demonstrating that they are selectively activated locally, and, suggesting specific recognition of tumor-associated antigens.

Conclusion: Altogether our results demonstrate that Treg are selectively recruited within breast tumors and are activated within lymphoid infiltrates containing mature dendritic cells (DC), resulting in immune escape through Tconv inhibition and ultimately tumor progression. As we previously described that plasmacytoid DC infiltration in human breast tumors was also correlated with an adverse clinical outcome (Treilleux et al, 2004), studies are in progress to investigate the interactions between PDC and Treg within breast tumors.

629 Poster

The WT1 antigen as a novel target for human leukemia-specific CD4+ T regulatory T cells

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Background: Recent studies demonstrated that regulatory T cells (Tregs) play an important role in regulating immune responses in cancer patients. The Wilms tumor antigen (WT1) is overexpressed in several cancers and it has been considered as a potential target for cancer immunotherapy. However, the generation of an effective anti-WT1-specific T cells has recently been shown to be largely affected by the presence of Tregs. We asked whether an anti-WT1 Tregs population exist in leukemia patients which may contribute to the impairment of anti-WT1 responses.

Materials & Methods: We used a pool of 110 WT1-derived peptides and a micro-scale WT1-peptide-set containing each peptide to identify an anti-WT1 Tregs epitope.

Results: We identified a Tregs population that specifically recognized a WT1-derived peptide (WT1-84) in an HLA-DRB1*0402/TCR-V beta 8-restricted fashion. These Tregs recognized HLA-DRB1*04-matched fresh leukemic cells expressing the WT1 antigen, exerted a Th2-cytokine profile, and had a CD4+CD25+Foxp3+GITR+CD127- Treg-phenotype. They significantly inhibited the proliferative activity of allogeneic MLR independently of cell-contact or cytokine production. Moreover, priming of allo-reactive T cells in the presence of Tregs strongly inhibited the expansion of NK; NK-T and CD8+ T cells; had an inhibitory effect on NK/NK-T cytotoxic activity but not on CD8+ T cells. The generated Tregs specifically produced Granzyme-B but not perforin and selectively induced apoptosis in WT1-84 pulsed-autologous APCs. Granzyme B produced by Tregs can induce apoptosis in target cells. Importantly, preliminary data indicated that anti-WT1-84 Tregs may exist in HLA-DR4-matched leukemia patients.

Conclusions: These findings will have important implications for the clinical manipulation of Tregs.

630 Poster

The prognostic value of intraepithelial and stromal innate immune system cells in non-small cell lung carcinoma

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Background: The major value of prognostic markers in potentially curable non-small cell lung carcinoma (NSCLC) should be to guide therapy after surgical resection. In this regard, the patient immune status at the time of resection may be important and also measurable. The immune system has paradoxical roles during cancer development. However, the prognostic significance of tumor infiltrating macrophages, natural killer (NK) cells and dendritic cells is controversial and not thoroughly studied, especially in the tumor stroma. The aim of this study is to elucidate the prognostic significance of these cells in the epithelial and stromal compartments of NSCLC.

Materials and Methods: Tissue microarrays from 335 resected NSCLC, stage I-IIIA were constructed from duplicate cores of viable and representative neoplastic epithelial and stromal areas. Immunohistochemistry was used to evaluate cells in epithelial and stromal areas with respect to CD68 (macrophage marker), CD56 (NK cell marker) and CD1a (dendritic cell marker).

Results: In univariate analysis, increasing numbers of stromal CD1a+ cells (P = 0.011) and CD56+ cells (P = 0.014) correlated significantly with an improved disease-specific survival (DSS). No such relation was noted for CD68+ cells or for epithelial CD1a+ and CD56+ cells. The prognostic significance of stromal CD56+ cells was an independent prognostic factor for DSS, P = 0.031 (HR 2.337, C.I. 1.081-5.049).

Conclusions: High density of stromal CD56+ cells is an independent factor associated with a better prognosis in resected NSCLC, suggesting that these cells might mediate a strong antitumor immune response in the tumor stroma.

631 Poster

CD44 promotes repopulation of thymus and T cell maturation in allogeneic bone marrow transplantation

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Background: Allogeneic bone marrow cell reconstitution (BMC) can provide an ultimate therapy in patients with hematological malignancies and solid tumors for which T progenitor cell homing into the thymus and maturation is of particular importance. In search for improving this protocol, we explored the impact of CD44 standard (pre T cell marker) and variant isoforms CD44v6 and CD44v7 on progenitor T cell homing and maturation.

Materials and methods: Progenitor cell homing into the bone marrow and the thymus was studied through short term (CFSE labelling) and long term reconstitution experiments. Prolifereation and apoptosis assays were performed with (H3)thymidine incorporation and Annexin V staining. In order to study the effect of CD44 on each subpopulation of thymocytes cells were sorted into double negative (CD4-CD8-), double positive (CD4+CD8+) and single positive (CD4+/CD8+) thymocytes with magnetic beads.

Results: CD44 has a major impact on progenitor cell homing into the bone marrow and the thymus. Antibody blocking studies and the transfer of CD44v7-deficient (CD44v7-/-) BMC provided evidence that bone marrow homing is also influenced by stromal cell CD44v7. Homing into the thymus was CD44v6 and CD44v7 independent. However, CD44v6 supported thymocyte expansion and apoptosis resistance. CD44v6 induced apoptosis resistance most strongly in double negative cells that was accompanied by Akt activation and Bcl-2 up regulation. In addition, CD44v6 induced proliferation of double negative thymocytes that proceeded via activation of the MAPK pathway. Distinct to early thymocytes, in double positive and single positive thymocytes CD44v6 only supported signal transduction via the TCR/CD3 complex.

Conclusions: Thus, CD44 plays a major role in hematopoietic stem cell homing and survival and is also required for thymus homing. CD44v6 in particular supports survival and expansion of early progenitor T cells. Accordingly, the transfer of CD44v6 transfected T progenitor cells can be expected to accelerate the reestablishment of a competent and host tolerant immune system.

632 Poster

Lentiviral TCR gene transfer for adoptive immunotherapy of cancer

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The immune system is often unable to mount effective T cell responses against tumours because tumour-associated antigens are poorly immunogenic. The introduction of alpha and beta chain genes of a specific TCR into T cells, has been shown a very promising therapy. The current clinical translation of this approach is based on gene transfer with retroviral vectors. However, TCR gene transfer using retroviral vectors can be achieved only after in vitro polyclonal stimulation of the target T cells, which may result in exhaustion and terminal differentiation. Lentiviral vectors are an attractive alternative to allow TCR gene transfer in the absence of polyclonal activation that may improve subsequent adoptive T cell therapy by maintaining naive phenotype and improved homing characteristics of gene modified T cells.

Lentiviral vector constructs have been generated containing both chains of an HLA-A*0201-restricted TCR specific for Wilms` tumour antigen 1 (WT1), myeloid leukaemias associated antigen. We analysed the effect of common gamma chain receptor cytokines IL2, IL7, IL15,IL21 on the transduction efficiency, proliferative potential, phenotype and functional activity of the WT1 TCR-transduced T cells. Primary T cells were successfully transduced after treatment with low-dose common gamma chain cytokines, either individually or in combination. All cytokines tested promoted the maintenance of a naïve phenotype as shown by expression of CD28 and CD62L. IL21 has shown to be important for homeostasis of naïve T phenotype of the transduced T cells. We hypothesize that more undifferentiated TCR-transduced T cells may demonstrate improved functional avidity in vivo than terminal differentiated T cell obtained by