Host-control of HIV

Balance between immunity and immunopathology

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CHAPTER 9

9

General Discussion

GENERAL DISCUSSION

HIV-1 infection is marked by the gradual loss of CD4 T cells as well as chronic activation. This chronic immune activation involves both the innate and adaptive immune system, resulting in overall immune dysfunction. The induction of an adequate antiviral immune response on one side and an overactive immune system on the other contributes to the highly variable outcome of HIV-1 infection. In this thesis, the innate and adaptive immune responses to HIV-1 infection in relation to disease progression were investigated.

Innate and adaptive immune responses in HIV-1 infection

Interferons (IFN) are amongst the first molecules excreted in response to viral infections, and subsequent IFN receptor signaling induces the transcription of interferon stimulated genes (ISGs) [1, 2]. The induction of type I IFNs and ISGs in the early stages of infection prevent viral replication and limit viral spread, and are important for the induction of the adaptive immune response [3-8]. However, when IFN responses persist this can also lead to chronic immune activation and disease progression [9-13]. Several studies have shown that in non-pathogenic simian immunodeficiency virus (SIV) infection the ISG expression is high during the acute phase of infection and declines during the chronic phase of infection when viral replication is controlled [9, 10, 14]. In pathogenic SIV infection, disease progression is linked to sustained upregulation of ISGs [9, 10, 14]. In HIV-1 infection, upregulation of ISG expression was linked to CD4 T cell depletion and disrupted T cell dynamics [11]. Further insights into the mechanisms behind ongoing immune activation observed in HIV-1 infection could provide novel targets to resolve the immune imbalance. In **chapter 2** we investigated the longitudinal IFN and ISG response in people with HIV (PWH) during HIV-1 infection and on ART, and compared this to expression levels before seroconversion. Previous studies have revealed increased expression of type I IFNs IFN-a and IFN-b in the acute phase of SIV infection [12, 15]. In contrast, we observed decreased levels of type I IFNs at 5-9 months after seroconversion. This discrepancy could be due to our relative late sampling and the use of PBMC rather than lymph node tissues. The observed increased expression of a cluster of type I and II ISGs, RSAD2, ISG15, IFI44L and IFI27, after HIV-1 infection, correlated positively with viral load during HIV-1 infection and predicted viral load during disease progression before the initiation of ART. The expression of these type I and II ISGs strongly correlated with sCD163, a monocyte activation marker, but not with T cell activation after HIV-1 infection. After ART initiation the expression levels of the cluster of type I and II ISGs returned to pre-seroconversion levels however, T cell activation remained elevated compared to pre-seroconversion levels indicating that the immune recovery is incomplete. In conclusion, the results presented in **chapter 2** indicate that induction of type I and II ISGs is mediated by monocyte activation and likely driven by viral replication. ART initiation

reduced plasma viremia to undetectable levels which normalized expression of type I and II ISGs.

In **chapter 3, 4, and 5** we studied how genetic variation can impact the immune response and aimed to identify the potential mechanisms involved. Individuals carrying two linked single nucleotide polymorphisms (SNPs), rs7262903 and rs7269320, in the gene encoding for mitochondrial antiviral-signaling protein (MAVS) experience a prolonged asymptomatic disease course [16]. MAVS is involved in the signaling pathway downstream from viral sensor RIG-I and has the potential to induce antiviral type I IFN and cytokine responses [17, 18]. However, HIV-1 is able to interfere with MAVS-signaling through polo-like kinase 1 (PLK1) binding to MAVS, impeding MAVS-induced antiviral responses [16, 19]. Individuals carrying two linked SNPs in MAVS (MAVS minors) have lower viral load in plasma at set-point and show delayed increase of viral load during infection [16]. This indicates that the HIV-1-induced PLK1-dependent suppression of the MAVS protein and the subsequent antiviral responses, are lifted in individuals carrying the MAVS minor genotype. In **chapter 3**, the mechanisms behind control of HIV-1 infection in individuals with the MAVS minor genotype were investigated during 7-year follow up. We observed that these individuals had more stable CD4 T cell counts and smaller HIV-1 reservoirs. However, no changes in immune activation, T cell exhaustion, senescence or cytokine production were observed. Individuals with the MAVS minor genotype showed lower naive CD4 T cells likely due to preservation of the memory/effector CD4 T cell pool. In addition, in vitro infection of PBMC from individuals with the MAVS minor genotype showed decreased HIV-1 replication. The protective effect of the MAVS minor genotype appears to be due to initiation of innate responses that affect viral replication and susceptibility of CD4 T cells to HIV-1 infection.

In **Chapter 4**, we showed that PWH carrying SNP rs4131564 in fucosyltransferase 8 (FUT8) have an accelerated disease progression. FUT8 is uniquely involved in the core fucosylation of N-glycan structures, which is an important post-translational modification [20, 21]. Core fucosylation plays an important role in T cell signaling and T cell activation as the T cell receptor is heavily core fucosylated [22-24]. Furthermore, the majority of glycans on IgG in plasma are fucosylated [25] and this affects IgG effector functions [26, 27]. Changes in fucosylation could thus impact the immune response to HIV-1 infection by the modulation of the T cell and antibody effector function [23, 26-28]. Investigations into the mechanisms behind the accelerated disease progression observed in PWH carrying the SNP in FUT8 revealed no major differences in T cell activation and differentiation. Moreover, antigen specific T cell responses and cytokine production was similar between PWH carrying SNP rs4131564 in FUT8 and those without. In addition, the SNP was not associated with IgG fucosylation and fucosylation levels of serum N-glycans were similar between carriers of the SNP and those without the SNP. The HIV-1 envelope itself is also a heavily glycosylated structure and could therefore be affected by the SNP in FUT8. However, no difference in susceptibility to viral replication was observed in donor PBMC with or without SNP

rs4131564 and viruses produced in these cells were equally sensitive to neutralization by broadly neutralizing antibodies. Altered core fucosylation can also affect gut fucosylation, which has been associated with a less diverse microbiome and increased inflammation [29]. Serum levels of gut integrity marker iFABP and monocyte activation marker sCD163 were similar in PWH with SNP rs4131564 and without. Although no major differences were found in the immunological and virological assays performed, it is possible that small functional differences in immune response or gut integrity may over time cause major differences in the disease outcome. Moreover, the naturally occurring genetic differences among individuals in the study population, may have masked the impact of the SNP on the immune response. Additional studies in which SNP rs4131564 in FUT8 is studied in the same genetic background by introduction or removal of the SNP using CRISPR-Cas, may reveal further insights in the functional consequences of this polymorphism.

Polymorphisms in human leukocyte antigen (HLA) class I alleles can also have a major influence on the course of HIV-1 infection [30, 31]. The HLA class I allele HLA-B*35 has been linked to accelerated disease progression [32-34] whereas alleles HLA-B*27 and HLA-B*57 are associated with long-term non-progression [30, 35, 36]. HLA molecules present viral peptides to CD8 T cells which will lead to the destruction of cells that express foreign proteins by cytotoxic CD8 T cells. In HIV-1 infection control by CD8 T cells is lost due to dysfunction [37-39]. However, in long-term non-progressors (LTNP) carrying protective HLA alleles strong HIV-specific CD8 T cell responses are observed throughout infection [40-42]. In **chapter 5** the transcriptional profiles of HIV-specific CD8 T cells from LTNP and progressors were characterized to better understand the mechanisms of CD8 T cell dysfunction. Our analysis revealed unique transcriptional profiles for each group. Based on the transcriptional analysis and pathway analysis, CD8 T cell dysfunction in progressors appeared to be associated with decreased functionality of RNA and protein metabolism. Previous studies analyzing HIV-specific CD8 T cells of progressors found upregulation of genes related to T cell exhaustion [43, 44]. In contrast, we did not observe increased expression of canonical hallmarks of exhaustion. However, in our study we selected samples from PWH during the asymptomatic phase of disease and relatively high CD4 T cell counts, at which time a clear upregulation of genes related to T cell exhaustion may not yet be evident. Exhausted T cells were previously shown to have an altered bioenergetics metabolism [45] and the transcriptional profile related to RNA and protein metabolism dysfunction, could thus be an indication of exhaustion. Our data indicate that dysfunctionality of HIV-specific CD8 T cells may be driven by changes in the energy demanding RNA and protein metabolism.

Natural immune control of HIV-1 infection

Natural immune control of HIV-1 is possible, although it is rare, and it appears to be at least in part mediated by protective HLA alleles. As mentioned in **chapter 1**, in addition to the

potent CD8 T cell responses observed in LTNPs carrying protective alleles, viral variants with escape mutations in HLA-restricted epitopes that cause viral attenuation are selected in these individuals. Natural control of HIV-1 is also observed in rare PWH that do not possess these protective HLA alleles. In **chapter 5** we included a group of individuals that did not carry the protective HLA-B*27 or HLA-B*57 alleles but showed prolonged control of HIV-1 infection. These non-B*57 LTNP formed a distinct unique cluster in a principal component analysis based on the transcriptional profile of HIV-specific CD8 T cells. The exact mechanism behind control in the non-B*57 LTNP is unclear but our data hints towards increased functionality of CD8 T cells. Furthermore, genetic polymorphisms in immune related genes might at least in part account for the increased CD8 T cell functionality. Apart from the spontaneous control observed in LTNP there are PWH that manage to control viral replication for extended time after the cessation of ART, the post-treatment controllers (PTCs). In most ART-treated PWH interruption of therapy will lead to rebound of viral levels due to the presence of the viral reservoir which is established early in infection. The initiation of ART in the acute phase of infection reduces reservoir size [46-48] and this is thought to be an important element in accomplishing post-treatment control [47, 49-51]. Furthermore, early initiation of ART likely also preserves the antiviral immune response leading to improved control of HIV-1 infection [52, 53]. Indeed, previous studies have implicated a role for NK and CD8 T cell responses in post-treatment control [54-56]. **Chapter 6** describes the immunological and virological characteristics of a PTC who has been successfully controlling HIV-1 infection for 23 years after discontinuing ART. This individual initiated treatment in the acute phase of infection after presenting with high viral loads. ART effectively suppressed viral replication but was discontinued after 26 months. The individual did not carry HLA-B*57 or B*27 alleles further confirming that this individual is not a LTNP. Interestingly, robust HIV-Gag-specific CD8 T cell proliferative responses were observed over the course of infection. Moreover, a mutation was found in a HLA-B*44:02-restricted Gag epitope which was associated with decreased viral replication. Reminiscent of the mechanism of control observed in LTNP carrying protective HLA alleles [40, 41, 57-59], HIV-1 is controlled by a strong CD8 T cell response in combination with attenuated viral replication in this PTC. Together the data of **chapters 5 and 6** strongly indicate that an adequate HIV-specific CD8 T cell response is an essential component in control of HIV-1 infection.

SARS-CoV-2 vaccination response in PWH

PWH are more susceptible to opportunistic infections due to the immunocompromised state of their immune system. Recently, it has been demonstrated that PWH are more at risk for having severe Coronavirus disease 2019 (COVID-19) outcome and mortality [60-62]. Approximately one year after the SARS-CoV-2 pandemic started, the first vaccines against

this virus became available and proved to be highly effective to prevent symptomatic and severe COVID-19 [63, 64]. However, concerns were raised about the immunogenicity of SARS-CoV-2 vaccines in PWH, as reduced responsiveness to a variety of vaccines against other pathogens has previously been observed in PWH [65, 66]. Studies on the SARS-CoV-2 vaccination response in PWH show discordant findings, with some finding comparable antibody and T cell responses comparing PWH to controls [67, 68] and others showing lower responses in PWH [69, 70]. The discrepancy between the observations is possibly due to differences in for instance age and sex, between PWH and control groups in studies reporting lower responses in PWH. Moreover, studies have shown that individuals with severe immunosuppression (CD4 T cell counts <200) have decreased antibody and T cell responses to SARS-CoV-2 vaccination [71, 72].

In **chapter 7** we compared the humoral and cellular response to SARS-CoV-2 vaccination between older PWH on successful ART and individuals without HIV-1 with similar demographics and lifestyle. The vaccine response in PWH and controls was comparable with regard to anti-S antibody levels, neutralizing antibody titers and memory B cell responses. Moreover, we observed higher SARS-CoV-2 specific IFN-γ responses in PBMC of PWH both before and after vaccination, however the number of SARS-CoV-2 reactive T cells were comparable between PWH and controls after vaccination. Interestingly, the higher IFN-γ response after vaccination was associated with increased expression of CD163 on classical monocytes, irrespective of HIV status. PWH had significantly higher expression of monocyte activation marker CD163 on classical monocytes compared to controls. This indicates that PWH have elevated immune activation, which may explain the higher IFN-γ release in the absence as well as presence of stimulation with SARS-CoV-2 peptides [73]. Durability of the vaccination response in PWH and response to booster vaccinations has not been assessed in our study. It is possible that the SARS-CoV-2 vaccination responses in PWH may wane more rapidly, as is observed with vaccinations against other pathogens [65], and that PWH would thus require more frequent booster immunizations.

Overall, the results presented in **chapter 7** show that PWH on ART with preserved CD4 T cell counts (>500/µl blood), mount a robust response to SARS-CoV-2 vaccination which underscores the importance of successful treatment. However, indications of immune activation remain present as indicated by the increased monocyte activation and the higher background IFN-γ response in PWH.

Novel treatment avenues

The development of novel ART medications is pivotal as resistance against current treatments occurs. Furthermore, new medications are more effective and better tolerated than older ones which improves treatment adherence further limiting the emergence of

resistant virus variants. One of the targets explored for new treatments is the p24 capsid protein (CA) of the Gag polyprotein, which is encoded by one of the more conserved regions of the HIV-1 genome and thus has limited variability providing a barrier to resistance [74]. The first capsid inhibitor that was approved for treatment by the EMA and FDA in 2022 has its effect both in the early and late stages of viral replication interfering with virion stability [75, 76]. However, the search for more novel CA inhibitors continues. In **chapter 8** we describe several compounds that were screened for their ability to inhibit CA assembly. One of the inhibitors showed a suppressive effect on HIV-1 replication. Further analysis revealed that reverse transcription of HIV-1 was not inhibited with the compound indicating that the suppressive effect takes place at a later stage of viral replication. Moreover, this inhibitor specifically binds monomeric CA likely at the interface between two monomers, possibly interfering with oligomerization of monomers into the mature capsid structure and/or transport of capsid. An advantage of this capsid inhibitor is that it likely inhibits HIV-1 relatively late in the viral replication cycle and is therefore active in cells that harbor an integrated HIV-1 provirus. Upon reactivation of viral transcription, this inhibitor does not block the production of viral proteins and allows viral peptide presentation by HLA molecules to activate antigen specific T cells responses. Moreover, the compound described can be synthesized at low cost, has high metabolic stability and has low toxicity making it an interesting molecule for further optimization as ART.

Concluding remarks and future directions

The outcome of untreated HIV-1 infection is determined by the fine balance between proper induction of a robust virus specific immune response and the aberrant overstimulation and dysfunction of the immune system. In this thesis we showed that HIV-1 infection induces a robust ISG response, which is linked to monocyte activation and driven by viral replication. This ISG response remains present over the course of untreated infection and may contribute to the overall immune dysfunction. Notably, impaired CD8 T cell functionality is already observed during the asymptomatic phase of disease as indicated by a transcriptomic profile related to immune metabolic changes. Moreover, we observed that polymorphisms in genes involved in the immune response influence the outcome of infection by shifting the balance resulting in better control of HIV-1 replication through innate as well as adaptive immune responses.

Initiation of ART at least in part restores the immune balance, as demonstrated by normalization of ISG levels and the induction of protective T cell and antibody responses upon COVID-19 vaccination. However, residual immune activation and exhaustion is still observed in PWH on effective ART [77, 78] and this increases the risk of age-related comorbidities [79]. Early ART treatment initiation or additional therapeutic strategies that

preserve or improve the immune system may thus further advance the health of PWH on effective ART.

Restoration of the immune balance should also be taken into consideration in current HIV cure research. The success of the 'shock and kill' strategy for HIV cure, which relies on the immune response for elimination of the viral reservoir upon latency reversal, has thus far been limited [80-82]. CD8 T cells are able to eliminate infected cells and provide long term control of HIV infection, as demonstrated in PWH carrying protective HLA alleles as well as in PTCs. Interestingly, we and others have shown that the cellular metabolism plays a crucial role in the maintenance of CD8 T cell functionality. Further research into drivers of metabolic dysfunction and dynamics of metabolic processes that regulate T cell effector function and exhaustion may provide insights into underlying mechanisms. Therapeutic strategies aimed at improving the metabolic function of virus-specific CD8 T cells could be a novel target for cure research to reverse immune dysfunction and induce control of HIV-1 infection. In addition, ART containing compounds like a capsid inhibitor described in this thesis, could induce virus specific CD8 T cell responses since residual viral protein production will allow presentation by HLA molecules to cytotoxic T cells. Therefore, future therapeutic cure strategies should be focused on both targeting of the viral reservoir as well as restoration of the immune balance.

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