ImmunoTools *special* Award 2014

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Study on the heterogeneity of CD16$^+$ myeloid cells in human blood that serve as precursors of inflammatory dermal dendritic cells

Immune activation in the skin can lead to the recruitment and development of inflammatory dermal dendritic cells (DCs) from human blood leukocytes; these may be CD14$^+$ monocytes or the ill-defined subset of CD16$^+$/CD14$^{+/-}$ monocytes. Recently, we provided evidence for a subtype of CD16$^+$ blood cells giving rise to inflammatory dermal DCs. In psoriasis slan(6-sulfo LacNAc)DCs circulate in the blood in an immature state, are found in and beneath the dermal vasculature and locally express IL-23, TNFα and iNOS. SlanDCs show parallels to a larger population of myeloid cells lacking the marker-antigen slan but sharing with slanDCs the expression of CD16 (FcγRIII). Further definition of these CD16$^+$ myeloid cells is hindered by the lack of specific markers and the rapid loss of CD16 expression after activation. To this end we set up a program to generate monoclonal antibodies with specificity for CD16$^+$ myeloid cells. BALB-c mice were repeatedly immunized with purified CD16$^+$ cells and >1x10^3 monoclonal antibodies were generated. Out of these the mAb clone DD3 turned out to be specific for a subset of CD16$^+$, CD14$^{low\text{-}negative}$ blood cells. Double staining with anti-slan (clone M-DC8) and the mAb DD3 revealed specificity for a CD16 expressing population overlapping but not identical with slanDCs. For the phenotypic analysis the CD16$^+$ CD14^{low+} myeloid cells were subdivided in three populations: 1) single mAb DD3$^+$ slan$^-$ cells, 2) DD3$^+$ and slan$^+$ cells and 3) single slan$^+$ DD3$^-$ cells.

We take whole blood from healthy donors and isolate peripheral blood mononuclear cells via Ficoll gradient separation. Subsequently we stain the cells with surface markers as HLA-DR, CD16, CD14, slan and DD3. For detailed phenotypical distinction of these populations we want to compare several additional surface markers as CD1a, CD3, CD54 and others via flow cytometry analysis. Therefore the markers from ImmunoTools would advance our project and could be very enlightening.

For functional studies we employed intracellular cytokine staining of PBMC stimulated for 18 hours with the TLR4-ligand LPS (100ng/ml) in the presence of Brefeldin (1µg/ml). For comparison $CD14^+$ monocytes, $CD1c^+$ DC and $CD141^+$ DCs were also studied. These studies revealed the highest IL-12p40/70 expression for slanDCs and the yet not further characterized population stained positive with the new mAb DD3. Now we aim to round off our functional studies with the help of ImmunoTools cytokine ELISAs.

Taken together with the mAb DD3 we describe a novel marker allowing for a more accurate functional, phenotypical and molecular definition of $CD16^+$ myeloid cells; a cell population among which the subset of slanDCs were already shown to be relevant to skin immunity. To complement our project the antibodies from ImmunoTools would be very helpful.

ImmunoTools *special* AWARD for **Priscila Wölbing** includes 23 reagents

FITC - conjugated anti-human CD1a, CD45RB, CD54, HLA-DP,

PE - conjugated anti-human CD44, CD52, CD105, CD177, IL-6,

PerCP - conjugated anti-human CD3, CD20, CD45,

APC -conjugated anti-human CD16, CD38,

human IL-4 ELISA-set, human IL-8 ELISA-set, human IL-12p40 ELISA-set
(each three reagents) DETAILS