66

Dentritic cells in AML and MDS-comparison of different FCS-free culture conditions

Schmetzer H., Pelka-Fleischer R., Kröll T., Hiddemann W. , Nüssler V.
Medical Department 3, Klinikum Großhadern, Universitiy of Munich, Germany

Insufficient presentation of tumor antigens may result in an insufficient elimination of leukemic cells by the immune system. Dendritic cells (DC) are professional antigen presenting cells and induce a T-cell mediated immune response. DC can be successfully generated from AML-cells presenting leukemic genotypes and antigens as well as DC markers. The aim of our study was to find optimal FCS-free culture conditions for the generation of DC from AML and MDS-PB-samples.We cultured mononuclear PB-cells from 2MDS and 8AML patients and 4 healthy donors in parallel in two different FCS-free media (X-vivo 15, Cellgro DC), supplemented with GM-CSF,IL-4 ,TNF-α ,± FL, ± 10% autologous plasma(Tab). The DC- and leukemic antigen expressions were evaluated at day (D) 0,7 or 14 using monoclonal DC markers (e.g. CD1a, CD83, HLA-Dr), costimulatory antibodies (e.g. CD80, CD86) together with myeloid and blast markers (e.g. CD33, CD117, 7.1, CD34).

Our data show, that DC can be grown in FCS-free conditions from normal or leukemic PB-samples with amounts depending from diagnosis and culture conditions:

1.) comparison Cellgro/X-vivo: amounts of DC and antigen profiles were similar, however with higher proportions of dying cells under Cellgro cultivation.

2.) culture ± FL: whereas in AML and healthy PB samples amounts of DC were not significantly different in the parallel cultures , in MDS higher DC proportions could be found if FL was added to the media.

3.) culture± autologous plasma: in most of the AML and healthy samples studied an inhibitory effect on DC generation was detectable, if plasma was added to the culture.

4.) proof of leukemic origin of DC: in AML patients with a high percentage of blasts positive for CD117, CD34 or 7.1 (marker for 11q23 aberrations) a coexpression of those antigens with DC antigens could be demonstrated pointing to the fact, that DC generated derived from clonal, leukemic cells.

We can conclude that optimal generation of DC in AML and healthy donors is possible with X-vivo without FL and autologous plasma; in cases with MDS the addition of FL and autologous plasma might improve the harvest of dendritic cells.

67

Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40L to induce high amounts of IL-12

Towarowski A., A. Krug, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg and G. Hartmann

Human plasmacytoid dendritic cells (PDC, CD123+) and myeloid dendritic cells (MDC, CD11c+) are able to discriminate between distinct classes of microbial molecules based on a different pattern of TLR expression. TLR9 which is critically involved in the recognition of CpG motifs in mice was present only in PDC. TLR4 which is required for the response to LPS was selectively expressed on MDC. Consistent with TLR, PDC were susceptible to CpG ODN but not to LPS, while MDC responded to LPS but not to CpG ODN. In PDC, CpG ODN supported survival, activation (CD80, CD86, CD40, MHC II), chemokine production (IL-8, IP-10) and maturation (CD83). CD40L and CpG ODN synergized to activate PDC and to stimulate the production of IFN-alpha and IL-12 including bioactive IL-12p70. Previous incubation of PDC with IL-3 decreased the amount of CpG-induced IFN-alpha and shifted the cytokine response in favor of IL-12. CpG ODN-activated PDC were more active to stimulate proliferation of naive allogeneic CD4 T cells, but Th1 polarization required simultaneus activation of PDC by CD40L and CpG ODN. CpG ODN-stimulated PDC expressed CCR7 which mediates homing to lymph nodes. In conclusion, our studies reveal that IL-12p70 production by PDC is under strict control of an adequate exogenous microbial stimulus such as CpG ODN and of CD40L on activated T cells. Thus CpG ODN acts as an enhancer of T cell help while T cell-controlled restriction to foreign antigens is maintained.

68

Phenotype characterisation of dendritic cells (DC) obtained from fresh or frozen precursors (PBMC) for human cancer vaccination.

A.Riccobon[1], R.Ridolfi[1], M. Stefanelli[2], A. Vecci[2], W. Zoli[1], M. Petrini[2], F. Ortolani[3], G. Migliori[3].
[1] Dept.of Medical Oncology, Pierantoni Hospital, Forlì, Italy.
[2] Istituto Oncologico Romagnolo, Forlì, Italy.
[3] Dept.of Transfusional Medicine, Pierantoni Hospital, Forlì, Italy.

DC were differentiated from peripheral blood lymphocytes (PBL) obtained by leukapheresis without pharmacological stimulation.After purification in ficoll, a quantity of PBL was utilised for DC generation (7-day culture of adherent cells with IL-4 and GM-CSF 1000 U/ml) and the rest was frozen in aliquots.On the 6th day of culture a part of DC were pulsed with autologous tumour lysate for 16-18 hours.A median of 6.8 litres of blood was processed in 12 leukapheresis procedures.Median WBC recovery was $7.6 \cdot 10^9$ (3.2-15), median post ficoll cells in culture was $1 \cdot 10^9$ (0.44-3),median DC obtained was $40.5 \cdot 10^6$ (4.12-317.7). DC obtained from apheresis product showed a 5% (0.4-10) differentiation in fresh PBMC (12 samples) compared to 2.3% (0.3-10) in frozen PBMC aliquots (25 samples).A systematic immunophenotype characterisation was performed on these two different types of DC and on pulsed and non pulsed DC.The cells displayed the characteristic immunophenotype of immature DC: high expression of HLA-DR and costimulatory and adhesion molecules (CD86, CD54), a variable CD1a expression and a lack of lineage specific markers (CD14, CD3, CD19). No significant differences were observed between pulsed and non pulsed DC or between DC from fresh and frozen PBMC. Functional experiments are ongoing. These results confirm the validity of clinical protocols based on sequential injection of fresh DC obtained from fresh or frozen PBMC .

69

M. Dauer[1], T. Meskendahl[1], J. Röbe[1], K. Pohl[1], M. Schnurr[1], K. Hallfeld[2], M. Schoenberg[3], S. Endres[1], A. Eigler[1]
[1]Abteilung für Klinische Pharmakologie, Medizinische Klinik Innenstadt, [2]Chirurgische Klinik Innenstadt, LMU München; [3]Abt für Chirurgie, Rotkreuz-Krankenhaus München

Immunotherapy of pancreatic carcinoma: effect of pancreatic carcinoma cell lysate on the survival of dendritic cells *in vitro*

Introduction: Incubation with lysate from pancreatic carcinoma (PC) cell lines can reduce the allostimulatory capacity of dendritic cells (DC) *in vitro*. In preparation of a phase II vaccination trial, we studied the effect of *primary* PC cell lysate on the survival and function of monocyte-derived DC *in vitro*. *Methods:* Lysates from PC cells were prepared by repeated freeze-thaw cycles and protein concentration was measured photometrically. Immature DC were incubated with PC cell lysate for 4h and subsequently stimulated with PGE_2 and TNF-α for 48h. DC survival and expression of surface markers were determined by flow cytometry. *Results:* Cell lysates were prepared from 13 specimens of histologically confirmed PC (weight: 150-4500 mg). Protein concentrations ranged from 97 to 2049 µg/ml. Incubation with one of the 13 tumor lysates reduced DC survival rate from 80 % (control: unpulsed DC) to 11%, 45% and 70% at protein concentrations of 150 µg/ml, 75 µg/ml and 37,5 µg/ml, respectively. Surviving DC showed equal expression of CD80, CD83, CD86 and MHC II compared to unpulsed controls. We are currently investigating the allostimulatory capacity and IL-12 production of DC incubated with the different primary PC lysates. *Conclusion:* Incubation with primary PC lysate can reduce the DC survival rate in *vitro*. This has to be considered in the use of primary PC cell lysate as a source of tumor associated antigen in DC-based vaccination strategies.