Viro-immunological studies on the role of Epstein-Barr virus in the development of AIDS-related non-Hodgekin's lymphoma
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Summary

In the studies presented in this thesis we aimed to obtain more insight into the role of the Epstein-Barr virus and EBV-specific immunity in the pathogenesis of AIDS-related NHL occurring in HIV-1 infected individuals. To understand how AIDS-NHL develops in HIV-infected individuals we performed both virological studies (part I), to define parameters that may predict the occurrence of an AIDS-NHL, and immunological studies (part II), to elucidate the role of (defective) EBV immunity in the pathogenesis of AIDS-NHL.

**Part I: virological studies**

No specific role for EBV type 2 in AIDS-related lymphomas

To investigate a possible relation between the less common EBV type 2 infection and the occurrence of AIDS-NHL, we investigated the prevalence of EBV type 2 in different subgroups of HIV-infected individuals using a newly developed sensitive nested-PCR assay enabling assessment of EBV types directly in PBMC.

In chapter 2, we showed that in HIV-1 infected individuals there is a high prevalence of EBV type 2 infection (55%) and superinfection with both type 1 and 2 (37%). Subgroup analysis did not show a difference in EBV type 2 prevalence between LTA (50%), other progressors (62%) and AIDS-NHL patients (53%). In addition, no difference was found in the prevalence of superinfection with both type 1 and 2 between LTA (24%), other progressors (40%) and AIDS-NHL patients (47%). EBV type analysis on PBMC samples obtained from AIDS-NHL patients in the course of HIV-1 infection revealed that EBV type 2 infection can be found already early in HIV-1 infection. Therefore, we concluded that EBV type 2 (super)infection is not associated with an increased risk for development of AIDS-NHL and hypothesized that EBV type 2 infection in HIV-infected individuals is merely a mirror of the EBV type 2 infection of the homosexual population in general.

**EBV type 2 is highly prevalent among homosexual men and this is caused by sexual transmission**

To test whether EBV type 2 infection is more prevalent among homosexual men in general, we subsequently determined the prevalence of EBV type 2 in the homosexual population as discussed in chapter 3 and compared this with prevalence of EBV type 2 in heterosexuals at high and low risk for sexual transmitted diseases (STD). In homosexual men indeed EBV type 2 prevalence was significantly higher than in heterosexual men (39% vs 6%), confirming our hypothesis. Interestingly, we found that in high risk (for STD) heterosexual men EBV type 2 prevalence was significantly higher than in low risk heterosexual men (15% vs 0%). This suggests that sexual behavior is involved in EBV type 2 prevalence in a non-endemic area. Indeed, because we found that EBV type 2 infection in homosexual men was significantly associated with increased numbers of intercourse
partners and HIV-seropositivity, we concluded that the high prevalence of EBV type 2 among Caucasian homosexual men is caused by sexual transmission of EBV type 2.

No predictive role for EBV load in development of AIDS-NHL

In chapter 4 we studied EBV load in several subpopulations using a quantitative real-time PCR assay to determine the number of EBV copies per $10^6$ PBMC. EBV load in HIV+ homosexual men was found to be ~100-fold higher than in HIV- homosexual men who had a higher EBV load than HIV- heterosexual individuals in PBMC. These differences were not related to degree of immunosuppression or EBV type(s) present, but could possibly be caused by chronic antigen stimulation and be related to sexual behavior.

In search for a parameter predicting the occurrence of an AIDS-NHL we studied EBV load in the course of HIV-1 infection. (chapter 4) In a longitudinal analysis, we compared EBV load in the course of HIV-1 infection in AIDS-NHL patients, HIV-1 infected individuals who progressed to AIDS without a lymphoma and long-term asymptomatic (LTA) individuals. In all individuals overall EBV load proved to be high and displayed considerable fluctuations over time. Absolute EBV load in PBMC was not predictive of the development of AIDS-NHL. We suggest that balance between load and EBV-specific immunity may be more predictive. Indeed, in several AIDS-NHL patients EBV load showed a progressive increase, suggestive of decreasing immune control, whereas in LTA and progressors to AIDS bursts of EBV load were followed by a decrease to baseline levels, suggestive of EBV-control.

Because EBV in serum (or plasma) has been reported to be predictive of lymphoma diagnosis in transplant recipients, we compared EBV load in PBMC with EBV load in serum. In most individuals tested EBV load was also detectable in serum at some time points, but at a much lower level. Overall, no correlation was found between EBV load in PBMC and serum.

Part II: Immunological studies

Functional loss of EBV-specific CD8+ T cells and increased EBV load in AIDS-NHL patients due to loss of CD4+ T cells

Our group demonstrated a loss in EBV-specific CTL precursors in a previous study. To investigate the underlying cause we studied whether the decrease in EBV-specific CTL is due to either physical loss or dysfunction using newly developed methods to measure CTL (chapter 5). In the same study we used the quantitative PCR assay to measure EBV load and correlated this with data on EBV-specific cellular immunity. The number of virus-specific T cells was detected using tetrameric HLA-EBV-peptide complexes; function of these EBV-specific T cells was determined using the INFy-ELISpot assay. We observed that EBV-specific CD8+ T cells were not physically lost. However, over time there was a considerable decrease in the capability to produce IFNy, a marker of loss of CTL function. In HIV-infection, this loss of function correlated with lower CD4+ T cell numbers,
suggesting that CD4+ T cells are important for maintaining the functional capacity of virus-specific CD8+ T cells. Furthermore, this loss of function was accompanied by increasing numbers of EBV virus particles. In long-term asymptomatic HIV-1 infected individuals, in whom CD4+ T cell numbers were stable, IFNγ producing EBV-specific T cells were stable and occasional bursts in EBV load were paralleled by increasing numbers of functional T cells, suggestive of immune control. We conclude that functional loss of EBV-specific CD8+ T cells with a concomitant increase in EBV load are important factors in the pathogenesis of AIDS-NHL.

Critical role for CD27- effector cells in maintaining EBV- and HIV-specific CD8+ T cell immunity in HIV-infected individuals

Besides studying the presence of EBV-specific T cells, we also performed phenotypical analysis of these EBV-specific CD8+ T cells using CD45RO and CD27 staining to discriminate between naïve (CD45RO−CD27+), memory (CD45RO−CD27+) and effector (CD45RO+/−CD27−) T cells. In chapter 6 results of phenotypic analysis are shown in comparison with a phenotypical analysis of HIV-specific CD8+ T cells. We show that differentiation into CD27− effector CD8+ T cells is essential in controlling chronic virus infections. HIV-infected individuals with an accumulation of HIV-specific CD27− T cells have delayed disease progression and HIV-infected individuals in whom EBV-specific T cells do differentiate into CD27− effector CD8+ T cells, do not develop an AIDS-NHL. Since a higher proportion of CD27− T cells produce IFNγ, lack of these effector T cells results in a relatively poorly functional HIV- or EBV-specific T cell population leading to faster progression to AIDS AIDS or progression to AIDS-NHL, respectively. We propose a model in which CD4+ T cell help is crucial to either establish efficient differentiation into CD27− T cells, or to maintain CD27− T cells, which have higher effector functions than CD27+ T cells.

Improved EBV-specific immunity after highly active anti-retroviral therapy (HAART)

To investigate if and how EBV-specific immunity changes after HAART, in chapter 7 we measured both presence and function of EBV-specific CD8+ T cells and EBV load in HIV-1-infected individuals receiving highly active anti-retroviral therapy (HAART). We compared EBV-specific CD8+ T cell kinetics with HIV-1-specific CD8+ T cell kinetics. EBV-specific immunity generally improved after initiation of HAART. In particular the number of IFNγ producing EBV-specific T cells increased, leading to a higher % of IFNγ producing tetramer+ T cells (IFNγ/tetramer ratio), especially when EBV load was high before start of therapy. The improved cellular immunity subsequently diminishes EBV load, after which EBV-specific T cells can decrease to "baseline". In contrast, HIV-specific immunity may initially improve (first month) probably due to redistribution. However, elimination of most HIV-antigen after initiation of HAART decreased HIV-specific immunity (both tetramer+ and IFNγ+ T cells).
Pathogenesis of AIDS-NHL

In chapter 8 we summarized the results of the studies described in this thesis and discussed both virological and immunological factors concerning EBV-infection in HIV-infected individuals. We propose a model which may explain our observations and give more insight into the mechanism by which the Epstein-Barr virus may cause lymphomas in immunocompromised individuals. Chronic antigen stimulation induced by HIV-infection and superinfection with additional EBV strains leads to an increased incidence of EBV-reactivation, causing an initial EBV-driven proliferation of B cells. Functional impairment of EBV-specific CD8+ T cells together with increased EBV load could lead to the outgrowth of a fully malignant lymphoma. Loss of function is caused by lack of differentiation into CD27− effector-type T cells and possibly due to lack of CD4+ T cell help. Outgrowth of malignant lymphoma depends on the acquisition of additional genetic change or stimulation by certain B-cell growth promoting cytokines. Thus, the balance between virus and the immune system may be a critical factor in the pathogenesis of AIDS-NHL. Since EBV-specific immunity improves after start of HAART in the majority of individuals studied, HAART is a good candidate therapy to reduce the risk of AIDS-NHL.