

STUDENT RESEARCH SYMPOSIUM

**Abstracts from the Tenth European Students'
Conference of the Charité*†**

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**A RECOMBINANT BISPECIFIC SINGLE
CHAIN ANTIBODY CD19xCD3 INDUCES
RAPID B CELL LYMPHOMA-DIRECTED
CYTOTOXICITY OF UNSTIMULATED
PRIMARY HUMAN T CELLS**A. Löffler, R. Lutterbüse, F. Zettl,
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Bispecific antibodies directed against malignant lymphoma have been shown to be effective *in vitro* and *in vivo*. So far, clinical use has been hampered by the fact that conventional approaches to produce these antibodies suffer from low yields, occurrence of ill-defined by-products, or laborious purification procedures. To overcome these problems we have generated a 60 kDa recombinant lymphoma-directed bispecific single-chain antibody. The antibody consists of two different single-chain Fv fragments joined through a Gly-Ser linker. One specificity is directed against the CD3 antigen of human T cells. The other antigen binding site is directed against the pan B cell marker, CD19, which is expressed with few exceptions in all B cell malignancies. The construct was expressed in CHO cells and purified from the supernatant by its C-terminal histidine tag. Specific binding to CD19 and CD3 was demonstrated by FACS analysis. Surprisingly, bispecific antibody binding activated even unstimulated

peripheral blood T cells and resulted in high cytotoxic activity against CD19-positive lymphoma cells, even at very low concentrations (10 – 100 pg/ml). Moreover, strong lymphoma directed cytotoxicity at very low antibody concentrations could be rapidly induced (after 4 h) even in experiments without any T cell restimulation. Addition of bscCD19xCD3 antibody to peripheral blood mononuclear cells derived from patients with chronic lymphocytic leukemia of the B cell type resulted in a dramatic decrease of autologous malignant B cells. Thus, this bscCD19xCD3 antibody is much more efficacious than previously described bispecific antibodies. It is unique in so far as it is active at very low concentrations and does not require preactivation of T cells. Therefore, clinical application of this novel reagent is warranted in patients with B cell malignancies refractory to conventional therapy.

**SYNERGISTIC EFFECT OF CYTOKINES IN
MALIGNANT MELANOMA GENE THERAPY
MURINE MODEL**P. Wysocki, M. Mackiewicz,
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For more than three years now, clinicians have been employing cytokine-modified tumor vaccines for treatment of malignant melanoma. However, we are still looking for genes or combination of genes that may display even greater effects in the stimulation of anti-tumor immunological responses in tumor rejection models. In our studies, we have tested several cytokines independently as well as in combination in a murine

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model of malignant melanoma. We have transduced the following genes into the murine melanoma cell line B78-H1: IL-6, H6 (hyper IL-6, a new designer cytokine comprising IL-6 and soluble IL-6 receptor joined by an artificial linker), IL-11, and GM-CSF. For the vector, recombinant retroviruses developed in our laboratory, DC CMV IRES Neo (dicistronic double copy vector based on Murine Stem Cell Virus) were used. Stable cell lines expressing the introduced gene were injected subcutaneously (5×10^5 cells) into C57BlxC3H mice. Each experimental group, consisting of 10 mice, was injected with one of the following: B78 mock transduced cells (control group), B78-IL6, B78-H6, B78-GMCSF, B78-IL11, B78-H6 + B78-GMCSF (1:1), or B78-IL6 + B78-GMCSF (1:1). During the 87 days of the experiment, we have evaluated the kinetics of tumor growth (mm as a mean tumor diameter of the whole group) and the mice survival rate. All the cytokines transduced into melanoma cells inhibited tumor growth. The most effective was the combination of B78-H6 + B78-GMCSF (1:1). In this group, the mean tumor diameter at the end of the experiment did not exceed 0.51 mm, whereas in other groups the mean tumor diameter was >11 mm. The survival rate was increased in all experimental groups compared to control, and the group that received B78-H6 + B78-GMCSF showed no mortality at the completion of experiment.

TIMING OF SOCIAL BEHAVIOUR DURING AND AFTER PREGNANCY

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Introduction: The timing of social behaviour during and after pregnancy is increasingly recognised as an important factor for the well-being and health of young families. Activity-rest patterns in adults are dominated by more or less stable circadian rhythms. In contrast, the activity of newborns is ultradian with alterations in the rhythmic structure of behaviour during the course of postnatal ontogenesis. The aim of the present study was to investigate the parental activity patterns during late pregnancy and, subsequently, the infant-parent interaction during the first four months after birth. **Methods:** Activity patterns of 7 families in Berlin, Germany, were recorded in a longitudinal design using actigraphy, which measures human movements continuously and non-invasively. Recordings of parental activity started at the beginning of the 32nd week of pregnancy and were continued in three series of three weeks sessions, each until four months after birth.

Informed consent was given by the parents. Activity-rest patterns were compared for differences in the timing of day/night phases (actogram), frequency composition (fast Fourier transformation, periodogram), and parent-child interaction (cross-correlation). Results: All pregnant women showed regular night awakenings. The nocturnal sleep duration in these women was lower than that of their partners. The mother's sleep period often shifted by several hours postpartally compared to prepartally. Six out of seven women exhibited a clear circadian period of 24 hours during pregnancy. In the first four weeks after birth, the amplitude of their circadian period was decreased. In five infants, the spectral composition exhibited a dominant or subdominant circadian period from the eighth day of life. The concordance of nocturnal movements as well as simultaneous morning activity was highest in mother-infant patterns. Conclusion: The timing of the mother's activity is shown to be altered during and after pregnancy. Preliminary data further suggest that mother-child interaction may act as a social time cue of likely relevance to the development of a diurnal rhythm during the first weeks of life and may thereby contribute to infant behaviour.

INTERLEUKIN-4 RECEPTOR GENE POLYMORPHISM AND ATOPY

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Interleukin-4 receptor (IL-4R) gene has been recently linked to the occurrence of atopic diseases. Hershey *et al.* (New England Journal of Medicine 337: 1720-1725; 1997) described a polymorphism within the IL-4R gene causing the substitution of glutamine to arginine in position 576 of the alpha subunit of IL-4R, which they suggested to be associated with atopy as a gain-of-function mutation. In the present study we attempted to analyse the frequency of the 576Arg allele in atopic patients and their families as well as the relation of this allele to the presence of atopy. We studied 35 families of patients with allergic diseases (140 persons). The control group comprised 30 healthy persons without familial occurrence of atopy. The Glu576Arg polymorphism of IL-4R gene was analysed by PCR-RFLP, using DdeI restriction enzyme. Total serum IgE was measured in all subjects by Pharmacia CAP assay and skin prick tests were performed in the group of atopic persons. Atopy was present in 68% of the family members. We found 127 homozygotes carrying the Glu576 allele, 35

heterozygotes and 7 homozygotes with the 576Arg allele. The allelic frequency of 576Arg in the investigated population was 0.20. There was no difference between the occurrence of the allele in atopic subjects (0.21), their healthy relatives (0.20) and controls (0.21). The total IgE level did not differ significantly between the groups, although the mean IgE concentration was lower in the 576Arg homozygotes (196 kU/L vs. 303 kU/L in others). Conclusions: 1) The allelic frequency of the 576Arg allele in the Polish population is higher than that described in the American population (0.20 vs. 0.10). 2) No significant association between the polymorphism in the interleukin-4 receptor gene and atopic status was observed.

PATHOGENESIS OF MALNUTRITION IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Introduction: Patients with liver cirrhosis and hepatocellular carcinoma (LC+HCC) are often malnourished. *De facto*, conflicting results have been reported regarding elevated or normal resting energy expenditure (REE) as a potential cause of malnutrition. To investigate this, REE, energy intake, and body composition were determined in patients with LC+HCC and in patients with liver cirrhosis only (LC). **Methods:** REE was measured by indirect calorimetry (REECALO) or it was calculated using the equations of Harris and Benedict (REEHB) for 22 patients with LC+HCC and for 40 patients with LC. Energy intake was quantified using standardized dietary recall (EBISTM). Body composition was assessed by means of anthropometry and bioelectric impedance analysis (BIA) to determine arm muscle area (AMA) and body cell mass (BCM). **Results:** LC+HCC patients were older (60.2 ± 7.5 years vs. 53.6 ± 11.7 years; $p < 0.05$), and had less severe liver disease (Child-Pugh score 6.5 ± 1.9 vs. 7.8 ± 2.1 ; $p < 0.05$) than LC patients. Muscle mass was severely reduced in both LC+HCC and LC patients. REEHB was not different between LC+HCC (1561 ± 203 Kcal/D) and LC (1539 ± 294 Kcal/D) patients. In absolute terms REECALO was increased in LC+HCC, but there was no difference when REECALO was related to BCM as the metabolically active compartment. When total energy expenditure was calculated as $1.3 \times$ REECALO, patients were in slightly

negative energy balance in both groups (LC+HCC: -36 ± 762 Kcal/D; LC: -130 ± 637 Kcal/D). **Conclusions:** Patients with LC are malnourished. Since LC patients had more advanced liver disease, those with LC+HCC were much more likely to be in a better nutritional state and to have higher REE in absolute terms. Accordingly, energy expenditure was not increased by HCC, when related to the metabolically relevant compartment, BCM. Our findings indicate that inadequate energy intake, rather than increased REE (Hypermetabolism), contributes to malnutrition in LC+HCC Patients.

ACTIVATED PERIPHERAL BLOOD MONOCYTES IN ESSENTIAL HYPERTENSION

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Background: Immunological factors seem to be involved in the aetiology of hypertension. We investigated the possible role of human monocytes in the pathology of hypertension. **Methods:** Blood samples were obtained from 22 patients with essential hypertension before any drug administration, and from 24 normotensive healthy blood donors as a control group. Both groups were matched for sex and age. Monocytes were separated by density gradient centrifugation and plastic adherence. Cells were divided into four groups. The first group remained unstimulated, the second was stimulated with lipopolysaccharide (LPS), the third group was incubated with angiotensin II, and the fourth group was stimulated by angiotensin II after preincubation with losartan, a specific angiotensin II receptor antagonist. After incubation, the supernatant concentration of the proinflammatory cytokines IL-1B, TNF- α , and IL-6 were determined by specific ELISA. Activation of the monocytes was also examined at the RNA level using semiquantitative RT-PCR. **Results:** Without stimulation there was no statistically significant difference in the *in vitro* secretion patterns of proinflammatory cytokines between controls and patients. We were able to demonstrate significantly increased levels of IL-1b ($p < 0.05$) and TNF- α ($p < 0.02$) after stimulation with LPS in patients with essential hypertension compared to the control group. Similar results were shown for IL-1B ($p < 0.05$) when monocytes were stimulated with angiotensin II. Preincubation with losartan diminished the secretion of IL-1B to comparable levels in both groups. Preactivation of monocytes in hypertensive

patients was also seen on RNA level. In summary, we suggest that activated monocytes may be of pathogenic importance in hypertensive patients.

EXTENT OF PRIMARY CYTOREDUCTIVE SURGERY AS PROGNOSTIC FACTOR IN PATIENTS WITH ADVANCED EPITHELIAL OVARIAN CARCINOMA: A RETROSPECTIVE ANALYSIS.

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Ovarian cancer in Poland accounts for 2000 deaths every year and morbidity in an additional 3000 patients. The management of advanced ovarian cancer consists of primary cytoreductive surgery (PCS) followed by chemotherapy. Radical surgical cytoreduction is regarded as standard primary treatment with the goal of leaving no residual pathologic tissue. The purpose of this study was to assess the extent of PCS performed by a variety of physician specialists in various hospital settings and its influence on the outcome of therapy. Medical records of 56 ovarian cancer patients in the advanced stages of the disease treated in the Oncology Centre in Gliwice were analysed. All patients underwent PCS followed by systemic platinum-based chemotherapy. Clinical data included the patient's clinical status prior to the initiation of chemotherapy, the time between PCS and the last follow-up, the operative report of the PCS and the identification of the hospital where PCS had been performed. Of the 56 patients with advanced ovarian cancer, 46 were operated in district hospitals and 10 in university hospitals. Out of the 56 patients, only eight underwent PCS with the removal of all visible tumor. Of these eight primary cytoreductive procedures, six were performed in university hospitals. The overall survival time in the radically operated patients ranged from 13 to 76 months with a median time of 68 months and a mean time of 58 months. In the non-radically operated patients, the survival time ranged from 4.5 to 102 months with a median time of 18 months and a mean time of 33 months. Patients who underwent PCS in university hospitals had a longer overall survival. Despite a well documented correlation between the extent (i.e. radicalness) of primary cytoreductive surgery and survival in patients with advanced ovarian carcinoma, only few undergo complete cytoreduction. Patients with advanced ovarian cancer should undergo PCS performed by an experienced team of gynecological oncologists in a specialised clinic.

ANALYSIS AND FUNCTIONAL CHARACTERIZATION OF ALTERNATIVELY SPLICED ANGIOTENSIN II TYPE 1 AND 2 RECEPTOR TRANSCRIPTS IN NORMAL AND FAILING HUMAN HEARTS

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Angiotensin II type 1 and 2 receptors (AT1 and AT2) are regarded as important antagonists in the pathophysiology of heart failure. Expression of AT1 and AT2 is regulated in cardiac diseases and may occur at the protein level or at the mRNA level. Aside from transcriptional mechanisms and the regulation of mRNA degradation rate, alternative splicing of the 5' untranslated region (5'UTR) may also play a role in mRNA regulation. The AT1 mRNA consists of at least 5 exons, while the AT2 mRNA consists of 3 exons; several differently spliced AT1 and AT2 transcripts have been identified in human tissues. We therefore searched for AT1 and AT2 mRNA splice patterns specific to chamber localization or to cardiac performance that might affect receptor expression. For this purpose, AT1 and AT2 transcripts in normal and diseased human hearts were analyzed using a RT-PCR followed by HPLC quantitation of the amplicates. Atria from patients with normal systolic cardiac function, endomyocardial biopsies, as well as ventricular and atrial samples from failing hearts were compared. AT1 transcripts with exon composition (Ex) 1/2/5 and 1/5 represented about 95% of all AT1 mRNAs. Ex 1/2/3/5 accounted for 2% in ventricles and for 8% in atria. Ex 1/2/5 was the most abundant splice variant in normal right atria (48%), but was expressed at lower levels in heart failure (41%). The ratio of Ex 1/2/5 to Ex 1/5 was 1.08 : 0.17 in normal atria (controls), 0.84 : 0.26 ($p < 0.05$ vs. controls) in atria from failing hearts, 0.64 : 0.12 ($p < 0.001$) in right ventricles and 0.56 : 0.11 ($p < 0.001$) in left ventricles with heart failure. Of the two identified AT2 transcripts, Ex 1/2/3 was the most abundant in the human heart (92% vs. 8% for Ex 1/3). To test the effect of different 5'UTRs of AT1 and AT2 mRNAs on protein expression, luciferase reporter gene assays were performed. Among the constructs, which contain the AT1 promoter/AT1 5'UTR, the plasmid Ex 1/5 expressed 29% higher lucifer activity than Ex 1/2/5, whereas Ex 1/2/3/5 exhibited the lowest luciferase expression (51% of Ex 1/2/5 activity). When we compared reporter gene plasmids with AT2

promoter/AT2 5'UTRs, the construct Ex 1/3 expressed 45% higher luciferase activity than the construct Ex 1/2/3. The AT1 splice variants containing exon 2 amount to 56% in normal atria but to only 39% in failing ventricles. These data strongly suggest there is a relative decrease in AT1 transcript 1/2/5 in favour of transcript 1/5 in cardiac diseases.

FOLLOW- UP OF PATIENTS WITH DEEP VENOUS THROMBOSIS TREATED WITH LOW MOLECULAR WEIGHT HEPARIN

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High-dose unfractionated heparin (UFH) and oral anticoagulant therapy are often used to treat deep venous thrombosis (DVT) but this treatment can cause major bleeding and, more rarely, severe thrombocytopenia. Many studies have recently suggested the use of low molecular weight heparin (LMWH) for the treatment of DVT. One hundred patients with DVT were included in this study; 48 of the patients were male and 52 were female. The mean age was 51 (range 16 – 82). The diagnoses were established by means of duplex sonography. The thrombus was located in the iliofemoral or iliofemoropopliteal vein in 89 patients, and solely in the popliteal vein in 11 patients. Enoxaparin, a LMWH, was initiated with a dose of 2 mg/kg/day (200 anti-factor Xa units) subcutaneously, divided into two injections, and continued for 5 days. On the second day of the treatment, warfarin sodium was initiated with a dose of 10 mg twice a day and was continued for three months so that the international normalized ratio (INR) would be 2.5 – 3. The patients were examined daily for symptoms and signs of pulmonary embolism and bleeding. Antithrombin III (AT III), protein C, protein S, and anticardiolipin antibodies were assessed in every patient. Patients were released from the hospital with socks covering below the knee and INR assays were performed every ten days. AT III was found to be low in 11 patients and protein C was low in 13 patients. Despite venous thromboembolectomy, limited amputation was necessary in one patient who developed phlegmia cerulae dolens. One patient died with dilated cardiomyopathy. Duplex sonography performed on day seven detected thrombus regression in 17 patients. All patients except one improved clinically and no bleeding due to enoxaparin was seen however bleeding due to warfarin was observed in five patients. We believe that LMWH is easier to administer than UFH, and that the

use of LMWH in the treatment of DVT does not require daily monitoring.

ANEMIA IN ROMANIAN CHILDREN

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The prevalence of anemia and its correlation with other factors were studied during the National Surveillance Program, a random longitudinal study of 20 counties (administrative districts) plus the city of Bucharest. In areas with a high mortality rate, the sample was overweighted. The local medical team performed measurements of each child's weight and height and also recorded data concerning feeding patterns. The iron status was assessed by determining the hemoglobin (Hb) level in one-year-old children. The program began in 1993 and by the end of 1997 46,838 children were monitored and Hb level was determined in 16,528 one-year-old children. The mean value of Hb was 10.8 g/dl with a standard deviation of 1.49, and with a significant difference between urban (10.9 g/dl) and rural (10.71 g/dl) regions ($p < 0.01$). Hb level under 11 g/dl was considered to be indicative of anemia. The prevalence of anemia was found to be 49.2%. The mean Hb level in children >2500 g birth weight was found to be significantly higher (10.9 g/dl) than the Hb level in children \leq 2500 g birth weight (10.41 g/dl) ($p < 0.01$). In children \leq 2500 g birth weight, the prevalence of anemia was 61.9% compared to 48% in children >2500 g birth weight. There was also a correlation between breast feeding duration and Hb level. In children with Hb level <7 g/dl, the mean age of weaning was 3.5 months; for Hb level between 7 and 8.9 g/dl, it was 5 months; for Hb level between 9 and 10.9 g/dl, it was 5.3 months; and for Hb level \geq 11g/dl, the mean age of weaning was 5.9 months. The level of maternal education also correlates with likelihood of anemia. Among children whose mother had only primary education (lowest level), prevalence of anemia was 56.8%; for incomplete secondary level of maternal education, the prevalence of anemia was 48%; for complete secondary level of maternal education, the prevalence of anemia was 46%; and for post-secondary level of maternal education (highest level), the prevalence of anemia was 43%. Conclusion: Anemia is a major public health problem and it is necessary to supplement the infant diet with iron. It will be necessary to prevent low birth weight and to improve feeding

habits for infants by increasing the age of weaning and by avoiding the early administration of cow milk. Training programs for mothers will improve maternal educational level.

THE COMPARISON OF ISOLATED GROWTH HORMONE DEFICIENCY AND MULTIPLE HORMONE DEFICIENCY ON CLINICAL AND ETIOLOGICAL BASES

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Growth hormone deficiency (GHD) is one of the most important causes of failure of normal growth. The diagnosis of GHD has classically been defined as peak serum growth hormone (GH) level <10 ng/ml in response to two pharmacologic stimulation tests in a child with an attenuated growth pattern. Patients with GHD may present with or without other hypothalamo-pituitary hormone deficiencies. The purpose of this study was to compare the clinical and etiological characteristics of 67 patients (44 boys, 23 girls) with isolated growth hormone deficiency (IGHD) and 91 patients (63 boys, 28 girls) with multiple hormone deficiency (MHD) diagnosed between 1980 – 1998. The mean age at diagnosis was 7.9 ± 4 years in the IGHD

group and 8.9 ± 4 years in MHD group. The birth history showed that in the IGHD group, the percentage of normal vaginal deliveries was higher than in the MHD group (89% vs. 68%, $p < 0.05$); in contrast, the percentage of breech deliveries was lower (2% vs. 17%, $p < 0.01$). Perinatal asphyxia was reported in 11% of IGHD and in 23% of MHD patients. At diagnosis, mean weight standard deviation score (SDS), height SDS, sitting height SDS, and bone age (Greulich-Pyle) were -2.5 ± 1.0 , -4.3 ± 1.7 , -4.2 ± 1.9 , 4.7 ± 3.3 years for the IGHD group and -1.9 ± 1.4 , -3.8 ± 1.9 , -4.5 ± 1.6 , 4.8 ± 2.9 years for the MHD group, respectively. Severe growth retardation (height SDS < -3) was found in 79% of IGHD and 83% of MHD patients. The ratio of complete GHD (peak GH < 5 ng/ml in two stimulations) was 60% in the IGHD group and 76% in the MHD group ($p < 0.05$). Cerebral imaging showed that 24% of IGHD patients and 55% of MHD patients had organic GHD, including congenital abnormalities (empty sella, hypoplastic or ectopic pituitary, agenesis of corpus callosum), craniopharyngioma, astrocytoma, microadenoma, etc.; the difference between the two groups was significant ($p < 0.001$). In conclusion, these findings show male predominance in both IGHD and MHD groups. Most of the children were diagnosed late and therefore had severe growth retardation. The high frequency of organic GHD, especially in the MHD group, warrant the need for neuroradiological imaging in these children. Complicated birth history is more frequently associated with an organic lesion causing hormone deficiencies in addition to GH.

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