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Blood 142 (2023) 4677–4678

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Distinct T Cell Receptor Gene Repertoires and T Cell Subset Distribution in Peripheral Blood and Bone Marrow of Patients with Multiple Myeloma

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Mounting evidence suggests that the T cell repertoire in multiple myeloma (MM) is actively shaped by antigen selection. However, the molecular underpinnings of this observation are not fully understood, while relevant studies have been mostly based on immunophenotypic characterization of T cell subsets, with considerably less information regarding T cell receptor (TR) gene repertoire profiles. Moreover, most available information derives from the analysis of a single tissue compartment [i.e. peripheral blood (PB) or bone marrow (BM)], thus hindering addressing the potential impact of local T cell responses. Here we sought to overcome these limitations through paired analysis of PB and BM aspirates of 20 newly diagnosed patients with MM before the administration of any treatment, from whom we isolated the PB and BM mononuclear cell (PBMC/BMMC) fraction, as well as CD4 $^{+}$ and CD8 $^{+}$ T cells. TR beta (TRB) chain gene rearrangements were RT-PCR amplified on RNA extracted from CD4 $^{+}$ and CD8 $^{+}$ T cells and then subjected to paired-end next generation sequencing (NGS). Raw reads (n=14,369,410 | median 179,618/sample) were processed through a purpose-built bioinformatics pipeline. Productive TRBV-TRBD-TRBJ gene rearrangements were taken into consideration (n=9,212,507 | median 115,156/sample) for the computation of clonotypes (i.e. TRB rearrangements with identical TRBV gene usage and amino acid complementarity-determining region 3 sequence). Overall, 1,005,150 TRB distinct clonotypes were assessed (median=12,565/sample). Both CD4 $^{+}$ and CD8 $^{+}$ T cell populations displayed skewed TRBV repertoires (7 TRBV genes and 3 TRBJ genes accounting for about ~40% and ~60% of the repertoire) in both BM and PB. All patients displayed oligoclonal T cell expansions in both CD4 $^{+}$ and CD8 $^{+}$ T cells, albeit these were significantly ($p<0.001$) more pronounced in CD8 $^{+}$ T cells for both BM and PB samples. That said, oligoclonality was more pronounced in BM vs. PB for both CD4 and CD8 T cells, reaching statistical significance in the former: in particular, the median cumulative frequency of the most expanded clonotypes/sample was ~40% higher in CD4 $^{+}$ BM T cells vs. CD4 $^{+}$ PB T cells ($p<0.001$). Additionally, a significant shift was noticed in the major clonotype repertoire of CD4 $^{+}$ and CD8 $^{+}$ T cells in both PB and BM. In more detail, the 10 most expanded clonotypes/sample in PB had significantly lower frequency in BM, and, vice versa, the 10 most expanded clonotypes in BM were represented at diminished frequency or were absent in the PB in 12/20 patients. Flow cytometry analysis revealed distinct T cell subset composition in CD4 $^{+}$ or CD8 $^{+}$ T cells, also in different tissue compartments (BM vs. PB). Significant ($p<0.001$) differences concerned the over-representation of: (i) effector T cells (CD45RA $^{+}$/CCR7 $^{-}$, Temra) in CD8 $^{+}$ vs. CD4 $^{+}$ T cells in both BM and PB (median frequencies in BM and PB: 22.5% and 7% for CD8 $^{+}$ T cells; 6% and 1.6% for CD4 $^{+}$ T cells); (ii) CD8 $^{+}$ Temra cells in BM vs. PB (median frequencies of 22.5% vs. 7% in BM and PB, respectively; $p<0.001$); (iii) CD4 $^{+}$ vs. CD8 $^{+}$ central memory T cells (CD45RO $^{+}$/CCR7 $^{+}$, Tcm) in both PB and BM (median frequencies in PB and BM: 42.7% and 49.1% for CD4 $^{+}$ Tcm and 14% and 18.8% for CD8 $^{+}$ Tcm); (iv) CD8 $^{+}$ effector memory T cells (CD45RO $^{+}$/CCR7 $^{-}$, Tem) in BM vs. PB (median frequencies of 21.8% vs. 2.7% in BM and PB, respectively); (v) CD8 $^{+}$ naïve T cells (CD45RA $^{+}$/CCR7 $^{+}$, Tn) in PB vs. BM (median frequencies of 67.7% vs. 24.2% in PB and BM, respectively). In conclusion, oligoclonal expansions of CD4 $^{+}$ and, particularly, CD8 $^{+}$ T cells in MM that are more pronounced in the BM (at least for CD4 $^{+}$ cells) argue for selection by BM-biased antigens, a claim also supported by the significant differences in the relative frequency of dominant clonotypes in BM vs. PB. The distinctive T cell subset distribution in the BM, characterized by

a significant increase of effector at the expense of memory T cells, likely reflects an antigen-driven albeit ineffective immune response.

Disclosures Chatzidimitriou: *Jansenn:* Other; *Novartis:* Other.

https://doi.org/10.1182/blood-2023-185872