Immune response to herpesvirus infections in immunocomprised children
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Chapter 1

General Introduction
GENERAL INTRODUCTION

Herpesviruses

Members of the *Herpesviridae* form a large and diverse family comprised of three subfamilies designated alpha-, beta-, and gammaherpesviruses. Virions are composed of a large DNA genome encased in an icosahedral capsid, which is in turn coated with a layer of proteins called the tegument and an envelope composed of about a dozen viral proteins and glycoproteins in a lipid bilayer. McGeoch et al. [1] indicated that the alpha-herpesviruses separated from the lineage that gave rise to the beta- and gamma-herpesviruses about 210 million years ago, and the beta- and gamma-herpesviruses diverged 180 million years ago (Fig. 1). Despite the differences resulting from long periods of separate evolution, alpha-, beta-, and gamma-herpesviruses share a substantial genetic heritage and thus may be presumed to have evolved from an ancestor that would have been clearly recognizable as a herpesvirus.

Common features of the herpesvirus family members include a high incidence of asymptomatic infections (except varicella-zoster virus (VZV)) and the establishment of latent infections which can be reactivated to cause recurrent or new episodes of disease. The human herpesviruses exhibit these common features as well as diversity in biology and pathogenesis. They include varicella-zoster virus (VZV) and herpes simplex virus (HSV) types 1 and 2 (α-herpesviruses); cytomegalovirus (CMV) and human herpesviruses (HHV) 6 and 7 (β-herpesviruses); and Epstein-Barr virus (EBV) and HHV-8 (γ-herpesviruses).

Figure 1 Phylogenetic tree for selected mammalian herpesviruses. Herpes simplex virus (HSV)-1 and -2, equine herpesvirus (EHV)-1, pseudorabies virus (PRV), and varicella-zoster virus (VZV) are representatives for the α-herpesviruses. Human cytomegalovirus (HCMV) and human herpesvirus (HHV)-6 are representatives for the β-herpesviruses. EHV-2, herpesvirus saimiri (HVS) and Epstein-Barr virus (EBV) are representatives for γ-herpesviruses. Adapted from McGeoch et al. (1995) [1].

Varicella-zoster virus

Varicella-zoster virus (VZV) is a human α-herpesvirus (HHV-3) that has a linear double-stranded DNA genome consisting of approximately 125 kb and encoding at least 69 unique proteins. VZV is highly species specific, having a natural host range that is restricted to humans. Primary infection usually results in chickenpox (varicella), characterized by a generalized vesicular rash (exanthem), fever, and malaise. Since VZV is highly contagious, more than 95% of people in temperate climates are infected during childhood. Whereas primary infections in children are usually benign and self-limiting, primary infections encountered during adulthood are associated with higher morbidity and mortality. VZV
infection shows a seasonal pattern, with most infections occurring in spring and late winter. Healthy individuals acquire VZV through contact with skin lesions of an infected person, followed by inoculation of respiratory mucosa, or by direct inoculation of virus in respiratory secretions. Upon infection, VZV has been presumed to be transferred to regional lymph nodes, where it is thought to initiate a primary viremic phase within a few days after infection [2]. A secondary viremia has been demonstrated to occur at the end of a 10- to 21-day incubation period, accompanied by the appearance of skin lesions. In the course of infection, VZV exhibits tropism for lymphocytes, monocyte-derived dendritic cells (DC) [3], skin [4], and neuronal and satellite cells of the sensory ganglia [5-8]. Infection of DC can lead to transmission of the virus to T cells [3]. T cell tropism is likely to be critical for the cell-associated viremia associated with primary VZV infection [9]. Although it is generally assumed that the viremia during VZV infections is highly cell-associated, viral DNA can be detected in plasma and serum from a large proportion of patients with acute varicella [4,10]. Upon resolution of primary infection, which normally occurs within one to two weeks after appearance of the rash [11], the virus develops latency within dorsal root ganglia [5-8]. Most, if not all, of the DNA molecule of VZV is present during latency in an extrachromosomal (nonintegrated) fashion [12,13]. Unlike herpes-simplex virus (HSV)-1, viral protein expression has been demonstrated during latency, in particular the ORF63 transcript [14-17]. The exact mechanism of establishing and maintaining latency are still largely unresolved. In clinical terms, virus reactivation upon waning immunity may lead to herpes zoster (shingles), characterized by a painful vesicular rash usually confined to one or more sensory dermatomes [5,18-20].

**Epstein-Barr virus**

Epstein-Barr virus (EBV) is a ubiquitous human γ-herpesvirus (HHV-4) with a genome of 172 kb. Primary infection generally occurs at an early age and is usually asymptomatic [21]. EBV carriers secrete the virus into the saliva, allowing transmission of EBV from one human to another through the oral route. Primary infection during adolescence or later can result in infectious mononucleosis (IM), which is a usually self-limiting disease characterized by fever, lymphadenopathy, splenomegaly, pharyngitis, and general malaise. The main primary target cells of EBV are B cells. During the early stages of IM, extremely large numbers of EBV-infected B cells circulate in the blood, but they are all resting memory cells. They are not proliferating blasts and do not enter the growth program [22,23]. After infection of B cells, the virus DNA forms a circle and persists as an episome in the nuclei of infected cells, thereby establishing a latent infection [24]. The current model of persistent EBV infection holds that the growth program of the virus activates B cells to become proliferating blasts so they can then differentiate into resting memory B cells through the process of the germinal-center reaction [25]. During latency, a range of EBV encoded genes are expressed, including Epstein-Barr virus-encoded nuclear antigen (EBNA)-1, -2, -3A, -3B, -3C, latent membrane protein (LMP)-1 and -2, and Epstein-Barr RNAs (EBER 1 and 2). The virus may remain latent under surveillance by EBV-specific T lymphocytes [26]. However, the virus can switch to a lytic cycle, in which new viruses are produced, finally resulting in death of the infected cells and the release of virus particles [24]. During the lytic cycle, the BZLF1 (ZEbra), BHRF1, BALF1, and BCRF1 genes are expressed. Although EBV usually behaves as a harmless passenger, in rare cases, the transforming capacities of the virus might promote the development of B cell lymphomas, T cell lymphomas and nasopharyngeal carcinoma [27].
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Cytomegalovirus

Cytomegalovirus (CMV) is a human β-herpesvirus (HHV-5) with a genome of 248 kb. The virus exhibits a ubiquitous pattern of distribution and efficient transmission via direct contact in all areas of the world regardless of socio-economic conditions. The virus is present in approximately 50% of the adult population. Primary CMV infection is most often asymptomatic in healthy individuals [28,29], and is correlated to a slow rise in cell-mediated immunity [30]. However, serious complications may occur in immunocompromised patients. Symptomatic CMV infection often presents as a systemic, nonspecific syndrome with symptoms of fever, leukopenia, and malaise. It can present as a hepatitis that mimics rejection, as well as interstitial pneumonia, or less frequently gastro-intestinal bleeding or diarrhea due to gastritis or colitis. CMV maintains its latency in endothelial cells and myeloid-lineage hematopoietic cells, including progenitors that give rise to granulocytes, macrophages, and DC's [31-34]. Primary infection, lifelong latency, and intermittent shedding resulting from reactivation commonly occur without any marked disease consequences in otherwise healthy individuals.

Immunity to herpesvirus infections

Resistance to herpesvirus infections critically depends on successful collaboration between the innate and adaptive immune systems [35-38]. Multiple components of the innate and antigen-specific immune response are activated during the course of a primary herpesvirus infection. Natural killer (NK) cells are key mediators in this first line of defense against invading pathogens, by restricting virus replication and spread [35,39,40]. Human NK cells are characterized by the expression of CD56 and lack of CD3 antigens, and comprise approximately 15% of peripheral blood lymphocytes. Development of NK cells is dependent on cell-cell contact between CD34+ progenitors and bone marrow stromal cells, as well as on cytokines such as IL-15, and growth factors like Flt3 ligand (FL) and stem cell factor (SCF) [41,42]. Under normal circumstances, NK cells are mostly confined to peripheral blood, spleen and bone marrow.

Although the precise mechanisms of activation of NK cells are as yet unresolved, it appears that the actions of NK cells are mediated by the integration of activating and inhibitory signals sent by cell surface receptors upon binding to ligands, the latter being dominant in steady-state [43,44]. Inhibitory signals are provided by interaction of inhibitory receptors and their ligands, mostly belonging to the MHC (-like) class I family [44-47]. The inhibitory receptors are characterized by their ability to recruit and activate SHP-1 and SHP-2 phosphatases through immunoreceptor tyrosine-based inhibitory motifs (ITIM) present in the cytoplasmic tails [44]. Activating signals are provided by binding of ligands to activating receptors, such as members of the recently described family of natural cytotoxicity receptors (NCRs) and NKG2D [48,49]. Activating receptors lack ITIMs and instead associate with the immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor molecule, DAP-12 or to the FceRγ and CD3ζ transmembrane adaptor proteins [50,51]. The ligands for the activating NK cell receptors are probably upregulated on target cells in response to infection, cellular activation or stress [52-54]. Upon activation, NK cells release cytokines and chemokines that induce inflammatory responses, control monocyte and granulocyte cell growth and function and influence the type of subsequent adaptive responses [55]. In addition to their immunomodulatory role, killing of virus-infected cells by NK cells is
accomplished by a variety of effector mechanisms, including release of granules containing perforin and granzymes, and ligation of FasL to its receptor on the target cell [56,57]. Complete containment of the virus is ensured by activation of components of the adaptive immune system, such as antigen-specific T cells and B cells. B cells are part of the humoral arm and activated upon triggering of the B cell receptor. Upon activation, the cells differentiate into antibody-producing plasma cells. The role of B cells in the control of primary herpesvirus infections seems to be of less importance than the role of T cells. T cells are part of the cellular arm and are activated within lymph nodes upon recognition of specific viral peptides presented in the context of either MHC class I (CD8+ T cells) or MHC class II (CD4+ T cells) by the T cell receptor (TcR), in combination with co-stimulatory signals. CD8+ T cells are specialized in killing infected cells, whereas CD4+ T cells are primarily involved in providing help to other cells of the immune system.

In response to viral infection, naive CD8+ T cells that recognize the viral peptide in the context of MHC-class I clonally expand and differentiate into effector cells, which eliminate or neutralize the virus, and memory cells, which provide enhanced immunity upon re-infection [58-60]. After the clonal-expansion phase, a large-scale apoptotic episode occurs also known as the contraction phase, resulting in a substantial reduction in the number of antigen-specific cells [61]. A subpopulation of T cells escapes death and remains as a stable population of memory T cells. Functionally distinct CD8+ T-cell subsets can be distinguished with combinations of phenotypic markers such as CD45RA/CD45R0, the costimulatory receptors CD28, CD27, and the chemokine receptor CCR7. Naive CD8+ T cells express CD45RA (but not CD45R0) as well as CD27, CD28 and CCR7. During acute infection the cells express CD45R0 [63,65,66], CD38 and HLA-DR, CD27, CD28, and CCR7. Later in infection CD27 and CD28 may become downregulated [67] and only a minority of virus-specific T cells express CCR7 [67-70]. Finally, during recovery, an increasing portion of the cells express CD45RA [67,69,71].

During acute infection, CD4+ T-helper cells that recognize the viral peptide in context of MHC class II, expand and differentiate from naive to effector and memory cells [72,73]. Effector and memory CD4+ T cells can be defined in two ways. First, effector cells express two isoforms of CD45 (i.e. CD45RA+CD45R0+), whereas memory cells only express the low-molecular isoform of CD45 (i.e. CD45RA+CD45R0) [72]. Second, cytokine production distinguishes Th1 cells (synthesizing IFN-γ and TNF-α) from Th2 cells (synthesizing IL-4, IL-5, IL-10 and IL-13) [74]. Virus-specific CD4+ T cells that are elicited during primary VZV, CMV or EBV infection are predominantly of the Th1 type [72,75,76] and function to produce high levels of IFN-γ, which potentiates the clonal expansion of antigen-specific T cells [40,77,78]. Although the classical CTL response is mediated by CD8+ T cells that recognize viral peptides in association with MHC class I molecules, virus-specific CTLs can also exhibit MHC class II (CD4+)-restricted killing of infected target cells [79-85].

Memory

Immunological memory is an exclusive property of the acquired immune system. The purpose of immunological memory is to protect the host from re-infection and to control persistent infections. Maintenance of immunological memory is the basis for the existence of typical "childhood diseases". The stronger responses on re-infection or reactivation are based on a higher level of antigen-specific T and B cells and to the adaptation of these cells to particular pathogens [59,86]. The frequency of virus-specific memory cells is to a large
extent determined by the initial clonal burst size [87,88]. The numbers of memory CD8+ T cells remain constant over time, whereas the numbers of memory CD4+ T cells seem to decline [89]. Memory cells are part of a network, which is continually evolving as immune responses composed of some cells alter the frequencies, distributions, and activities of others. This network is composed of a diverse repertoire of naïve and memory cells, which compete with each other for available space and sources [90-92]. Homeostatic proliferation, boosting by antigen, or cross-reactivity are considered as key factors in long-term maintenance of immunological memory [90,93-96]. Memory CD8+ T cells require IL-7 and IL-15 to persist, whereas the cytokines required for memory CD4+ T cells are not yet defined [97-102].

A special role for NK cells in herpesvirus infection

VZV, CMV and EBV are able to reduce the usual constitutive MHC class I expression on the surface of infected cells in order to evade immune recognition by antigen-specific CD8+ T cells [103-105]. Virus-infected cells with downmodulated MHC class I expression could be rendered susceptible to attack by NK cells, similar to tumor cells, because the inhibitory NK receptors for MHC class I are no longer engaged (Fig. 2) [106,107]. NK cells may therefore not only play an important role in the initial stages of infection, but also as a back-up mechanism in the immune response to herpesviruses. The importance of functional NK cells in controlling herpesvirus infections has been demonstrated in the past [108]. Furthermore, the severe inherited immunodeficiency disorder termed X-linked lymphoproliferative disease (XLP) is characterized by critical mutations in the gene encoding SAP, which is normally bound to the cytoplasmic tails of the NK cell coreceptors 2B4 and NTB-A [109-111]. The ligand for 2B4 is CD48, a broadly expressed protein whose expression is significantly upregulated on EBV-transformed B cells. Instead of activation, the NK cells are inactivated due to the mutated SAP and patients frequently die of fulminant infectious mononucleosis (70-80% mortality) upon exposure to EBV [109].

Figure 2 NK cells are activated in accordance with “the missing-self hypothesis”. (A) Normal body cells are MHC class I positive. The interaction of MHC class I (like) molecules with inhibitory receptors silences the NK cell. (B) Herpesviruses are able to downmodulate MHC class I on infected cells, in order to evade CD8+ T cell immune responses. Because of the absence of MCH class I, inhibitory signals are not provided to the cell and NK cells are activated by interaction of cellular ligands to activating receptors, thereby killing the infected cells by release of perforin and granzymes.

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**IL-15 and IL-21 in differentiation and maintenance of NK cells and CD8⁺ T cells**

A network of cytokines is involved in directing the immune response. In the past years, it became clear that two of the common γc cytokines, IL-15 and IL-21, are critical for differentiation and maintenance of NK cells and CD8⁺ T cells. IL-15 shares homology with IL-2, but in contrast to IL-2- is hardly detectable in T cells, but produced by a variety of tissues, monocytes and DCs. The cytokine is mainly involved in maintenance of CD8⁺ T cell memory, apart from its role in naïve CD8⁺ T cell development or survival [112-114]. IL-15 can not only induce T cell proliferation, as well as co-stimulate TCR-mediated proliferation, but also mediate T cell survival [115,116]. Furthermore, IL-15 is an important factor for the generation and survival of NK cells [117], since mice lacking IL-15 or its unique receptor component (i.e. IL-15α) are highly deficient for NK cells [114,118].

IL-21 is produced by activated CD4⁺ T cells and shares significant homology with IL-2, IL-4 and IL-15 [119]. Its receptor is expressed on lymphoid tissues, DCs, macrophages, and endothelial cells [119-121]. The widespread lymphoid distribution of IL-21R suggests that IL-21 may potentially play a substantial role in immune regulation. Indeed, IL-21 has pleiotropic roles in the lymphoid lineages, including co-stimulation of T cell proliferation, and the inhibition of IgE production in B cells [119,122-124]. Within the NK cell lineage, IL-21 promotes expansion and differentiation of NK cells from bone marrow progenitors in vitro in synergy with Flt3 ligand and IL-15, and enhances lytic effector function against target cells in lysis assays, but it also limits ongoing NK cells expansion as demonstrated in mouse models [119,122,125].

**IMMUNITY UNDER IMMUNOCOMPROMISED CONDITIONS**

Since clearance of primary herpesvirus infections and maintenance of latency seem to be critically dependent on the intact immune system, it is not surprising that these viruses can cause severe clinical problems in immunocompromised patients. Reduced functioning of the immune system may result from immunosuppressive drug treatment after bone marrow or solid organ transplantation, chemotherapy, inherited disorders, or HIV-infection. In this thesis we focussed on complications from VZV and EBV infections in the immunocompromised patients, whereas other herpesviruses such as CMV and HSV also form a threat to these patients [126,127].

**Complications of VZV infection**

Although varicella is considered to be a benign and self-limiting disease, complications may occur. VZV affected about 4 million children every year in the United States before the vaccine was introduced. The disease burden attributable to primary VZV infection included hospitalization of 5 per 1000 cases and a case fatality rate of 0.7 per 100,000 in the 1- to 4-year age group [128]. Varicella morbidity and mortality are associated most often with invasive group A Streptococcus and Staphylococcus aureus infections, neurological syndromes, including encephalitis and cerebellar ataxia, pneumonia, and hepatitis [129,130]. These complications are rare in otherwise healthy children, whereas the incidence of morbidity and mortality are highly increased in case of primary infection of the fetus in utero (congenital varicella syndrome), neonates (perinatal varicella), adults and immunocompromised children [128].
**EBV-induced lymphoproliferation**

With the growing number of powerful anti-rejection medications available, it is important to recognize that these drugs predispose transplant recipients to the risk of opportunistic infections. These infections can be a major cause of mortality and morbidity after transplantation. The link between sustained immunosuppression of transplant recipients and increased evidence of posttransplant lymphoproliferative disease (PTLD), has long been apparent and the association with EBV is now widely recognized [131,132]. PTLD encompasses a range of lesions, from polyclonal, polymorphic B lymphoproliferation to monoclonal B cell lymphomas, which arise because of inadequate T cell control of latent EBV infection [131,133,134]. One major factor influencing PTLD risk, the intensity of T-cell suppression, underlies most of the differences seen in PTLD incidence in different transplantation settings. A second major influence on PTLD risk is the patient’s EBV status at the time of transplantation. A 20-fold higher incidence is observed in EBV-seronegative recipients [135], explaining why young children are at particular risk [136-140].

**Treatment of herpesvirus infection**

The vast majority of primary herpesvirus infections are asymptomatic, with the exception of varicella. Therefore, these infections generally remain untreated. However, in case of complicated courses of primary infection or reactivation, anti-viral treatment can be administered. Broad-spectrum immunoglobulins, and nucleoside analogues such as acyclovir and gancyclovir may inhibit viral replication and spread.

**Prevention of herpesvirus illness**

Antiviral therapy has to be administered upon each viral contact or shortly after the onset of the disease. Prevention of severe courses of disease due to herpesvirus infection in the immunocompromised may be pursued by induction of immunity upon vaccination which should provide long-term protection. In particular in case of oncological treatment, the anxiety of patients and their parents in case of exposure will be significantly reduced. Currently, the only vaccine approved to prevent illness from herpesvirus infection is varicella vaccine. In the early 1970’s, wild-type Oka strain was isolated from a patient and attenuated using the empiric approach of growth in non-human cells, taking advantage of the fact that VZV replicates in guinea pig embryo fibroblasts. Subcutaneous inoculation of V-Oka did not cause illness in children, indicating that viremia did not occur or was subclinical, and seroconversion was elicited reliably [141]. The vaccine was approved by the Food and Drug Administration in 1995 for routine use in healthy persons older than one year of age who are susceptible to varicella. Japan, Korea and the USA included varicella vaccination in their routine schedule. Since the implementation of VZV vaccination, the USA has seen a marked decline in the number of cases of varicella and a trend towards less hospitalizations due to chickenpox [142].

Prelicensure clinical studies in the USA demonstrated that V-Oka elicited adaptive immunity when administered subcutaneously to healthy children [143]. One dose induced humoral and cell-mediated immunity in more than 95% of vaccine recipients. The vaccine was less immunogenic in children more than 12 years old and in adults, but a two-dose regimen induced humoral and cellular responses to VZV comparable to those observed in younger children given a single dose. The vaccine was protective in about 85% of children evaluated. The severity of disease is usually modified when vaccinated children and adults develop
breakthrough varicella after exposure to wild-type VZV [144,145]. Susceptibility to breakthrough varicella was associated with low or undetectable VZV-IgG antibody titers at 6 weeks after immunization [143]. Several other studies showed that the vaccine induces long-term humoral and cellular immunity in children and adults [146,147]. Efficacy of the vaccine in leukemic patients was investigated in another prelicensure study, a collaborative study of immunization of approximately 600 varicella-susceptible American and Canadian children with leukemia in remission; extensive and prolonged severe varicelliform or zosteriform skin reactions due to the Oka strain were infrequent [148-150]. The clinical markers of V-Oka attenuation are that vaccine-associated rashes, either at the site of injection or at distant sites, occur in only about 5% of healthy children and adults and the replication of V-Oka is restricted in vaccinees who have leukemia or other immunosuppressive conditions that would predispose them to life-threatening pneumonia, hepatitis, and encephalitis with wild-type VZV infection [143,151].

**SCOPE OF THE THESIS**

Immunocompromised individuals are often incapable to protect themselves from developing severe courses of disease upon herpesvirus infections. Not only primary infections are dangerous to these individuals, but also reactivation of the viruses from latency. The social and economical problems associated with herpesvirus infections in this particular patient group may be underestimated. The scope of this thesis is to acquire knowledge on the factors important to control primary infection, re-infection and latency of herpesvirus infections in children with impaired immunity due to transplantation or chemotherapeutic treatment. These insights will help us to identify patients at risk for developing severe courses of disease, as well as to provide patient-tailored treatment strategies.

It is well established that CD8\(^+\) T cells are pivotal in anti-viral immunity. More insight into virus-induced CD8\(^+\) T cell development and functional properties of the respective CD8\(^+\) T cell subpopulations will be of great meaning to clinical diagnostics in viral disease. In healthy individuals, a high degree of variation in the subset composition of the circulating CD8\(^+\) T cell population is observed. Although the way in which this variability is generated is unknown, recent studies have suggested that particular viruses may preferentially be associated with certain phenotypes of virus-specific T cells in latency [152]. In Chapter 2 we determined in a large cohort of children whether the presence of particular circulating CD8\(^+\) T cell subsets could be correlated to previous herpesvirus infections such as CMV, EBV, and VZV, or to MMR vaccination.

Not only primary infection, but also latent EBV infection is controlled by virus-specific cytotoxic CD8\(^+\) T cells. Without these cells, EBV-infected B cells would proliferate in an unlimited fashion, resulting in lymphoproliferative disorders [153]. In transplant recipients who receive immunosuppressive drugs, the balance between host immunity and viral replication is disturbed. Given the high EBV genome levels that develop in the PBMCs of immunosuppressed patients, it becomes important to know whether increasing loads might be used as a prognostic indicator of PTLD risk [154,155-159]. The interaction between EBV and host immune factors can be evaluated by assessing spontaneous EBV transformation of B cells [160]. In Chapter 3 we designed a prospective study in a cohort of pediatric renal transplant patients on different regimens of immunosuppressive drugs to evaluate the value
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of the spontaneous EBV B cell transformation (SET) assay as a monitor of EBV-specific immunity in pediatric recipients of kidney allografts.

Although antiviral strategies may be applied in the immunocompromised upon contact with herpesviruses to prevent the development of complications, it would be of great benefit to vaccinate patients at risk in order to prevent -rather than treat- serious disease. Varicella vaccination is nowadays administered to the majority of children in the USA and Japan. This vaccine has not been implemented in the standard vaccination program in The Netherlands. Although malignancies are contra-indications for the vaccine, we describe in Chapter 4 the safety and protective potential of varicella vaccination in a cohort of pediatric oncology patients. These studies have been performed before, with the vaccine administered either before the start of chemotherapeutic treatment, or during maintenance therapy. The required delay of chemotherapeutic treatment of the malignancy in these children could have a negative effect on the clinical outcome. Furthermore, patients are at highest risk during their chemotherapy and administration of the vaccine during maintenance therapy may simply be too late. We therefore administered the vaccine in a relatively early phase of treatment and studied the efficacy of varicella vaccination.

The existence of typical childhood diseases is based on immunological memory. Reinfections usually occur asymptomatic, due to the memory response that ensures a stronger and faster control of the virus. The role of antigenic stimulation (boosting) in maintenance of immunological memory remains controversial. In Chapter 5 we provide a detailed analysis of a human memory response to a herpesvirus infection (VZV) in comparison to the primary immune response.

Herpesvirus infections may give rise to severe, and sometimes life-threatening problems in immunocompromised patients. Therefore, these patients are highly controlled and immediately treated upon known contacts with herpesviruses in order to reduce the severity of the disease. However, severe to life-threatening courses of disease also occur in otherwise healthy children. Intensive immunophenotyping in these cases may lead to the identification of important factors determining the outcome of the disease, upon which new treatment strategies can be based. In Chapter 6 we describe five otherwise healthy children who developed life-threatening varicella. In Chapter 7 we describe a 4-year-old boy with no known immunodeficiency with a chronic course of varicella.

NK cells are key players in the control of herpesvirus infections. Until recently, NK cells were considered to be a homogeneous population. It is now clear that human NK cells can be divided into two functional subsets by the differential expression of CD56 (Fig. 3) [161,162]. However, little is known on the differentiation and migration of NK cells, or special characteristics that may be linked to protection from severe infection. Since CD27 has been shown to discriminate functional T cell subsets [163], we investigated in Chapter 8 whether this TNF-receptor could also be used to identify functional NK cell subsets.
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**Figure 3 Schema of human NK cell subsets.** CD56\textsuperscript{bright} NK cells produce high levels of cytokines following stimulation with monokines. This subset is CD16\textsuperscript{low} and exhibits potent lymphokine-activated killer (LAK) activity. CD56\textsuperscript{bright} NK cells have high-level expression of the inhibitory CD94/NKG2A complex but have low-level expression of KIRs. This NK cell subset expresses a number of cytokine and chemokine receptors constitutively, including the high-affinity IL-2R (IL2-Rαβγ), c-kit, CCR7 and L-selectin. By contrast, CD56\textsuperscript{dim} NK cells produce low levels of NK-derived cytokines but are potent mediators of ADCC, LAK activity and natural cytotoxicity, and have more granular morphology than CD56\textsuperscript{bright} NK cells. The CD56\textsuperscript{dim} NK cell subset is KIR\textsuperscript{high}. These cells have distinct expression of cytokine and chemokine receptors. CD56\textsuperscript{dim} NK cells lack L-selectin but highly express PSGL-1. Adapted from Cooper et al. [161].

**References**


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