinical Applications With High Accuracy

This may also soon apply to the antibody which recognizes a deletion mutant of the protein E-cadherin. E-cadherin is a protein which is partly responsible for maintaining the contact of the cells. Deleted E-cadherin, which is only found on cancer cells, particularly frequently with diffuse gastric cancer, is recognized by a monoclonal antibody. If α-radiators are coupled with the antibody, only the cancer cells will die, because the antibody binds to them exclusively. What can already be done at the laboratory, will hopefully also soon cure cancer patients in the clinics.

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The first main project was started in the seventies with the aim of developing an internationally binding, uniform study protocol for the measurement of the important risk factors, such as smoking, high blood pressure and overweight. Twenty-eight countries from four continents participated in this MONICA study of the World Health Organization, which was planned for a period of ten years. MONICA stands for Monitoring of Trends and Determinants in Cardiovascular Disease. It was for a good reason that Dr. Ulrich Keil, head of the working group Epidemiology of what was then the GSF Institute of Medical Computer Science and System Research, chose the Augsburg region as a location for one of the four German MONICA centers: Augsburg has a large modern hospital as a treatment center, the population structure corresponds to conditions in Germany, and there are particularly few people entering and leaving the region.

Fighting Widespread Diseases
The Health Platforms MONICA and KORA

The GSF has been running a health research platform in the Augsburg area for just over 20 years. Physicians, epidemiologists, statistics and genetics experts take a close look at widespread diseases, such as diabetes, cardiovascular diseases or allergies. In large study populations selected to be representative for the population as a whole, the scientists investigate not only the classical risk factors, but also dietary habits, physical activity, psychosocial factors and the utilization of medical care.
In the so called acute coronary event register of Augsburg, its head Dr. Hannelore Löwel from the GSF Institute of Epidemiology, has been recording all patients with a fatal or non-fatal acute myocardial infarction as well as all those who died suddenly before reaching a hospital since 1985.

**Health Awareness Still Low**

“For men the risk of suffering a myocardial infarction starts to rise considerably from about 40 years of age, for women from 55,” says Löwel. “For women this risk is principally much lower. However, the still very low risk of younger women is rising continuously – probably because they increasingly start smoking at a much younger age and many also take oral contraceptives. But on the whole the rate of myocardial infarctions in men and older women has gone down. Health awareness, however, is still incredibly low. This often reminds one of gambling behavior: “Most people hope not to become ill despite the risk,” Löwel criticizes. This passive attitude is found particularly frequently among patients with high blood pressure, who show an unchanged high number of myocardial infarctions. Only about half of these people know about their blood pressure, and of these only about 50 per cent receive drug treatment, and only about half of these go down to normal blood pressure as a result.

**Big-Style Cooperative Health Research**

In 1996, when the WHO’s MONICA study ended, the GSF decided to continue its health research in the Augsburg Region in KORA (Cooperative Health Research in the Augsburg Region). Thus, the first German cohort study, comprising nearly 14,000 persons, on the question what the connection between typical risk factors and myocardial infarction is was established as a long-term project. As for MONICA, the GSF has the scientific control over the overall project, Klinikum Augsburg continues to be the partner for most laboratory analyses. KORA received its own study center which is headed by Dr. Christa Meisinger. Prof. H.-Erich Wichmann, Director of the GSF Institute of Epidemiology, acts as spokesman of KORA.

For the assessment of the time trends of the risk factors for cardiovascular diseases, KORA can use the MONICA data. “Due to this unique and excellent data quality management we can evaluate the stored biosamples and data according to new aspects even today. Other partners can join the project on the basis of scientific cooperation assignments,” says Löwel.
Thus, scientists from many research institutes, clinics, universities and other institutions also participate in the KORA study with a look to assessing care structures and processes, providing scientific support for decision-makers and supporting health research in the fields of epidemiology and health economics. The successful cooperation is also facilitated by the great financial commitment of the GSF and further funds from the Federal government, the DFG, the EU and other international sponsors. The success of this research platform is seen not least in the numerous publications in top-class international journals.

In the frame of KORA, the cardiovascular MONICA research is ongoing. However, extended by a broader field of chronic diseases and sub-clinical outcomes, another 6000 citizens were randomly selected from the address file of the registration offices and invited to participate in the study, examined thoroughly and interviewed between 1999 and 2001. They gave information on habits, such as smoking, diet, alcohol consumption, sports activities, their occupational environment as well as chronic diseases. All blood samples, from which the scientists obtain numerous laboratory parameters, were frozen for future analyses, as had been the case since the beginning of the study. Thus, GSF scientists have meanwhile gathered data of about 18,000 people, who they want to continue to track with interviews and follow-up studies.

The more than 30 studies in the genome research network show that genetic research benefits greatly from the KORA data. In myocardial infarction research the specific combination of genetic parameters with more and more individual factors opens up the vision of formulations increasingly made to measure. “Today we do not want to make just general statements any more, we want to assess the risk of individuals to suffer a myocardial infarction and enable people who are at risk to identify and reduce their very personal risk,” says Löwel.

Another result of international relevance found by the scientists in the KORA Health Platform was that they confirmed that air pollution and arteriosclerosis and myocardial infarctions are connected. This result was met with extreme skepticism at first, but meanwhile it has become the basis for worldwide research activities. It was seen that during phases of strong air pollution – mainly with ultrafine and respirable particles – more myocardial infarctions occur. “The genes could also be of importance for this elevated risk,” Löwel suspects. Therefore, in the ongoing AIRGENE Study on the complex “Air Pollution and Inflammatory Response,” which is sponsored by the EU, particularly susceptible persons will be defined on the basis of genotyping in six big European cities. Thus, inflammation markers were determined regularly in the patients concerned and the times of the measurements were related to the air pollution values. This project is also coordinated at the GSF: Dr. Annette Peters, GSF Institute of Epidemiology, coordinates the international project.

20 years of MONICA / KORA mainly means detailed scientific work, which, however, resulted in spectacular success. “Several years ago it was found that arteriosclerosis is not only the deposition of plaques in the vascular system, but an inflammatory disease. This was a scientific breakthrough to which the GSF scientists from the KORA team made a big contribution,” Löwel recalls. With its complexity the long-term observation of the MONICA/KORA test subjects provides a unique possibility to determine the new pro- or anti-inflammatory and genetic parameters considered to be relevant from frozen blood samples of the basic examination at short notice and to relate them to the diseases which have meanwhile occurred. The KORA studies are also increasingly included in international meta-analyses, which makes Germany as more visible a research site.

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The development of a vaccine stimulating the immune system to fight HIV is one of the greatest challenges in AIDS research. Vaccine research is faced with the big task of developing a therapeutic vaccine against an already existing infection. At the GSF Institute of Molecular Virology scientists have now achieved some first success with a vaccine on the basis of a genetically modified vaccinia virus, which is now going to be combined with another vaccine to produce a powerful combination. Using such combination vaccines, scientists hope to one day be able to protect healthy people from infection.
Despite all information campaigns, AIDS is advancing throughout the world. It is mainly in southern Africa that the disease has taken disastrous dimensions, but in numerous other countries the number of new infections is also rising rapidly. Although there are meanwhile highly effective antiviral therapies, these are unaffordable, particularly in third-world countries – only five per cent of all those who are infected worldwide have access to these drugs. Apart from that modern therapies (HAART – highly active anti-viral therapy) keep the viral load low for a long time and thereby prevent an outbreak of the disease, but they cannot remove the virus from the body altogether.

Vector Vaccine with Special Design

“The purpose of vaccinating must be to stimulate the immune system of patients who are already infected with HIV, so that the outbreak of AIDS will be delayed or even prevented,” explains Professor Volker Erfle, the recently retired Director of the GSF Institute of Molecular Virology. The scientists from the institute first developed a vector vaccine on the basis of harmless genetically modified MVA viruses (modified vaccinia virus Ankara), into which the design for the HIV protein Nef was introduced. Nef was chosen, because it is of decisive significance in the life cycle of the virus: Nef is produced by infected cells soon after the infection and causes an effective reproduction of the virus. In the absence of Nef AIDS does not break out. If the vaccination works, it will beat the pathogen with its own weapons: the inoculated vectors invade body cells and stimulate them to produce Nef, so that the immune response against Nef is stimulated and Nef is deactivated. In this process both antibodies against Nef are produced (= humoral defense) and specific defense cells are activated, which destroy the infected cells (= cellular defense). “Our idea was to look for a therapeutic vaccination which stimulates the immune response, so that the number of virus-specific CD4-positive T-cells remains high,” explains Dr. Antonio Cosma from the GSF Institute of Molecular Virology.

As a first practical test Erfle and Professor Frank Goebel, the head of the outpatient AIDS department at the Munich Innenstadt-kliniken, and scientists from the Munich Klinikum rechts der Isar, conducted a clinical phase I study on vaccination with MVA-Nef.
The result was encouraging even after just one immunization: in four out of the ten patients vaccinated the number of Nef-specific CD4 cells increased. “This cell type had not been identified in any of the patients before, so that a clear immune reaction to the target structure HIV-Nef was seen,” Cosma explains. All subjects had been infected with HIV for some time and were undergoing treatment with HAART, which was at first continued during the clinical study. After the vaccination seven of the ten patients agreed to stop the antiviral therapy. The number of HIV viruses increased following this, but the immune response to Nef also increased – evidence that the patient benefits from the vaccination. Nevertheless six patients had to resume drug therapy after several weeks.

**Practical Vaccine Test**

The patient with the best response to the vaccination has been able to control the virus himself for nearly three years. Not only is the number of CD4 cells high and stable in this patient, he also shows a strong CD8 immune response. CD8-T-cells are cytotoxic cells.
which recognize and destroy infected cells. CD4-T-cells produce growth and signal factors which cause the production and maintenance of CD8-T-cells. For successful immune defense there must be sufficient quantities of both cell types. “This patient is, of course, very interesting for us, because if we find out why his immune response is so strong, we might be also able to help other patients more,” Cosma explains.

Keeping an Eye on Immune Response

Since there is often little change in the clinical condition of vaccinated patients, it is important to observe the condition of the immune system by good immune monitoring. To assess the success of the vaccination and find markers for the immune response, the scientists developed new methods which allow a deeper insight into immunological processes. “Using new methods of flow cytometry, for example, we can determine the phenotype of individual T-cells and define exactly which markers express these cells — up to 13 markers at the same time,” Cosma explains. Thus, the scientists can analyze how T-cells react to the vaccination and characterize the immune response of individual patients. “In order to find markers for a particularly good immune response, we want to find out, for example, how the immune response of the patient with the best response to the HIV-Nef vaccination differs from that of other patients,” says Cosma.

Better Characterization of T-Cells

An essential breakthrough for the monitoring of immune therapies was the establishment of the MHC tetramer technology by the Clinical Cooperation Group “Antigen-Specific Immunotherapy,” headed by Prof. Dr. Dirk Busch. This method for the characterization of antigen-specific T-cell populations uses so-called MHC restriction: T-cells only recognize antigens, if they are presented by an MHC molecule. Individual MHC-antigen complexes are, however, only weakly bound and dissociate quickly. Therefore, the scientists crosslink several complexes with each other, so that structures with more stable binding are produced. As a rule four complexes are combined to so-called tetramers. Tetramers bound to T-cells, in turn, can be made directly visible using fluorescence dye. “Tetramer technology is a very useful instrument for finding virus-specific T-cells, which will then be further examined,” Busch says.

Hopes for Combination Vaccine

One problem of all HIV vaccines developed so far is that the virus changes: it mutates very fast and even minor changes in its surface proteins may mean that it is no longer recognized by T-cells. In order to obtain an even...
more effective vaccine for the future, the scientists, therefore, plan to combine different HIV vaccines which attack the virus in different ways: some vaccines contain structural virus components, others act through regulatory proteins which control the reproductive cycle of the virus or the expression of its genes. If the new vaccine contains both structural and regulatory components, it will offer the immune system a broad range of points of attack, since the body builds up an immune defense against all injected components.

The GSF scientists will test the combination of MVA-Nef with another vaccine which confronts the immune system with the HIV envelope protein Env in a new clinical study as soon as in 2006. “This way we activate the immune system on the one hand via Nef to develop cellular defense mechanisms and on the other hand we increase antibody production via the Env protein, because the envelope protein is expressed on the surface and is, therefore, a good target for specific antibodies,” Cosma explains. In cooperation with Professor Goebel the clinical study will start out with 50 subjects who will be divided up into small groups, in order to be able to investigate all possible combinations of the vaccines. “If both are injected together, one vaccine tends to be dominating, while the other becomes ineffective,” Cosma regrets, “therefore, it is better to administer the different vaccines one after the other: the next vaccination follows, when there has been a response to one vaccine.”

The new combination vaccination will first be tested on healthy subjects. While MVA-Nef was intended to be used as a therapeutic vaccine for those who are already infected with HIV, the scientists hope to be able one day to use the new combination vaccinations to protect healthy people from infection. Whether such effective vaccine protection against HIV will actually be developed soon, is as yet uncertain. But even a vaccine which activates the immune system to such an extent that the risk of infection is reduced would be great help in the fight against the AIDS pandemic – mainly in third-world countries, where drug treatment for HIV is usually no option for most of the infected patients for financial and logistics reasons.
Renal cell carcinoma is the most frequent malignant kidney tumor. There are approx. 14,000 new cases per year in Germany, most of which are discovered by coincidence. There has been hardly any therapy for patients with metastatic renal cell carcinoma, since the tumor cells respond poorly to radiation or chemotherapy. Scientists at the GSF Institute of Molecular Immunology are developing new therapies to activate the patient’s immune system to effectively fight tumor cells.

Renal cell carcinoma, or RCC, is an extremely aggressive type of cancer. “At the time of the first diagnosis of a renal cell carcinoma there are often metastases already, e.g. in bones, lungs or brain,” says Dr. Bernhard Frankenberger from the GSF Institute of Molecular Immunology. The prognosis is unfavorable — more than three quarters of the patients with metastases die within two years. Together with the Director of the Institute, Professor Dolores Schendel, he pins great hopes on immunotherapy, because there are indications that the tumor basically can trigger an immune response: it is often infiltrated by defense cells of the immune system, and in about two per cent of
the patients the tumor recedes spontaneously. The activation of the immune system with cytokines, such as interferon-alpha and/or interleukin-2 as a classical immunotherapy can sometimes result in partial or even complete remission, but is often associated with severe side-effects for the patient.

Long-Term Survivors at the Service of Research

Therefore, the scientists are working on a therapeutic vaccine which is supposed to stimulate the immune system with the help of genetically modified tumor cells. Tumor cells of the cell line RCC26 are used for the vaccination, which comes from a patient whose immune system could apparently fight the tumor particularly well, since – being a long-term survivor – he has survived the outbreak for more than ten years. “These tumor cells may present immunodominant tumor antigens on their surface, which are particularly good at boosting the immune system,” Frankenberger explains. Tumor antigens are specific proteins which distinguish cancer cells from healthy cells. The immune system should really be able to recognize the cancer cells as degenerate cells by these antigens and destroy them. The problem: the tumors have developed various mechanisms helping them escape the immune cells.

Thus, the RCC26 cells do not have certain costimulatory molecules required to trigger an immune response. In the absence of these molecules defense cells – mainly cytotoxic CD8-positive T-cells – migrate to the tumor cells, where they are deactivated, however, instead of being activated for the fight against the tumor cells.

Therefore, in order to improve the immune reaction against the tumor antigens Prof. Schendel and her colleagues together with Prof. Blankenstein and his group at the Max-Delbrück-Center of Molecular Medicine in Berlin introduced not only the gene for the costimulatory molecule B7.1, but also genes for certain cytokines (such as interleukin-2 and –7) into RCC26 tumor cells to try and ensure that tumor-specific T-cells can proliferate better and maintain a long-lasting immune reaction.

Tumor Antigens on Display

The actual tumor antigens of the cell line RCC26, which can “train” the T-cells for the tumor, remain unknown with this type of therapeutic vaccination, because they are presented by the tumor cell itself. Fortunately the tumor antigens presented by the cell line RCC26 also seem to be present on renal cell tumors of other patients, because RCC26 cells were also recognized by T-cells of other patients. However, T-cells only recognize tumor-specific antigens, if the tumor cell offers them on certain MHC molecules. RCC26 cells present several antigens on an MHC molecule type HLA-A2. For the communication between the antigen-presenting tumor cell and the T-cell to work, the T-cells of the patients must be so-called HLA-A2-restricted cells which recognize this molecule together with a fragment of a tumor antigen.

The scientists benefit from a coincidence: “Fortunately the HLA-A2 molecule is expressed in about 50 per cent of the Caucasian population, so that our genetically modified RCC26 vaccine can be used in about half of...
all patients,” Frankenberger explains. Apart from the cytotoxic T-cells of the adaptive immune defense, RCC26 cells can also activate natural killer cells (NKs) and non-MHC-restricted (NK-like) T-cells of the innate immune system. NK cells can even recognize tumor cells, when they down-regulate their MHC molecules. Therefore, they are also important for the elimination of degenerate tumor cells. The RCC26 cells apparently have a natural immunogenity, so that they can activate various effector cells of the immune system.

First Steps to the Clinic

In a clinical phase I/II study started last year twelve patients are treated with the RCC26 vaccine at the Urological Clinic of Ludwig-Maximilians-University in cooperation with Dr. Heike Pohla’s Clinical Cooperation Group. “In these patients the vaccine has not shown any toxic side-effects yet. But a final conclusion as to the effect of the vaccination is not possible yet, since the immune monitoring accompanying the study is still continuing,” says Frankenberger.

Despite the comparatively good immunogenic properties of the RCC26 vaccine, vaccination with tumor cells may also have disadvantages, because tumor cells generally tend to be weak antigen-presenting cells. Since in many cases there are no MHC class II molecules on tumor cells, they often cannot activate any CD4-positive helper T-cells. Therefore, the scientists follow another approach in parallel with the vaccination with genetically modified RCC26 cells: “professional antigen-presenting cells, such as dendritic cells (DCs) can stimulate the immune system much better: they induce both CD8 and CD4 T-cells, they carry costimulatory molecules on their surface, and they produce substances which tend to be beneficial to the immune system,” Frankenberger explains. Therefore, DCs should also be applied in the fight against renal cell carcinoma. As opposed to the vaccination with RCC26, however, it is advantageous for the development of a DC-based vaccine, if the RCC-associated tumor antigens are known. Then the RNA coding for these tumor antigens could specifically be introduced into the DCs. “The DCs produce the antigens coded by the RNA and present them on their surface, where they are ideally recognized by tumor-specific T-cells. This can stimulate the immune system and allow a specific reaction to the tumor,” says Frankenberger.

New Sleuths at Work

This therapeutic approach is substantially driven by great progress in the methods by which potential tumor-associated antigens (TAAs) can be identified. Prof. Schendel’s group investigates which antigens are presented by the tumor cells in cooperation with laboratories in Tübingen and in the US, where the peptides presented by the tumor cells on HLA-A2 are isolated and sequenced. Differential transcriptome analyses, in which the transcript quantities of the potential

Schematic diagram of the regulation of MHC-restricted and non-MHC-restricted T-lymphocytes. With their specific T-cell receptor (TCR) classical cytotoxic T-cells recognize the HLA/peptide complex expressed on tumor cells. This interaction is a prerequisite for the activation of these killer cells. Non-MHC-restricted T-cells, in turn, are regulated by MHC/peptide complexes in a mirror image: the binding to inhibitory receptors (KIRs) induces negative signals which deactivate non-MHC-restricted T-cells.
The production of dendritic cells (DCs) loaded with RCC antigens is pursued within a Collaborative Research Center. “We are currently testing the production and loading of the DCs in accordance with “Good Manufacturing Practice” guidelines, with the aim of using their application in a clinical study early in 2007,” says Iris Bigalke, head of the new GMP laboratory of the GSF, which is being set up. Her task will be to generate the DCs for application in patients under the conditions of the GMP with her working group: all pharmaceutical drugs to be used in patients are subject to strict legal regulations. Since cell therapy products are sterile, they must be manufactured in cleanroom conditions, i.e. in an environment free from bacterial contamination and particles. A pressure cascade ensures that no external contaminated air can enter the cleanrooms.

Preparations for commissioning such a cleanroom system for the production of cell preparations have been under way at the GSF for two years. The production protocols and the standard operating procedures (SOPs) for the DCs are developed in parallel at a preparatory laboratory. It often takes one to two years until a laboratory method can be implemented into GMP practice, since not all methods and reagents used in research are also suitable for GMP production. Apart from that, much larger numbers of cells are required for a therapeutic application than are needed for scientific purposes, so that sometimes existing methods must be established almost from scratch with different reagents or equipment. In addition extensive documentation and quality control are required.

All pharmaceutical drugs to be used with patients must be produced in an environment free from bacterial contamination and particles. With her group Iris Bigalke, head of GMP laboratory of the GSF which is being set up, will produce cell drugs in such cleanroom conditions in the future. An overpressure system ensures that no contaminated air enters the room.

The planned GMP laboratory (GMP = Good Manufacturing Practice) at the Innovations- und Gründerzentrum Biotechnologie (IZB, Innovation Center for Young Entrepreneurs in Biotechnology) will accommodate an area for production of cell drugs and a separate quality control area.
TAAs in tumor cells and in normal renal cells are compared, help to discover overexpressed tumor-specific antigens which are not found in normal cells at all or only in small quantities. The samples for such studies were obtained by the scientists from the GSF Institute of Pathology. "Our aim is to find as many tumor-associated antigens as possible and to introduce them into the DCs in the form of RNA, in order to offer the immune system many different points of attack against the tumor," Frankenberger explains.

The same antigens can also be used to track the success following a vaccination of the patient: if the patient responds to the vaccination, antigen-specific cytotoxic T-cells must be present in the patient’s blood, which are stimulated to proliferate upon contact with the respective antigens and produce immunological messenger molecules.

“As a third pillar in the immunotherapy of renal cell carcinoma we also want to develop a therapy with tumor-specific transgenic T-cells at our institute in the near future,” Frankenberger elaborates. The scientists introduce tumor-specific receptors into T-cells by genetic engineering, which then – as is the case with passive immunization with antibodies – specifically trace and fight isolated tumor cells in the patient’s blood or micrometastases.

In parallel with the vaccination with genetically modified RCC26 cells, the GSF immunologists pursue another new approach in the fight against renal cell carcinoma: they use dendritic cells from the patient’s blood, which have an important function in immune defense due to effective antigen presentation.

**New Collaborative Research Center for Immunity Research**

Fundamental work by Prof. Kolb had shown that the infusion of donor lymphocytes following bone marrow transplants can cause the elimination of leukemia cells, that this is due to T-cell-mediated immunity and that, therefore, the immune system is capable of curing cancer. On the basis of this discovery a new transregional Collaborative Research Center of two Helmholtz Centers – the GSF and the Max-Delbrück-Center in Berlin, the two Munich Universities, Humboldt University Berlin as well as Charité in Berlin – was established.

The aim of this work is to understand the foundations of the immunity mediated by specific T-lymphocytes as well as to develop new forms of the therapy of malignant diseases and chronic infections by transferring such T-cells into the patient.

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Treating cancer is one of the big challenges for modern science. The causes of tumor formation must be understood and methods must be developed allowing degenerate cells to reform to their healthy predecessors or to kill them selectively. In the last few years it has turned out that the packaging of the genetic material DNA in chromatin is of central significance to processes involved in cancer. Scientists at the GSF Institute of Toxicology study a class of enzymes — histone deacetylases — which considerably influence the packaging of DNA and are, thus, a potential point of attack for a cancer therapy.

For more than 30 years a drug has been on the market which can be used for the successful treatment of patients suffering from epilepsy. This is valproic acid, a small organic molecule, which results in the stable absence of seizures in more than 60 per cent of the patients. Valproic acid is well tolerated and has few side-effects, apart from one prominent exception: the substance is teratogenic, which means that its application during pregnancy may seriously damage the embryo. The embryo’s spinal canal is not closed properly and the skull’s development is out of proportion.

It was long unclear why valproic acid has such a fatal effect on the embryonic development, but does not cause any serious dam-
age in the adult organism. Together with his colleagues at the time at the Karlsruhe Research Center and at Georg-Speyer-Haus in Frankfurt/Main the toxicologist Prof. Dr. Martin Göttlicher studied the molecular biology processes underlying the teratogenic effect of valproic acid. The results of this work were quite sensational. The scientists did not only find the explanation as to why the substance interferes with the embryonic development, at the same time they showed possibilities of how this active substance could be used to treat certain forms of cancer.

It’s the Packaging That Counts

“Our studies at the time showed that valproic acid changes the packaging density of the genetic material DNA, which allows increased activity of many genes,” Göttlicher explains, who has headed the GSF Institute of Toxicology since 2003 and has the Chair of Toxicology at the Technical University of Munich. “In embryonic development this elevated gene expression causes malformations, but with certain cancers the activation of genes seems to have an advantageous effect: the growth of tumor cells is inhibited or they die due to the induction of programmed cell death.”

DNA molecules are in the nucleus of the cell — together with a number of proteins — in a highly organized structure called chromatin. At the first level of packaging the DNA double helix is packaged into so-called nucleosomes: sets of about 200 base pairs of the DNA are wound round a nucleus of eight proteins, the histones. Due to their amino acid composition the histone proteins are positively charged, but they can be modified by enzymes, so that their total charge changes. Thus, a certain class of enzymes — histone acetyl transferases — cause acetyl moieties to be attached, thereby neutralizing the intrinsic charge of the histones. Another class of enzymes — histone deacetylases — can remove these acetyl moieties again and thereby restore the positive intrinsic charge of the histones. Since the components of the DNA — the nucleotides — are negatively charged, the charge state of the histones assumed to have considerable influence on the packaging density of the DNA in the chromatin and thus also on the accessibility of genes for transcription. In other words: genes in a loosely packaged DNA on acetylated histones are more active than genes in a densely packaged DNA on non-acetylated histones.

In their earlier studies Göttlicher and his colleagues could show that the antiepileptic valproic acid inhibits the activity of the histone deacetylase enzymes. Therefore, the acetyl moieties can no longer be removed from the histones, the histones are uncharged, the chromatin is packaged less densely, and many genes whose control depends on histone acetylation are, therefore, more active. In embryonic development, which is characterized by a sensitive balance of active and passive genes, this ‘unplanned’ gene activation causes malformations of the medullary tube and the skull. The adult organism is apparently more capable of compensating for the inhibition of the histone deacetylases within certain limits compared to the embryo.

Gene Activation to Control Cancer Cells

The gene activation caused by the inhibition of histone deacetylases could — the scientists speculated — however, also be a promising concept for the therapy of certain tumor diseases. In all body cells a highly complex program
controls which genes must be activated and deactivated at which time. This program is, however, susceptible to interferences. Environmental influences or defects in the genetic material may cause genes to be deactivated, although their expression is really important, e.g., for protecting the cell from uncontrolled growth, for its differentiation in accordance with its purpose or for inducing programmed cell death.

When such genes are wrongly deactivated, a cancer cell can develop from a healthy body cell, which may then proliferate unhampered.

It was known from tumor research that the modification of chromatin is disturbed in many types of cancer cells and that, therefore, the expression of numerous genes is dysregulated. For example, in certain leukemia cells the mechanism for the acetylation and deacetylation of histones is faulty – too much histone deacetylase activity is apparently bound to certain genes. This changes the chromatin structure, so that important regulatory genes remain ‘silent’.

For the treatment of such tumors it would be ideal, if changes in the chromatin, which cause irregular silencing of genes due to histone deacetylases could be reversed. There are a number of natural and synthetic substances which can inhibit these enzymes, including valproic acid. Its special advantage as a therapeutic drug would be that it has long been approved as a pharmaceutical drug and there is extensive experience with effects and side-effects – albeit for a different application.

The scientists first studied the antitumor effect of valproic acid on animal models for breast and colon cancer, on carcinoma cell cultures and on human leukemia cells. The results were extremely promising. Many tumor cells reformed, redifferentiated to more normal cells or were eliminated by programmed cell death. There were, however, also cancer cells which did not react to the inhibition of histone deacetylases.

**Promising Building Block for Therapy**

Meanwhile clinical partners have also started studies with valproic acid as an antitumor drug. At Krankenhaus Nordwest in Frankfurt/Main a phase I/II study on dosaging and tolerance is being conducted, and at the University of Ulm the AML study group (AMLSG) is conducting a phase II and phase III study on patients with acute myeloid leukemia to find out what the effect of valproic acid is in combination with conventional and other innovative antitumor drugs.

**Solid Tumors**

In cooperation with the Karlsruhe Research Center Göttlicher and his colleagues from the GSF Institute of Toxicology recently also found a connection between a malfunction of histone deacetylases and the development of solid colon tumors. Such tumors often develop on the basis of a defect in the so-called APC (adenomatosis polyposis coli) tumor suppressor gene. Among other things failure of this gene results in the increased production of a certain histone deacetylase, HDAC-2. Apparently elevated HDAC-2 amounts have great significance to the further destiny of the cancer cells. Isolated colon tumor cells have been observed to only be able to survive, if there are increased amounts of HDAC-2. Recent research investigates the question why an inhibition of histone deacetylases results in the death of these tumor cells. The genome-wide search for genes which are activated by the inhibition of histone deacetylases in colon tumor cells shows that the balance between inductors and inhibitors of programmed cell death is apparently decisively controlled by the histone deacetylase activity. On top of that components involved in the recognition of tumor
cells by the immune system also depend on the activity of histone deacetylases.

The greater occurrence of histone deacetylase HDAC-2 in colon tumors raises the question whether this enzyme makes a vital contribution to tumor development and whether any tumors can develop in the absence of HDAC-2. The availability of genetically modified mice now allows us to investigate this question with the help of mouse tumor models.

In view of the future possibility of inhibiting HDAC-2 for therapeutic purposes it is important that the organism as a whole can tolerate this inhibition of the enzyme to a certain extent. The genetically modified mice already provide important information here: they are viable despite the absence of HDAC-2. Although they are smaller than their unmodified siblings from birth and do not go through their development without any defects, these analyses are enough reason to hope that the inhibition of HDAC-2 in the adult organism which has developed a tumor does not result in any serious damage. The development of colon tumors is reduced in mice without HDAC-2, although it is not completely absent – up to the stage of benign polyps. The question remains unanswered to what extent the further development of such adenomas all the way to the malignant carcinoma is influenced by the absence of HDAC-2, and whether it is a worthwhile perspective to develop selectively effective inhibitors for HDAC-2.

In cooperation with the Technical University of Munich and Klinikum rechts der Isar the concepts developed in fundamental research are reviewed in studies on tumor patients. The scientists use samples from human colon tumors to investigate whether the predictions based on cell culture and mouse experiments can be confirmed and there is actually a connection between increased histone deacetylase amounts and the reduction of certain growth inhibitors and triggers of cell death. They expect these studies to provide indicators for those tumors which respond to treatment with inhibitors for histone deacetylases. These studies are funded by the Deutsche Forschungsgemeinschaft (German Research Foundation) within the framework of the Collaborative Research Center 456 “Target Structures for Selective Tumor Interventions.”

Cancer Award

In March 2006 Prof. Martin Göttlicher and Prof. Thorsten Heinzel, formerly at Georg-Speyer-Haus in Frankfurt/Main, now at the University of Jena, were awarded the experimental Cancer Prize 2006 of the Deutsche Krebsgesellschaft (German Cancer Society). Excerpt from the reasons: “The work done by the award-winners resulted in the better understanding of a fundamental mechanism of the development of cancer and – based on this knowledge – (…) the approval of a new cancer drug in the foreseeable future.”
In 2001 each German citizen is estimated to have received a mean effective X-ray dose of about two millisievert (mSv) in medical examinations on average. This means that with a mean total exposure of 4.7 mSv X-rays make up the lion’s share of radiation exposure. According to the Federal Agency for Radiation Protection nearly 40 per cent of the two mSv comes from computed tomography (CT) scans, which, however, are only about five per cent of all X-ray scans. In computed tomography images of slices of the

A broken leg, a painful tooth root, suspected breast cancer or pneumonia – the doctor takes X-ray pictures. The high-energy radiation has become indispensable in everyday medical practice. Nearly 40 per cent of medical radiation exposure comes from computed tomography scans and the number of CT scans will continue to rise in the future – and radiation exposure with it. Scientists at the GSF Institute of Radiation Protection have developed a new process which provides high-quality scans with a clearly reduced radiation dose.
In computed tomography images of slices of the body are shot. Reassembled they give the physician an accurate representation of the interior of the body. Multislice CT, which was introduced a few years ago, allows the simultaneous calculation of several adjacent layers of the body.

It is a disadvantage that CT implies comparatively high radiation exposure: as opposed to classical X-rays regions of the body which are not of interest are more difficult to shield, all parts of the body in the direction of the scan are irradiated; on top of that the radiation dose at the point of entry into the body is slightly higher. Scans of one to two CT layers carry a similar radiation exposure to that of a conventional full-size X-ray of the same region of the body.

Technical improvements have so far been able to reduce the dose per CT by a maximum of 30 per cent. With a further reduction of the radiation dose, however, the image quality would suffer, mainly due to greater image noise. The goal of the GSF Working Group Medical Physics is to find a way to generate high-quality scans despite a lower dose. The head of the Working Group, Dr. Christoph Hoeschen, outlines its work: “For the necessary reconstruction of the images from CT data we apply a novel algorithm, which makes better use of the information contained in the raw data.” This means that the scientists can reconstruct images of at least the same quality as with the standard procedure used in the past, “filtered back projection” (FBP) with a comparable extent of computing with half the data, i.e. half the radiation exposure. On the basis of the new algorithm they have also developed various new scanning geometries which could help to reduce the dose even further without losing any of the quality.
The algorithm of FBP consists of two main steps: back projection and filtering. "The data are recorded as projection radiographies from different angles. For simplification we assume that the rays belonging to one angle run parallel," Hoeschen explains. "Then the total data record forms a so-called sinogram. After that we project the result back: we assign an absorption value to all elements on the respective projection line, so that the total absorption value measured would result. If we do this for all projection radiographies, we get an approximation to the original image." The more angles are used to "shoot" the rays, i.e. the more projection radiographies are produced, the better will the image be. But a point object will appear as the shape of a star. Filtering turns it back into a point, but also blurs the image to a certain extent. To minimize this effect, very high-definition measuring is required using photon-rich rays, so that the image noise does not get to great. I.e.: high radiation exposure must be accepted.

Together with colleagues from the University of Oregon in Eugene, USA, the GSF scientists have created a novel reconstruction algorithm, which can calculate image data from the raw data of a CT scan.

**New: Algorithm OPED**

Its principle, an “orthogonal polynomial expansion on the disc” (OPED), is not easy to...
understand for non-physicists. The principle is based on the fact that a function describing the object can be represented approximately by a polynomial. By carefully selecting the required basic functions the experts manage to reconstruct an extremely accurate approximation of the actual properties of the object fairly easily with relatively little computing. Correct data input is a prerequisite – for the CT this means: enough photons must penetrate the absorbing medium to generate detectable signals.

Reconstruction using FBP implies yet another problem apart from that of a blurred image, which is caused by the indispensable filtering: the rays which do not run in parallel, but like a fan. But since most conventional FBP versions necessitate parallel rays, they must be calculated from the fan beam, which may result in mistakes in the reconstruction. With the OPED algorithm raw data from rays at different distances can be used: the required data can be obtained right from the scans shot with fan beam. The data just have to be resorted, then the reconstruction can be started immediately.

Better Images With Half the Radiation Dose

The GSF scientists prove in test simulations that OPED need not fear the comparison with FBP: using technical objects and layers of the voxel models of human bodies made by them they simulate original data as an actual CT would produce them. These data are then reconstructed using FBP once and OPED once from half of the FBP data. These images already give the impression that OPED does very well – the OPED image reproduces details more clearly without considerably greater noise. Quantitative evaluations of technical phantoms show: with OPED at least the same signal-to-noise ratio is possible with half the dose as it is with FBP with the full dose. So using the new algorithm the dose can be halved without sacrificing quality.

 Advantageous Geometries

One aim of medical imaging is to record more and more layers of the body at the same time in order to minimize artifacts caused by movements, e.g. in heart scans. For this purpose it would be desirable to combine CT with modern digital X-ray image detectors, so-called flat-panel detectors. This, however, involves two serious problems. One is associated with the fact that the detector elements are no longer arranged on a circle, but in a flat surface; this means that they should be of different sizes – getting bigger towards the edges. Since when in the conventional process the fan beam impinges on detector elements of equal size and in a circular arrangement, after it has penetrated the patient, all part beams arriving there are of the same width. With the flat-panel detector, however, the part beams are of different widths. “This causes many problems,” says Hoeschen. “But we can solve them, because OPED does not have to rely on beams of equal width – in particular, because the beams need not be arranged at equal distances. So we vary their distances and thereby their widths. If this is done skillfully, it can be arranged in such a way that beams of equal width impinge on the detector after passage
through the patient after all – so that there is an optimal utilization of the detector.”

The second difficulty is that a large portion of the patient’s body surface is irradiated with flat-panel CT. This generates much scattered radiation, which superimposes the actual measured signal. While with conventional CT the scattered radiation makes up one quarter of the direct ray, it is four times its size with flat-panel CT. “So we have a sort of scattered ray image. But we will get a grip on this, we will use masks to blank the rays which are not required. This can drastically reduce the portion of scattered radiation,” says Hoeschen.

There is yet another disadvantage of current CT systems which could be compensated for by OPED – by coupling the algorithm with a completely new geometry. To minimize scanning times, the CT tubes do not only emit radiation while the detectors are reading out, but generally continuously. Since fewer rays are sufficient for OPED and, in particular, rays at variable distances, a fixed inner ring could be installed in an existing CT system, which has two functions: it is both a mask and a detector for a second scanning level. This would provide two data records for reconstruction. Apart from that, all the radiation to which the patient is exposed would be used for imaging, which would allow a further considerable dose reduction without a reduction in quality. A prototype of this kind is currently being built by the GSF scientists.

The new OPED process has yet to go on to commercial application. But tests have started, and there are ample indications that OPED will pass the evaluation phase successfully. So hopes that a milder computed tomography can enter medical practices and clinics in the foreseeable future seem to be justified.
Allergy
Specific change in immunity in terms of pathogenic hypersensitivity. Allergies are directed against foreign substances (allergens) affecting the body.

Allogen
Originating from a genetically unrelated individual of the same species.

Alveolae
Air sacs in the lungs, located at the end of the bronchial tree in the pulmonary tissue on the bronchioles.

Autologous
Belonging to the same individual.

Antigens
Molecules bound by a specific antibody. Antibodies are produced by the immune system upon first contact of the body with the antigen recognized as “foreign.”

Antibodies
Proteins with a specific binding site for antigens. Antibodies are usually dissolved in the blood, but may also be bound to membranes. Upon contact of the body with antigens antibodies are synthesized in a complex immune reaction.

Bronchoalveolar lavage
Irrigation of the lungs with physiological salt solution to obtain lung fluid with its protein and cell material.

Chemokines
Chemotactically acting cytokines, which can be secreted by many cell types. Chemokines can attract and activate, e.g., leukocytes.

Cytokines
Proteins produced and released by immune cells, but also by non-immunological cells. The cytokines serve as “messenger substances” for the immune cells, they control and coordinate the defense of pathogens. Cytokines are partly responsible for the successful result of an immune reaction.

DNA
Deoxyribonucleic acid, chemical name of the carrier of hereditary information. It consists of the bases adenine, thymine, cytosine and guanine, which are connected by a sugar-phosphate backbone. The linear sequence of the bases is the “sequence” of the DNA. The DNA’s spatial structure is that of a double helix.

Emphysema
Also: pulmonary emphysema, abnormally increased air volume of the lungs due to overdistension or swelling, which can cause alveoli to burst.

Epithelium
Group of cells covering the inner or outer body surfaces.

Gene
A gene is a section of the DNA coding for a particular protein and also containing regulatory elements.

Genome
Totality of the genetic material of a cell or an individual.

Granulocytes
One of three types of white blood cells. Granulocytes – like monocytes – are produced in the bone marrow.

Histamine
Messenger substance released after contact with allergens and IgE antibodies. It is responsible for symptoms such as itching or rhinitis.

Histones
Proteins round which the thread of hereditary material – the DNA – is wound.

Hybridoma
Cell created by the fusion of two cell populations, which produces monoclonal antibodies and is capable of proliferating without any time limit.

IgE
Abbreviation of immunoglobulin E. Class of antibodies responsible for mediating immediate allergic reactions. They are found in higher concentrations in the blood of allergic patients and are suitable for diagnosis (e.g. RAST).

Immunoglobulins (Ig)
Proteins usually acting as antibodies. They are divided up into different classes, depending on their shape and function (e.g. IgM, IgE).
Immunomodulation
Manipulation of the immune system.

Interferons
Cell glycoproteins produced especially by leukocytes and fibroblasts upon infection with a virus. They have unspecifically antiviral properties. Human interferons for medical purposes can already be produced by genetic engineering.

Lipids
Fats: mixed group of biomolecules, which are of importance for the building of cell walls, energy storage and nutrition and for the communication between cells.

Liposomes
Inclusion in L. can transport drugs to their exact place of action, thereby improving their effect.

Lymphocytes
One of three groups of white blood cells.

Macrophages
Eater cells. Macrophages are important mediators of immune defense and can eliminate microorganisms and cell components after ingestion (so-called phagocytosis).

Nucleosome
Complex of DNA and histones, the first packaging stage of DNA.

PCR
Polymerase chain reaction. Method for the replication (amplification) of small quantities of a DNA for diagnostic, analytical or forensic purposes.

Proliferation
Cell division, cell growth.

Protein
Molecule produced by the connection of numerous amino acids by peptide binding. Proteins are of decisive significance, e.g., as structural proteins or as biocatalysts (enzymes) in the metabolism of living cells.

RNA (ribonucleic acid)
Nucleic acid molecules consisting of a sugar-phosphate chain, to which the bases adenine, uracil, guanine and cytosine are bound. RNAs transfer the hereditary information from the DNA to proteins (translation).

Sequencing
1. Sequencing of nucleic acids: process for the determination of the sequence of bases.
2. Sequencing of peptides/proteins: process for the determination of the amino acid sequence.

Sputum
Expectoration; secretions of the bronchi; analysis of the sputum helps diagnose respiratory diseases.

T helper cells (T lymphocytes)
Cell type partly responsible for the cellular immune response.

Transcription
Transfer of the genetic code from the double-stranded DNA to a single RNA strand with the involvement of numerous enzymes (transcriptases); first step of gene expression.

Tumor necrosis factor (TNF)
Endogenic messenger substance of the cells of the immune system, a cytokine.

Tumor virus
Virus interfering with the growth control of a cell and resulting in uncontrolled proliferation of the cell, e.g. the Epstein-Barr virus, which triggers a fast growing tumor (Burkitt lymphoma).

Virus
Non-cellular biological unit; consists of nucleic acids (DNA or RNA) not bound to chromosomes in a protective envelope of proteins, lipids etc. Viruses can only reproduce in a host cell using the enzymes of the host.
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Pictures:
The GSF contributes to the foundation of future medicine and health care as well as ecosystems, which are of critical importance for health. Our focus is on chronic degenerative diseases like lung diseases, allergies, cancer, and cardiovascular diseases that are influenced to a large extent by environmental conditions, personal risk factors, and lifestyle. Therefore, in order to generate a knowledge base, we analyse interactions between genetic disposition, biological systems, and environmental factors. This will lead to the development of new personalised approaches in prevention, diagnostics, and causal therapy – the future direction of medicine. Our strategy is based on translational approaches that bridge basic research with clinical application to provide an immediate benefit to the patient.

The research projects of the GSF are focussed on four complementary areas:

- Environmental factors and health,
- Mechanisms of health and disease,
- Infection and immunity, and
- Ecosystems and health.

Networking across disciplines facilitates exchange of knowledge and value creation. In addition, GSF scientists ensure that the most recent research results can flow into guidelines and new legislation by participating in national and international advisory commissions. The GSF is highly committed to scientific and technical excellence in its institutes and departments, as well as the promotion of young scientists.

The GSF is a research institution of the Federal Government and the State of Bavaria within the Helmholtz Association of German Research Centres with approximately 1700 associates in 24 institutes and departments.

The main research site is located in the north of Munich. Several groups are closely linked to the universities and hospitals.