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A B S T R A C T S O F T H E M E E T I N G : BALKAN ENDEMIC NEPHROPATHY: AN UPDATE

RENAL INTERSTITIAL FIBROSIS IS A MAJOR FEATURE OF BALKAN ENDEMIC NEPHROPATHY (BEN)

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Balkan Nephropathy is a common renal disease in the Balkan region which often leads to endstage renal failure. The pathogenesis of this disease is still unknown, however, the pathway to terminal renal insufficiency is similar to other renal diseases and is characterized by tubulointerstitial disorders. These tubulo-interstitial changes are histomorpholically characterized by mononuclear cell infiltration, tubular atrophy, reduced numbers of peritubular capillaries and renal interstitial fibrosis. Thus renal excretory function correlates negatively with the increase of tubulointerstitial disorders, especially with the occurrence of renal interstitial fibrosis. Tubular epithelial cells (TECs) as well as renal interstitial fibroblasts (RIFs) play a major role in the pathogenesis of renal fibrosis. TECs get activated either by the glomerular ultafiltrate from their apical side or by mononuclear cells from their basolateral side. They initiate the scarring process by secreting chemokines which in return attract mononuclear cells as well as growth factors that stimulate RIFs. In later phases of renal fibrogenesis, cellular changes of tubular epithelial cells contribute to the chronic impairment of renal function. Recently it has been demonstrated that these RIFs belong to a fibroblast stem cell system presenting with heterogeneous cell types at different differentiation stages. Cytokines such as FGF-2, TGFß and EGF have been shown to be pro-fibrogenic whereas HGF may inhibit fibrogenesis. Very recently we showed that BMP-7 in animal experiments also inhibits the fibrogenic process and furthermore reduces scarring in established renal fibrosis. Further investigations are necessary to analyse the pathways of renal interstitial fibrosis in order to develop new therapeutic approaches for this disease.

PROTEIN PROFILING AS A POSSIBLE NEW TOOL IN BEN

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Despite great advances in genomics it is well known that the cellular concentration of a specific mRNA is poorly correlated with the actual abundance of that protein, in particular since the expressed proteins from a single gene in reality may contain large amounts of micro-heterogeneity. Post-translational modifications also dramatically influence the complexity of proteins expressed in cells and tissues. Thus, in most instances it has been difficult to implicate specific proteins deduced from genomics in diseases such as BEN. The proteome defined set of all expressed proteins in cells or tissues is thought to contain protein variants which may be representative for specific pathological conditions. Overcoming the diversity and dynamics of protein expression in cells also for lower abundance proteins represents a significant challenge for proteome analysis with the aim to identify marker proteins for diseases and later analyze their potential functional significance.
Up to now proteome analyses are mostly based on a combination of the techniques of two- dimensional gel electrophoresis (2-DE) and mass spectrometry. Proteins separated in distinctive spots in the two-dimensional SDS-gels are digested in-gel and the fragments sequenced by mass-spectrometry. Matrix-assisted laser desorption /ionisation time-of-flight (MALDI-TOF) MS is used to create a unique mass-fingerprint of the fragments which can be applied as unique mass-identifiers of the protein in computer sequence database matching. However, 2-DE strategies are usually not suitable to detect proteins of low abundance in a mixture of thousands of other proteins and variants. Attractive

solutions to these problems have been developed which allow more powerful and specific protein separations before direct MS identification. Protein-chips or microarrays are produced which contain specific surface chemistriesy to attach proteins of interest (i.e. SELDI). Alternative approaches integrate nanoscale microcapillary liquid-chromatography with tandem mass-spectrometry (LC/MS/MS) with subsequent online mass-spectrometrical analysis. Thus several limitations of 2-DE can be overcome.
The presentation will address experiences in the application of reversed-phase HPLC and subsequent MALDI-PSD identification of HLA associated peptides as relevant immunogenic structures for T cell activation in tissues, as well as show examples of the application and prospectives of SELDI for differential proteomic analyses of cells and tissues. Combining the analytical and technical advances for proteins with the new developments in cell selection directly from tissues. By techniques such as laser-microdissection it can be expected that new insights into local pathological conditions of complex diseases such as BEN can be gained in interdisciplinary collaborations of cell biologist, pathologists and biochemists.

PREVALENCE AND INCIDENCE TRENDS OF BALKAN ENDEMIC NEPHROPATHY IN THE VRATZA DISTRICT IN BULGARIA FROM 1964 TO 1987

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Balkan Endemic Nephropathy, first described in 1956 in Vratza region, Bulgaria, may result from prolonged, chronic exposure to environmental toxicants, but the underlying etiologic factors remain elusive. There has been no recent systematic characterization of the epidemiology of this disease. Recently, it has been suggested that the incidence of the disease is decreasing. We therefore abstracted data from registers of patients in 21 affected villages and the town of Vratza, Bulgaria maintained from 1964 through 1987. In 1964, the prevalence of BEN was 6.0 per 1000 inhabitants; if the town of Vratza is excluded, the prevalence was 12.3 per 1000. From 1965 to 1975 the incidence rate was 0.7 per 1000 person-years, and from 1976 to 1987 the incidence rate was 0.3 per 1000 person years (rate ratio 0.43; $p<0.001$). Incidence was much lower in Vratza town; among village residents, the period-specific rates were 1.7 and 0.8 per 1000 per year, respectively (rate ratio 0.47; $p<0.01$). These trends were consistent across all villages for which registers were maintained.
However, the study also demonstrates under-recording of BEN cases and less complete case identification, especially after 1979. Migration of population might also have contributed to an apparent decline in registered cases. We recommended a rigorous monitoring of BEN in all afflicted countries, before concluding that the incidence of BEN is decreasing.

ASSOCIATION STUDY OF 3Q MICROSATELLITE LOCI IN BULGARIAN PATIENTS WITH BALKAN ENDEMIC NEPHROPATHY

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Background: Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial kidney disease characterized by development of urinary tract tumours in about 38-40% of BEN patients. The etiology of the disease has not been fully understood yet. A hypothesis for genetic predisposition to BEN has been accepted. The aim of the study was to investigate the association of BEN with 3q polymorphic markers based on our previous cytogenetic data showing 3q24-3q26.3 region to be frequently involved in chromosomal aberrations in blood samples from BEN patients.

Methods: We analyzed 55 BEN patients and 55 controls by PCR-PAGE. An association study of the allele frequencies in four microsatellite loci (ACPP, D3S1282, D3S1509, D3S1212) at chromosome region 3q22.1-3q26.2 in Bulgarian BEN patients as well as in healthy individuals (control group) was carried out.
Results: Significant differences in the frequencies of alleles, respectively C4 (144bp) in ACPP locus (χ^2=9.39; p=0.002), a2 (184bp) in D3S1509 (χ^2=6.26; p=0.010) and A6 (148bp) in D3S1282 (χ^2=9.30; p=0.002) between both studied groups were observed.
Conclusions: Alleles C4 (ACPP) and A6 (D3S1282) could be assumed to be positively associated with BEN, whereas a2 (D3S1509) - negatively. The results support our previous cytogenetic data on the significance of 3q24-3q26.3 region for BEN. The data from the present study provide a good basis for more detailed molecular analysis of this chromosome region.

MOLECULAR GENETIC STUDIES OF BEN TUMORS

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Background: Balkan endemic nephropathy is spread in certain regions of the Balkan Peninsula. The patients are predisposed to epithelial cell tumors of the urinary tract. These tumors have not been genetically investigated so far.
Methods: We studied the loss of heterozygosity (LOH) in 3 BEN associated tumors at 7 microsatellite loci at 3q21.3 - 3q27.3. Comparative genomic hybridization (CGH) was also performed on these tumors, one of which was in addition investigated by 24 color FISH.
Results: LOH in locus D3S1299 (3q24) was established in one case. The results of CGH revealed genetic gains at 1q, 3q, 7p, 7q, 15q, and 19q in at least two of the three tumors analyzed. Genetic loss was found in one case at 4q. The 24 color FISH revealed extremely complex chromosomal rearrangements. Most frequent aberrations were der(X), der(X)t(X;18), der(16), der(3)t(3;15) and der(12).
Conclusion: The LOH supposes the presence of a new, so far not reported tumor-suppressor gene at 3q24. In pTa BEN tumor extremely high genome instability was revealed by CGH. Frequent rearrangements of chromosome X could be somehow associated with the female predominance. Chromosome 3 anomalies support our previous data on 3q24 - 3q26.3 association with BEN.

GENOTYPING OF CYP2D6 MUTANT ALLELES IN BEN PATIENTS

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Background: The concept of multifactorial etiology of BEN anticipates that a combination of polymorphic gene variants with various environmental factors may result in an increased risk for the disease. CYP2D6 variants with complete deficiency or ultra-high enzyme activity have been associated with cancerogenesis. We suppose that they may predispose to uroepithelial cancer development in BEN patients. The aim of the study was to investigate the frequency of different CYP2D6 polimorphic variants among BEN patients and controls (case-control study) in an attempt to clarify the role of CYP2D6 polymorphisms in urinary tract tumor predisposition in BEN.
Methods: We determined the frequency of CYP2D6 alleles *3, *4 and *5 in 55 Bulgarian BEN patients and in 70 healthy individuals (control group) by allele-specific PCR. Multiduplicated CYP2D6 alleles were determined in 87 BEN patients and in 109 healthy individuals by long PCR. All statistical analyses were done with Chi-Square Test available on (http:// quantrm2.psy.ohio-state.edu/kris/chisq/chisq.htm)

Results: Our results showed that the frequency of homozygotes for mutant alleles (*3, *4, *5), responsible for poor metabolisers phenotype (PM), was 5.45% in BEN patients versus 10% in the healthy group. Significant differences were found between the frequency of heterozygotes in the BEN group (12.73%) and in the healthy persons (31.43%)($p<0.01$). The frequency of homozygotes for the wild type allele was significantly higher in the group of BEN patients (81.82%) compared to the controls (58.57%)($p<0.01$). Our data for the frequency of homozygous carriers of these mutant alleles in the control group corroborate previous data for PM phenotype in Caucasians (7-10%). Our findings show that duplication of CYP2D6, causing ultrarapid metabolise of debrisoquine occurred in 4.6% of BEN patients and in 6.42% of the controls.
Conclusions: Different CYP2D6 allele distribution in the group of BEN patients from healthy individuals may suggest that individual polymorphism in this xenobiotic –metabolising enzyme system may be used as possible marker for BEN susceptibility.

ASSOCIATION OF COMMON GENETIC VARIANTS OF XENOBIOTIC METABOLIZING ENZYMES (CYPS AND GSTS) WITH BALKAN ENDEMIC NEPHROPATHY: A STUDY IN THE VRATZA'S DISTRICT OF BULGARIA

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The xenobiotic metabolizing enzymes are classified as phase I activating enzymes (e.g., cytochrome P450, CYP) and phase II detoxifying enzymes (e.g., glutathione S-transferases, GST). It has recently become clear that many of their genes are polymorphic and can have profound effects on increasing or reducing the metabolic capabilities of the enzymes. It has been hypothesized that environmental toxicants (mycotoxins, PAHs, etc.) damage renal and urothelial cells in genetically susceptible individuals, which might be the cause of BEN. To evaluate this hypothesis, we launched a case-control study utilizing PCR-based methods for genotyping of polymorphisms in common variants of xenobiotic metabolizing enzymes: CYP1A1, CYP1A2, CYP3A4, CYP2E1, CYP2C9, CYP2D6, CYP17, CYP19, GSTM1, GSTT1, GSTP1, NAT1, and NAT2. Genomic DNA was extracted from white blood cells of 64 unrelated patients with BEN and 104 age- and sex-matched controls from the region of Vratza, Bulgaria. Differences in the proportion of genotypes between the two groups were tested with χ^2-test (for 3×2 contingency tables) and Fisher's exact test (two-tailed) for pairwise comparisons (SPSS 10.0). The significance level was set at $p<0.05$. Selected results at this stage of our study include: (1) No significant differences for CYP1A2, CYP2D6*4, GSTT1, and GSTP1 between the two groups were observed, though the differences for some of them (e.g., CYP1A2) approached significance level. (2) The comparison for GSTM1 revealed significantly lower frequency of GSTM1 del/del genotype in the BEN group ($p=0.003$). (3) Homo- and heterozygous carriers of the common CYP1A2 allele, who simultaneously carry the GSTM1 del/del genotype occurred less frequently among the BEN-patients ($\chi^2=7.64$; d.f.=1; $p<0.01$).

GENETIC POLYMORPHISMS OF XENOBIOTIC ENZYMES AND TRANSPORTER PROTEINS IN BULGARIAN PATIENTS WITH BALKAN ENDEMIC NEPHROPATHY

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Background: Many environmental factors and mycotoxins are thought to be involved in the etiology of BEN and may also be related to the frequent development of uroepithelial tumors in BEN patients. Cytochrome P450 and N-acetyltransferases (NATs) play an important role in the activation of many procarcinogenes or chemicals. The multidrug resistance gene (MDR) is associated with multixenobiotic resistance, and its product P-glycoprotein (Pgp) transports

xenobiotics from mesangial and tubule cells in the kidney. Genetic variants in xenobiotic metabolising enzymes and transporters might be responsible for a higher susceptibility of BEN patients to exogenous factors.
Methods: We determined the following polymorphisms in Cyp3A4, Cyp3A5, Nat1, Nat2 and MDR genes in 96 **Bulgarian** BEN patients and 112 healthy volunteers (as a control group): Cyp3A4(A -290G), Cyp3A5 (A6986G; G14690A; 5H30Y; K34X; T398N), Nat2 (T341C; C282T), Nat1 (C1095A; T1088A; C559T; C560A; T640G) and MDR (C3435T; S2677T). The polymorphic sites were detected using cycle amplification with allele-specific probes and melting curve analyses. To increase the throughput of genotyping, probes were designed for temperature multiplexing where possible. For the first time the frequency of different alleles in xenobiotic metabolising enzymes and MDR gene in patients with BEN and in a healthy Bulgarian population was established.
Results: The study of the polymorphisms showed significant prevalence of homozygous rapid NAT2 acetylators in the control group in relation to BEN patient group ($p<0.05$). Significant differences were observed also between BEN patients and healthy controls for C3435T in MDR gene - higher frequencies of heterozygous individuals (0.625) in BEN patients than in the healthy population (0.420)($p<0.05$).
Conclusions: The results suppose that genetic variants in NAT2 and MDR could be involved in the genetic background of Balkan Endemic Nephropathy.

HIGH-THROUGHPUT TISSUE MICROARRAY TECHNOLOGY FOR MOLECULAR PROFILING OF A LARGE NUMBER OF BLADDER CANCER

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Background: Tissue microarray (TMA) is a new technology providing a unique opportunity for hundreds of tissue samples to be brought into one recipient paraffin block, sections of which can be further used for all kinds of in situ analysis – hematoxylin/eosin staining (H&E), immunohistochemistry and FISH. By this approach rapid screening for molecular alterations in a huge number of malignancies simultaneously is facilitated. 11q13 is one of the candidate regions revealed by CGH to be frequently amplified in bladder cancer. The best candidate oncogene located in the region is Cyclin D1 (CCND1). CCND1 with its cyclin dependent kinase is involved in the phosphorylation of pRB and in case of amplification drives the cell to tumor development.
Methods: A TMA technology was used to evaluate the importance of cyclin D1 in a preexisting TMA (Institute of Pathology, University of Basel, Switzerland) containing 2317 bladder tumor samples from 1853 patients by FISH using combined gene specific and 11 centromere probe. The samples included 277 pTaG1, 567 pTaG2, 107 pTaG3, 206 pT1G2, 309 pT1G3, 186 pT2-4G2, and 551 pT2-4G3 TCC bladder tumors.
Results: FISH was successful in 1188 cases (64,1%). Amplifications for CCND1 were found in 128 tumors (10.77%) while genetic gains were observed in 131 (11,03%). Both amplifications and gains increased significantly from pTa to pT1-4 and from G1 to G3. Amplification was 6,9% in pTa and 14,5% in pT1-4. Gains ranged from 5,0% in pTa to 16,1% in pT1-4. Both gains and amplifications were significantly related to patient survival with the tumors of all stages ($p<0.0001$) but not in the subgroup of pT2-4 carcinomas alone ($p=0.5139$). The copy number changes were not associated with recurrence in pTa carcinomas ($p=0.4376$) whereas they were associated with progression in pT1 tumors ($p=0.0010$).
Conclusion: Cyclin D1 is a bad prognostic factor for survival of the patients with tumors of all stages and for progression of pT1 bladder cancer tumors. TMA allowed us to evaluate the phenotype-genotype correlations and we consider it suitable for similar studies of tumors associated with Balkan Endemic Nephropathy.

FISH STUDY OF 11Q13 AMPLIFICATION ON TISSUE MICROARRAY (TMA) OF BEN TUMORS

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Background: 11q13 amplification has been observed in numerous tumors including transitional cell carcinoma of the urinary bladder. It is assumed that the amplified DNA includes a critical gene (or genes) whose overexpression drives tumor development. At 11q13 are located cyclin D1 (CCDN1), fibroblast growth factor 3 and 4 (FGF3, FgF4) and EMS1. Cyclin D1 together with its cyclin dependent kinase (cdk) partner is responsible for transition of G1 to the S phase of the cell cycle by phosphorylating inactivate pRB, which then releases transcription factors important in the initiation of DNA replication. FGF3 and FGF4 are members of the fibroblast growth factors gene family. EMS1 protein has shown high identity with chicken cortactin. Cortactin contains (i) a filamentous actin binding tandem repeat domain, (ii) a proline-rich SH3-binding and (iii) a SH3 domain that is common in proteins involved in signal transduction. It has a function in signal transmission between cell contact sites and the cytoskeleton.
Methods: A tissue microarray (TMA) of 207 urinary tract tumor samples from Bulgarian patients was constructed. Three of the tumors were associated with Balkan Endemic Nephropathy (BEN) and 204 were from patients living in non-endemic regions. There were 168 uroepithelial tumors of the bladder, 31 of the kidney pyelon, and 6 – of the ureter. According histology the TMA contained 183 transitional cell carcinomas (TCC), 12 TCC with squamous cell carcinoma, 2 TCC with adenocarcinoma, 6 squamous cell carcinomas, 2 renal cell carcinomas, 1 rhabdomyosarcoma and 1 fibrosarcoma. The series contained 10 pTaG1, 1 pTaG2, 60 pT1G1, 50 pT1G2, 2 pT1G3, 9 pT2G1, 2 pT3G1, 41 pT2-4G2, 22 pT2-4G3 TCC, and 4 pT2-4G4 TCC. The BEN tumors were classified as follows: BEN tumor 1 - TCC, pT3, grade 3; BEN tumor 2 - TCC, pTa, grade 2; BEN tumor 3 - TCC, pT3, grade 2-3. Of the 207 patients, 163 were males and 44 females. The array was studied by FISH for chromosome 11q13 amplification using 4 different DNA probes - CCND1, FGF3/FGF4, FGF3 and EMS1. All probes were applied in combination with alternatively labeled chromosome 11 centromeric probe.
Results: FISH was successful for CCND1 in 154 (75,5%), for FGF3/FGF4 in 119 (58,4%), for FGF3 in 113 (55,4%), and for EMS1 in 144 tumors (71,6%). All 4 genes were successfully analysed in 106 tumors. 3 of these tumors (2,83%) had the 4 genes amplified. 3 tumors (2,83%) had amplifications for CCND1, FGF3/FGF4 and FGF3 together (2 of which had gain for EMS1 and 1 was normal for EMS1). No tumor had amplification of a single gene. 8 of the tumors had gain for all 4 genes (7,5%), 3 tumors (2,83%) had gain only for CCND1. 2 tumors (1,9%) had gain only for CCND1 and FGF4 and 1 tumor had gain for all 3 genes without CCND1.
Interestingly, two of the three BEN tumors studied had genetic gain at 11q13, the third did not react.
Conclusion: For the first time TMA from Bulgarian patients with urinary tract tumors was constructed. The results revealed coamplification of CCND1, FGF4, FGF3 and EMS1 on 11q13. The BEN tumors showed gain for the 4 genes while the frequency of these gains in the tumors from non-endemic regions was lower (only 7.5%).

EVALUATION OF COAL LEACHATE CONTAMINATION OF WATER SUPPLIES AS A HYPOTHESIS FOR THE OCCURRENCE OF BALKAN ENDEMIC NEPHROPATHY IN BULGARIA

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It has been suggested that coal-related materials can contaminate groundwater with toxic organic chemicals such as polynuclear aromatic hydrocarbons (PAHs). PAHs are known to have high levels of carcinogenic and mutagenic properties; because of this the possibility of their presence in water supplies has long been a concern in drinking water regulation. It has been hypothesized that long-term exposure to PAHs and other organic chemicals present in water supplies in rural regions of Bulgaria might be the cause of Balkan Endemic Nephropathy (BEN), a rare kidney disease that is often fatal to those who contract it.

To evaluate this hypothesis, we developed a screening method that could be used in remote locations for the presence of coal-derived compounds in drinking water at the lowest possible detection limits. The method employs solid phase extraction (SPE) technology, followed by HPLC separation and fluorescence and ultraviolet detection of organic chemicals suspected to have leached from coal. The method is capable of screening water contaminated by coal leachate for the 16 EPA priority PAHs at detection limits at or below currently accepted methodologies. This method was verified by leaching PAHs and other organic compounds from coal samples of different geologic origins. All but one of the 16 EPA priority PAHs were found to leach from the samples into water. Numerous other compounds were detected in the leachate samples.
Utilizing this method, water supplies were sampled in 13 endemic villages and 14 non-endemic villages, located in two different districts in Bulgaria. Samples included regional water distribution systems and local springs and wells. Numerous chromatographic peaks were detected in nearly all of the samples analyzed. No statistically significant (95% confidence) differences between BEN and non-BEN samples were observed. Because HPLC alone does not provide for compound identification, the presence of coal-derived compounds was neither confirmed nor rejected by this result. However, the absence of a clear difference between samples from the two environments in a test capable of detecting leachate compounds, does raise questions as to the strength of this hypothesis.

OCHRATOXIN A CONCENTRATIONS IN FOOD AND FEED FROM A REGION WITH BALKAN ENDEMIC NEPHROPATHY

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Balkan endemic nephropathy (BEN), a chronic renal disease of unknown etiology, is found in geographically near-by areas of Bulgaria, Romania and Serbia. Ochratoxin A (OTA), a secondary metabolite of *Aspergillus* and *Penicillum* species and a natural contaminant of food and feed, is a putative cause of BEN. Some studies have found a geographic covariation between OTA content in food/feed and BEN manifestation; others have not. In May 2000, using a competitive direct ELISA assay for OTA (sensitivity 1 pg/kg), we investigated OTA contamination in 165 samples of home-produced food (beans, potatoes, corn, wheat, flour) and feed from households in villages from the BEN region (Vratza District) of northwestern Bulgaria. Samples were collected from: a) BEN villages (8), and therein from BEN households (20) and BEN-free households (16) (within-village controls, WVC); b) BEN-free villages (7) and therein BEN-free households (22) (between-village controls, BVC). BEN households consistently had a higher proportion of OTA-positive samples than WVC households, but similar (for some foods) or lower (for other foods) proportions to BVC households. The proportion of OTA-positive samples was also higher in BVC than in WVC households. Furthermore, BEN households had a similar proportion of OTA-positive samples to the pooled, WVC and BVC, group of households. OTA-exposure estimates, derived from our OTA-concentration findings and the reported average per capita monthly consumption of basic foods in rural Bulgaria, showed the highest OTA intake in BEN households (1.21 μg/day), versus 1.03 μg/day in BVC and 0.71 μg/day in WVC households. These OTA intakes are higher than those in the European Union, and are close to the upper limits acceptable to several food-safety organizations. Our results indicate that OTA may not alone cause BEN; only synergistically with other environmental toxicants and/or predisposing genotypes may it do so.

STUDIES OF THE HOST IMMUNE SYSTEM IN PATIENTS WITH BEN

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Background: Immunological studies based on the measurement of the immunoglobulin serum levels (1975-83), complement components determination (1976-1997) and some investigations on cellular immunity functions (1977-83) failed to detect any significant changes.
Methods: Lymphocytic populations were characterized through Flow cytometric analysis by using a set of monoclonal antibodies (MoAbs) vs. CD3/HLA-DR, CD3/CD4, CD3/CD8, CD3/CD19, CD19, CD57/CD8, CD3/CD16+CD56. Lymphocyte (Ly) gate was set by physical parameter FSC/SSC and by using the combination of CD45-F/CD14-PE. The isotypic control was applied to put the fluorescence markers. The probes were collected by the flow cytometer FACS Calibur (Beckton Dickinson) and software CellQuest and analysed by the program Simulset. Test kit PHAGOTEST (Opregen Pharma, for the quantication of phagocytic activity of monocytes (Mo) and granulocytes (Gr) was applied. The overall percentage of Mo and Gr showing phagocytosis in general - ingestion of one or more fluorescein (FITC)-labelled opsonized bacteria (E. coli) per cell, were measured by flow cytometry. The measurements were carried out in the flow cytometer as pointed above using the blue-green excitation light (488 nm argon-ion laser) and CellQuest software.
Results: Analysis of Ly population data in the 32 BEN patients (a mean age of 64.1) and 60 healthy persons (mean age of 59.2±7.9 years) demonstrated an insignificant decrease of the total T Ly fraction (%) in contrast to the substantial diminishment of the absolute Ly number. At the same time, an increase of both activated Ly percentage and immune potential were recorded. Moreover, a marked diminishment of T suppressor/cytotoxic cells and an insignificant increase of the part of total NK cells were registered. A more detailed view on these immunotyping data rearranged the BEN patients in three groups, as follows: a. persons with increased NK cells as compared to the control group (n = 12); b. persons with an increase in the activated T Ly (n = 9); c. the rest of the patients with an insignificant increase of B Ly (n = 11). A decreased phagocytic activity of Mo and Gr was found in BEN 22 patients out of the total number of 32 tested.
Conclusion: The data obtained demonstrated significant changes in the Ly population phenotype of the patients with BEN. Moreover, a decreased phagocytic activity of Mo and Gr was recorded in the majority of them.

NEOPTERIN LEVELS IN THE URINE OF BEN PATIENTS

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Background: The monitoring of neopterin concentrations in body fluids is a sensitive way to detect Th-1 type immune response initiated by various causes. Measurements of neopterin concentrations in urine, serum and cerebrospinal fluid are employed as a laboratory diagnostic tool, e.g. to earlier detect immunologic complications in transplant recipients, to predict prognosis in HIV infection and malignancies or to mark the onset of antibody seroconversion following an acute viral infection.
Methods: In urine samples the concentration of the low molecular weight component neopterin is compared to creatinine and the results are given as a N/C ratio (μmol neopterin/mol creatinine). The neopterin concentration in urine samples (100 μl) was determined by means of HPLC in the reversed phase by fluorescence measurement (maximum excitation at 353 nm, maximum emission at 438 nm) and creatinine was simultaneously quantified by UV adsorption measurement at 235 nm.
Results: Neopterin concentrations were determined in the individual urine samples from 48 patients with BEN or suspected for BEN living in the endemic area. Data obtained demonstrated markedly increased N/C ratio values in 50% exactly of the urine samples from the total number of 48 BEN patients, as compared to the normal ranges for the urinary neopterin concentration. The mean N/C ration value of 263.1 was evaluated, exceeding all normal N/C ratio values established for different ages both for men and women. Higher neopterin values were recorded in BEN patients aged over 65 as compared to the 56-65 year group, 285.4 and 242.6, respectively.
Conclusion: In the present study urine samples from BEN patients showed elevated neopterin concentrations compared to healthy controls. The result of the investigation correlates well with rate and progression of the BEN tested.