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Immune Restoration in Cancer Patients by Cyclooxygenase-Inhibition In Vitro and In Vivo

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Introduction: Inhibition of cyclooxygenase and thus prostaglandin E2 (PGE_2) synthesis via non-steroid antiinflammatory drugs (NSAIDs) has been described to inhibit the tumor growth of head and neck carcinomas (SCCHN) *in vitro* and *in vivo*. However, the molecular mechanism of the antineoplastic action of NSAIDs remained unclear.

Material and Methods/Results: We could demonstrate that various SCCHN lines significantly produced PGE_2. SCCHN-supernatants (SN) as well as purified PGE_2 downregulated the expression of the chemokine receptor CCR5 and the adhesion molecule Mac-1 on monocytes, thus resulting in a reduced migration and adhesion *in vitro*. These impaired monocyte functions could be restored by inhibiting cyclooxygenase activity in tumor cells with Aspirin or Indomethacin. Additionally, CCR5 and Mac-1 were downregulated on peripheral monocytes of SCCHN patients *in vivo*, causing a significantly reduced monocyte adhesion. Both, our *in vitro* and *in vivo* data (i) elucidate the molecular mechanism and (ii) prompted us to preoperatively apply Indomethacin in head and neck cancer patients. This resulted in a significant restoration of suppressed monocyte function, i.e., CCR5 expression and adhesion.

Conclusion: Our data provide the rationale for the use of NSAIDs in chemoprevention or immunoadjuvant therapy of head and neck cancer.

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Expression of cytokine receptors on AML blasts

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Haematopoetic cytokines regulate haematopoetic cell functions via specific cell surface receptors. There is evidence to suggest, that those receptors could have a role in leukemia. We have studied the expression of cytokine receptors on mononuclear bone marrow (BM-) cells of 93 patients with acute myeloid leukemia (AML) at first diagnosis by FACS – analysis using directly Fluorescein conjugated antibodies: CD 114 (hG-CSF-R), CD 116 (hGM-CSF-R), CD 117 (hSCF-R), CD 123 (hIL3-R), CD 130 (gp130 subunit), CD 135 (hFL-R).

Our findings indicate, that most of patients with FAB-type M4/M5 express CD116, whereas most of the patients with FAB-type M2 express CD114. CD117 and CD 123 were found in nearly all patients with FAB-type M0-M5. Most of patients with FAB-type M1, M4 and M5, but only a part of patients with FAB-type M2 and M3 expressed CD 135. Separating our patient cohorts in cytogenetic risk groups we could not detect differences in the cytokine receptor profiles of the 'bad risk group' compared with the 'good' and 'intermediate' risk groups.

We can conclude, that cytokine receptor expression in AML patients is very variable. This reveals the great diversitiy of immunophenotypes in AML and might contribute to identify individual blast phenotypes in order to detect residual disease in remission. On the other hand the detection of specific cytokine receptors on leucemic cells might be important for individual cytokine therapies in AML-patients. The prognostic value for the clinical follow up of the patients remains to be evaluated.

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Low CD62L- expression in Acute Myeloid Leukemia (AML) correlates with a bad cytogenetic risk

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Interactions between haematopoetic cells and the stromal microenvironment or immunoreactive cells are mediated by specific cell surface receptors. The expression of those molecules may alter the adhesive qualities (mobility, homing) as well as immune response behaviour of leukemic blasts. L-Selectin is suggested to play a role in the redistribution and homing of hematopoetic progenitor cells to the bone marrow (BM). Downregulation of L-Selectin is responsible for mobilization of blasts from the BM into the circulation and ligation of L-Selectin stimulates proliferation of progenitor cells. This could have an influence on the process of leukemia.

We have studied the expression of CD62L on mononuclear BM-cells of 36 AML-patients at first diagnosis by FACS-analysis using directly Fluorescein conjugated antibodies. On average the patients presented with 69% cytological blasts in the BM. L-Selectin CD62L was very heterogenously expressed in all FAB-groups. Highest expression was found in cases with AML-M4 with 4 of 9 cases presenting with an inv(16)-karyotype. Separating our patient cohorts in cytogenetic risk groups we could detect a high expression of CD62L in cases with a 'good risk' karyotype and very low expression in cases with 'bad risk' karyotype. The expression tended to be higher in primary AML than in secondary AML.

We can conclude, that 1) expression of CD62L on AML blasts is very variable. This reveals the great diversitiy of immunophenotypes in AML and might contribute to identify individual blast phenotypes in order to detect residual disease in remission. 2) Low L-Selectin expression correlates with a bad cytogenetic risk and might reflect a decreased homing of the blasts to the BM as well as an impaired T-cell cytotoxic reaction against leukemic cells. The expression of CD62L on leukemic blasts might be influenced by individual cytokine therapies and result in an altered hematological reconstitution after cytotoxic therapies as well as in an altered immunological recognition of blasts.

The prognostic value for the *clinical* follow up of the patients remains to be evaluated.

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***In vitro*-generation of potent antigen-presenting cells for immunotherapy: IL-4 is more effective than IFN-α**

Introduction: The use of IL-4 has become routine for the generation of monocyte-derived dendritic cells (DC). However, IL-4 may have negative effects on DC function, e.g. by interference with prostaglandin metabolism. To evaluate an alternative strategy for the generation of potent antigen-presenting cells *in vitro*, we compared IL-4-generated DC with DC matured in the presence of interferon-alpha (IFN-α).

Methods: The adherent cell fraction from PBMC was cultured in medium containing GM-CSF (1000 U/ml) and either IL-4 (500 U/ml) or IFN-α (500 U/ml) for 6 days. After incubation for 48 h with different stimuli known to induce DC maturation, expression of surface markers was determined by flowcytometry and IL-12 production was measured.

Results: After 6 days of culture, both cell types showed similar pinocytotic uptake of dextran. IFN-α-treated cells consistently expresssed higher levels of CD80 and CD14, but lower levels of CD86, CD83 and MHCII. After stimulation, IFN-α-treated cells showed less upregulation of CD86, CD83 and MHCII and produced less IL-12 independent of maturation stimuli. Of all stimuli tested, the combination of IL-1β, IL-6, TNF-α and PGE_2 lead to a maximal increase in surface marker expression and IL-12 production in both DC subtypes. Currently, we investigate the allostimulatory capacitiy of the two DC-subtypes.

Conclusion: We found no evidence for negative effects of IL-4 on the function of monocyte-derived DC and conclude that IL-4 currently remains optimal for the generation of potent APC *in vitro*.