Summary

Macrophages are widely believed to be among the first cells to become infected following exposure to HIV-1, to be important for the establishment of infection, and to contribute significantly to both the latent as well as the anatomical viral reservoir in HIV-1-infected individuals. Both these reservoirs may form major obstacles in the complete eradication of HIV-1 from an infected individual with currently available combination antiretroviral therapy (cART) and most likely contribute to residual replication during cART and to viral rebound after cessation of cART. In addition, HIV-1-infected monocytes/macrophages play an important role in the etiology of HIV-1-associated dementia (HAD), as well as in the onset of other pathologies such as HIV-1-associated cardiovascular disease and AIDS-related lymphomas. Previous *in vitro* studies reported differences in HIV-1 replication between monocyte-derived macrophages (MDM) from different individuals, suggesting that host genetic factors may influence HIV-1 replication in this cell type. This could be relevant to the above mentioned clinical aspects that have been associated with HIV-1-infected monocytes/macrophages. To identify novel host factors that may contribute to this variation, we investigated whether host genetic variation was associated with differences in HIV-1 replication in MDM of different individuals. By strictly controlling viral and environmental factors we could assume that the observed variation in HIV-1 replication in MDM was caused by genetic differences between hosts such as single nucleotide polymorphisms (SNPs), the most common form of genetic variation. An association between a SNP and *in vitro* HIV-1 replication in MDM may be indicative of the importance of the genetic locus tagged by the SNP for HIV-1 replication in these cells.

We developed a standardized method for determining variation in *in vitro* HIV-1 replication in MDM and subsequently used blood samples from over 500 healthy blood donors to quantify this between-person variation (*Chapter 2*). A more than 3 log difference in *in vitro* HIV-1 replication in MDM was observed which could be explained in part by the presence of the previously described 32 base pair deletion in *CCR5*, encoding an important co-receptor for HIV-1. However, strong evidence was also obtained that other – non-entry related – host factors influence replication of HIV-1 in MDM. We subsequently performed a genome-wide association study (GWAS) using the variable *in vitro* HIV-1 replication in MDM as a phenotype (*Chapter 3*). DNA was used from Caucasian donors whose MDM ranked in the quartile of donors with lowest or the quartile of donors with highest HIV-1 Gag p24 production (a measure for HIV-1 replication) for genotyping more than 500,000 SNPs. For analysis, an additive relationship between the genetic variant and the phenotype was assumed. Using linear regression we found associations between the minor alleles of intronic SNPs in *DYRK1A* and *PDE8A*, and lower HIV-1 Gag p24 levels in MDM cultures.
Multivariate analysis showed that these associations were independent of the *CCR5* Δ32 genotype. However, none of the associations were genome-wide significant, meaning statistically significant after correction for multiple testing. To obtain independent validation of these findings, both *in vitro* and *in vivo* approaches were used. The association between *DYRK1A* SNP rs12483205 genotype and variation in HIV-1 replication was again observed after HIV-1 infection of MDM from 31 additional and independent donors. In addition, the minor allele of *DYRK1A* SNP rs12483205 was found to be associated with slower disease progression in two of four cohorts of HIV-1-infected individuals. No effect of this SNP on *DYRK1A* pre-mRNA splicing or *DYRK1A* mRNA levels was found and further studies are needed to delineate its effect on *DYRK1A* function as well as on HIV-1 replication. The minor allele of SNP rs2304418 in *PDE8A*, a gene previously identified to affect HIV-1 replication in genome scale RNAi studies reporting several hundred novel HIV-1 dependency factors (Chapter 4), was associated with lower mRNA levels of the various *PDE8A* transcript variants and lower levels of HIV-1 replication in MDM. In agreement with this, knocking-down *PDE8A* mRNA resulted in reduced HIV-1 replication *in vitro*. While sequencing *PDE8A* gene regions generally known to be involved in gene expression (promoter region and 5’ UTR) or mRNA stability (3’ UTR) did not reveal more likely causal SNPs, multiple intronic *PDE8A* SNPs in high linkage disequilibrium with SNP rs2304418 were predicted to affect putative transcription factor binding sites.

In Chapter 5 we determined whether SNPs that affected *in vitro* HIV-1 replication in macrophages, as identified by the GWAS in Chapter 3, contributed to the pathology of HAD. In addition, all common genetic variants previously investigated for their relationship with the etiology of HAD, as well as a SNP in *PREP1*, coding for a transcription factor recently shown to bind the *MCP-1* promoter, were included for this candidate SNP analysis. In this case-control study, genotype frequencies were compared between 72 patients with HAD (cases) and 241 AIDS patients without HAD (controls). While no difference in genotype distribution between cases and controls was found for the SNP in *MCP-1* or any of the other SNPs tested, a significant difference was found for an intronic SNP in *PREP1*. Interestingly, this locus has been previously associated with cholesterol levels and cognitive test performance by two independent recently GWAS, supporting the hypothesis that genetic variation in this gene may contribute to the etiology of HAD.

In Chapter 6 host genetic variation contributing to HIV-1 susceptibility *in vivo* was studied. DNA from 60 seronegative individuals that reported high-risk sexual behavior was compared with DNA from over 400 HIV-1-infected patients. The subsequent GWAS identified multiple SNPs, although none of these associations reached genome-wide significance. For some of the SNPs that showed the strongest association with HIV-1 susceptibility, similar differences in genotype distributions were observed in an independent French cohort, where HIV-1-infected patients were compared with DNA from healthy controls. Obviously, larger study populations, ideally also from other ethnicities, or functional follow-up experiments
will be required to determine if these associations indeed affect susceptibility to HIV-1 infection.

All together, the data derived from these genetic studies on HIV-1 replication in macrophages, the etiology of HIV-1-associated dementia, and on differences in susceptibility to HIV-1 infection resulted in the identification of several interesting novel loci not previously associated with these phenotypes, and may offer new leads for the development of novel antiretroviral medications.