Immunization with HCs induced activation of proliferating and cytotoxic T cells and significantly retarded tumor growth, also confirmed by upregulated expression of distinct cytokines genes. The same observations accented by vaccination with HCs in the tumor bearing host. Finally, when T cells from HCs vaccinated mice were transferred into naive tumor-bearing mice, tumor growth was most strongly retarded and an efficient proliferative and cytotoxic T cell response was observed. Tumor growth was reduced by over 50%, and tumor development was significantly delayed.
Taken together, we demonstrate that HCs offer for an effective immunotherapy of poorly immunogenic carcinomas. This is independent of whether the HCs are taken for adoptive transfer or as a vaccine.

542 POSTER

Combination of hybrid-primed lymphocytes and hybrid vaccination prevent tumor growth of Lewis Lung Carcinoma in mice

R. Savai[1], R. Schermuly[2], M. Schneider[1], S. Pullamsetti[2], S. Greschus[3], F. Grimminger[2], W. Seeger[2], G. Banat[1]. *[1]Justus-Liebig-University, Department of Hematology & Oncology, Giessen, Germany; [2]Justus-Liebig-University, Department of Internal Medicine II, Giessen, Germany; [3]Justus-Liebig-University, Department of Neuroradiology, Giessen, Germany*

Immunotherapy with tumor cell-dendritic cell fusion hybrids has been shown to induce immune response against multiple tumor antigens including unknown tumor antigens. The aim of this study was to explore the possibility of optimizing the host protective anti tumor immunity by combined immunization strategies of tumor cell-dendritic cell fusion hybrids. Further, the effects of combined immunization strategies on tumors were evaluated by flat-panel volumetric Computer tomography (fpvCT) and immunohistochemical (IHC) analysis. As previously shown fusion of C57BL/6 mice bone marrow derived dendritic cells with Lewis Lung Carcinoma (LLC1) cells were effective against poorly immunogenic carcinomas with all three potential tumor-therapeutic strategies applied: protective immunization, vaccination and adoptive cellular therapy.
Interestingly, in this study combination of hybrid-primed lymphocytes and hybrid vaccination induced activation of proliferating and cytotoxic T cells and significantly retardation tumor growth (85%). In addition, a significant delay in tumor development, a reduction in the number of pulmonary metastases and survival times were observed. Further, the tumor bearing mice treated with hybrids displayed significant morphological changes of apoptosis compared to LLC1 and dendritic cell treated groups shown by IHC analysis and Tunel assay. An increased CD3 expression was also observed in these hybrid treated tumors, which was accompanied by strong involvement of tumor infiltrating T cells.
These findings were underlined by clearly increased spleen size compared to other treatment regimens. Thereby, these results demonstrate that the combination therapy of fusion hybrids is an effective immunotherapeutic regimen against poorly immunogenic carcinomas.

543 POSTER

Expression of survivin, a novel inhibitor of apoptosis, in advanced rectal cancer with preoperative chemoradiotherapy

Y.J. Choi[1], Y.T. Kim[2], E.J. Chung[3], S.M. Park[4], J.G. Kang[5]. *[1]National Health Insurance Corporation Ilsan Hospital, Pathology, Koyang, Korea; [2]National Health Insurance Corporation Ilsan Hospital, Internal Medicine, Koyang, Korea; [3]National Health Insurance Corporation Ilsan Hospital, Radiation Oncology, Koyang, Korea; [4]National Health Insurance Corporation Ilsan Hospital, Diagnostic Radiology, Koyang, Korea; [5]National Health Insurance Corporation Ilsan Hospital, Surgery, Koyang, Korea*

Background: Survivin is a recently described member of the family of inhibitor of apoptosis protein. We investigated the association of survivin expression with prognosis and other apoptosis-related biological factors in advanced rectal cancer with preoperative chemoradiotherapy.
Material and methods: We examined 16 patients with rectal cancer, who were preoperatively staged as at least T3 or T4 (determined by MRI). Enrolled patients were given by 5-FU 425 mg/m^2/day and leucovorin 20 mg/m^2/day intravenously for 3 days during weeks 1 and 5 of pelvic radiotherapy (45 Gy). Surgical resection was performed 4–6 weeks after completion of the scheduled treatment and the patients were followed for up to 55 months after operation. Tumor response was divided as CR (complete response), PR (partial response; over 50% diminution of tumor volume) and NR (no/minimal response). Immunohistochemical staining of paraffin sections using monoclonal antibodies for survivin, bcl-2, p53 and ki-67 was performed on pretreatment biopsy and surgically resected tissues.
Results: No CR was achieved. PR was obtained in 10 patients (62.5%) and NR in 6 patients (37.5%). Survivin expression was found in cytoplasm or nucleus of tumor cells but not in nonneoplastic cells on pretreatment biopsy. After preoperative treatment, survivin expression tended to be decreased in tumor cells (62.5% to 31.3%) and slightly increased in adjacent normal mucosa. The NR cases showed high survivin expression on pretreatment biopsy (5/6). Survivin positivity on pretreatment biopsy showed the tendency of low apoptotic index and low median time to progression. But we failed to find any significant relationship between survivin expression and any of the parameters examined.
Conclusions: In this study, the immunohistochemical assessment of survivin status does not seem to be helpful in the prognostic characterization of rectal cancer. Further studies including more cases with sufficient follow-up period are needed in order to provide survivin as a prognostic and therapeutic target in rectal cancer.

Publication
Cytokines/immunobiology/immunotherapy

544 PUBLICATION

Tumor-associated antigens in rheumatoid arthritis

E. Szekanecz[1], P. Antal-Szalmás[2], Z. Sándor[3], Z. Szekanecz[4]. *[1]Univ. of Debrecen Medical and Health Science Cente, Oncology, Debrecen, Hungary; [2]D, .Department of Clinical Biochemistry and Molecular Pathology; [3]D, .Department of Pathology and Division of Rheumatology; [4]Third Department of Medicine, Division of Rheumatology, University of Debrecen Medical Center, Debrecen, Hungary*

Background: There have been scattered reports, that some tumor-associated antigens (TAA) may, apart from cancer cells, become expressed ont he surface of inflammatory cells. Carcinoembryonic antigen (CEA) is present mostly on colorectal and gastric carcinomas, CA 15–3 on breast carcinoma, CA 19–9 on pancreatic carcinoma, CA 125 on ovarian carcinoma, CA 72–4 on gastric and mucinous ovarian carcinoma and neuron-specific enolase (NSE) on small-cell lung carcinoma and neuroblastoma. However, recent studies revealed that soluble carcinoembryonic antigen (CEA), as well as CA 19–9, CA 125 and CA 15–3 TAAs may be detected in the sera or on synovial cells of patients with rheumatoid arthritis (RA), as well as in the sera of patients with scleroderma, lupus and Sjögren's syndrome.
Objectives: In this study, we assessed levels of various TAAs in the sera of RA patients and healthy subjects. Serum TAA levels were correlated with markers of disease activity.
Methods: TAAs including CEA, CA 15–3, CA 72–4, CA 125, CA 19–9 and NSE were assessed by ELISA in the sera of 78 patients with established, treated RA (disease duration >2 years) and 50 age- and sex-matched healthy controls. Normal upper limits for these TAAs were 3.4 μg/l, 25 kU/l, 6.9 kU/l, 35 kU/l, 34 kU/l and 16.3 μg/l, respectively. TAA concentrations were correlated with serum rheumatoid factor (RF; <50 U/ml), anti-CCP (<25 U/ml) and CRP (<5 mg/l). DAS28 indicating clinical disease activity was also assessed.
Results: There were more RA patients showing abnormally high levels of TAAs in comparison to controls (CEA: 12.8% vs 6%; CA 125: 11.5% vs 4%; CA 19–9: 7.7% vs 6%; CA 15–3: 15.4% vs 4%; CA 72–4: 3.8% vs 0%; NSE: 20.1% vs 8%). Significant differences were found in the case of CEA, CA 125, CA 15–3, CA 72–4 and NSE ($p < 0.05$). Among RA patients, serum NSE levels showed significant correlation with CRP ($r = 0.42$, $p < 0.05$), as well as anti-CCP levels ($r = 0.62$, $p < 0.05$). None of the assessed TAAs showed any correlation with DAS28.
Conclusion: The concentration of some TAAs may be elevated in the sera of patients with established RA in comparison to healthy subjects. Furthermore, some TAAs, such as NSE, may also correlate with laboratory markers of RA.

545 PUBLICATION

Investigation of TNF-alpha activity on new cell line from patients with myelodisplastic syndroma

V. Jurisic[1], G. Bogdanovic[2], V. Kojic[2], J. Mrdjanovic[2], D. Jakimov[2], N. Kraguljac. *[1]School of Medicine, University of Kragujevac, Physiopathology, Kragujevac, Serbia; [2]Institute of Oncology, Exp Laboratory, Novi Sad, Serbia; [3]Institute of Hematology, Flow cytometry laboratory, Belgrade, Serbia*

TNF-alpa is a pleiotropic cytokine which can induce apoptosis is sensitive cells, but also regulated cell proliferation, cellular activation and differentiation. To be better estimated TNF-alpha effects on new establisched cell line, entitled PC, originally developed from patients with myelodisplastic syndrome at Institute of Oncology Sremska, Kamenica, Novi Sad. In this research we monitored the kinetics of changes after in

vitro treatment with or without TNF-alpha in presence anti-CD45 and CD95 MoAb, FLT3, IL-3 and GM-CSF.
Cell viability were analyzed by cell enumeration; intracellular metabolic activity by determination of total LDH activity after sonification, cell proliferation by 3H thymidine incorporation into DNA, cell membrane molecule expression, apoptosis and necrosis using flow cytometry (Becton Dickinson) on gated cell population. Analyses were performed 2, 6, 8 and 24 h after treatment under some experimental conditions.
Our results showed that in comparison with untreated cells, TNF-alpha induced significantly increase in apoptosis and necrosis, in PC cells, which expressed high level of CD95 and TNF alpha receptors. Pretreatment of PC cell with anti-CD45 and anti CD95 monoclonal antibodies modulated cell death induced by TNF. In addition, presence of TNF in cell culture medium induced significantly decrease in cell proliferation, stimulated by IL-3, FLT3, GM-CSF, TNF-alpha, or its combination. However, no changes in CD13 and CD33 antigen expression following cell proliferation, determined after 4 days stimulation with cytokine combination in comparison to percentage expression before treatment. No changes in intracellular LDH activity before and after cell proliferation induced with different cytokines.
We conclude that sensitivity to apoptosis limited cell proliferation estimated on this cell line.

546 PUBLICATION

The immunoreactivity of serum immunoglobulins with gliadin in patients with myeloma multiplex and non-Hodgkin's lymphoma

A. Konic-Ristic[1], Z. Juranic[2], I. Besu[2], S. Jelic[2], N. Milanovic[2], M. Marinkovic[2], D. Milosevic[2], I. Stankovic[1], N. Stanojevic-Bakic[2].
[1]Faculty of Pharmacy, Insitute of Bromatology, Belgrade, Serbia and Montenegro; [2]Institute for Oncology and Radiology of Serbia, Belgrade, Serbia and Montenegro

Background: Gluten intolerance is system immunological disorder which is characterized in part by the presence of antigliadin antibodies, which sometimes are also directed to calreticulin. The results from previous work showed high intensity of the interaction of serum IgA with gliadin in two patients with IgA plasmacytoma (only in patients with IgA, M component in the serum). Antigliadin IgA immunoreactivity was also found in 1out of 4 patients with IgG plasmacytoma and in 1 patient with non-Hodgkin's lymphoma. Patient with two M components, showed IgA immunoreactivity with the blocker, bovine albumin, but not with gliadin.
Therefore, the aim of this work was to determine is there any immunoreactivity of serum immunoglobulins with gliadin, in group of patients with myeloma multiplex, non-Hodgkin's lymphoma and in healthy controls.
Patients, material and methods: Six patients with IgA plasmacytoma, 10 patients with IgG plasmacytoma, 8 patients with non-Hodgkin's lymphoma and 16 healthy people were included in the study. For determination of the level of the immunoreactivity of antigliadin IgA or IgG antibodies two ELISA tests were used: a home made ELISA test with 5 micrograms of crude gliadin (SIGMA) as the antigen, while 1% bovine serum albumin was used as blocker, and commercial ELISA test (Binding Site). The absorbance of sample was divided by absorbance of positive control serum and multiplied by 100, providing arbitrary units, in the aim of standardization of the results. The cut off values, calculated as Xav+2SD of arbitrary units for healthy controls were 14.31 for IgA reactivity and 18.88 for IgG reactivity.
Results: The antigliadin IgA immunoreactivity was higher then cut off value for 3 of 6 patients with IgA plasmacytoma (27.4, 63.6, 72.5), 2 of 8 patients with non-Hodgkin's lymphoma (16.4, 20.9) and less then cut off for all patients with IgG plasmacytoma. Antigliadin IgG immunoreactivity was higher then cut off for 2 of 6 patients with IgA plasmacytoma (26.4, 19.4), for 2 of 10 patients with IgG plasmacytoma (26.4, 22) and 4 of 8 patients with non-Hodgkin's lymphoma (22.3, 21.2, 92.5, 23.9). Two patients with IgA plasmacytoma showed high IgA reactivity to BSA.
Conclusion: These preliminary results are the first showing antigliadin IgA and IgG immunoreactivity in patients with IgA and IgG plasmacytoma and non-Hodgkin's lymphoma; they set up a question on the importance of gluten intolerance in the emergence and development of myeloma multiplex.

Epidemiology, Prevention and Public Health

Oral presentations (Wed, 2 Nov, 9.15–11.15) Epidemiology

547 ORAL

Long-term risk of non-germ cell malignancies in 5-year survivors of testicular cancer

F.E. Van Leeuwen[1], A.W. Van den Belt-Dusebout[1], G.M. Ouwens[1], R. De Wit[2], J.A. Gietema[3], S. Horenblas[4], J.G. Ribot[5], W.J. Louwman[6], B.M.P. Aleman[7]. *[1]The Netherlands Cancer Institute, Department of Epidemiology, Amsterdam, The Netherlands; [2]Erasmus MC-Daniel den Hoed Cancer Center, Rotterdam, Department of Medical Oncology, Rotterdam, The Netherlands; [3]University Medical Center Groningen, Department of Medical Oncology, Groningen, The Netherlands; [4]The Netherlands Cancer Institute, Department of Urology, Amsterdam, The Netherlands; [5]Catharina Hospital, Eindhoven, Department of Radiotherapy, Eindhoven, The Netherlands; [6]Eindhoven Cancer Registry, Comprehensive Cancer Center South, Cancer Registry, Eindhoven, The Netherlands; [7]The Netherlands Cancer Institute, Department of Radiotherapy, Amsterdam, The Netherlands*

Background: To assess long-term risk of non-germ cell malignancies (NGCMs) in 5-year survivors of testicular cancer.
Patients and methods: We conducted a nation-wide cohort study comprising 2707 5-year survivors treated for testicular cancer between 1965 and 1995. Complete medical follow-up information until at least January 1, 2000 was available for 90% of all patients. The number of non germ-cell malignancies was compared with general population rates to assess relative risk (RR) and absolute excess risk (AER) of non-germ cell tumors.
Results: After a median follow-up of 16.3 years, 255 NGCMs were observed. The risk of NGCM overall was 1.7-fold (95%CI: 1.5–1.9) increased compared to the general population. Among survivors treated before age 30 the risk of any NGCM was even 3.9-fold (95%CI: 2.9–5.3) increased compared to the general population. When survivors grew older, the RR for NGCM overall decreased from 3.2 (95%CI: 2.4–4.1) for patients with attained age 50 or younger to 1.3 (95%CI: 1.0–1.7) for patients with attained age of 70 or older. There was an increase of RRs with longer follow-up time for all gastro-intestinal cancers combined, stomach cancer, urinary bladder cancer and especially prostate cancer, consistent with a radiation effect, whereas this time-trend was not found for all NGCMs combined. However, due to a rising background incidence of cancer with increasing age, the AERs for NGCM overall increased strongly with follow-up time till 25 years after testicular cancer diagnosis, with 86 excess cases per 10,000 person-years in the 20–25 year follow-up interval, and slightly decreased thereafter. NGCM risk overall was rather constant over treatment periods, whereas RRs of stomach, bladder and kidney cancer decreased with more recent treatment eras. Patients treated with chemotherapy alone had increased risks of urinary bladder cancer and melanoma (RRs of 5.0 (95%CI: 0.9–14.8) and 6.2 (95%CI: 2.0–14.7), respectively). Patients who received combined modality treatment had a 2.7-fold (95%CI: 1.9–3.9) increased risk of NGCM overall compared to the general population.
Conclusion: Survivors of testicular cancer, especially of nonseminomatous testicular cancer, were still at significantly elevated risk of developing NGCMs more than 20 years after testicular cancer diagnosis. Excess risks of NGCMs were mainly attributable to radiotherapy, but to a lesser extent also to chemotherapy.

548 ORAL

Second non-breast malignancies following breast cancer

Y. Kirova[1], L. Gambotti[2], J.R. Vilcoq[1], B. Asselain[2], F. Campana[1], R. Dendale[1], M. Bollet[1], A. Fourquet[1]. *[1]Institut Curie, Radiation Oncology, Paris, France; [2]Institut Curie, Biostatistics, Paris, France*

Background: The place of the adjuvant radiotherapy (RT) for breast cancer (BC) in the increasing the incidence of second primary cancers is debatable.
Purpose: To estimate the risk of second non-breast malignancies (SNBM) following radiotherapy (RT) for breast cancer in one institutional homogeneous cohort of patients.
Patients and methods: We reviewed the records of 16 705 patients (pts) with non-metastatic breast cancers treated at the Institut Curie between 1981 and 1997. Of them, 13 471 (81%) received radiotherapy and 3 234 (19%) did not. SNBM included all first cancers occurring after treatment of the primary breast cancer, except contalateral breast