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CpG ODNs enhance recall and prmary specific human CTL responses
V. Hornung, S. Rothenfusser, S. Britsch, A. Towarowski, S. Endres, D. Speiser, and G. Hartmann
In humans we identified two distinct types of CpG ODN which differ in their ability to activate B cells and to induce IFN -α/-β production in plasmacytoid dendritic cells: CpG-A ODN (high IFN -α/-β induction, low B cell stimulation) and CpG-B ODN (low IFN -α/-β induction, high B cell stimulation). It is not known whether CpG ODN promote CTL responses in the human system. We tested the effect of CpG-A ODN and CpG-B ODN on the activation of antigen-specific naive and memory CD8+ T cells in vitro. PBMC from HLA-A*0201 positive donors (used as antigen presenting cells) were incubated with MHC I-peptides and autologous CD8+ T cells in the presence or absence of different CpG ODN. HLA-A*0201-restricted peptides derived from the influenza matrix protein (GILGFVFTL) and the tumor antigen Melan A / MART-1 (ELAGIGILTV) were used. After 14 days antigen-specific CTLs were quantified both by intracellular IFN-γ staining of CD8+ T cells after 6 hours restimulation with the corresponding peptide or an unrelated control peptide and by MHC-tetramer staining. CpG-B ODN increased the frequency of CTLs specific for the recall antigen influenza matrix protein up to 4.5 fold (mean increase: 2,6 fold; n=12; p<0,05) and the frequency of CTLs specific for the primary tumor antigen Melan-A up to 12-fold (mean increase: 2,9 fold; n=11; p=0,9). In contrast CpG-A ODN only increased the frequency of recall CTLs. These findings have important implications for peptide vaccination protocols in cancer therapy.

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Membrane expression of heat shock protein 70 (Hsp70) in hematological malignancies
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By multiparameter flow cytometry using lineage specific markers together with the Hsp70 specific antibody RPN1197, an Hsp70 plasma membrane expression was demonstrated on freshly isolated bone-marrow specimens of patients suffering from primary, and secondary acute myeloid leukemia (AML), multiple myeloma, myelodysplastic syndrome, acute lymphoblastic leukemia and non-Hodgkin lymphoma (NHL). Bone marrow cells of healthy human individuals and CD34 enriched cells did not express Hsp70 on the cell surface. In 77% of all AML cases (47 out of 61) Hsp70 expression was associated with an intermediate, in 78% (18 out of 23) with an unfavorable and in 55% (6 out of 11) with a good prognostic karyotype.
Previously we have shown that membrane-bound Hsp70 acts as a tumor-selective recognition structure for allogeneic NK cells. A preincubation of NK cells with an Hsp70 peptide further enhances this reactivity. The present study indicates that the anti-leukemic effect of patient-derived NK cells is inducible following stimulation with the Hsp70 peptide. Taken together our data provide evidence that Hsp70 acts as a target structure for autologous and allogeneic NK cells.

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Expression of HLA-Class I and II molecules is maintained on leukemic blasts from patients with acute myelogenous leukemia (AML) and the costimulatory molecule CD 86 is upregulated
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Recently, leukemia associated antigens (LAA) like WT-1, PRAME, MAZ and RHAMM have been identified by our group and others. For the feasibility of specific immunotherapies employing antigen peptides alone or pulsed on dendritic cells, it is of crucial importance that the targeted cells, i.e. leukemic blasts express molecules HLA-class I and II. Immunotherapies for patients with melanoma or prostate cancer have been hampered by the loss of HLA-class I molecules in up to 50 % of the patients. To determine the potential of cells for the presentation of LAA, expression of HLA-class I and II, and costimulatory molecules like CD40, CD80 and CD86 were examined by fluorescence associate cell sorting (FACS) using double staining against CD45 in 10 healthy volunteers and 10 patients with AML. A dim expression of CD45 in leukemia vs. a high ex-pression on normal leukocytes as described by others could not be detected. To dissect the subpopulations of lymphocytes form normal hematopoesis vs. leukemic blasts, a second FACS analysis with double staining against CD34 was performed in AML patients positive for CD34. The expression of HLA-ABC, HLA-A2, HLA-, CD40 and CD80 was similar in normal lymphocytes and leukemic blasts. The expression of CD86 was 0.5 to 1 log higher in leukemic blasts than in normal lymphocytes.This demonstrates that antigen presentation in leukemic blasts is not impaired by a loss of HLA- or costimulatory molecules.