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S77. Proffered paper: In vitro induced response patterns of antileukemic T-cells – characterization by combination of functional assays, spectratyping and next generation sequencing

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Background

T-cell receptor (TCR) diversity is characterised by somatic alterations in the complementary determining region 3 (CDR3) of the human TCR β-chain. Complemented with the TCR alpha-chain, TCR diversity can hypothetically result in up to 10^{18} different TCR molecules.

Myeloid leukaemic cells can be induced to differentiate into leukaemia-derived dendritic cells (DC_{leu}) regaining the stimulatory capacity of professional DCs while presenting the whole leukaemic antigen repertoire.

Our aim was to identify TCR Vβ-chain-rearrangements in T-cells stimulated with leukaemic blasts and DC_{leu} in 3 patients with AML and furthermore to detect, amplify or monitor T-cell clones with defined Vβ-profiles in correlation with antileukaemic function, in vitro and in vivo.

Material and methods

HLA matched or HLA haplo-identical (allogeneic) donor- or autologous T-cells were repeatedly stimulated, either with leukaemic blasts or the corresponding DC_{leu} from 3 different AML-patients. Cytotoxicity assay was carried out for measuring the lytic activity of effector T-cells, spectratyping was performed to identify the restriction of the TCR Vβ-repertoire in unstimulated and stimulated T-cells and Sanger sequencing to analyse the β-chain sequence information including the CDR3 regions. Additionally next generation sequencing (NGS) was established to analyse the accurate TCR sequence information of thousands of TCR β-chains with high coverage.

Results

No significant differences in T-cell proliferation were observed. The T-cell mediated cytolytic response patterns showed blast lysis (n=1) and blast proliferation (n=2).

Spectratyping revealed a remarkable TCR Vβ-restriction of the $CD4^+$- or $CD8^+$-TCR repertoire of blast- or DC/DC_{leu}-stimulated T-cells, independently of blast or DC/DC_{leu} used as stimulators. Although in absolute terms, DC/DC_{leu} stimulation induced the highest grade of restriction in the $CD8^+$ T-cell subset, the $CD4^+$ T-cells seemed to be relatively more affected.

In vitro stimulation with DC/DC_{leu} resulted in an identical TCR (β-chain restriction pattern) as identified in vivo in a patient sample 3 months after allogeneic stem cell transplantation (SCT).

Conclusion

A combined strategy using spectratyping and NGS with functional tests may provide useful information about the specificity and efficacy of the intra-individual variable induced T-cell response.

Spectratyping allows the identification of a restricted Vβ-repertoire by Gaussian-like distribution, NGS allows sequencing of TCR repertoires with high coverage, novel software allows the analysis of the exact length and sequence composition (the combination of the Vβ- and Jβ-genes) of the β-chains, especially of the CDR3, and the exclusion of non-functional transcripts.

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The identification of defined Vβ-T-cell clones may lead to selection procedures generating Graft-versus-Leukaemia reaction- but not Graft-versus-Host disease-mediating T-cells for adoptive immunotherapy after SCT.

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