transplantation. In 16 of the 34 patients the fungal infection was suspected at the beginning. According to the EORTC diagnostic criteria for IFI, 12 patients (75%), had a possible IFI and 4 cases (25%) presented a probable IFI. There were no cases with a proven IFI before death. *Results.* The autopsy demonstrated the presence of fungal infection in 10 patients: in 7 cases there was a clinical suspicion of fungal infection while in three cases it was an unexpected discovery in the autopsy. The organs shown up by the autopsy to be affected by the fungal infection were: lung (9 cases), digestive (6 cases), heart (2 cases), kidney (2 cases), CNS (2 cases) liver (2 cases) spleen (1 case), mediastinic mass (1 case), and pancreas (1 case). It is relevant that in most patients, the organic involvement other that lung was not suspected before their death, and it was responsible for very outstanding clinical manifestations during the end stage of the illness: superior vena cava syndrome (1 case), serious heart arrythmias (1 case), profuse diarrhea (1 case), renal failure (1 case), and hepatic failure (1 case). *Conclusion*: Our study shows high incidence of clinical suspected IFI at the end-stage disease not confirmed with the autopsy, and the complexity of the clinical manifestations associated to this type of infections.

1253

CD40 LIGAND AND CALCIUM IONOPHORE TREATMENT OF DENDRITIC CELLS FROM HEALTHY DONORS AND PATIENTS WITH MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE AND MULTIPLE MYELOMA

L. Kovarova, J. Michalek, M. Penka, R. Hajek

University Hospital, BRNO, Czech Republic

Backgrounds. Dendritic cells (DC) are the most potent antigen-presenting cells that can initiate adaptive immune response. They can differentiate from peripheral blood precursors and as an immature dendritic cells react to wide range of stimuli. Upon the activation/maturation process they change their phenotypic, morfologic and functional characteristics. The ability to acquire and activate blood DCs makes them a valuable source for future immunotherapy trials, but there are inconsistent reports about the functional state of dendritic cells from patiens with multiple myeloma (MM). *Aims.* Comparison of 48h treatment of immature dendritic cells with different stimuli as CD40 ligand (CD40L) and calcium ionophore (CI). Searching for differences in phenotype of DCs from healthy donors and patiens with MGUS and MM after stimulation. *Methods.* Ficoll-Hypaque-separated peripheral blood mononuclear cells (PBMC) from 10 healthy donors and 12 patients (7 MM and 5 MGUS) were used. Adherent precursors of DCs were cultured with GM-CSF and IL-4. CD40L and/or CI were added in day 1 or 4 to generate mature DCs. Multicolor flowcytometric analysis was done in day 0 and after harvest of DCs in day 3 or 6. Following monoclonal antibodies were used: CD11c, CD80, CD83, CD86, lineage mixture, CCR2, CCR5, CCR7, IL-12, MIP-1a, HLA-DR. *Results.* The highest percentage of CD83, characteristic marker of mature DCs, was found in 3rd day of culture after stimulation CD40L and also CI. In the 6th day was the average percentage of CD83 decreased to the half of 3rd day. There was found no differences between donors and patients. Expression of HLA-DR was relatively constant, independent on the time of the harvest or type of the stimulation and again without differences between groups of patients and donors. Expression of costimulation molecule CD80 slowly increased in 6th day of culture after CD40L stimulation, but CD86 was higher after CI stimulation. Chemokines receptors CCR2 and CCR5, markers of immature DCs, were expressed in low density as well as CCR7, marker of mature DCs. There was some evidence, that CCR7 was increased in healthy donors. Production of cytokine IL-12 and chemokine MIP-1a were also low. *Summary/conclusion*. Addition of CD40L and/or CI to an immature DCs obviously didn´t evoke their maturation, because there were found no strong expression of CCR7, IL-12 and MIP-1a. We didn´t found significant diferences between DCs generated from healthy volunteers and patients.

Funding: Supported by grant MZCR NR/8081-3.

1254

THE PREPARATION OF MYELOMA-SPECIFIC CYTOTOXIC T CELLS BASED ON INTERFERON γ PRODUCTION

D. Ocadlikova,[1] L. Zahradova,[1] L. Kovarova,[1] M. Penka,[2] R. Hajek,[1] J. Michalek[3]

[1]*LEHABI, BRNO, Czech Republic;* [2]*Department of Clinical Hematology, BRNO, Czech Republic;* [3]*Cancer Immunobiology Center, DALLAS, USA*

Backgrounds. Autologous hematopoietic stem cell transplantation has been considered recently as part of a standard treatment strategy in patients with multiple myeloma (MM). Here we attempted to enhance the immunotherapeutic potential of autologous T cells based on selection of myeloma-reactive lymphocytes *in vitro*. *Aims.* The aim of this study was to identify and characterize autologous myeloma-reactive T cells *in vitro* and to evaluate their cytotoxic effect. *Methods.* Irradiated myeloma cell line ARH 77 or patient's myeloma cells were used as tumor antigen for dendritic cells loading. Peripheral blood mononuclear cells of 8 healthy volunteers and 10 MM patients were used for repeated stimulation of T lymphocytes. Activated T cells producing interferon γ were isolated using immunomagnetic separation (MACS) (Miltenyi Biotech) and expanded *in vitro* by phytohemaglutinin and high concentrations of interleukin 2. A specific cytotoxicity against myeloma cells was tested after the expansion with propidium iodide or 7-amino actinomycin D. Activated T cells were labeled with CFSE. Allogeneic T cells and interferon γ negative fraction of T cells served as controls. *Results.* In an allogeneic setting with ARH 77 cells the enrichment of interferon γ positive T cells by magnetic beads in healthy donors started from a median of 2.83% (1.97-4.58%) to 48.57% (15.14- 82.98%) after MACS and from 1.91% (1.14-3.4%) to 73.14% (3.9-88.75%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were further expanded *in vitro* from 0.5×10^6 to a median of 160×10^6 (150×10^6-420×10^6) T cells within 4 weeks and the test of cytotoxicity has demonstrated a high degree of specific killing of ARH 77 myeloma cells 69.17% (38.04-78.23%). Cytotoxicity of expanded interferon γ negative T cells was negligible. In an autologous setting with autologous myeloma cells used as an antigen, the enrichment of interferon γ positive T cells from MM patients started from 1.12% (0.27-6.2%) to 7.85% (0.42-12.6%) after MACS and from 1.9% (0.37-14.4%) to 14.7% (1.28-71.4%) after MACS in $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells, respectively. Interferon γ positive T cells were expanded in vitro from 0.12×10^6 (0.05×10^6-0.4×10^6) to 88.5×10^6 (35×10^6-226×10^6) within 8-12 weeks and the test of cytotoxicity has demonstrated only a modest specific killing of autologous multiple myeloma cells (18.88%) and allogeneic ARH 77 cells (18,21%). *Conclusions.* These data demonstrate a promising tumor-specific effect of allogeneic myeloma-reactive T cells but only a modest effect in an autologous setting in patients with MM. Whether that is due to a low MACS enrichment or low immunogenicity of autologous myeloma cell needs to be further clarified.

Funding: Supported by the grant: IGA MZCR 1A/8907-5.

1255

AMINO ACID SEQUENCES OF T CELL RECEPTOR REACTING AGAINST MULTIPLE MYELOMA

S. Dudova,[1] L.K. Kovarova,[2] R.H. Horak,[2] R.H. Horvath,[3] M.P. Penka,[4] R.H. Hajek,[5] D. Ostroveanu[6]

[1]*FN Brno, BRNO, Czech Republic;* [2]*LEHABI, FN Brno, BRNO, Czech Republic;* [3]*Genex CZ, BRNO, Czech Republic;* [4]*OKH, FN Brno, BRNO, Czech Republic;* [5]*IHOK, FN Brno, BRNO, Czech Republic;;* [6]*Fundeni Clinical Institute, BUCHAREST, Romania*

Backgrounds. Multiple myeloma (MM) is a disease caused by malignant proliferation of B lymphocytes in the bone marrow. Recently, high-dose chemotherapy with autologous hematopoietic transplantation has been considered a standard treatment for patients with advanced stages of MM. Such treatment delays relapse but it is not curative and almost all patients ultimately develop recurrent disease. Based on preclinical and clinical studies it is evident that myeloma-reactive T lymphocytes play an important role in immunologic response to this malignant disease. Myeloma-reactive T lymphocytes have been shown to be a promising approach in adoptive cellular immunotherapy aside autologous transplantation of bone marrow graft. *Aims.* Our aim was to analyse T cell receptor (TCR) sequences reacting against multiple myeloma. Experimental study was performed in 10 patients to provide information on the specificity and spectrum of recognized antigens. *Methods.* Dendritic cells loaded with apoptotic bodies from magnetically isolated myeloma cells have been used to stimulate autologous T lymphocytes. Activated myeloma-specific T cells were identified and expanded. After mRNA isolation the anchored reverse transcription using modified version of SMART method was done. PCR product was cloned into plasmid vector, transformed in bacterial cells and individual clonotypes were sequenced. *Results.* Oligoclonality of TCR receptor was demonstrated in myeloma specific *in vitro* expanded T lymphocytes, in one case monoclonal population of tumor specific T cells was found. These findings support the assumption of myeloma specific antigens stimulating only certain autologous T lymphocytes. *Conclusions.* Structural characterization of TCR receptor of myeloma specific clones provides further evidence for the role of these T lymphocytes in immunotherapy. Receptor