Analysis of influenza-specific T cells in humans

PBMCs of vaccinated donors were isolated as described. The principle gating strategy is shown in Fig. 1A. Because of donor specific variabilities the gating of $CD8^+$ T cells against a dump channel containing an anti-CD14, anti-CD19, and anti-CD56 staining had to be adjusted for each donor (Supplementary Fig. 1). Depending on the individual HLA isotype, 7 different HLA-multimer reagents (Supplementary Table 1) were used to detect influenza-specific T cells within the $CD8^+$ T cell population. All samples stained with the same HLA-multimer were analyzed as a group with a fixed gate for influenza-specific T cells of $CD8^+$ T cells (Supplementary Fig. 1). Therefore, donor-specific variances can be seen in percentage values, while a subjective impact during gating is avoided. To compare different HLA incompatible donors with high variances with each other the fold change of influenza-specific $CD8^+$ T cells was calculated for each donor (Fig. 1 D).

Deep sequencing and pathway analysis

C57BL/6 and IFNAR-B mice were infected with 10^5 pfu MVA. After 24 h B cells were isolated from spleens using the untouched magnetic B cell separation kit (Miltenyi). FACS sorting of B8-specific $CD8^+$ T cells from spleens was conducted using a MoFlo XDP cell sorter (Becton Dickinson). RNA isolation was conducted using the NucleoSpin RNA kit (Macherey-Nagel). mRNA sequencing was performed at TRON (Translational Oncology Mainz, Germany). After sequencing, the raw reads that were generated by sequencers, were saved in the fastq format. To obtain reliable clean reads, the dirty raw reads were filtered according to the following criteria based on Trimmomatic software [1]: (i) reads with sequence adaptors were removed; (ii) reads with more than 5% 'N' bases were removed; (iii) reads with length < 20 bases were removed; (iv) 3' end of Q (Q= -10 logerror ratio) less than 10 of the base quality were removed; (v) low- quality reads, in which less than 50% of the quality were > 20 bases were removed. All subsequent analyses were based on clean reads. Tophat v2.0.9 (http://tophat.cbcb.umd.edu/) spliced mapping was used to map the cleaned reads to the mouse mm10 reference genome with two mismatches. After genome mapping, Htseq-count [2] was used to calculate the raw-gene count and the count matrix was used for differential expression analysis using DESeq2 [3] in R package [4]. The log2 fold change from the normalized expression was calculated with respect to mock controls. The genes satisfying the criteria of absolute log2 fold change value of greater and equal to 1 were considered as differentially expressed. Moreover, to compensate for false-positive at this significance threshold, an adjusted p-value correcting for multiple testing of less than 0.05 were finally considered as significantly differentially expressed genes. Overall, a gene is said to be significantly differentially expressed if it follows the criteria of adjusted p-value <= 0.05 and absolute fold change of >= 2.

References:

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