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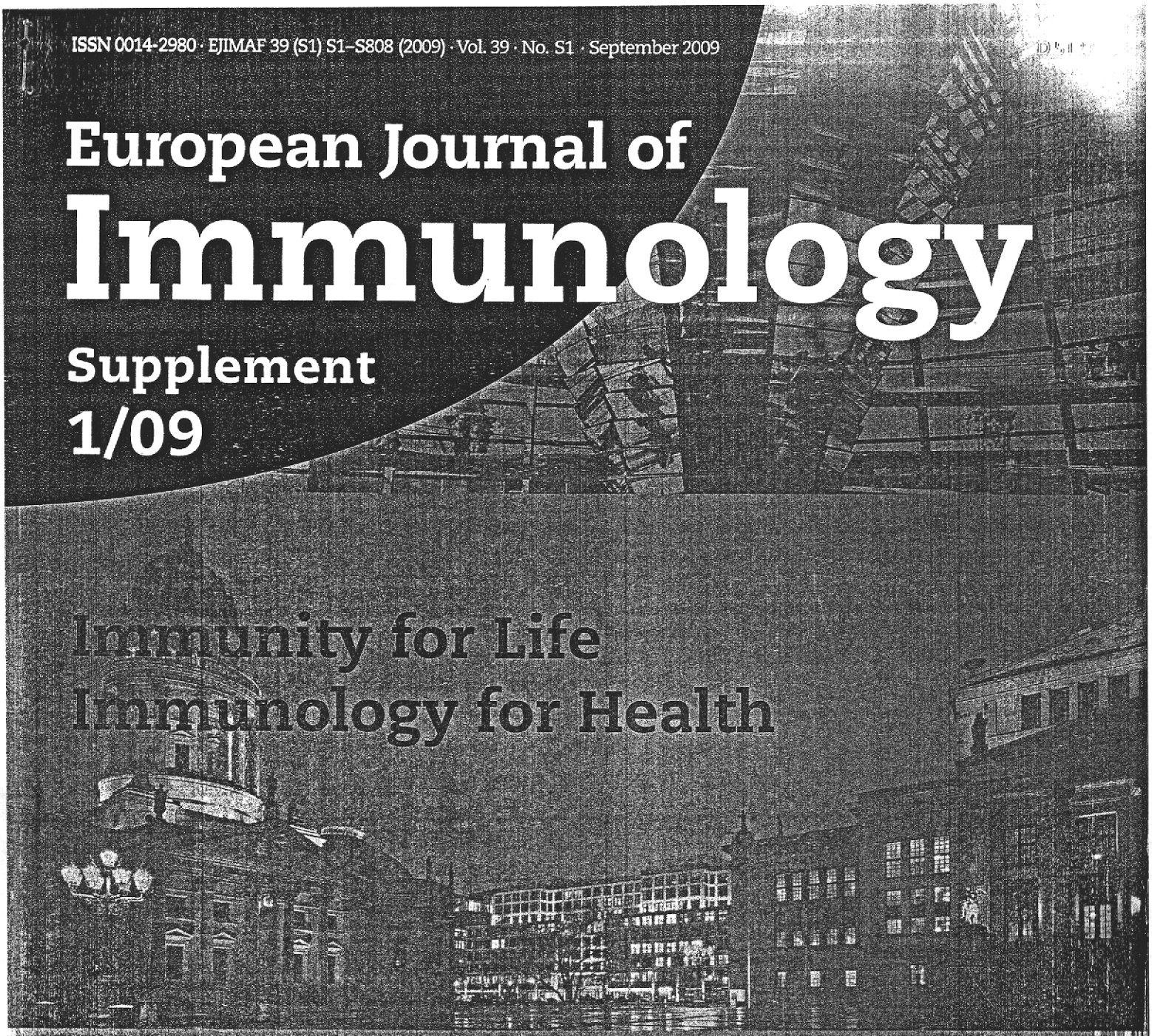

Immunity for Life
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Abstracts

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Methods: Immunoinflammatory T1D was induced in C57BL/6 male mice with streptozotocin (40 mg/kg, given i. p. for 5 consecutive days). To evaluate the impact on the disease development, DOLE was administered for 21 day as a continuous i.p. treatment (40 mg/kg/day). Infiltration of immune cells into pancreatic islets was analyzed on pancreatic tissue sections after staining with Mayer's hematoxilin. Blood glucose and body mass were monitored weekly, whereas *ex vivo* analyses by RT-PCR and ELISA of insulin and cytokine production in pancreas, spleen, pancreatic lymph nodes, peritoneal cells and serum were performed on day 15 of diabetes post-induction (p.i.). iNOS expression was detected by RT-PCR, whereas NO production was determined by Griess reaction.
Results: *In vivo* DOLE administration significantly reduced clinical signs of MLDS-induced diabetes (hyperglycemia and body weight loss) and led to complete suppression of histopathological changes in pancreatic islets. In line with these, insulin expression and release were restored in DOLE-treated mice. Protection from diabetes correlated with reduced expression and production of proinflammatory cytokines (IFN-gamma, TNF-alpha, IL-1beta, IL-6, IL-2), and up-regulation of anti-inflammatory cytokines (IL-10 and IL-4) in spleen, peritoneal cells and pancreatic lymph nodes. Interestingly, iNOS expression and NO production were significantly elevated in peripheral tissues, but were down-regulated within the local environment of endocrine pancreas.
Conclusion: DOLE interferes with autoimmune diabetes development by down-regulating production of proinflammatory and cytotoxic mediators and shifting towards protective Th2 response. DOLE may thus be considered as a potent therapeutic approach in the early stages of T1D.
Funded by Serbian Ministry of Science and Technological Development (grant No 143029).

PC11/18 EFFECTS OF DIETARY FACTORS ON CYTOKINES SECRETIONS OF 3T3-L1 CELLS DIFFERENTIATED FROM PREADIPOCTES TO ADIPOCYTES AND THEIR *IN VIVO* EFFECTS ON INSULIN RESISTANCE IN MICE FED HIGH-FAT DIET

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Objectives: It has been suggested that adipose tissue macrophages infiltrate adipose tissue during obesity and the inflammatory mediators contribute to insulin resistance. Recent report showed that adipose tissue macrophages from lean animals show an alternatively activated, M2 phenotype, which is normally induced by Th2 cytokines, such as interleukin-4 (IL-4) and IL-13. These M2 macrophages produce IL-10 to suppress inflammation. In this study, we investigate whether dietary factors such as bitter gourd and some fractions from *Ganoderma*, including protein, polysaccharide, and triterpenoids have effects on cytokine secretions from 3T3-L1 adipocytes cell line, and further on the development of insulin resistance in mice fed high fat diet.
Methods: 3T3-L1 adipocyte were differentiated in a cocktail DMEM, 10% bovine serum containing insulin, isobutylmethylxanthine, dexamethasone and biotin for 12 days. 3T3-L1 preadipocytes were treated with bitter gourd and some fractions from *Ganoderma*, including protein, polysaccharide, and triterpenoids. During the differentiation supernatants were collected for cytokines assays. *In vivo* anti-diebetic effect was carried using C57BL/6 mice fed with high-fat diet supplemented without (control), or with those dietary factors. After 8 weeks' feeding, OGTT was performed.
Results: The result showed that bitter gourd power promoted IL-4 secretions by 3T3-L1 adipocytes in a dose-dependent manner. In vivo results showed that the BGP group had significantly lower serum glucose level and area under curve compared to the control. The BGP group had significantly higher serum insulin level of oral glucose stimulation and lower insulin resistance index than the control group. Further data including indicators of metabolic syndrome will be investigated and demonstrated.
Conclusion: Dietary factors such as bitter guard may affect the cytokines secretions by adipocytes and then influence the insulin resistance.

PC11/19 INTRAVENOUS IMMUNOGLOBULINS ATTENUATE DIABETES INDUCTION IN MICE

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Type 1 diabetes mellitus is an autoimmune disease in which pathologic, autoreactive T cells of the immune system attack the insulin-secreting pancreatic islets of Langerhans. It was shown that intravenous immunoglobulins may have a therapeutic effect in some autoimmune diseases. Therapeutic effect of immunoglobulins in the models of type 1 diabetes mellitus has not been tested.
Objectives: We examined the effect of intravenous immunoglobulins (i.v.Igs) on the development of immune mediated diabetes induced with multiple low doses of streptozotocin in susceptible C57BL6 male mice.
Methods: Diabetes was induced by five daily injections of streptozotocin. Two doses of i.v.Igs were used (50 mg/kg and 200 mg/kg body weight) daily for 15 days. Control animals received same doses of human serum albumin. Glycemia and glycosuria were evaluated daily while serum level of TNF and IL-17 as well as HbA1c and the pancreatic lymph nodes $CD4^+$ $CD25$ $Foxp3^+$ cells determined by day 21.
Results: Treatment with i.v.Igs in the higher doses (200 mg/kg) induced significant attenuation of diabetes induction as evaluated by glycemia ($p< 0.001$) glycosuria ($p< 0.01$) and HbA1c level. Pearson correlation indicated inverse correlation between HbA1c level and iv Igs dose ($p< 0.04$). Finally, serum level of TNF-a and IL-17 was significantly lower ($p< 0.05$) and $CD4^+$ $CD25^+$ $Foxp3^+$ higher in the pancreatic lymph nodes by day 21 after disease induction with high dose.
Conclusion: Our results show for the first time that i.v. Igs may downregulate diabetes induction possibly by favoring induction of T regulatory cells.

PC11/20 IL2: A NEW IMMUNOSSUPRESSIVE DRUG IN TYPE 1 DIABETES

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CD4+CD25+ regulatory T cells (Treg) control type 1 diabetes (T1D). We tested whether IL2, which is capable of boosting endogenous Treg, could be used as a new therapy of T1D. When pre-diabetic NOD mice were treated with low doses IL2, reduced diabetes incidence was observed. Moreover, when spontaneously diabetic mice were treated during 5 days with low dose IL2, an important proportion of mice reverted clinical diabetes and in some of them, long-term remission was attained. This treatment did not modify basal homeostasis of CD4+, CD8+, Foxp3+, NK, NKT, B, dendritic cells and macrophages in lymphoid organs. Remarkably, IL2 did specifically augment the proportion of Treg among the pancreas infiltrating cells, and enhanced their expression of CD25 and Foxp3. Gene array analysis of Treg and effector T cells (Teff), before and after in vivo IL2 treatment, revealed that Treg and Teff differentially upregulate genes involved in various pathways, notably the canonical pathways of IL2/IL2R signalling. These results indicate that low level IL-2 treatment control T1D development by acting locally in the pancreas, specifically favouring the survival or expansion of Treg in pancreatic islets.

PC11/21 ALTERING THE PEPTIDE LANDSCAPE ON THE ROAD TO DIABETES

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Objectives: To analyse changes in antigen processing and presentation of beta cell associated peptides during the course of diabetes.
Methods: Pulse chase analysis of NIT-1 cells was used to examine maturation of class I MHC in basal and inflammatory states. Concurrent western blot analysis of NIT-1 and primary islets was used to examine changes in proteins associated with antigen processing. These studies were coupled with proteomic analysis of NIT-1 and NOD islets using 2D-DIGE. In addition we have studied the impact of inflammatory changes on the peptides presented by beta cells. The expression of soluble K^d and D^b molecules in NIT cells, as reporters of peptide supply, combined with iTRAQ for quantitative mass spectral analysis allows characterisation of the peptide repertoire under multiple conditions.
Results: Metabolic labelling of NIT-1 demonstrated minimal de novo synthesis of MHC molecules which was elevated following cytokine treatment. We have found low levels of tapasin and proteasomal subunits in NIT-1 and islets under basal conditions; however cytokine treatment dramatically increased expression. To uncouple the effect of co-ordinated upregulation of class I MHC and components of the peptide loading complex in response to inflammation, we have generated NIT-1 cells overexpressing individual molecules of interest. Of note, overexpression of tapasin retains class I molecules, consistent with its role as a peptide editor retaining poorly loaded MHC molecules in the ER. H-$2K^d$ and -D^b are being harvested from cells overexpressing tapasin to biochemically characterise the influence of tapasin on the peptides presented by these molecules.
Conclusion: We hypothesise under basal conditions the beta cell is a poor target for T cell mediated cytotoxicity. This is supported by the relative resistance of beta cells to T cell killing in vitro without cytokine treatment and low de novo synthesis/maturation of class I MHC. During the course of diabetes, the beta cell is exposed to an increasingly inflammatory environment. Our data support the hypothesis that this leads to dramatic changes in antigen processing and presentation, revealing the beta cell to the immune system as a highly desirable target.

PC11/22 EVALUATION OF THE PERFORMANCE OF TIME-RESOLVED IMMUNOFLUOROMETRIC ASSAYS IN SCREENING FOR AUTOANTIBODIES TO GAD65 AND IA-2 IN A COHORT OF CHILDREN AT GENETIC RISK FOR TYPE 1 DIABETES

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To assess the performance of newly established time-resolved immunofluorometric assays (TR-IFMAs) for the detection of autoantibodies to glutamic acid decarboxylase (GAD65) and the protein tyrosine phosphatase-like IA-2 molecule in screening serum samples from children at genetic risk for Type 1 diabetes, compared to conventional radiobinding assays (RBAs).
1572 follow-up samples from a cohort of 100 islet cell antibody (ICA)-positive children, and single samples of 100 ICA-negative children and 100 healthy control children were analysed for the presence of circulating autoantibodies to GAD65 (GADA) and IA-2 (IA-2A). The TR-IFMA and RBA methods gave highly concordant results in most cases, and most discrepancies between the methods were caused by borderline values. There were, however, a few sera where clear differences were seen. At least two of the follow-up samples of a child were GADA-positive in 34 of the 100 children with both methods. Additionally, four cases scored positive in

involved in cytotoxicity inhibition (SPI-CI). SPI-CI works as a specific inhibitor of granzyme M, a major component of the cytotoxic effector molecules stored in cytotoxic granules of NK cells. Knock down of SPI-CI by stable lentiviral expression of SPI-CI-specific shRNA (ES/shSPI-CI) sensitized ES cells for lysis by NK cells. Conversely, overexpression of SPI-CI in ES cells increased resistance to NK cell-mediated lysis. After i.v. injection into syngeneic mice, ES/shSPI-CI cells were eliminated from the spleen more effectively than parental wt ES cells. ES/shSPI-CI cells implanted s.c. into syngeneic mice developed in only about 60% of the recipients very slowly growing tumors, barely detectable at 21 days post injection with average diameters below 1mm. This is in stark contrast to wt ES cells that gave rise to fast growing teratoma in all recipients with a mean diameter of more than 5mm at 21 days post injection.Our data suggest that constitutive expression of SPI-CI protects murine ES cells against lysis by NK cells *in vitro* and *in vivo* and thereby contributes to the tumorigenicity of ES cells.

PD10/8 VIRUS-INDUCED HEPATOCELLULAR CARCINOMA 'SNEAK THROUGH' DESPITE PERSISTENTLY FUNCTIONAL CTLS

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Virus-associated tumors are often under effective T-cell surveillance because of their high immunogenicity. It is not clear why surveillance occasionally fails, e.g. against hepatitis virus-associated hepatocellular carcinoma (HCC). Reasons could be an initial failure to induce effective T cells, tumor-induced tolerance or immune escape by losing immunogenicity. We established a transgenic model for virus-induced HCC based on Cre recombinase-encoding adenovirus infection of liver cells, thereby activating the dormant oncogene SV40 large T (Tag). Infection induced cytotoxic T lymphocytes (CTLs) against the virus and Tag leading to clearance of the majority of infected cells. Then, few surviving Tag^+ cells progressed to large tumors that remained immunogenic and even up-regulated Tag-specific CTLs rather than inducing (systemic) tolerance. Together, virus-induced tumors 'sneak through' despite the induction of CTLs at the time of infection.
*First two authors contributed equally to this work.

PD10/9 ATTENUATION OF PRIMARY BREAST TUMOR GROWTH AND LUNG METASTASES IN ST2 DEFICIENT MICE

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ST2 is selectively expressed on the surface of Th-2 cells. We have shown recently that deletion of IL-33-ST2 signaling favours the expansion of Th-1 and Th-17 cells. Th-1 mediated rather than Th-2 mediated immunity is required for antitumor immune response. We used 4T1 mouse mammary tumor cell line as a breast cancer model with the capacity to metastasize efficiently to sites affected in human breast cancer in order to evaluate the role of ST2 in tumorogenesis.
Objectives: We tested *in vivo* whether IL-33/ST2 signaling downregulated antitumor immunity in experimental breast cancer in BALB/C mice.
Methods: Female BALB/C type and ST2-/- mice on BALB/C background were injected with 5×10^4 4T1 tumor cells into 4th mammary fat-pad. Tumor size was evaluated daily and number and size of tumor metastases, volume of primary tumor and serum level of proinflammatory cytokines determined by day 36. Lymphocyte cytotoxicity was tested in group of animals by day 13 after tumor inoculation.
Results: The appereance of palpable tumor was significantly delayed in ST2 -/- mice ($p=0{,}017$). On day 36 volume of the primary tumor was significantly lower in ST2 -/- mice (10047.24 mm^3 vs 517,31 mm^3; $p=0{,}006$). Further incidence of metastases was significantly higher in "wild type" mice ($p< 0{,}05$). TNF-α and IL-17 levels were higher in ST2 -/- mice ($p< 0{,}05$). Finally, MTT test indicated higher cytotoxic activity in the draining lymph node cells in ST2 deficient mice ($p< 0.05$).
Conclusion: We provide evidence suggesting that blocking ST2 signaling may enhance antitumor response in a model of primary breast tumor and pulmonary metastases.

PD10/10 IFN-G- MEDIATED UPMODULATION OF MHC CLASS I EXPRESSION ACTIVATES TUMOR-SPECIFIC IMMUNE RESPONSE IN A MOUSE MODEL OF PROSTATE CANCER

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The mouse Prostatic adenocarcinoma tumorigenic cell lines TRAMP-C1 and TRAMP-C2 represent a suitable animal model to study the influence of Major Histocompatibility class-I (MHC-I) molecules on protection against tumor development and progression *in vivo*. In these cell lines, MHC-I expression decreases after *in vitro* passaging, but it can be restored by treatment with IFN-a and IFN-g, without modifying their tumorigenicity.
We have transduced TRAMP-C2 cells with the cDNA of the co-stimulatory molecule B7-1 (TRAMP-C2/B7 transfectants). TRAMP-C2/B7 cells showed impaired growth *in vivo*, but they did not elicit a protective response against TRAMP-C2 parental tumor, unless by treating TRAMP-C2 and TRAMP-C2/B7 cells with IFN-g prior to injection. No such effect was obtained with IFN-a. IFN-g resulted a better inducer of peptide transporters TAP-1/TAP-2 and of proteosome subunits LMP-2/LMP-7 than IFN-a. In addition, IFN-g antagonizes the down-regulation of MHC-I mediated by TGF-b largely produced by TRAMP-C2. Preventive immunization of syngeneic C57BL/6J animals with TRAMP-C2/B7 conferred protection against TRAMP-C2-derived tumors in function of the IFN-g-mediated fine-tuned modulation of either the MHC-I antigen processing and presentation machinery or TGF-b signaling.
To explore possible clinical traslation of these results we attempted delivery of IFN-g to TRAMP-C2 tumor growth site by means of genetically engineered mesenchymal stem cells (MSCs) secreting IFN-g. This approach produced encouraging results matching those obtained with IFN-g-treated TRAMP-C2 cells.

PD10/11 A LONGITUDINAL ANALYSIS OF ANTIBODY RESPONSES AGAINST CANCER-TESTIS ANTIGENS IN MULTIPLE MYELOMA PATIENTS

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Objectives: Cancer-testis (CT) antigens represent attractive targets for cancer immunotherapy based on their tumor-restricted expression. We and others have recently described that the CT antigens MAGE-A3 and MAGE-C1/CT7 are frequently and specifically expressed in malignant plasma cells of patients with multiple myeloma (MM). Since CT antigens are known to elicit spontaneous and therapy-induced immune responses, we set out to perform the first longitudinal analysis of antibody responses against a variety of CT antigens in MM patients. We asked the question if and under which conditions humoral responses against CT antigens would occur in the patients and if the existence of such immune responses had an influence on the patient's clinical outcome.
Methods: Antibodies directed against CT antigens (MAGE-C2/CT10, MAGE-A1, MAGE-A3, MAGE-A4, MAGE-A8, MAGE-A11, SSX-2, SSX-4, NY-ESO-1, PRAME) were analyzed by an ELISA using full-length recombinant proteins. The specificity of those antibodies was confirmed by Western blot analysis.
Results: We screened 1100 sera of 194 patients with MM at different time points for antibody responses against the 10 CT antigens. Sera of 100 healthy volunteers were used as controls. Importantly, we identified a number of patients with significant titers against CT antigens, some of them consistently showing high-tittered responses. 51% of all myeloma patients were at least at one point of time antibody positive for MAGE-C2/CT10 or MAGE-A11, 33% for MAGE-A8, 26,8% for MAGE-A1, 26,8% for PRAME, 16,5% for SSX-2, 15,5% for MAGE-A3, 15,5% for SSX-4 and 8,2% for NYESO-1. Control samples were all negative for antibodies directed against those antigens. Remarkably, in many cases titers were only induced by allogeneic stem cell transplantation.
Conclusions: We show in this first comprehensive and longitudinal analyses of humoral responses against CT antigens in myeloma that spontaneous occur and often persist over the course of the disease. Furthermore, allogeneic stem cell transplantation can induce high-titer humoral responses targeting CT antigens in multiple myeloma patients. We plan to further investigate these responses on the T cell level and we will analyze if the stimulation of the adaptive immune system is able to reverse the negative effect of CT antigen expression on the patients' prognosis.

PD10/12 TRANSDIFFERENTIATION OF DENDRITIC CELLS INTO ENDOTHELIAL CELLS AS A NEW MECHANISM OF TUMOR EVASION IN PANCREATIC CARCINOMA

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Pancreatic carcinoma is a very aggressive neoplasm with extremely poor prognosis. Despite advances in single/combined surgical/radiation/chemotherapy protocols, about 90% of patients die within one year after diagnosis; five-year survival rate is < 3%. Two crucial mechanisms for cancer development and progression are the formation of new vessels and paralysis of the effector arm of the anti-tumour immune response.
Dendritic cells (DC), specialising in capturing antigens and processing and presenting them to T lymphocytes, act as "natural adjuvants" in the immune response against antigens, potentially also against tumour-associated ones. However, DC have been shown to lose this stimulatory ability in tumours, explaining host tolerance to cancer. It has recently been observed that monocytes and DC of myeloid origin present wide phenotypic superimposition with specific microvascular endothelial cells within inflamed tissue, and that, under particular conditions of exposure to pro-angiogenic cytokines, they may become endothelial cells. The role of DC plasticity in the context of pancreatic cancer has not yet been investigated. Our research was thus designed to explore this important issue. Using the classical in-vitro model to generate immature DC from peripheral purified CD14+, we aimed to evaluate the ability of the stabilised human-pancreatic-carcinoma cell line PT-45 supernatant to induce the transdifferentiation of DC to endothelial cells. Using real-time PCR, flow cytometry and functional tests, we showed that, in the presence of PT-45 cell supernatant and of pro-angiogenic cytokines including VEGF, TGF-beta1, angiopoietin 1 and IL-8, DC not only change morphologically, but lose their allostimulatory ability in MLR and acquire endothelial-type functional and phenotypic features, confirmed by expression of VE-cadherin, von Willebrand

Factor and VEGF receptor 1 and 2, and by their in-vitro ability to form cord-like structures. The results show for the first time that, by releasing cytokines with pro angiogenic and immunomodulatory activities, the tumour not only promotes traditional neoangiogenesis mechanisms but can also transform DC, which should play a decisive role in the immune response against tumour, into active endothelial cells.

PD10/13 CLINICALLY COMPATIBLE DOSES OF ZOLEDRONIC ACID REPOLARIZE TUMOR-ASSOCIATED MACROPHAGES AND INHIBIT MAMMARY CARCINOGENESIS BY TARGETING THE MEVALONATE PATHWAY

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Objectives: Zoledronic acid (ZA) is the most potent aminobisphosphonate (NBP) clinically available to treat bone disease in cancer. ZA induces osteoclast apoptosis by specifically targeting the enzyme farnesyl pyrophosphate (FPP) synthase in the mevalonate (Mev) pathway. Since FPP synthase is an ubiquitous enzyme ZA has the potential to affect the survival and function of almost any cell type. ZA has thus become an attractive tool to concurrently target tumor cells and the local microenvironment. So far, most studies have investigated the antitumor activity of ZA in mice models of bone metastatization or used ZA at concentrations far exceeding those recommended for clinical use. It is unknown whether ZA at clinically relevant doses is active against tumors not located in bone.
Methods: Seven-week-old mice transgenic for the activated ErbB-2 oncogene displaying atypical mammary hyperplasia were treated with 100 μg/kg ZA intravenously (equivalent to the 4 mg standard clinical dose) weekly for 4 weeks followed by a 3-week rest. This schedule was reiterated throughout the mice life for a cumulative mean number of doses equivalent to that recommended in humans by main clinical societies.
Results: A significant reduction in the mean number of tumors per mouse and a reduction of their growth rate was observed in ZA-treated mice, resulting in a significant increase in tumor-free and overall survival. ZA did not exert a direct effect on tumor cell growth, but it clearly modulated the Mev pathway and affected protein prenylation in both tumor cells and tumor-associated macrophages (TAMs). Histological and immunohistochemical analysis showed that both the number of TAMs and tumor vascularization were strongly reduced in ZA-treated mice. These effects were paralleled by a strong reduction in the local production of VEGF and IL-10 and by a triggered secretion of IFN-g. Moreover, ZA exposed macrophages recovered an antitumoral M1 phenotype, as shown by nuclear translocation of NF-kB, iNOS expression, and NO production.
Conclusion: These data show that ZA at clinically achievable doses has a significant impact on disease-free survival, and overall survival in tumors not located in bone by targeting the Mev pathway of tumor cells and TAMs.

PD10/14 THE ALPHA$_E$(CD103)BETA$_7$ INTEGRIN, AN ESSENTIAL MEDIATOR OF ANTITUMOR CYTOTOXIC T LYMPHOCYTE ACTIVITY

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We have recently demonstrated that the interaction of epithelial cell marker E-cadherin with alpha$_E$(CD103)beta$_7$ integrin, often expressed on tumor-infiltrating lymphocytes (TIL), plays a major role in effective tumor cell lysis. Indeed, we found that although tumor-specific CD103$^+$ TIL clones are able to kill E-cadherin$^+$/ICAM-1$^-$ autologous tumor cells, CD103$^-$ PBL-derived counterparts are inefficient. Furthermore, tumor cell killing is abrogated after treatment of the TIL clones with blocking anti-CD103 monoclonal antibody or after targeting E-cadherin in the tumor cells using ribonucleic acid interference. Confocal microscopy analysis also demonstrated that alpha$_E$beta$_7$ integrin is recruited at the immunological synapse and that its interaction with E-cadherin is required for cytolytic granule polarization and subsequent exocytosis. In this report we show that although CD103$^+$ TIL are able to form the same number of stable conjugates with tumor cells expressing or not E-cadherin, electron microscopy analyses of the contact zone revealed that the synapses are very different. Indeed, alpha$_E$beta$_7$ integrin engagement is necessary to induce close apposition surfaces between the killer cell and its target cell. Moreover, we show that CD103 induces cell spreading on recombinant E-cadherin-coated coverslips, and interaction of this integrin with its ligand leads to the phosphorylation of signalling molecules required for effective target cell lysis. Therefore, CD103 engagement could provide costimulatory signals necessary for activation of TIL lytic functions. Collectively, our data emphasize a key role for alpha$_E$beta$_7$ integrin interaction with E-cadherin in promoting cytotoxic immunological synapse maturation and are consistent with a function in adhesive costimulation. Thus, CD8$^+$/CD103$^+$ tumor-reactive T lymphocytes infiltrating epithelial tumors most likely play a major role in antitumor cytotoxic response through alpha$_E$beta$_7$ integrin/E-cadherin interactions.

PD10/15 MECHANISMS OF IMMUNOSUPPRESSION IN PROSTATE CANCER

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Objective: Immunotherapy has recently emerged as a complementary and alternative approach for prostate cancer (PC), one of the leading causes of cancer related death in the male population. Despite evidence for the induction of tumor-specific immune responses in vaccinated PC patients, clinical responses are still below expectation. A reason for such a limited success resides in the mechanisms adopted by the tumor to escape immune surveillance. Aim of the study was to investigate mechanisms of immunosuppression in transgenic adenocarcinoma of the mouse prostate (TRAMP) males, a reliable model of spontaneous PC development that closely resemble the human pathology.
Methods: In TRAMP mice, either healthy or affected by PIN or PC, we investigated the dynamic of the immune response against Tag, a tissue-specific tumor associated antigen, and potential mechanisms of immunosuppression.
Results: PC development and progression was accompanied by induction of a profound state of tumor-specific tolerance, which hampered immunotherapy. No obvious function for natural CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells (CD4$^+$Treg), myeloid derived suppressor cells or cells producing indoleamine 2,3-dyoxigenase could be found in tumor-bearing TRAMP mice. Rather, we obtained evidence that a yet uncharacterized population of bone-marrow derived cells exerts important immunosuppressive activities in these mice.
Conclusions: We postulate that such immunoregulatory cells are responsible for tolerance induction in the TRAMP model and possibly in PC patients.

PD10/16 INTERLEUKIN-12 INITIATES TUMOR REJECTION INDEPENDENT OF NK CELLS AND ADAPTIVE IMMUNITY

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Lymphocytes and their secreted cytokines are considered to play a critical role in tumor elimination. In a variety of tumor models, Interleukin-12 (IL-12) has been shown to repress tumor growth. The tumoricidal activity of IL-12 is widely held to be mediated by the activation and polarization of NK and TH1 cells respectively. Using gene-therapy, we found drastic growth repression in vivo of B16 melanocytes constantly secreting low amounts of IL-12 (B16-IL12), while the parental B16 cells form a massive subcutaneous tumor. The usage of IL-12Rβ2 deficient mice revealed that the tumor derived IL-12 acts on the host rather than the tumor itself and BM-chimeras showed that IL-12 engages a hematopoietic cell population. Surprisingly B16-IL12 also failed to grow subcutaneously in RAG-1$^{-/-}$ mice, demonstrating that neither MHC-restriction nor B and T lymphocytes are involved in IL-12-mediated tumor elimination. We could also show that NK cell depletion in RAG-1-/- mice as well as the use of IL-15Ralpha deficient mice did not render the repression of the subcutaneous tumor growth after challenge with B16-IL12 melanocytes. In summary our data clearly demonstrate that the IL-12-mediated tumor suppression acts independent of NK cells and adaptive immunity. The induced immune response does not act systemically but in a local highly efficient manner. This novel, unprecedented IL-12-mediated pathway of immune action reveals a potential therapeutic mode of IL-12 in tumor suppression.

PD10/17 DELETION OF GALECTIN-3 *IN VIVO* DOWNREGULATES LUNG SPECIFIC METASTASIS OF MELANOMA CELLS

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Adhesive interaction between the molecules on cancer cells and the target organ is one of the key determinants of metastatic outcome. Galectin-3, a b galactoside-binding lectin is a multifunctional protein which regulates cell adhesion, growth and proliferation as well as angiogenesis and apoptosis. It had been suggested that galectin-3 on vascular endothelium facilitate lung specific metastases.
Objectives: We used metastatic variant of B16 melanoma (B16F$_1$) to study lung colonization and tumor cell adhesion in galectin-3$^{-/-}$ mice in order to directly demonstrate its relevance for disease progression.
Methods: Metastases were induced by injecting 5×10^4 B16F$_1$ cell i.v in Gal-3$^{-/-}$ and "wild-type" C$_{57}$BL/6 male mice. Animals were sacrificed by day 21 and number and size of metastatic colonies examined microscopically and specific binding of the malignant cells to lung tissue evaluated by adhesion assay on tissue slides.
Results: When compared with "wild-type" C$_{57}$BL/6 mice, Galectin-3$^{-/-}$ mice exhibited significant resistance to lung colonization of B16F$_1$ melanoma cells as evaluated by number and size of metastatic colonies ($p < 0.003$). Accordingly, adhesion assay *in vitro* indicated that number of attached B16F$_1$ malignant cells was significantly higher in the tissue section derived from "wild-type" C$_{57}$BL/6 mice ($p < 0.001$).
Conclusion: We provided direct evidence that Galectin-3 is required for melanoma metastasis in the lung and could therefore be considered as therapeutic target.