Abstract

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Analysis of association of MHC and KIR variants with the course of lentivirus induced disease by next-generation sequencing

Thesis

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Background: The aim of this work was to use the SIV infection in macaques (the only available model of human HIV infection) to show potential differences in MHC I haplotypes in macaques experimentally infected with SIVmac239 (or SHIV clade C) and their correlation with low or high viral loads, using pyrosequencing.

Methods: The RNA samples from leukocytes from experimental macaques were reversely transcribed into cDNA and subjected to PCR of the 190 bp fragment of MHC I using fusion primers containing MIDs and FastStart High Fidelity Taq DNA polymerase. Subsequently the PCR generated samples were subjected to pyrosequencing using GS Junior System (Roche) according to the manufacturer`s protocol. The generated data were analyzed by a series of data filtration steps using scripts in Python and subsequently by DNAStar Lasergene 11. Final alleles were identified by ncbi-blast+ 2.25.

Results: A total of 98 known aleles and 11 new aleles of MHC class I were found. The analysis identified those aleles, which correlate with fast or slow disease course. The aleles associated with slow disease course are: Mamu - A1*004, A1*007, A1*019, B*029, B*041, B*047, B*048, B*065, B*069. Conversely, aleles associated with fast progression are Mamu - A1*003, A1*003:02, A1*007:03, A1*023, A1*024, B*005, B*028, B*040, B*052, B*055, B*058. Sequencing errors were found in 0,31 % of bases.

Conclusion: The comparison of MHC I expression in the two groups of macaques identified individual alleles, which correlate with the disease course. Further experiments should focus on these alleles to confirm our data. The pyrosequencing method allowed for a detailed analysis of the MHC class I alleles in macaques. The sensitivity of this method allows for an identification of the allelic variants expressed at low levels and gives a detailed picture of alleles in the individual organism. The error rate of this method, however, cannot be disregarded and lead to difficulties in the discovery of single-nucleotide substitutions.