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Human Cytomegalovirus and Dendritic Cell Interaction: Role in Immunosuppression and Autoimmunity

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ABSTRACT

Human cytomegalovirus (HCMV) infection is a major cause of morbidity and mortality in patients with an impaired immune system. The virus itself can cause transitory but significant immunosuppression in immunocompetent as well as immunocompromised infected individuals. Besides immunosuppression, HCMV infected patients often develop other signs of immune dysfunction, such as autoimmune phenomena.

The aim of this thesis was to analyze the immunological dysfunctions observed in HCMV-infected patients and to further examine the underlying mechanisms of these immunological abnormalities. In our first study, we observed the presence of anti-endothelial cell, anti-smooth muscle cell and anti-nuclear autoantibodies in the sera of 43% of liver transplant recipients undergoing an acute HCMV infection and we found a correlation between the appearance of these autoantibodies and the presence of high amounts of virus in the blood. A strategy that may be effective for the virus in inducing general immunomodulation is subverting the function of cells that play a key role in the generation of both the innate and the adaptive immunity, i.e. dendritic cells (DC). It has been shown that HCMV inhibits DC functions *in vitro*. We examined the effect of HCMV on DC functions *in vitro* and *in vivo*. We compared myeloid DC (MDC) obtained from heart transplant patients undergoing an acute HCMV infection with MDC from HCMV-negative transplant recipients. We found an impaired immunophenotype in immature MDC and a reduction in the ability of mature MDC to stimulate an allogenic T cell response in cells obtained from patients undergoing an active HCMV infection. Similar results were observed in immunocompetent individuals undergoing a symptomatic HCMV infection (i.e., HCMV mononucleosis), who are not subjected to immunosuppressive therapy. Mature DC obtained from patients with HCMV mononucleosis were inefficient in stimulating proliferation of allogenic lymphocytes. We also analyzed the pattern of cyto-chemokines secreted by DC obtained from HCMV-infected patients and observed a pro-inflammatory profile. We therefore concluded that HCMV impairs the MDC immunophenotype and functions during infection in both immunocompetent and immunocompromised hosts. These strategies may assist the virus to interfere with early functions of the host immune system and may lead to immunosuppression.

The peculiar trafficking properties of DC is a key event in the early host response to pathogens and may also be targeted by the virus to hamper DC functions. We therefore examined the viral effect on DC migration in response to inflammatory chemokines upon HCMV infection *in vitro*. HCMV inhibited the migration of immature MDC in response to inflammatory chemokines by 95% at 1 day after infection. HCMV infection significantly reduced the cell-surface expression of CCR1 and CCR5, but left expression of the lymphoid chemokine receptor CCR7 unchanged. HCMV infection also induced secretion of the inflammatory chemokines CCL3, CCL4 and CCL5 and neutralizing antibodies specific for these chemokines reduced the effects of HCMV on chemokine receptor expression and DC migration. We therefore suggest that HCMV may downregulate the chemokine receptor expression on DC by inducing the secretion of inflammatory chemokines and thereby hamper DC migration. The interference of HCMV with the migratory ability of DC may prevent DC trafficking *in vivo* and contribute to immune suppression in infected individuals.

Finally, we examined the effects of HCMV on the recently discovered plasmacytoid DC (PDC) in an *in vitro* model of infection. Through the production of type I IFN PDC exert a dual role in antiviral responses by directly inhibiting viral replication and by determining the initiation and regulation of downstream B cell and T cell responses. Although HCMV only infected 1-3% of PDC, we observed viral induced activation of PDC that resulted in secretion of high amounts of IFN-α through engagement of TLR9. PDC interaction with HCMV resulted in opposite downstream effects for B cell and T cell subsets. HCMV-activated PDC secreted soluble factors that stimulated B cell activation and proliferation and we suggest that B cell hyperactivation may contribute to the development of humoral autoimmunity, as was observed in the first study of this thesis. Conversely, HCMV inhibited the allostimulatory ability of PDC, leading to depressed proliferation of $CD4^+$ and $CD8^+$ lymphocytes. Subverting the immunostimulatory ability of these potent antigen presenting cells may considerably support a condition of immunosuppression.

In conclusion, a viral attack on cells that are crucial in initiation and orchestration of innate and adaptive immune responses such as the DC, may result as an effective strategy for the virus to escape the host immune response. A side effect of this strategy may be the induction of significant immunosuppression and the generation of autoimmune phenomena in HCMV-infected patients.

AIMS OF THE STUDY

Human cytomegalovirus (HCMV) can cause transitory but significant immunosuppression in immunocompetent as well as immunocompromised infected individuals. Besides immunosuppression, HCMV infected patients often develop other immune dysfunctions, such as autoimmune phenomena. The overall aim of this thesis was to analyze the immunological dysfunctions observed in HCMV-infected patients and to further examine the mechanisms underlying these immunological abnormalities. To achieve this goal I focused on the following specific objectives:

1. To examine the influence of HCMV infection on the generation of humoral autoimmunity in solid organ transplant patients (paper I).

2. To study the influence of active HCMV infection on the function of dendritic cells (DC) in solid organ transplant recipients and in immunocompetent individuals (paper II and IV).

3. To analyze the effect of HCMV on the migration of monocyte-derived DC *in vitro* (paper III).

4. To investigate the interactions between HCMV and plasmacytoid DC *in vitro,* and the effects of such interactions on the innate and adaptive immune responses (paper V).

LIST OF PUBLICATIONS

This thesis is based on the following papers:

I. Varani S., Muratori L., De Ruvo N., Vivarelli M., Lazzarotto T., Gabrielli L., Bianchi F.B., Bellusci R., and Landini M.P. Autoantibody appearance in cytomegalovirus-infected liver transplant recipients: correlation with antigenemia. *Journal of Medical Virology* 2002; 66: 56-62.

II. Varani S.⋆, Frascaroli G.⋆, Gibellini D., Potena L., Lazzarotto T., Lemoli R.M., Magelli C., Söderberg-Naucler C., and Landini M.P. Impaired dendritic cell immunophenotype and function in heart transplant patients undergoing active cytomegalovirus infection. *Transplantation* 2005; 79: 219-227.

III. Varani S., Frascaroli G., Homman-Loudiyi M., Feld S., Landini M.P., and Söderberg-Naucler C. Human cytomegalovirus inhibits the migration of immature dendritic cells by downregulating cell-surface CCR1 and CCR5. *Journal of Leukocyte Biology* 2005; 77:219-28.

IV. Frascaroli G.⋆, Varani S.⋆, Mastroianni A., Britton S., Gibellini D., Rossini G., Landini M.P., and Söderberg-Nauclér C. Dendritic cell function in cytomegalovirus-infected patients with mononucleosis. *Manuscript under final revision, Journal of Leukocyte Biology.*

V. Varani S., Frederich M., Feld S., Tammik C., Frascaroli G., Landini M.P., and Söderberg-Nauclér C. Human cytomegalovirus differentially controls B-cell and T–cell responses through effects on plasmacytoid dendritic cells. *Manuscript.*

⋆ These authors share primary authorship.

LIST OF ABBREVIATIONS

APC	antigen presenting cells
BCR	B cell receptor
BMT	bone marrow transplant patients
CD40L	CD40 ligand
CLR	C-lectin receptor
cmvIL-10	HCMV-encoded IL-10
CTL	cytotoxic T lymphocytes
DC	dendritic cells
DC-CK1	DC chemokine 1
E	early
EBV	Epstein-Barr virus
EC	endothelial cells
ELISA	enzyme linked immunosorbent assay
FACS	fluorescence-activated cell sorting
GM-CSF	granulocyte-macrophage colony-stimulating factor
gp	glycoprotein
GVHD	graft-versus-host-disease
HAART	highly active antiretroviral therapy
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HHV6	human herpesvirus 6
HHV7	human herpesvirus 7
HHV8	human herpesvirus 8
HLA	human leukocyte antigen
HSV	herpes simplex virus
HT	heart transplant patients
HUVEC	human umbilical vein endothelial cells
ICAM-1	intercellular adhesion molecule-1
IE	immediate early
IFN	interferon/s
IL	interleukin
IP10	IFN inducible protein 10
IRF	interferon regulatory factor
IRL	internal repetitive long sequence
IRS	internal repetitive short sequence

L	late
LPS	lipopolysaccharide
LT	liver transplant patients
MCMV	murine cytomegalovirus
MCP	monocyte chemoattractant protein
MDC	myeloid dendritic cells
MHC	major histocompatibility complex
MIP	macrophage inflammatory protein
MLR	mixed leukocyte reaction
NK cell	natural killer cells
ODN	oligodeoxynucleotide/s
PCR	polymerase chain reaction
PDC	plasmacytoid dendritic cells
PMNL	polymorphonuclear leukocytes
pp	phosphoprotein
RANTES	regulated on activation, normal T expressed and secreted
RSV	respiratory syncytial virus
SDF-1α	stromal cell-derived factor-1α
SLC	secondary lymphoid tissue chemokine
TAP	transporter of antigenic peptides
TARC	thymus and activation–regulated chemokine
TGF	transforming growth factor
Th	T helper
TLR	toll-like receptor
TNF	tumor necrosis factor
TRAIL	TNF-related apoptosis inducing ligand
TRL	terminal repetitive long sequence
TRS	terminal repetitive short sequence
UL	unique long
US	unique short
VZV	varicella zoster virus

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1. HUMAN CYTOMEGALOVIRUS (HCMV)

1.1 Characteristic features of the herpesviruses

Human cytomegalovirus (HCMV) is a highly species specific herpes virus that infects and is carried by the majority of the human population (1). Herpesviruses are highly prevalent, affecting an estimated 70–100% of the world's population. To this date, the human herpesvirus family consists of eight different members: herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), human cytomegalovirus (HCMV) and human herpesviruses 6A, 6B, 7, and 8 (HHV6A, HHV6B, HHV7, and HHV8) (as reviewed in (2)). The criteria for including a virus in the herpesvirus family is based on the architecture of the virion. A herpesvirus particle is composed of a core containing a linear double-stranded DNA molecule, which is enclosed within an icosadeltahedral capsid of 100 nm, containing 162 capsomeres. The capsid is surrounded by a tegument layer which consists of amorphous protein material. In the mature virion, these structures are enclosed in a lipid bilayer envelope, which contains a number of viral glycoprotein on its surface. The mature virions range in size from 150-200 nm.

All herpesviruses share four significant common properties: 1) they encode a large array of enzymes involved in nucleic acid metabolism, DNA synthesis, and processing of proteins such as thymidine kinase, DNA polymerase, helicase, primase and protein kinases; 2) the synthesis of viral DNA and the capsid assembly occurs in the nucleus of infected cells. Capsids are enveloped as they transit through the nuclear membrane and mature within the cytoplasm and intracellular compartments; 3) production of infectious progeny virus is mainly accompanied by the destruction of the infected cell; 4) after primary infection, all herpesviruses persist in a latent state in their host and may be reactivated later in life. In cells harboring latent virus, the viral genome appears in the form of a closed circular structure, and few if any viral proteins are produced.

Herpesvirus latency is established in different cell types; HSV-1, HSV-2 and VZV remain in a latent state in neurons of dorsal root ganglia, whereas latent EBV has been found primarily in memory B cells. HCMV establishes latency mainly in the myeloid cell linage (3). For a herpesvirus to persist in its host, some general conditions must be fulfilled: the virus must be able to infect cells without producing infectious progeny and therefore the virus infection cannot be cytopathic, host mechanisms must permit long-term maintenance of the viral genome, and the virus must be able to avoid elimination by the immune system. A delicate balance must therefore exist between the virus and its host to avoid virus elimination by the immune system or host destruction by the infection. Persistence in an immunocompetent host has put an enormous evolutionary pressure on these viruses to develop strategies to avoid immune recognition.

1.2 HCMV biology

The HCMV genome is the largest and most complex among the members of the *Herpesviridae* family, and consists of a linear double-stranded DNA genome ranging in size from 230 to 240 kbp. The viral genome consists of an unique short (US) and an unique long (UL) gene region, which are flanked by internal repetitive (IRS and IRL) and terminal repetitive (TRS and TRL) sequences (4). The genome has a high G+C content (57.2%) and viral proteins are expressed at immediate early (IE), early (E), and late (L) times of infec-

tion. Recent characterization of clinical HCMV strains suggests that the viral genome has 252 open reading frames that may encode potential proteins (5). However, genome analyses and mutagenesis studies show that only 45 to 57 of the viral genes are committed to essential tasks of replication. Hence, the vast majority of viral proteins are probably devoted to modulating the cellular and immunological responses of the host.

The HCMV virion has been structurally divided into three regions: the capsid, the tegument, and the envelope (as reviewed in (4) and (6)). The capsid is composed of at least seven proteins. The major capsid protein is encoded by UL86 and constitutes approximately 90% of the total protein content of the capsid. The minor capsid protein is encoded by UL85, the minor capsid binding protein is encoded by UL46 and the smallest capsid protein is encoded by UL48.5 (also called UL48/49). In addition, three distinct assembling/assembly-related proteins encoded by UL80, UL80a, UL80.5 are associated with capsids.

At least 25 proteins are located in the tegument layer between the capsid shell and the envelope. Many of these proteins are phosphorylated as denoted by the prefix pp. The functions of all the tegument proteins are not clear, but some of the proteins are most likely participating in the assembly and egress of the virion, while others are important in regulating viral and cellular processes. The two major tegument proteins are pp150 (ppUL32) and pp65 (ppUL83), which are both highly immunogenic. Other known tegument proteins include pp71 (ppUL82, that can transactivate IE viral promoters) and the highly immunogenic protein pp28 (ppUL99).

The envelope of HCMV consists of host lipids, host incorporated proteins and viral glycoproteins. Host proteins that have been found in the viral membrane include CD13 (7), β2-microglobulin (8), annexin II (9), topoisomerase II (10), CD55 and CD59 (11). To date three major viral glycoproteins complexes have been identified; gCI (gB), gCII (gM/gN), gCIII (gH, gL, gO). The gCI complex consists of glycoprotein B (gpUL55). The HCMV gB protein has highly immunogenic properties in humans for production of neutralizing antibodies (12) and participates in virus binding to target cells, as well as in cell to cell spread of the virus (13). The gCII complex is formed by disulfide-linkage of the two glycoproteins gM (gpUL100) and gN (gpUL73), is a major structural component of the viral envelope and is essential for the production of infectious virions (14). The gCIII complex consists of glycoprotein H (gpUL75), glycoprotein L (gpUL115), and glycoprotein O (gpUL74) (15, 16). Neutralizing antibodies against HCMV gH have been shown to prevent virus penetration, but not virus attachment, indicating that gH plays a role in membrane fusion during infection (17). Recent data also suggest that the gH/gL complex but not gH or gL alone induce cell-to-cell fusion (18).

1.3. HCMV entry and the initial steps of infection in the host

HCMV infection requires viral envelope glycoproteins and the specific cellular receptors to bind to host cells. After stable attachment to the cell surface, HCMV fuses with the cell membrane in a pH-independent fashion (19). The primary interaction between the virus and the cell is mediated by binding of viral gB to heparan sulfate proteoglycans present on the cell surface (20). HCMV most likely interacts with multiple membrane glycoproteins on the cell surface, and several different glycoproteins have been postulated to act as receptors for HCMV; β2 microglobulin (8), a 92,5 kDa protein (21), CD13 (22), epithelial growth factor receptor (23), DC-SIGN (24) and annexin II. However, the function of annexin II in viral entry is controversial (25).

Epidemiological evidence suggests that entry of HCMV into its host follows contact

between infectious virus and a mucosal surface. The site of viral entry appears to be epithelium of upper alimentary tract, genitourinary tract, or respiratory tract. However, initial infection of mucosal epithelial cells is not required, as HCMV infection can be established by blood transfusion and transplanted organs as well as by placental transmission during congenital infection.

1.4. Spread of the virus within the host

Although the key pathways for spread of the virus from the initial site of replication are not completely known, leukocytes and vascular endothelial cells (EC) appear to play a crucial role in this event. The importance of leukocytes in viral dissemination is suggested by the constant presence of HCMV in both polymorphonuclear leukocytes (PMNL) and monocytes from immunocompromised patients with disseminated HCMV infection (26) and by the evidence that transmission of virus through blood products from seropositive donors can be prevented by removal of leukocytes (27).

Virus-encoded chemokines may also facilitate dissemination of HCMV from the site of viral entry by attracting leukocytes. In the mouse model, a β-chemokine homologue produced by murine cytomegalovirus, MCK-2, is an important determinant of viral dissemination through its ability to recruit mononuclear leukocytes (28).

The role of EC in spreading HCMV infection is documented by the detection of viral antigens in EC obtained from nearly all organs of AIDS patients who have died from disseminated HCMV disease. HCMV-infected EC in AIDS patients are often cytomegalic and can be observed along the vessel tree throughout the body. Infected EC can detach from the basal membrane, enter the blood stream and may then constitute a major vehicle for dissemination of virus to distant tissues; this phenomenon has been defined as circulating cytomegalic EC viremia (29).

It has been suggested that the interplay between EC and leukocytes represents the pathogenetic basis for viral spread within the host and for many clinical syndromes originating during disseminated HCMV infections (30). The capacity of both PMNL and monocytes to propagate the viral infection *in vivo* after contact with infected EC has been shown by the recovery of HCMV from *ex vivo* leukocytes after coculture with EC. In addition, these leukocytes are capable of transmitting the infectious virus to susceptible cells, indicating that virus infectivity must be somehow preserved inside PMNL and monocytes, as these cells do not support a complete virus replication cycle (31). These findings suggest that inside blood vessels both PMNL and monocytes may disseminate the infection from infected to uninfected EC. At the same time, monocytes may also migrate to and infiltrate organ tissues, where these cells can differentiate into macrophages and become highly permissive to virus replication.

1.5. HCMV cell tropism

During the acute phase of HCMV disease, many cell types in virtually any organ system can be infected, including monocytes/macrophages, EC, epithelial cells, smooth muscle cells, fibroblasts, neuronal cells, and hepatocytes (as reviewed in (32)). Although the susceptibility of these cells to HCMV infection has been confirmed *in vitro*, the ability of the virus to grow varies in different cell types. For example, viral replication is considerably less efficient in macrophages, dendritic cells (DC), EC, and epithelial cells than in fibrob-

lasts—the prototype cell for growth of virus *in vitro*.

Expression of HCMV can be restricted to IE and E genes in certain cell types, and is then referred to as a nonproductive or nonpermissive infection. The presence of HCMV has been demonstrated in both T cells and monocytes, but gene expression is thought to be restricted to early events (33). In contrast to monocytes, infected differentiated macrophages expressing L viral genes are commonly detected in tissue samples obtained from patients suffering from HCMV disease (34) and during *in vitro* infection (35). These observations indicate that the ability of HCMV to replicate is dependent on the state of cellular differentiation, an hypothesis that has been supported by additional experimental studies in monocytic cell lines and embryonal carcinoma cells *in vitro* (36, 37). Similarly, myeloid $CD34^+$ DC progenitors are a site of HCMV latency during natural persistence, and there is a critical linkage between their differentiation to mature DC ex vivo and reactivation of virus from latency (38).

Fibroblast-adapted HCMV strains, such as AD169 and Towne, are not capable of infecting macrophages, DC, and EC (39). Clinical isolates can grow initially in EC and fibroblasts, but viral passage in fibroblasts consistently reduce viral replication in EC, suggesting that HCMV cell tropism might be lost by passage of the virus *in vitro* in an inappropriate cell type (40). Endotheliotropism is defined as the property of HCMV isolates to productively infect human umbilical vein EC (HUVEC) (41), while leukotropism is defined as the property of HCMV isolates to be transferred to leukocytes following co-culture with infected cells (42). Evidence suggests a genetic difference between clinical isolates and laboratory strains of HCMV in terms of endotheliotropism and leukotropism (43). Recently, the viral genes that determine EC tropism have been discovered; the UL131–128 genes confer the genetic determinants of endotheliotropism and leukotropism (44). Passage of clinical isolates in fibroblasts appears to cause disabling mutations in the UL128, UL130, and UL131A gene regions that leads to a loss of endotheliotropic properties (45). The same region is also responsible for viral tropism in monocyte-derived DC (46). Therefore, endotheliotropic and leukotropic strains of HCMV, such as TB40/E and VR1814, are also DC-tropic, i.e. capable of productively infecting DC (46, 47).

1.6 Epidemiology of HCMV infection and its emerging importance

Seroepidemiological studies show that HCMV is universally distributed among human populations from industrial societies to isolated aboriginal groups (1, 48). In general, the prevalence is higher and HCMV is acquired earlier in life in developing countries. From a global perspective, the frequency of seropositivity ranges from 60-80% in developed countries whereas the corresponding figure for developing countries approaches 100% (1, 49, 50). The seroprevalence of HCMV infection increases with age in every group that has been studied.

HCMV can be transmitted by body fluids such as saliva, tears, urine, breast milk (51), cervical (52) and vaginal excretions (53) and semen. Blood products, solid organ and bone marrow transplants can also transmit active as well as latent HCMV (54). The infection is generally acquired during childhood, with an incidence of 30–40% during the first year of life, mainly by breast milk, and subsequently by close personal contact in day care centers and schools (55, 56). In addition, transmission of HCMV through sexual contact is an important means of spread (57, 58). Most of the HCMV infections in the immunocompetent host appear to be clinically unnoticed (1, 59), which makes it difficult to date the acquisition of the infection. The systemic phase of primary HCMV infection is accom-

panied by persistent viral shedding in body fluids for months to years, which becomes relevant for viral transmission (1). A primary HCMV infection is followed by a life long persistence of the virus in a latent phase.

Reactivation of latent HCMV is often observed in patients with immunodeficiency. Therefore, the clinical importance of HCMV has risen during the last decade due to an increasing number of AIDS patients and patients undergoing immunosuppressive therapy following organ or bone marrow transplantation. The detailed molecular mechanism by which HCMV is reactivated from latency remains at present unclear, but increasing evidence suggests that inflammation is a key event in this process. In its latent state, HCMV resides in cells of the myeloid lineage (3, 60) and immune activation and differentiation of these cells into macrophages appears to be required for viral reactivation and replication (61).

Current evidence suggests that interferon (IFN)-γ and tumor necrosis factor (TNF)-α are necessary for the development of HCMV-permissive macrophages (62) and this finding has important clinical implications since immune-mediated processes involving activation of T cells and production of these cytokines may facilitate the reactivation of latent HCMV from monocytes *in vivo*. Thus, reactivation of HCMV is not only caused by immunosuppression but also appears to be linked to the activation of the immune system. In support of this hypothesis, subclinical activation of latent HCMV has been demonstrated in association with elevated serum levels of TNF-α in patients with atopic dermatitis (63) or sepsis (64, 65). Furthermore, HCMV infection is commonly reactivated following acute rejection of organ transplants as well as after acute graft-versus-host disease (GVHD) in bone marrow transplant recipients (BMT) in whom elevated levels of TNF-α have been shown to involve an increased risk for HCMV infection (66-69).

The HCMV genome and viral antigens have more recently also been detected in a majority of cancer cells in tumors of the colon (70) and prostate (71) as well as in malignant tumors of the brain (glioblastomas) (72). Reactivation of HCMV in malignancies may be due to an inflammatory reaction that is often associated with certain forms of cancers. Whether HCMV infection is causative or simply an epiphenomenon or whether it aggravates the existing inflammation in patients with cancer or autoimmune diseases (see below) requires further elucidation.

Interestingly, patients suffering from acute myocardial infarction have also been found to reactivate HCMV, as a result of a highly stressful event in the absence of systemic inflammation (73).

1.7 Clinical significance of HCMV infection

1.7.1.Congenital HCMV infection

In pregnant women, active HCMV infection can result in transplacental transmission of the virus to the fetus. HCMV is an important cause of congenital infection that occurs in 0.3% to 2% of all live births. More than 10% of congenitally infected neonates have symptoms at birth, such as central nervous system involvement, hematologic abnormalities, hepatosplenomegaly, and 10-15% of the infected newborns without symptoms at birth will develop long-term sequelae, such as mental retardation, deafness and visual impairment (1). It is generally accepted that symptomatic congenital HCMV infection occurs mainly after primary infection during pregnancy (74, 75). Nevertheless, increasing evidence shows that the outcome of nonprimary maternal infection may be symptomatic and severe (76, 77).

1.7.2. HCMV infection in the immunocompetent host (paper IV)

Primary HCMV infection is usually asymptomatic in immunocompetent individuals (59), but the virus occasionally gives rise to clinical illness, i.e. a self-limited mononucleosis-like syndrome (1). It has been estimated that HCMV is responsible for 20% to 50% of heterophile-negative mononucleosis and that this accounts for approximately 8% of all cases of mononucleosis (78). Mononucleosis due to HCMV is clinically similar to the more common EBV mononucleosis. Malaise, headache and high fever are typical features and may persist for weeks. Lymphoadenopathy, pharyngitis and splenomegaly are each seen in around 30% of patients and are more frequent findings in EBV mononucleosis. Peripheral blood lymphocytosis with atypical lymphocytes and slight elevation of hepatic transaminases are typical laboratory hallmarks of mononucleosis and constantly present in HCMV-infected symptomatic patients (which we also observed in our study of HCMV infection in immunocompetent individuals, **paper IV**). A variety of other clinical abnormalities have been associated with HCMV infection in the normal host, including the Guillan-Barré syndrome, peripheral neuropathy, meningoencephalitis, myocarditis, hepatitis, interstitial pneumonitis, hemolytic anemia, and thrombocytopenia. In our study, we detected two cases of interstitial pneumonia among the 14 cases of HCMV-mononucleosis (**paper IV**). All patients of our study group fully recovered from the disease without developing sequelae.

A high proportion of immunocompetent patients with HCMV infection appear to have viruria that persists >6 months after the onset of infection (79) and virus can be detected in the blood by polymerase chain reaction (PCR) within 6 months after the onset of symptoms (80). The pattern of virus excretion in adolescents with asymptomatic primary HCMV infection reveals a shorter duration of urine excretion and less viral shedding from saliva and the genital tract than in HCMV-infected patients with mononucleosis (59).

1.7.3 HCMV infection in the immunocompromised host

HCMV is one of the most common opportunistic pathogens that complicate the care of immunocompromised patients. Infection can occur by reactivation of latent virus, by re-infection in patients who are already infected, or by primary infection (1). HCMV infection in immunocompromised individuals cause different clinical syndromes in different groups of patients and the severity of the infection parallels the degree of the immunosuppression. The most severe infections are seen in recipients of allogeneic bone marrow or stem cell transplant and in AIDS patients with low $CD4^+$ counts. HCMV symptomatic infections are also often observed in solid organ transplant recipients and in patients receiving immunosuppressive chemotherapy for cancer.

HCMV retinitis and colitis are common features among AIDS patients with a $CD4^+$ T cell count below 100 cells/mm^3. Prior to the use of combination regimens of highly active antiretroviral agents (HAART), it was estimated that around 40% of adults and 9% of children with AIDS would develop HCMV disease (81). The incidence of HCMV retinitis and disseminated disease has dramatically decreased with the advent of HAART, as a result of the increase in HCMV-specific immunity coupled with the decrease in HCMV reactivation (82, 83). Consequently, after the introduction of HAART in the developed world, HCMV disease afflicts only a limited number of HIV-infected patients, when $CD4^+$ cell counts fail to rise or when reconstitution of specific HCMV-immune response does not occur. However, nearly 40 million of people are currently infected with HIV in developing countries and, as antiretroviral therapy has not made its way to these countries, between 10% and 20% of these patients can be expected to lose vision or die due to HCMV infection (84, 85).

HCMV infection causes severe morbidity and mortality in transplant recipients and remains the single most important pathogen affecting these patients. In BMT the most common clinical manifestations of HCMV infection are pneumonitis and gastrointestinal disease. HCMV-infected solid organ recipients instead generally develop fever, leukopenia, malaise, arthralgia, and macular rash (1). The effects of HCMV infection in transplant patients can be divided into two categories: the direct effect of the infection, which causes the clinical syndromes described above; and the indirect effects, which include how the virus influences the net state of immunosuppression and contributes to allograft injury (86). Increasing evidence suggests that HCMV is a strong immunosuppressive agent, and a rising incidence of bacterial and fungal infections have been observed in both BMT and solid organ transplant patients suffering from active HCMV infection (87-89). In addition, evidence from several cohort studies shows that HCMV infection is associated with an increased risk of acute graft rejection in solid organ transplant patients and with long term complications, such as chronic graft rejection and chronic GVHD in solid organ recipients (90-92) and in BMT (93-95), respectively.

After the introduction of antiviral drugs, the incidence of symptomatic HCMV infection has decreased, but clinical experience suggests that antiviral drugs delay rather than prevent the onset of disease in predisposed patients. The two main antiviral strategies to prevent HCMV infection after transplant are prophylaxis that starts at engraftment and is continued at least for 100 days post-transplant, or pre-emptive therapy, which relies on the detection of early active infection and prompt treatment before disease onset (96, 97). Ganciclovir is the most widely used drug against HCMV.

1.7.4 HCMV infection in patients with autoimmune diseases

Laboratory signs of acute HCMV infection and anti-HCMV antibodies have been observed and associated with a number of autoimmune diseases.

HCMV antigens are detected in 85% and 90% of biopsies from patients with ulcerative colitis and Crohn`s disease, respectively (98), and the presence of HCMV infection is associated with poor clinical outcome in these patients (99, 100). Subclinical HCMV infection has also been detected in psoriatic patients and has been correlated to high levels of TNF-α expression (101). Furthermore, HCMV DNA, specific antigens and infectious virus particles have been detected in synovial tissue and fluid obtained from patients with rheumatoid arthritis (102-105). In addition, active HCMV infection is frequent in children with systemic lupus erythematosus (106), and the virus has been implicated in the development and/or exacerbation of the disease (107-109). Occasionally, HCMV infection may mimic or aggravate this disease and antiviral treatment may be beneficial in such cases (108, 110). Finally, autoantibodies specific for systemic sclerosis recognize the late HCMV protein UL94 and are associated with the diffuse form of the disease but not with the limited form, suggesting a viral correlation with the severity of systemic sclerosis (111, 112).

The hypothetical mechanisms by which HCMV may contribute to the development of autoimmune phenomena will be discussed later in this thesis. Chronic inflammation associated with autoimmune diseases would provide an ideal microenvironment for reactivation of latent HCMV in inflammatory macrophages (61). However, it remains to be seen whether the virus contributes to the pathogenesis of autoimmune diseases or whether viral reactivation is merely an epiphenomenon induced by the predisposing inflammation.

1.8. Laboratory diagnosis of HCMV infection

As HCMV infection is either asymptomatic or accompanied by symptoms that are not specific to HCMV, laboratory techniques are the sole means of diagnosing acute HCMV infection.

1.8.1 Serological tests: HCMV diagnosis in the normal host

Serological tests for detection of HCMV specific antibodies are useful to determine whether a patient had a HCMV infection in the past, which is of high clinical importance for organ and blood donors. In addition, serological tests for determining seroconversion and for detection of HCMV specific IgM are commonly used to establish a diagnosis of ongoing HCMV infection in the immunocompetent host, i.e., patients with HCMV mononucleosis (113-118). Tests for HCMV specific IgM often exibit a moderate frequency of false-positive results. However, the more recently developed automated enzyme linked immunosorbent assays (ELISA) using recombinant viral antigens have been reported as a sensitive and specific assay for the detection of anti-HCMV IgM (119). Detection of specific IgM is a sensitive indicator of an ongoing or recent HCMV infection, but is not a specific indicator of primary infection, as IgM antibodies are often produced during active nonprimary infections (120, 121). The detection of primary HCMV infection is of crucial importance in pregnant women, where primary infections may have a greater clinical impact than recurrent or exogeneous reinfections (74, 75). Diagnosis of primary HCMV infection is accomplished by demonstration of seroconversion and/or detection of low specific IgG avidity, this being indicative of a low functional affinity of IgG class antibodies (122, 123). Conversely, antibody tests are not useful in the diagnosis of HCMV infection and disease in the immunocompromised host, and therefore other virological methods must be utilized for these patients.

1.8.2. Virological tests: diagnosis of HCMV in the immunocompromised host

In the immunocompromised host, the most critical period for developing disseminated HCMV disease is at the time of systemic diffusion of the virus. HCMV-infected leukocytes contribute to the spreading of HCMV by infecting vascular EC (30). Conversely, HCMV-infected EC are thought to be the most likely source of both infectious virus and pp65 for leukocytes (42).

In recent years, several assays have been developed for quantitation of HCMV in the blood of immunocompromised patients. It is currently accepted that the only reliable indication of the degree of dissemination of HCMV infection/disease is the measurement of virus load in the blood. The most important diagnostic assays for HCMV quantification in the blood are; (1) viremia, which quantifies infectious HCMV carried by leukocytes; (2) antigenemia, which quantifies the number of leukocytes positive for viral pp65 in the nucleus; (3) circulating cytomegalic EC viremia, that measures the number of circulating cytomegalic EC (occasionally detected during the antigenemia assay); (4) leukoDNAemia, which quantifies the number of HCMV genome copies present in leukocytes by quantitative PCR (124). Both antigenemia and leuko-DNAemia are more sensitive methods than viremia and have been extensively correlated with risk and severity of HCMV disease in AIDS patients and transplant recipients (125-128). Therefore, these two methods are the most widely used techniques for monitoring HCMV infection in immunocompromised patients.

The antigenemia assay is based on the detection of HCMV structural phosphoprotein

pp65 in the nuclei of peripheral blood leukocytes or PMNL. The original methodology was described by van der Bij (129) and comprises three specific steps: isolation of leukocytes by dextran sedimentation, fixation, and immunocytochemical detection by indirect immunoperoxidase staining. The assay has since then undergone some improvement and in particular Revello et al. (130) proposed using immunofluorescence detection. The advantage of the antigenemia test is its good level of specificity and sensitivity, whereas its drawbacks are its technical complexity and the fact that samples must be tested within a few hours of collection.

Several methods have been developed in the past few years for DNA quantification by PCR (131-133). Quantitative PCR tests are less labor-intensive than the antigenemia test and previously frozen samples can be analyzed. The major disadvantage of PCR is the high risk of contamination.

Monitoring of HCMV infection in transplant recipients and AIDS patients has established threshold values for different assays above which HCMV-related clinical symptoms are likely to appear. These values are approximately 50-100 positive cells among 200,000 cells for antigenemia, and 1,000 HCMV genomes among 200,000 leukocytes for leukoDNAemia in solid organ transplant recipients and AIDS patients, whereas lower values are considered as threshold for starting a pre-emptive therapy in BMT (124, 134, 135). In addition, the presence of a single circulating cytomegalic EC at the antigenemia test is sufficient to suggest a disseminated HCMV infection with potential organ involvement (124).

1.9 HCMV and the immune system

1.9.1 The immune system

Sensing and defeating microbial infections is essential for host survival. These functions are accomplished by the immune system, which is divided into the innate and the adaptive branches. The innate immune system provides the first line of defense against microorganisms and innate immune recognition relies on a limited number of germline-encoded receptors. These receptors evolved to recognize conserved products of the microbial world, but not host products. Conversely, adaptive immunity can provide specific recognition of foreign antigens, immunological memory of infection and pathogen-specific proteins. However, the adaptive immune response is also responsible for allergy, autoimmunity, and the rejection of tissue grafts in transplant patients (136).

Among the cells that bear innate immune or germline-encoded recognition receptors are macrophages, DC, mast cells, neutrophils, eosinophils, and natural killer (NK) cells. These cells become activated during infection with a pathogenic microbe and rapidly differentiate into short-lived effector cells whose main role is to get rid of the infection or keep it in control until adaptive immunity is developed. When the innate immune system is unable to deal with the infection, activation of an adaptive immune response becomes necessary. In these cases, the innate immune system stimulates and instructs the adaptive immune system about the nature of the pathogenic challenge. It does so by inducing secretion of appropriate cytokines and chemokines and by controlling the expression of costimulatory molecules, such as CD80 and CD86, on the surface of specialized antigen presenting cells (APC), the most important of which are DC, that guard against infection in all tissues (137). Together, innate and adaptive immunity function to provide an optimal host defense and DC are the key cells that couple innate recognition to the initiation of T cell and B cell activation.

1.9.2. The host defense against HCMV

Innate immunity (paper V)

NK cells represent a distinct population of lymphocytes that can contribute to protective innate responses against a variety of infections by mediating perforin-dependent lysis and the production of cytokines (138). NK cell activity is increased in children that are infected with HCMV (139). In addition, NK cells attack HCMV-infected cells *in vitro* (140, 141) and secretion of type I IFN by human leukocyte antigen (HLA)-DR^+ cells contributes to NK cell-mediated lysis of HCMV-infected target cells (142). Although NK cells are believed to be important in combating HCMV infection, only one case report has implied the importance of these cells in clearing HCMV infection *in vivo* (143).

The engagement of plasmacytoid DC (PDC) may also be involved in sensing and combating HCMV infection. PDC are the main producers of type I IFN in response to viral infection and possess a dual role in antiviral responses by directly inhibiting viral replication and by determining the initiation and orchestration of downstream B and T cell responses (144-148). A crucial role for PDC in the activation of NK cells has been implied in the murine model. In response to murine cytomegalovirus infection (MCMV), PDC secrete high levels of type I IFN and acquire an ability to rapidly activate NK cells, which in turn produce IFN-γ and gain cytotoxic effector functions. Thus, during MCMV infection *in vivo*, PDC appear to be specialized to activate the innate immune response (145).

However, little is known about the role of PDC during the innate response to cytomegalovirus infection in humans. We therefore decided to investigate the effect of HCMV on PDC in an *in vitro* model of infection. In **paper V**, we demonstrated that HCMV can activate and cause partial maturation of PDC. In our experiments, we found that PDC up-regulated the expression of major histocompatibility complex (MHC) class II and CD83 molecules but not the costimulatory molecules CD80 and CD86 after contact with HCMV. In addition, PDC secreted interleukin (IL)-6, IL-10, TNF-α, CCL3/macrophage inflammatory protein (MIP)-1α, and high amounts of IFN-α within 24 hours after the contact with the virus. Type I IFN released by HCMV-infected PDC may activate NK cell cytolytic activity (138) and induce downstream T cell and B cell activation (146, 149).

Adaptive immunity: the humoral response to HCMV

B cells carry immunoglobulin surface receptors and are responsible for specific antibody production. Humoral antibody responses are either T-cell dependent or T-cell independent. Bacterial lipopolysaccharide (LPS) interacts with both Ig-receptors and a Toll-like receptor (TLR, see Chapter 2) on B cells and induces T-cell independent responses. However, specific immune reactivity against most viral proteins is T-cell dependent. Antibodies produced by B cells can act to inhibit the binding of viruses to the corresponding receptor on host cells, causing neutralization of the viral particles before target cells are infected. Antibodies may also help to kill infected cells, either by antibody-dependent cellular cytotoxicity or by antibody-complement-mediated lysis of infected cells.

HCMV specific antibodies do not clear an infection and do not protect against re-infection of the virus or reactivation of latent virus. However, antibodies play an important role in reducing viral spread and in protecting the host from disease. In fact, pre-existing antibodies have been shown to limit symptomatic infections in transplant recipients, and maternal antibodies reduce the risk of disease in congenitally infected fetuses (1, 74).

Primary HCMV infection is accompanied by a transient IgM response followed by persistent elevated IgG levels (150). HCMV infected individuals produce antibodies against at least 15 HCMV proteins, and different individuals show different patterns of reactivi-

ty (151, 152). The humoral response is directed against both non-structural and structural HCMV proteins. The most immunogenic protein is pp150, and nearly 100% of HCMV seropositive individuals have developed antibodies against this protein. In addition, the most abundant structural protein of HCMV, pp65, is also an important immunogen, as the antibody response to this protein is very high during the acute phase of infection, but decreases rapidly after early convalescence (152). Antibodies to structural glycoproteins may be clinically relevant due to their location on the virus envelope. They are targets for neutralizing antibodies and are therefore the best candidate in vaccine development. Neutralizing antibodies against gB can be detected in 50-70% of HCMV seropositive individuals (153) and neutralizing antibodies to gH and gN are also produced (154, 155).

Adaptive immunity: the cellular response to HCMV

Cellular immunity to HCMV plays a crucial role in eliminating infection and restoring the balance between the virus and the immune system. Both $CD4^+$ and $CD8^+$ T cells are essential for combating HCMV infections. $CD4^+$ T cells regulate the immune response by producing cytokines that are necessary for $CD8^+$ T cell activation and provide costimulatory signals for B cell differentiation. Activated $CD8^+$ T cells develop into cytotoxic effector lymphocytes (CTL) and are capable of directly destroying viral infected cells by the release of cytolytic proteins.

CTL specific for HCMV mediate protective immunity and clearance of detectable levels of the virus in the host. HCMV-specific $CD8^+$ T cells have been isolated from seropositive individuals (156, 157), and an HCMV-specific $CD8^+$ T cell response is critical for protection and recovery from HCMV infection (158, 159). Patients undergoing bone marrow transplantation with T cell-depleted grafts also have an increased incidence of HCMV infections and fatal interstitial pneumonitis (160). In these patients, reactivation of virus also occurs earlier and is not affected by prophylactic treatment with ganciclovir (161).

HCMV is one of the most immunodominant antigens encountered by the immune system. There is a remarkably high frequency of HCMV-specific $CD8^+$ T cells in the peripheral blood of normal virus carriers (162), with a few T cell clones specific for a viral peptide that undergo massive expansion and are maintained for years (163). The huge proportion of $CD8^+$ T cell memory devoted to this single persistent virus may imply that these T cells play an important role in controlling the infection throughout the host's life. Such expanded virus-specific clones increase with age. In fact, the $CD8^+$ T cell response against HCMV in healthy elderly persons, which occurs as large expanded clones of a few viral epitopes, may dominate the whole T cell repertoire and may constitute up to 50% of the $CD8^+$ repertoire (164, 165).

Functional $CD8^+$ T cell and antibody responses however appear to be insufficient to control HCMV replication completely. In otherwise healthy children who have acquired cytomegalovirus, prolonged viral shedding is associated with persistent deficiency of virus-specific $CD4^+$ T cells and not with a deficient virus-specific $CD8^+$ T cell response (166, 167). Evidence suggests that formation of an effective memory $CD4^+$ T cell response is necessary for recovery from primary HCMV infection in renal transplant patients (168, 169). In the murine model, $CD8^+$ T cells are dispensable for clearance of MCMV, and mice depleted of the $CD8^+$ T cell subset eliminate infectious virus through cooperation between $CD4^+$ T cells and other immune cells (170). Therefore, $CD4^+$ T cells appear to play a crucial role in clearing cytomegalovirus infections. Generally, $CD4^+$ T cells recognize viral peptides expressed in the context of MHC class II molecules and inhibit viral replication mainly by producing antiviral cytokines. HCMV-specific $CD4^+$ T cells display antiviral activity *in vitro* through the secretion of TNF-α and IFN-γ (171).

Different nonstructural and structural HCMV peptides derived from IE, E, or L proteins that are expressed in the context of MHC class I or class II molecules can trigger an immune response in infected individuals. HCMV-specific T cells have been found against many HCMV proteins such as IE (156, 172), gB (156) and pp65 (173, 174). To date, both pp65 and IE-1 are considered dominant T cell targets (175). Recently, the role of the IE- and pp65-specific $CD8^+$ T cell clones in preventing HCMV disease and inducing recovery from symptomatic infection has been better characterized. While a high frequency of IE-1-specific $CD8^+$ T cells correlates with protection from HCMV disease, symptoms appear to develop exclusively in HCMV-infected solid organ transplant patients with a dominant pp65-specific $CD8^+$ T cell response (176). Similarly, an essential feature of recovery from HCMV disease is the increased $CD8^+$ T cell responses against the IE1 protein during the early recovery period of acute HCMV infection in HIV-positive patients. In these patients, immune restoration after HCMV disease is associated with an enlarged antigenic repertoire and diversity of HCMV-specific $CD8^+$ responses (177). A schematic drawing of cellular interactions involved in the induction of the adaptive immune response to HCMV is depicted in **Figure 1.**

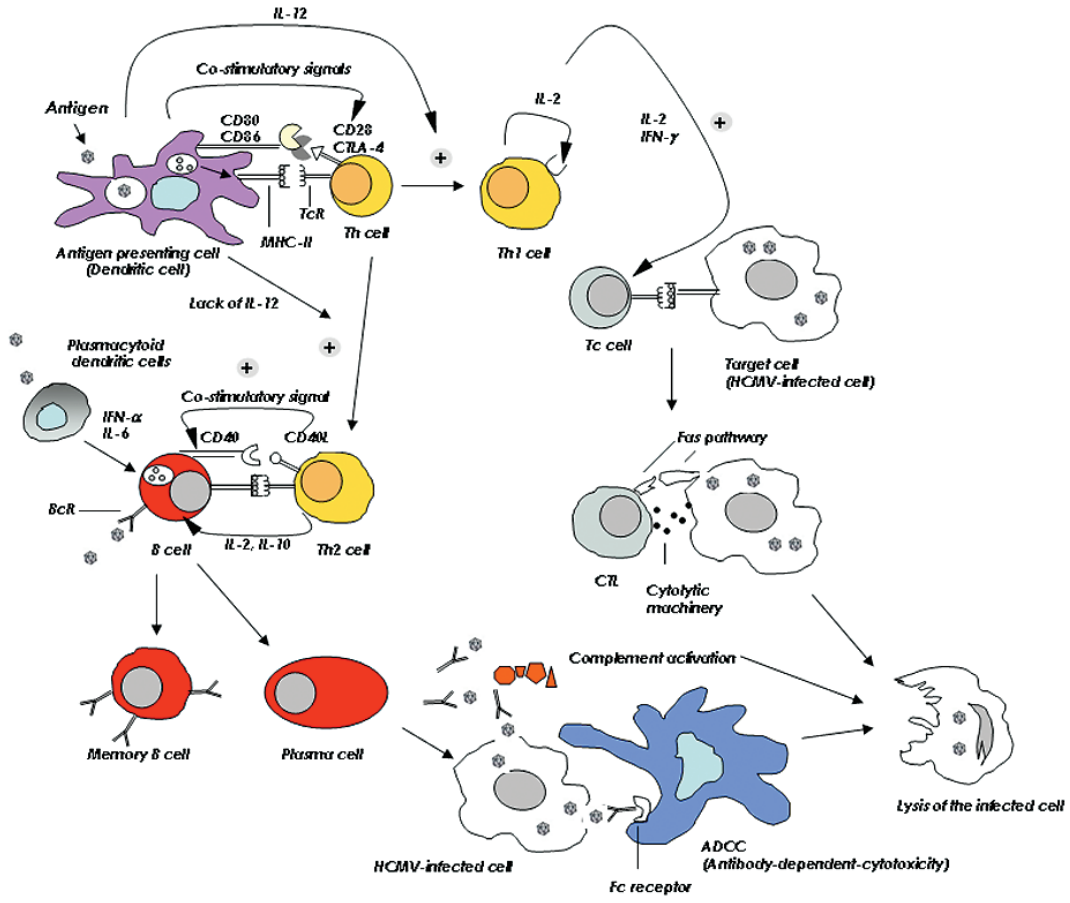

Figure 1. **Cellular interactions involved in the induction of humoral and cellular immune response against HCMV.** Th, $CD4^+$ T helper cells; Tc, $CD8^+$ cytotoxic T cells; CTL, cytotoxic effector cells.

1.9.3 HCMV immune evasion strategies (paper III and V)

HCMV is characterized by the persistence of productive infection and the life-long survival of the latent virus in the immunocompetent host despite a normal immune response. To achieve these goals, the virus has developed a number of mechanisms that involve multiple gene products and are designed to avoid and interfere with host immune responses.

HCMV can evade recognition by the innate immune system escaping NK cell-mediated cytolysis. The destruction of target cells by NK cells is regulated by interactions between receptors on the surface of the NK cells and molecules on the target cells; in particular absence or loss of MHC class I molecules on target cells triggers NK activation through "the missing self" hypothesis. Although HCMV infected cells express reduced levels of MHC class I molecules (see below), infected cells are not as susceptible to NK-mediated lysis as uninfected cells are (178-180). In order to resist NK cell attack, HCMV can block the recognition of infected cells by inhibiting positive signals or by delivering negative signals to NK cells. As an additional strategy, the virus can protect infected cells from the cytolytic activity of NK cells by membrane-stabilizing mechanisms. The HCMV encoded MHC class I homologue UL18 is believed to mediate resistance to NK cells by blocking NK cell recognition of infected cells (181). However, cells infected with a mutant strain of HCMV from which the UL18 gene has been deleted are still protected against lysis by NK cells (180), implying that additional pathways must contribute to NK cell resistance. In infected cells, the HCMV-encoded glycoprotein gpUL40 has been reported to facilitate the expression of HLA-E, which blocks NK cell-mediated lysis by interacting with the inhibitory receptor CD94/NKG2A on the NK cell surface (182, 183). In addition, the HCMV-encoded UL16 gene product is a decoy protein capable of blocking the stimulation of an NK cell activation receptor (184, 185). As an alternative mechanism of NK cell-mediated lysis resistance, the UL16 protein stabilizes the plasma membrane of HCMV-infected cells. HCMV-infected cells as well as transfected cells expressing UL16 exhibit increased resistance to the actions of NK cell-produced lytic proteins, including perforin and granzyme B (179). This novel viral strategy for increasing resistance to NK cell attack may also work on cells targeted by cytotoxic T cells, which destroy cells by releasing cytotoxic peptides.

As part of the innate immune response, virus-infected cells respond to infection by the induction of cellular signaling pathways that lead to the transcription and production of antiviral cytokines, such as type I IFN. These cytokines act in an autocrine and paracrine fashion to limit viral replication. However, HCMV has developed strategies to block the antiviral response induced by type I IFN. The HCMV immediate early 2 gene product IE86 can efficiently inhibit the transcription and secretion of IFN-β in HCMV infected fibroblasts (186). In addition, the viral tegument protein pp65 interferes with IFN signaling in cells where it is expressed. pp65 may prevent the activation of the interferon regulatory factor (IRF)3, which is a key transcriptional regulator of cellular IFN responses, leading to a block in the response to type I IFN (187). In contrast, other authors have observed that the virion-associated pp65 appears to block the induction of pathways that leads to the activation of NF-kB and IRF1, but not IRF3 (188). The ultimate result is that HCMV triggers, but then totally or partially blocks, the innate immune response pathways that lead to the induction of IFN-responsive genes.

HCMV evades recognition by the adaptive branch of the immune system by utilizing a number of different strategies. HCMV infection of DC and viral induced impairment of their function in stimulating T cell responses will be extensively discussed in Chapter 3. Antigen presentation by DC and other APC may be subverted by the virus through a

number of mechanisms. For example, HCMV interferes with multiple steps in class I antigen-presentation pathways. During viral infections, viral proteins synthesized in the cytoplasm of infected cells are targeted for degradation and presented by MHC class I molecules to CTL, which can kill virus infected cells. Several HCMV-encoded proteins interfere specifically with antigen presentation, thereby allowing HCMV infected cells to avoid detection by CTL. HCMV is able to block the presentation of MHC class I peptide complexes by producing at least four different gene products; gpUS2, gpUS3, gpUS6 and gpUS11 (189). Two such proteins gpUS2 and gpUS11, independently reduce the level of expression of MHC class I molecules on the surface of infected cells by accelerating their degradation. Another protein, gpUS3 retains peptide-loaded MHC class I molecules in the endoplasmic reticulum compartment impeding their expression on the cell surface. Finally, the viral gpUS6 protein binds the endoplasmic reticulum part of the transporter of antigenic peptides (TAP) and inhibits its function. Since only peptide-loaded MHC class I molecules can leave the endoplasmic reticulum for transport to the cell surface, inhibiting the function of TAP also results in intracellular retention of MHC class I molecules.

HCMV has also developed powerful strategies that cause inhibition of the expression of MHC class II molecules on infected cells and therefore avoid recognition by $CD4^+$ T cells. The US2 and US3 proteins also downregulate the surface expression of MHC class II molecules, inhibiting antigen recognition by $CD4^+$ T cells (190, 191). Furthermore, the pp65 protein mediates accumulation of HLA-DR molecules in lysosomes, where the HLA-DR alpha chain is degraded (192). HCMV infection also inhibits IFN-γ induced expression of MHC class II molecules by interfering with signal transduction pathways involving Jak/Stat (193).

HCMV has also designed strategies to avoid recognition by the humoral immune response. In infected cells, HCMV encodes for two Fc receptor homologues, UL119-118 and TRL11/IRL11 (194, 195). HCMV-encoded Fc receptors may hide viral antigens by coating the virus-infected cell surface with IgG antibodies. This may also inhibit complement interaction with the Fc portion of antibodies and protect the infected cell from antibody-mediated cellular cytotoxicity, thereby rendering anti-HCMV antibodies ineffective. In addition, HCMV induces the surface expression of cellular regulators of complement activation, such as CD46 and CD55 and may thereby increase resistance of infected cells to complement-mediated lysis (196).

Soluble factors such as cytokines and chemokines orchestrate the initiation and maintenance of both the innate and adaptive immune responses to viral infection and HCMV can influence the production of cyto-chemokines. A number of different cytokines and chemokines including IL-1β, IL-6, IL-8, IL-10, TGF-β, TNF-α, CCL2/monocyte chemoattractant protein (MCP)-1, CCL3/MIP-1α, CCL5/regulated on activation, normal T cell expressed and secreted (RANTES) and CXCL10/IFN inducible protein 10 (IP10) are induced during HCMV infection (197-207). We have also observed in *in vitro* studies that HCMV induced the secretion of CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES in monocyte-derived DC cultures and of IFN-α, IL-6, IL-10, TNF-α, and CCL3/MIP-1α in PDC cultures within 24 hours of infection (**paper III** and **paper V**).

In addition, HCMV encodes a homologue of the anti-inflammatory cytokine IL-10 (cmvIL-10), that has a 27% sequence homology with human IL-10 (208). cmvIL-10 exhibits potent immunomodulatory activity *in vitro* and inhibits mitogen stimulation of peripheral blood mononuclear cells, the expression of MHC class I and II molecules, and production of pro-inflammatory cytokines (209). This viral induced cytokine is also capable of subverting the immunostimulatory functions of DC, as described in Chapter 3.2.2.

A viral transcript with homology to human and viral IL-10 is expressed during the latent phase of HCMV infection. It has been suggested that the expression of the transcript-corresponding protein during latency would further help the virus to avoid immune recognition and clearance (210). The HCMV genome also encodes two chemokine homologues, UL146 and UL147, of which UL146 is a functional CXC chemokine that induces calcium mobilization, chemotaxis and degranulation of neutrophils *in vitro*. Hence, this viral protein may increase neutrophil recruitment to sites of HCMV infection *in vivo* (211).

HCMV can regulate the functions of cyto-chemokines by additional mechanisms such as influencing the expression levels of their cognate receptors. For example, the levels of one of the TNF-α receptors on the surface of HCMV-infected cells is reduced (212), which may explain, at least in part, the insensitivity of these cells to TNF-α (62). Complementing this phenomenon, clinical isolates of HCMV encode a TNF receptor homologue, UL144, but the function of this protein has not yet been determined (213). Furthermore, the HCMV genome encodes four chemokine receptor homologues (UL33, UL78, US27 and US28) (214, 215). US28 has been shown to be functionally active as a receptor for the β-chemokines CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES and CCL7/MCP-3 with a selectivity that is distinct from that of known mammalian β-chemokine receptors (214, 216). US28 has been proposed to reduce extracellular levels of inflammatory chemokines and may thereby inhibit the recruitment of inflammatory cells to infected tissues. Although HCMV induces RANTES production in infected cells, the amount of this protein and of the constitutively expressed chemokine CCL2/MCP-1 are decreased at late time points after infection by binding to US28 (198, 217, 218). The ability of US28 to internalize RANTES does not strictly correlate with US28 expression and the US27-encoded receptor may also contribute to this process (217). Thus, HCMV can modify the chemokine environment of infected cells through intense sequestering of CC-chemokines. It has been shown that US28 mRNA is found *in vivo* in peripheral blood monocytes and is expressed at immediate early times (219) in latently HCMV-infected monocytic cells *in vitro* (220). In addition, US28-transfected cells bind firmly to immobilized CX3CL1/fractalkine under flow conditions (221), suggesting that US28 expressed by latently HCMV-infected monocytes may enable adhesion of these cells to fractalkine-exposing endothelium. US28 may thus facilitate adhesion of circulating latently infected monocytes to EC and subsequent cell-to-cell transmission of HCMV or transendothelial migration, leading to viral spread *in vivo*, through dissemination of viral particles sheltered from the immune system *via* cell-to cell passage (222).

1.9.4 HCMV-induced immunopathology

HCMV-induced immunosuppression (paper II, III, IV, V)

HCMV has developed a number of strategies to subvert the host immune system and escape immune surveillance, enabling the virus to co-exist with its host. Such viral disruption of key pathways in the immune response also leads to a general immunosuppression that inhibits the immune response against the virus itself as well as against unrelated pathogens in the infected individuals (223). HCMV-induced suppression of the immune system, particularly evident during primary infections, is transitory but substantial.

Reactivation of previously controlled viral infections has been reported during the acute phase of HCMV infection (224) and active HCMV replication enhances pre-existing immunosuppression in both solid organ and stem cell transplant patients, making them at high risk for invasive bacterial and fungal infections (87, 88). Immunological impairments

that could be responsible for the depressed host immune function have been described in studies of HCMV infection both *in vivo* and *in vitro*. For example, a loss of delayed-type hypersensitivity reactions to recall antigens (224), and a reduced lymphoproliferative response to mitogens (225) and to specific antigens (226) have been detected in patients with HCMV mononucleosis. In immunocompetent adolescents undergoing an asymptomatic primary HCMV infection, the lymphocyte proliferative response to HCMV is low compared with those of seropositive controls (59). Impaired specific cell-mediated immunity is also present in children with congenital (227) or acquired (228) HCMV infection. *In vitro*, HCMV has been shown to cause depressed lymphocyte proliferative responses to T cell mitogens and to inhibit cytotoxic and NK cell activities (229, 230). In addition, HCMV results in suppression of bone marrow myelopoiesis, possibly by infecting stromal cells and by altering the marrow microenvironment (231, 232).

We considered that an effective strategy for HCMV to escape the immune system and thereby to generate immunosuppression in the host would be to attack cells that are crucial in the initiation and control of the immune response, i.e. DC. Therefore, we focused our interest on the effect of *in vivo* and *in vitro* HCMV infection of myeloid DC. We found that HCMV impaired DC functions in both heart transplant recipients (HT) and normal individuals undergoing an acute infection (**paper II** and **IV**). In addition, *in vitro* HCMV infection of immature DC paralyzed their ability to migrate in response to inflammatory chemokines (**paper III**). We then examined the effect of HCMV on a recently discovered DC subset, i.e., PDC, in an *in vitro* model of infection. We observed that the virus inhibited the allogenic immunostimulatory properties also of these APC (**paper V**). Our findings and those of other investigators in relation to HCMV's ability to blunt DC functionality will be extensively reviewed and discussed in Chapter 3. Importantly, the impairment of DC functions during *in vivo* and *in vitro* infections may contribute to the immunodepression that is often observed in HCMV-infected patients.

Attacking and manipulating key cells that initiate and orchestrate the immune responses, such as DC, would provide an extremely effective strategy for the virus to escape the host immune response. A side effect of this strategy may include the significant immunosuppression that HCMV infection often causes in its host. Such immunosuppression was previously not evident in individuals with a normal immune system. However, since large patient groups with a suppressed immune system have appeared in society, such viral aggravated immunodepression often leads to patients becoming at high risk for the development of invasive bacterial and fungal infections thereby becoming manifest clinically.

HCMV-induced autoimmune phenomena (paper I and V)

Apart from immunosuppression, HCMV-infected individuals often develop other immune dysfunctions, such as autoimmune phenomena. Natural and anti-CD13-specific autoantibodies have been found in BMT undergoing HCMV infection (233, 234). Anti-EC autoantibodies associated with HCMV infection have also been detected in HT and renal transplant patients and may contribute to the development of acute and chronic allograft rejection (235, 236). In addition, autoantibody production is a feature of HCMV-induced mononucleosis and post-perfusion syndrome (237, 238). As mentioned earlier, laboratory signs of acute HCMV infection are also detected in a number of autoimmune diseases. We have also observed anti-EC, anti-smooth muscle cell and anti-nucleus autoantibodies in sequential sera of 10 out of 23 liver transplant patients (LT) undergoing an active HCMV infection, as diagnosed by the antigenemia test. Conversely, none of the 17 antigenemia-negative transplant patients developed autoantibodies (**paper I**). The specificity

of autoantibodies that we detected in HCMV-infected patients was unknown and we cannot rule out the possibility that they were anti-HLA antibodies.

The initiation of autoimmune diseases can be simplified as a three-stage process involving both genetic and environmental influences (239). First, a repertoire of immune cells with the capacity for autoreactivity has to be established. Appropriate MHC and T cell receptor alleles, the presence of which is genetically determined, must be able to present and to recognize self-antigens, thereby generating an autoreactive response. Second, potentially autoreactive cells must be activated. Viral infection may be responsible for this stage of the induction of autoimmunity, as explained below for HCMV. Third, a failure of the immune system to counter-regulate the autoreactive response would result in chronicity of the autoimmune response. The lack of effective regulation of such responses could also be genetically determined.

Different mechanisms have been proposed to explain how the virus might contribute to the development of autoimmune phenomena. All individuals appear to harbor quiescent, potentially autoreactive lymphocytes, but these cells remain innocuous until they somehow become activated. These cells have low affinity for their antigens, which is the reason why they may be able to escape thymic deletion and these cells may also exibit a high activation threshold. Viral mimicry is one of the mechanisms that might be responsible for their activation (240). As described earlier, the HCMV genome encodes a series of genes that are homologous to cellular genes (**Figure 2**). An antigenic determinant of HCMV that is structurally similar to a determinant of a self-protein may be sufficiently different to be recognized as foreign by the host's immune system. The immune response to the viral determinant would thereby crossreact with the host tissue and eventually lead to autoimmunity.

Molecular mimicry has been observed between HCMV UL94 and an EC integrin. In the majority of sera obtained from patients suffering from systemic sclerosis, antibodies directed against an epitope contained within the HCMV-encoded protein UL94 have been detected (111, 112). The UL94 epitope shows homology with NAG-2, a cell surface mole-cule highly expressed on EC and associated with integrins. In addition, purified anti-UL94 peptide antibodies induce apoptosis of EC upon engagement of the NAG-2-integrin complex (111). These findings indicate that during HCMV infection, a subset of

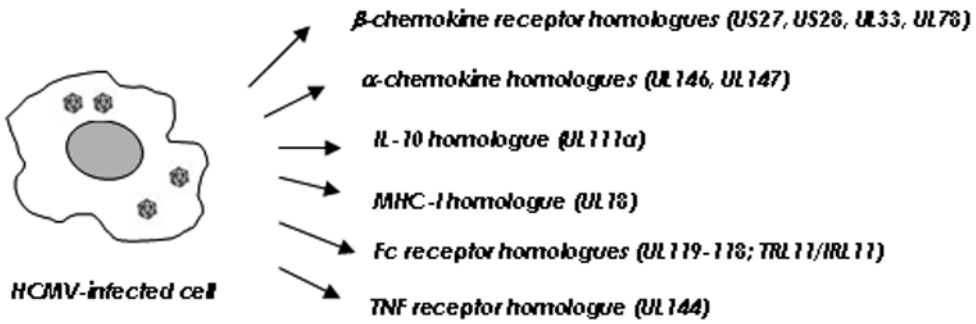

Figure 2. Viral mimicry. The HCMV genome has evolved with its host and has hijacked or incorporated several genes that are homologous to, or can functionally mimic cellular genes. Many of these genes appear to modify and evade the host immune response. They may also play a role in immunopathology because of cross-reactivity with host cell proteins.

antibodies directed against the viral protein UL94 crossreacts with surface molecules normally expressed on EC and cause apoptosis of EC through a mechanism of molecular mimicry (241). Interestingly, EC damage is a primary pathogenic event that characterizes the early stages of systemic sclerosis.

Activation of autoreactive lymphocytes may also be induced by cell lysis and exposure of intracellular proteins not recognized as part of the self-repertoire, or by EC damage with disclosure of a self-antigen normally expressed by tissue cells, but generally invisible to the immune system through protection by the EC barrier (242). Autoreactive T cells specific for hidden epitopes generally ignore these self-peptides, because the amount presented by APC is insufficient to induce either T cell activation or tolerance. During viral infections, the generation of tissue- or cell-segregated self-epitopes may be enhanced by viral cell lysis or by indirect tissue damage due to inflammatory or immune reactions. HCMV-infected cells could also produce a number of different cytokines and chemokines, e.g., TNF-α, IL-1β, IL-6, IL-8, MCP-1 and RANTES (198-200, 202) that may further aggravate inflammation. Inflammatory cytokines may also induce maturation of professional APC, such as DC, that may become more effective in presenting the exposed autoantigens to the quiescent specific autoreactive T cells, leading to their activation. If sufficient amounts of previously hidden self-peptides are presented to inactive T cells in the periphery, they may proliferate and induce autoimmunity. Destruction of specific tissues, release of sequestered antigens and increased local inflammation are grouped under the term "bystander activation" to define the antigen-nonspecific theory of autoimmunity induction (239). We propose such a mechanism to explain the development of autoantibodies in transplant recipients undergoing HCMV infection (**paper I**).

In our study, all but one case of autoantibody detection were observed in patients with very high viral load in the blood and by coincidence with or just after the viremic peak of the infection. The viremic phase was monitored by the antigenemia test that detects and quantifies the viral protein pp65 in the nucleus of PMNL, as described in Chapter 1.8.2. Current evidence suggests that HCMV-infected EC are the source of virus and pp65 in infected PMNL (42, 243). Severely infected EC can also detach from the basal membrane, enter the blood and be detected by the antigenemia test, which is a clear indicator of disseminated HCMV infection (124, 127, 135). Consequently, antigenemia may indirectly reveal the severity of EC infection and patients with high HCMV load in the blood most likely have a severe infection of EC that can lead to EC damage. Thus, the production of autoantibodies may be due to HCMV-induced damage of the EC barrier and to the resulting exposure of EC intracellular self-antigens and/or tissue-privileged self-epitopes. Exposure of high amounts of generally unrevealed self-antigens may activate low-affinity autoreactive T cell clones which in turn could stimulate self-reactive B cells to differentiate into autoantibody-producing plasma cells.

The expression of host cell proteins at the surface of the viral envelope may render the host proteins immunogeneic providing an additional mechanism for induction of autoimmunity. CD13-specific autoantibodies have been detected in BMT with either HCMV disease or HCMV viremia. These autoantibodies appeared in the patients' sera at the same time that HCMV was detected and were correlated to the development of the autoimmune-like chronic GVHD in these patients (93, 233). CD13 serves as a receptor molecule for the entry of HCMV into cells (22) and is present on cells that are susceptible to HCMV infection, as well as on infectious virus particles. These observations suggest that CD13 molecules associated with HCMV in some way become immunogenic during HCMV infection in BMT. The production of autoantibodies to CD13 may result from activation of T cell clones with specificity for HCMV-derived peptides expressed on APC. This process could

then stimulate CD13-reactive B cells that have taken up HCMV particles containing CD13, leading to the processing and presentation of viral peptides in association with MHC class II molecules and subsequent stimulation of helper T cells. Such a scenario could result in production of CD13-specific autoantibodies (244). Continuous production of antibodies against CD13 could mediate antibody-dependent lesions, such as cellular cytotoxicity. Specific autoantibodies may also cause non-immunological damage of target cells, e.g., by inducing apoptosis or interfering with physiological cellular functions.

Finally, humoral autoimmune phenomena may also be generated by nonspecific B cell hyperactivation caused by HCMV. It has been shown that HCMV is a polyclonal B-cell activator *in vitro* , and that the B cell overresponse does not require virus replication (245). In the last project of this thesis, we observed that HCMV prompted B cell activation through stimulation of a specific DC subset (**paper V**). *In vitro*, contact of the virus with PDC induced the secretion of soluble factors, such as IL-6, IL-10, TNF-α and IFN-α , known to be critical for B cell activation and differentiation (146, 246, 247). When virus-free supernatants from HCMV infected PDC was used to culture B cells in the presence of B cell receptor (BCR) stimulation, we observed B cell activation and proliferation. In a B cell and PDC co-culture model, HCMV but not influenza virus induced B cell activation after 3 and 7 days of incubation in the presence of BCR stimulation, indicating that the activation of B cells *via* PDC is an HCMV-specific phenomenon. Therefore, HCMV-induced hyperactivation of B cells through PDC may contribute to the development of autoantibodies often observed in HCMV-infected patients.

Our initial working hypothesis was that HCMV may act as a T cell independent polyclonal B cell activator, and that development of humoral autoimmune phenomena may be facilitated by the lack of control generally exerted by T cells. However, in our experiments we found that HCMV-activated B cells did not induce antibody production in a T cell-independent manner. In order to better elucidate the mechanism by which antibodies and autoantibodies are produced during HCMV infection we are now trying to generate an *in vitro* model to test whether HCMV under any condition can induce B cells to differentiate into antibody-producing plasma cells.

2. DENDRITIC CELLS (DC)

DC are the sentinels of the immune system. These cells are the most potent APC and play a pivotal role in the induction of adaptive immune responses to infection. This is, in part, related to their apparently unique capacity to prime naïve T cells and thus trigger differentiation of an activated T cell population (248, 249). In addition, through their release of specific cytokines and chemokines, DC influence the type of T cell response and participate in the recruitment and activation of other effector cells, including NK cells, macrophages, and B cells (250, 251). In the absence of ongoing inflammatory responses, DC constitutively patrol through the blood, peripheral tissues, lymph nodes and secondary lymphoid organs. Upon inflammation or infection, circulating immature DC enter tissues and efficiently uptake and process antigens to form MHC-peptide complexes. Antigen capture in tissues induces functional maturation of DC. Mature DC have a reduced capacity to endocytose antigens, but instead express high amounts of MHC and costimulatory molecules, which make them excellent presenters of antigens to T cells. The same antigenic and inflammatory signals that stimulate DC to shift from a "processing" to a "presenting" stage, induce their mobilization from the periphery to lymph nodes or to spleen T cell areas, where DC prime T cell responses.

Distinct DC subsets exist that are endowed with different properties and lead to different types of immune responses. In the human, two major DC subsets have been identified based on the differential expression of CD11c, a cell surface protein: the myeloid DC (MDC), which are $CD11c^+$, and the plasmacytoid DC (PDC) which are $CD11c^-$ (**Figure 3**).

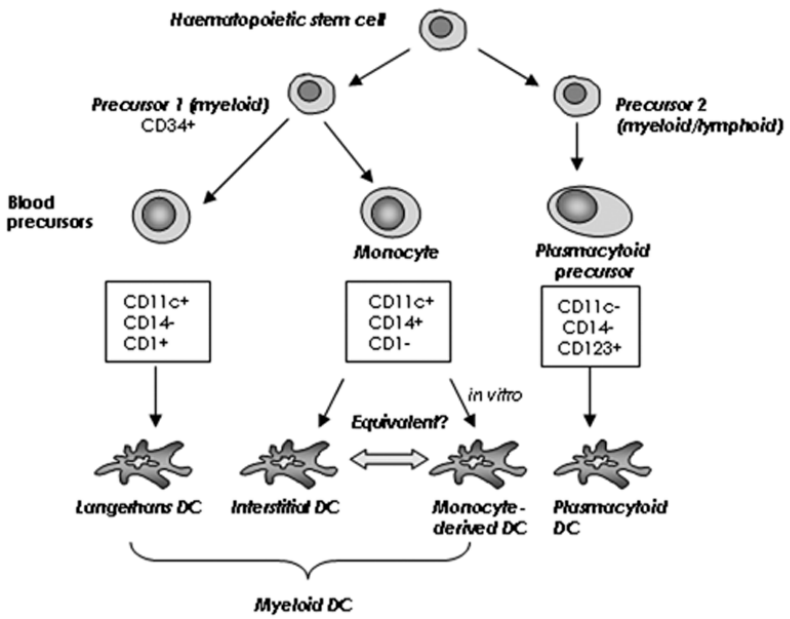

Figure 3. Pathways of human dendritic cells (DC) development. Myeloid $CD34^+$ progenitors differentiate into monocytes ($CD14^+$ $CD11c^+$ DC precursors) that yield immature DC in response to GM-CSF and IL-4 (the interstitial pathway). Myeloid progenitors also differentiate into $CD11c^+$ $CD14^-$ precursors, which yield Langerhans cells in response to GM-CSF, IL-4 and TGF-β. The $CD14^-$ $CD11c^-$ $CD123^+$ precursors may originate from myeloid or lymphoid progenitors and differentiate into plasmacytoid DC.

2.1 Myeloid dendritic cells (MDC)

The myeloid differentiation pathway for DC includes Langerhans cells that reside in stratified epithelia such as the skin, and $CD14^+$-derived DC, which include interstitial DC and circulating DC (252, 253). In human peripheral blood, circulating MDC express the β2-integrin CD11c as well as other myeloid markers (CD13, CD33 and CD11b). Blood DC are believed to have two main functions: (1) to replenish the immature DC that sit within tissues and that have migrated out of the tissue either in the steady state or in response to inflammatory stimuli, and (2) to capture microbes that reach the bloodstream.

Several protocols have been established to obtain purified MDC from precursors *in vitro* (254-256). Cord blood $CD34^+$-derived DC differentiate along two independent pathways in response to granulocyte-macrophage colony-stimulating factor (GM-CSF) and TNF-α. After 5-6 days, two subsets (one $CD1a^+CD14^-$ and one $CD1a^-CD14^+$) can be observed; after 12 days of culture, all cells are $CD1a^+CD14^-$. The $CD1a^+$ precursors differentiate into Langerhans cells that contain Birbeck granules, whereas the $CD14^+$ precursors lead to $CD1a^+$ MDC that do not produce Birbeck granules and that possess the characteristics of interstitial DC (253). It is also believed that monocytes represent a pool of circulatory precursor cells that are capable of differentiating into MDC that resemble the features of interstitial DC (248) (see **Figure 3**). *In vitro*, monocyte-derived DC with the typical phenotype of $CD1a^+CD14^-$ cells can be obtained by stimulation with IL-4 and GM-CSF for 6 days (256). *In vitro*-generated MDC from human peripheral blood precursors (bone marrow progenitors and monocytes) are considered to be immature and have a high capacity to endocytose soluble antigens. These cells can also acquire the ability to present antigens to T cells upon maturation stimuli, such as bacterial LPS, inflammatory cytokines (TNF-α and IL-1β) and T cell signals (e.g. CD40 ligand, CD40L).

MDC have the unique capacity to prime naïve $CD4^+$ T cells (248) and through fine modulation of IL-12 secretion they orchestrate the type of adaptive immune response (257). In fact, IL-12 producing MDC skew the T helper (Th) cell profile towards Th1, whereas MDC that fail to secrete IL-12 prime Th2 responses (258-260).

While all MDC can secrete IL-12 and induce strong T cell proliferation, $CD14^+$-derived DC capture soluble antigens more efficiently than Langerhans cells and are the sole MDC subpopulation capable of stimulating naïve B cell to differentiate into IgM-secreting plasma cells in response to IL-2 (252). Therefore, $CD14^+$-derived DC are involved in launching primary B cell responses. B cell priming is induced by a two-step process, through the secretion of IL-12 by MDC first, followed by IL-6 production (261, 262).

2.2 Plasmacytoid dendritic cells (PDC)

The other main human DC subset is represented by PDC. PDC precursors isolated from blood are small cells that morphologically resemble plasma cells with rich endoplasmic reticulum. These cells express CD4, CD123 and high levels of MHC class II molecules, but lack myeloid markers, such as CD11c, CD13, CD33, and for this reason these cells were considered to have a lymphoid origin (263, 264). However, recent studies have shown that PDC precursors can derive from both common lymphoid and myeloid progenitors in humans (265) (see **Figure 3**). PDC present the same properties of MDC in terms of antigen presentation, maturation and trafficking abilities, but can be distinguished from MDC for being an essential component of innate immunity by secreting high amounts of type I IFN in response to viral DNA or RNA stimulation (266).

The release of type I IFN initiates a cascade of events that leads to elimination of the virus.

In particular, IFN-αβ acts directly on most cell types to turn on biochemical pathways that restrict viral replication and render host cells resistant to further viral infection (148). Beyond cytokine secretion, PDC can also act as APC and trigger adaptive immunity by directing T cell responses (267-269). Immature PDC exhibit poor T cell stimulatory capacity and this function is acquired after maturation of these cells (263, 270). Thus, activated PDC can present antigens and induce considerable expansion of T cell populations, although less efficiently than MDC (147, 271, 272). Whether PDC have the capacity, like MDC, to prime naïve $CD4^+$ T cells and to produce IL-12 is still under debate (267, 273).

On the other hand, recent evidence suggests that PDC are essential in the development of an antiviral humoral responses. Depletion of PDC from leukocytes abrogates both influenza-specific antibody production and associated polyclonal Ig secretion, and the coculture of purified B cells, T cells and PDC, challenged with influenza virus, yield influenza specific antibodies (146). Under certain circumstances, plasma cell differentiation from B cells in the presence of PDC can proceed in a T cell independent manner. In fact, cocultures of PDC and B cells stimulated through BCR and CpG DNA (see paragraph 2.3.1), induce the production of IL-6, IL-10 and IFN-α and stimulate the differentiation of B cells into Ig-secreting plasma cells (246). These observations suggest a crucial role for PDC in innate immunity as well as in triggering T cell responses and antibody production.

2.3 MDC and PDC in innate immunity: the role of Toll-like receptors

Recognition of invading pathogens is mediated by a set of germ line-encoded receptors (pattern recognition receptors) that have evolved to recognize conserved pathogen-associated molecular patterns shared by large groups of organisms (136). One of the major families of these receptors is the Toll-like receptor (TLR) family. The recognition of pathogen-associated molecular patterns by cells of the innate immune system through TLR is the essential step toward the induction of innate immune responses and subsequent adaptive immune responses (274). To date, 10 different (TLR1-10) human TLR have been described. The pattern-recognition strategy is based on the detection of a limited set of conserved molecular patterns that are unique to the microbial world and invariant among entire classes of pathogens.

TLR 1, 2, 4, 5 and 6 (275, 276) seem to be specialized in the recognition of mainly bacterial products that are unique to the microorganisms and not made by the host. Their detection therefore affords a straightforward self-non-self discrimination. Conversely, TLR 3, 7, 8 and 9 mainly specialize in viral detection and recognize nucleic acids (277-279), which are not unique to the microbial world. In this case, the discrimination of non-self is not mediated by the molecular nature of the ligands, but by their accessibility to the TLR. In fact, these TLR are mainly located in intracellular compartments and detect viral nucleic acids in late endosomes-lysosomes. Because host nucleic acids are not normally accessible in these compartments, they do not trigger TLR (274). However, in certain conditions, such as deficient clearance of apoptotic cells, the host-derived nucleic acids (often in complex with DNA or RNA binding proteins) may become available for activating TLR, which may break tolerance and lead to autoimmunity (280).

Several different viruses have been shown to interact with TLR, including respiratory syncytial virus (RSV), measles virus, vaccinia virus, HSV type 1 and type 2, HCMV and MCMV (281). In particular, innate immune defenses against MCMV are triggered by engaging TLR 3 and TLR9 in mice, and activation of both TLR pathways by the virus lead to type I IFN production but neither of them offers full protection against MCMV in

the absence of the other (282). TLR2 appears to have broad ligand recognition properties. Beside various bacterial components, such as peptidoglycan and lipoteichoic acid, TLR2 has been found to be engaged in the innate response against measles virus and HCMV (283, 284). Measles virus hemagglutinin signals through TLR2 and HCMV virion-mediated activation of human cells involves both TLR2 and CD14.

Different human DC subsets exhibit a different expression profile of TLR (274, 285). Circulating MDC express TLR1, TLR2, TLR3, TLR5, TLR6 and TLR8. Monocytes and *in vitro* differentiated monocyte-derived DC also express TLR4, by which these cells strongly respond to LPS. On the other hand, PDC express a unique set of TLR, which includes TLR7 and TLR9 in humans. Consequently, different DC subsets recognize a variety of microbial components through different TLR expression, as shown in **Figure 4**. MDC recognize bacterial or fungal components as well as double and single stranded RNA viruses, and produce the proinflammatory cytokines TNF-α, IL-6 and IL-12, whereas PDC mainly recognize viral and other intracellular parasites and produce large amounts of type I IFN (273, 286). Thus, different DC subsets may induce different modes of innate immunity suitable for eliminating different microorganisms.

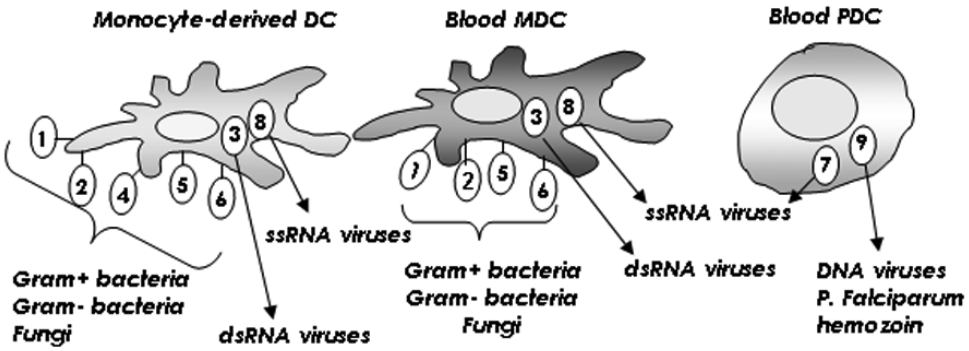

Figure 4. DC populations express different sets of TLR. Monocyte-derived DC, human peripheral blood MDC and PDC express distinct sets of TLR on their cell surfaces or in their endosomal/lysosomal compartments, which bind to different ligands. dsRNA, double-stranded RNA; ssRNA, single-stranded RNA; P.falciparum, *Plasmodium falciparum.*

2.3.1 Immunostimulatory DNA and TLR9

Aside from its function of encoding genetic information, DNA exhibits direct immune activities. The immune system has evolved a defense mechanism that is able to distinguish bacterial DNA from our own because of differences in the frequency and methylation of CpG dinucleotides. In contrast to bacterial DNA, mammalian DNA has a low frequency of CpG dinucleotides, and these are mostly methylated; therefore, mammalian DNA does not have immunostimulatory activity (287). Similar to bacterial DNA, HSV and other herpesviruses, such as HCMV, have a genome enriched with CpG unmethylated dinucleotides and HSV type I DNA has been shown to exhibit immunostimulatory properties both *in vitro* and *in vivo* (288).

Innate host immune response to CpG DNA is mediated by TLR9 (278). Interestingly, the immunostimulatory features of microbial DNA can be mimicked by synthetic oligodeoxynucleotides (ODN) that contain unmethylated CpG dinucleotides (289). Immune stimulatory CpG ODN are potent inducers of both innate and adaptive immunity and specific motifs of CpG ODN can vary in their ability to induce individual immune effects. Consistent with TLR9 expression, PDC and B cells are susceptible to stimulation by CpG ODN.

Three different classes of CpG ODN have been proposed based on their distinct immunological effects on PDC and B cells, CpG-A, CpG-B and CpG-C. The prototype sequence of a CpG-A is ODN 2216 that induces large amounts of IFN-α in PDC and has little or no effect on B cells (290). ODN 2006 is the prototype of a CpG-B ODN that stimulates B cells and provides potent polyclonal activation of memory B cells with very limited effects on PDC (291). CpG-C combines properties of the two other CpG classes, potently activating PDC and B cells at the same time (292). By combining both direct and indirect activation of B cells, CpG-C ODN are superior to CpG-A (lacking direct activation of B cells) and to CpG-B (weak IFN-α production in PDC) in regards to B cell stimulation. Specific DNA sequences have also been identified that are able to inhibit TLR9-mediated activation in human cells as well as in mouse cells, and the prototype of these sequences is ODN 2088 (293, 294). The mechanisms of action of these inhibitory sequences is not known, however, they do not interfere with cellular uptake, which is critical for the activity of immunostimulatory ODN, nor do they compete for binding of the receptor for the stimulatory sequences. Recent data suggest that immunoinhibitory CpG ODN most likely interfere with components of the very early events in TLR9 signaling (293).

2.4 Cytokine secretion by DC links innate and adaptive immunity

DC recognition of pathogens through TLR and the stimulation of these APC *via* factors produced by tissue cells in response to microorganisms immediately trigger immature DC to secrete large amounts of cytokines. At the same time, activation of DC initiates a program of DC maturation, leading to transformation of immature DC into potent effector DC that are capable of initiating and driving specific Th1 or Th2 immune responses (136). Naïve $CD4^+$ T cells can be induced to differentiate into either Th1 or Th2 cells, which differ in cytokine production and functions. In brief, Th1 cells produce IFN-γ and are mainly involved in cell-mediated immune responses. Th2 cells produce IL-4, IL-5 and IL-10 and are important for humoral and anti-parasitic immune responses. Cytokines produced by activated DC contribute to the regulation of inflammation, link innate with adaptive immunity and are T cell-polarizing factors.

Myeloid DC stimulated by CD40L, cytokines or bacterial components produce proinflammatory cytokines, such as TNF-α, IL-1α, IL-1β, IL-6 and IL-12 (257, 295, 296). IL-1, IL-6 and TNF-α are critical factors in the inflammatory response, as a part of natural immunity. However, a role for these cytokines has also been pointed out in antigen-specific immunity. IL-1 appears to be required for normal $CD4^+$ T cell clonal expansion and for appropriate regulation of Th1/Th2 responses (297, 298). TNF-α is able to promote DC differentiation from monocytes in the presence of stromal cells (299) and contributes to PDC and MDC maturation (147, 256). Nevertheless, TNF-induced maturation of MDC is only partial since TNF-stimulated MDC fail to secrete IL-12 in the absence of additional maturation stimuli (257). The ability of directing the differentiation of monocytes towards MDC and to induce maturation of different DC subsets render TNF-α a

crucial cytokine for the initiation of adaptive immunity. Finally, IL-6 promotes Th2 differentiation and simultaneously inhibits Th1 polarization in $CD4^+$ T cells (300, 301). This cytokine is also involved in terminal differentiation of B cells (146, 302). In addition, IL-6 switches the differentiation of monocytes from DC to macrophages *in vitro* , and therefore appears to be an essential factor in the control of APC development (303).

MDC also secrete IL-12 and other members of the IL-12 family, such as IL-23 and IL-27, which differ in their ability to be induced by different pathogens (304). IL-12 exhibits a critical role in activating and controlling Th1 immune responses (257, 305-307). In fact, through fine modulation of IL-12 secretion, which is regulated by microenvironmental stimuli and by the kinetics of the secretion itself, mature MDC orchestrate the type of adaptive immune response (257). IL-12 producing MDC skew the T helper cell profile towards Th1, whereas MDC that fail to secrete IL-12 instead prime Th2 responses (258-260). Beside activating naïve T cells, IL-12 enhances the activation of cytotoxic T lymphocytes (308). In addition, IL-12 is a key molecule involved in the IL-2-dependent differentiation of naïve B cells into IgM-secreting plasma cells. Thus, IL-12 may play a pivotal role not only in controlling cell-mediate immunity, but also in launching primary B cell responses (262). Finally, IL-12 is capable of triggering cells of innate immunity and induces NK cells to secrete IFN-γ and to display strong cytolytic activity (309, 310).

Beside the secretion of pro-inflammatory cytokines, interstitial DC and monocyte-derived DC, as well as circulating MDC (including all MDC populations derived from $CD14^+$ precursors) are capable of producing the anti-inflammatory cytokine IL-10 upon CD40L and LPS stimulation. However, the $CD14^-$ Langerhans cells fail to secrete this cytokine (295). Endogenous IL-10 controls the levels of T cell activation induced by MDC (311), reduces the capacity of MDC to initiate Th1 responses (312) and acts on T cells both directly and through DC to induce a state of anergy (313, 314). Thus, the production of IL-10 by $CD14^+$-derived DC might have a specific role in inhibiting Th1 responses and in the induction of T cell tolerance. In addition, this cytokine inhibits MDC maturation and secretion of proinflammatory cytokines (312). $CD14^+$-derived DC have the exclusive ability to produce IL-10 and to prime B cell responses as well as having a greater ability to endocytose antigens and this strongly support the hypothesis that $CD14^+$-derived cells and Langerhans cells represent two independent pathways of DC development addressed to accomplish different functions.

In contrast to MDC, PDC precursors mainly recognize viral components and produce large amounts of type I IFN. Type I IFN released by human PDC activate NK cell cytolytic activity but protect uninfected cells from NK cell-mediated lysis (138, 315). Type I IFN can also affect T cell functions, inducing early activation markers such as CD69, long-term T cell survival, IFN-γ production and Th1 differentiation (149). In addition, type I IFN promote differentiation, maturation and immunostimulatory activity of different DC subsets. *In vivo* and *in vitro* studies have demonstrated cross-talk between PDC and MDC through IFN-α that leads to MDC maturation and an increased ability to activate T cell responses (316-319). Moreover, PDC activated by type I IFN in an autocrine manner differentiate into mature DC that are themselves able to trigger adaptive T cell-mediated immunity (147, 271, 272). Finally, through the secretion of type I IFN, virus- or CpG-activated PDC play a critical role in the differentiation of B cells into Ig-secreting plasma cells (146, 246). Thus, by acting on NK cells and PDC precursors, type I IFN are capable of initiating an innate immune response, while by activating T cells, B cells, MDC and PDC they also contribute in triggering and controlling the adaptive immune response.

2.5 Functional plasticity of DC subsets in inducing Th1/Th2 adaptive immune responses

The fate of naïve Th cells is determined by three signals that are provided by pathogen-primed mature DC. The first stimulatory signal results from the ligation of T cell receptors by pathogen-derived peptides, presented by MHC class II molecules on the cell surface of DC, and determines the antigen-specificity of the response. The initiation of protective immunity also requires T cell co-stimulation. In the absence of this co-stimulation that provides the second signal, Th cells become anergic, which might lead to tolerance. T cell receptor stimulation and co-stimulation allow naïve Th cells to develop into protective effector Th1 or Th2 cells, which is accompanied by high expression levels of selective sets of cytokines. The polarization of the resulting T cell response is dependent on DC (signal 3). DC not only play a role in the promotion of effector Th1 or Th2 cell responses, but also control the generation of regulatory T cells (320).

It was previously accepted that different DC subsets are preprogrammed to direct the Th1/Th2 differentiation of $CD4^+$ T cells and it has been suggested that MDC (previously named DC1) induce $CD4^+$ T cell differentiation into IFN-γ-producing Th1 cells, while PDC (previously described as DC2) stimulate the generation of IL-4-producing Th2 cells (321-324). However, when freshly isolated from blood, both DC subsets direct Th1 cell development (325). These observations suggest that MDC and PDC subsets may not have an intrinsic capacity to direct either Th1 or Th2 cell development, but that the response rather might be modified by the microenvironment.

In support of this hypothesis, murine MDC and PDC have been shown to be influenced by a range of factors including the antigen dose, the state of DC maturation and the pathogen-derived products, that are able to determine whether naïve $CD4^+$ T cells develop into Th1 or Th2 cells (326). In addition, an *in vitro* study of human MDC has shown that Th1/Th2 polarization *via* IL-12 is adapted to the nature of the microbial compounds and that Th1 immune responses are induced against intracellular bacteria in an IL-12-dependent manner. In this model Th2 development was shown to be favored in response to stimuli associated with parasites or allergens through inhibition of IL-12 secretion (327). Finally, human PDC have been demonstrated to induce either Th1- or Th2-type immune responses upon exposure to viruses or IL-3 respectively, and Th1 differentiation involves the secretion of type I IFN by activated PDC (328). Thus, DC are considerably flexible in directing T cell responses and the Th1/Th2 polarization induced by DC is influenced by the stage of DC maturation and the nature and concentration of the antigen.

DC select the type of effector immune response by expressing a selective set of T cell polarizing factors (signal 3). These molecules are either soluble or membrane-bound and determine the balance between Th1, Th2 responses and the development of regulatory T cells (329). Various DC-derived factors which exhibit Th-cell-polarizing capacities have been identified. As previously described, IL-12 and type I IFN are examples of factors that determine the polarization of T cells towards Th1. In addition, cell-surface expressed intercellular adhesion molecule 1 (ICAM-1) is a Th1 polarizing molecule (330). Among Th2-cell-polarizing factors, OX40 ligand has been identified in human (331) and mice (332). In addition, CCL-2/MCP-1 has been found to be a Th2 determining factor in mice (333). Finally, IL-10 and programmed death-ligand 1, which is a member of the B7 family, have been recognized as regulatory T cell-polarizing factors (329, 334).

Fully matured DC do not appear to be able to modify their ability to polarize a certain type of T cell response following microbial (335) or cytokine (257, 336) restimulation.

This observation implies that effector DC primed by a certain microbe are not subjected to subsequent cross-modulation by the priming abilities of other pathogens, thereby acquiring a stable functional DC phenotype that triggers a specific type of T cell response.

2.6 DC trafficking properties and chemokine secretion (paper III)

The capacity of DC to migrate first to sites of inflammation and then to the spleen and the draining lymph nodes is a crucial component of their immunobiology, and this ability is tightly regulated by changes in the expression of surface chemokine receptors (337-339). Immature MDC arise from blood precursors and are capable of migrating towards inflamed tissues in response to chemotactic stimuli (i.e., inflammatory chemokines such as CCL-2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES). These cells express receptors for such inflammatory chemokines, including CCR1, CCR2, and CCR5, which guide them to inflammatory sites where antigen sampling can take place (339). After antigen uptake, DC undergo functional maturation, which results in rapid changes in their expression of chemokine receptors, including upregulation of CCR7 and loss of receptors for inflammatory chemokines. The upregulation of CCR7 is critical for the homing of mature DC because the CCR7 ligands CCL19/MIP-3β and CCL21/secondary lymphoid tissue chemokine (SLC) are produced in lymphoid organs (337-340) (**Figure 5**).

In contrast to MDC, circulating PDC fail to migrate in response to inflammatory chemokines, even though they express high amounts of the inflammatory chemokine receptors CCR2 and CCR5 (341). Thus, it appears that the inflammatory receptors expressed on PDC are not functional and that additional signals, such as TLR activation or virus infection, are required for coupling of migration with chemokine receptor expression in PDC. These additional stimuli may provide PDC with the capacity to migrate to inflamed tissues, as demonstrated by their accumulation in sites of inflammation *in vivo* (342, 343). The modulation of chemokine receptor expression upon maturation in PDC follows the pattern exhibited by MDC, with downregulation of CCR5 and a strong upregulation of CCR7. Upon maturation, upregulation of CCR7 is coupled to migration, allowing proper positioning of PDC in the T cell areas of secondary lymphoid tissues in response to CCL17/thymus and activation–regulated chemokine (TARC), and CCL19/MIP3β. Like MDC, PDC thus respond to lymph node-homing chemokines following maturation. (344). In addition, both immature MDC and PDC constitutively express the chemokine receptor CXCR4 and are able to migrate in response to its ligand CXCL12/stromal cell-derived factor (SDF)-1α, which is produced in lymph nodes (345), but migration of MDC and PDC to CXCL12/SDF-1α, is regulated differently by maturation, being maintained in the former and lost in the latter cell subset (344).

DC do not only respond to, but also produce chemokines. Chemokine secretion is instrumental for DC to regulate their own migratory capacities and to orchestrate recruitment of different cell types of both innate and adaptive immune responses (346). Different DC subsets disclose dissimilar pattern of chemokine secretion. Two general modes of chemokine production can be defined: constitutive/homeostatic and inducible.

Resting immature monocyte-derived DC constitutively release low levels of chemokines, such as CCL17/TARC, CCL18/DC chemokine 1 (DC-CK1) and CCL22/macrophage-derived chemokine. At early stages of maturation, monocyte-derived DC have an initial burst of inflammatory chemokines, such as CCL3/MIP-1α, CCL4/MIP-1β and CXCL8/IL-8, which stop within a few hours. CCL2/MCP-1 and CCL5/RANTES are also induced, but in a more steady fashion. At later time points of maturation MDC mainly secrete lym-

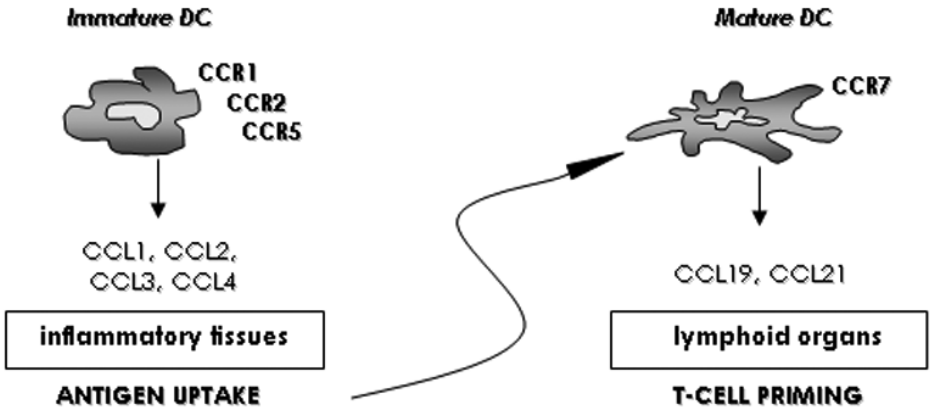

Figure 5. Myeloid DC trafficking properties. Immature MDC express receptors for inflammatory chemokines, including CCR1, CCR2, and CCR5, which guide them to inflammatory sites where antigen sampling can take place. After antigen uptake, DC undergo maturation, which results in upregulation of CCR7 and loss of receptors for inflammatory chemokines. Upregulation of CCR7 is critical for the homing of mature DC because the CCR7 ligands CCL19/MIP-3β and CCL21/SLC are produced in lymphoid organs, where mature MDC can prime T cell responses.

phoid chemokines, including CCL17/TARC, CCL19/MIP3β, and CCL22/ macrophage-derived chemokine (346). The production of such high levels of chemokines in a precise time-ordered fashion suggests an important role for these chemokines in regulating DC functions. Hence, on one hand, inflammatory chemokines produced early during an infection enhance the recruitment of circulating monocytes, immature DC, NK cells and T cells to inflamed tissues. On the other hand, the late production of lymphoid chemokines that attract naïve and memory T cells may facilitate cell positioning and cell-to-cell interactions within the lymph node microenvironment (347). In addition, chemokines produced by MDC appear to have a crucial role in organizing the architecture of lymphoid organs. In support of this hypothesis, mice lacking mature MDC have a profoundly disorganized T cell area, a defect that can be partially restored by reconstitution with DC (348).

Chemokine production by MDC has consequences not only for recruitment of other cell types, but also for the orientated migration capacity of the maturing DC themselves, since it results in binding and down-regulation of the cognate receptors in an autocrine fashion. Triggering of inflammatory chemokine receptors with their subsequent desensitization may allow DC to exit from inflammation sites upon DC maturation (337, 346). In our studies, we found that, in a similar manner, HCMV causes downregulation of CCR1 and CCR5 on the surface of immature MDC by inducing the secretion of inflammatory chemokines, which bind to their cognate receptors (**paper III**). Inflammatory chemokine production caused by HCMV may be a powerful strategy utilized by the virus to reduce the expression of chemokine receptors on the surface of MDC and thereby impair the migratory capacity of these cells.

Chemokine production represents an additional level of diversity between human DC subsets. In fact, in contrast to MDC, PDC do not secrete CCL17/TARC, CCL18/DC-CK1 and CCL22/ macrophage-derived chemokines, neither constitutively nor after stimulation. Conversely, PDC produce high levels of the inflammatory chemokines CCL3/MIP-1α, CCL4/MIP-1β and CXCL10/IP-10 in response to bacterial, viral and T cell-derived

stimuli (349, 350). The secretion of inflammatory chemokines then influence the recruitment of different immune cells and it has been shown that PDC infected with HSV, through the secretion of CCL4/MIP-1β and CXCL10/IP-10, induce the recruitment of NK cells and activated T cells (350). CpG DNA simulate PDC to secrete different inflammatory soluble factors, such as CXCL8/IL-8, that is known to recruit neutrophils, and CXCL10/IP-10, which recruits activated T cells (351). Thus, different DC subsets exibit the capacity to secrete different chemokines in response to different stimuli, with consequently distinctive abilities to recruit immune cells.

3. INTERACTION BETWEEN DC AND HCMV

3.1 Interactions between DC and viruses (paper V)

The presence of DC within the skin, the blood and mucosal surfaces identifies them as one of the cell populations that most likely have the earliest contact with viruses during infection. DC exhibit a double role in viral infections. On one hand, DC are involved in triggering a primary antiviral defense as potent APC. On the other hand, when the infection is caused by a DC-tropic virus, these cells contribute to virus invasion and dissemination through direct infection. In some viral infections, DC may also facilitate virus escape of immune responses through virus-induced inhibition of their functions (352).

As shown in **Table 1**, several viruses are known to induce infection of these APC and among them, HIV, measles virus, influenza virus and several herpesviruses cause a productive infection of DC. Interestingly, some viruses, such as HSV, HIV and influenza virus that induce death of infected MDC can trigger PDC survival, most likely inducing type I IFN secretion by these cells (353).

DC express many molecules that are also used by viruses to invade host cells. Among such hijacked receptors, CD4, CCR5, CXCR4, expressed by MDC and PDC are used by HIV for viral entry (354). Viral components are recognized by DC *via* pattern recognition receptors, including TLR and sugar-binding-C-lectin receptors (CLR). HSV-1 and HSV-2 DNA stimulate murine PDC to secrete IFN-α *via* TLR9 activation (355, 356) and influenza virus genome binds to endosomal TLR7 in murine PDC (357). In **paper V**, we showed that HCMV induced type I IFN secretion by human PDC through engagement of TLR9. Measles virus hemagglutinin and HCMV virions have been shown to signal through TLR2 (283, 284), and TLR4 mediates the response to RSV (358). However, whether TLR recognition of these viruses occurs in the MDC population has so far not been determined.

CLR are pathogen receptors with broad specificities. A range of CLR are expressed by DC and they bind carbohydrate containing structures on microbes. Among them, DC-SIGN, that is expressed on MDC, is required for infection of DC by HCMV, hepatitis C virus (HCV), dengue virus and Ebola virus (24, 359-361). In addition, DC-SIGN is a *trans*-receptor on DC for HIV, which means that DC-SIGN binds the envelope glycoprotein gp120 on HIV but, instead of causing viral entry, facilitates efficient transmission of the virus to neighboring permissive T cells (362). DC-SIGN can be used for *trans*-infection also by Ebola virus, HCMV and HCV, thereby starting the infection of DC and facilitating transmission of the viral particles to other permissive cells. In contrast to TLR triggering, triggering of DC-SIGN does not result in upregulation of costimulatory molecules. The role of this pattern recognition receptor in antigen processing and presentation of viral antigens remains to be elucidated.

Various RNA viruses, such as dengue, measles and influenza virus, infect MDC and induce maturation of these cells (363-365). HIV does not cause direct maturation of MDC, but stimulates PDC to induce their bystander maturation (317). In contrast, DNA viruses, such as HHV6, vaccinia virus, HSV and HCMV inhibit maturation of MDC (366, 367, 368, 369). Both RNA and DNA viruses instead induce activation and maturation of PDC and when these cells are exposed to influenza virus, HIV, HSV or MCMV, they secrete IFN-α and TNF- α, that mediates their maturation (145, 272, 355, 370, 371).

Viruses can exert an inhibitory effect on MDC's ability to stimulate T cell proliferation. By suppressing the maturation of MDC (as for HSV, HCMV and HHV6) or by infect-

Family	Virus	Binding Receptor(s)	Interaction with human DC		
			Subtypes		Replication
			in vivo	*in vitro*	
Herpesviridae	HSV	HVEM; Prr-2; Prr-1; TLR9		MDC PDC	Productive in iMDC; PDC?
	VZV			MDC	Productive in iMDC and mMDC
	EBV	CD21		Mono-cytes	Non permissive
	HCMV	DC-SIGN, TLR9 (**paper V**)	MDC (**paper II**)	MDC LC PDC (**paper V**)	Productive in MDC; inefficient in PDC (**paperV**)
	HHV6	CD46		iMDC	Productive in MDC
Poxviridae	Vaccinia virus	Heparan sulfate; chondroitin sulfate		MDC	Abortive in iMDC and mMDC
Orthomyxoviri-dae	Influenza virus	Sialic acid TLR7		MDC PDC	Productive in MDC and PDC
Paramyxoviri-dae	Measles virus	CD46; CD150		MDC LC	Productive in MDC
	RSV	Surface glycoproteins		MDC	Productive in MDC
Filiviridae	Ebola virus	DC-SIGN; L-SIGN		MDC	Productive in MDC
Flaviviridae	Dengue virus	DC-SIGN	LC	MDC LC PDC	Productive in MDC; PDC?
	HCV	DC-SIGN	MDC PDC		HCV-RNA detected in MDC
Retroviridae	HIV	CD4; CCR5; CXCR4; DC-SIGN; langerin	MDC PDC	MDC PDC	Productive in MDC and PDC

Table 1. Interactions of DNA and RNA viruses with human dendritic cells. HVEM, herpesvirus entry mediator; iMDC, immature myeloid dendritic cells; mMDC, mature myeloid dendritic cells; LC, Langerhans cells. Modified from Larsson et al. (353).

ing already matured MDC (as for VZV), viruses may block the upregulation of MHC class I and II molecules or reduce their expression levels and thereby interfere with antigen presentation to T cells (366, 368, 372, 373). In addition, measles virus blunts T cell responses by expressing a viral glycoprotein complex on the surface of infected DC that leads to induction of apoptosis of T cells due to increased TNF-related apoptosis inducing ligand (TRAIL) expression (374). Furthermore, HIV subverts the immunostimulatory ability of MDC by decreasing surface expression of CD4 (375). Conversely, contact with viruses activates PDC and while immature PDC only exhibit a weak T cell-stimulatory capacity, PDC matured by HSV, HIV or influenza virus can efficiently expand allogenic naïve T cells. (147, 271, 272).

Finally, viruses often affect the cytokine production by DC. PDC in contact with viruses secrete high amounts of type I IFN (145, 272, 355, 370, 371), TNF-α (317), IL-6 and IL-10 (**paper V**). MDC produce IL-12 when infected with influenza virus, but other viruses, such as HIV, HHV-6, HCMV and HSV, are capable of inhibiting IL-12 secretion upon maturation (366, 368, 372, 376).

3.2 The interplay between DC and HCMV: multilayered viral-induced functional paralysis of DC

HCMV endotheliotropic strains and clinical isolates can productively infect monocyte-derived DC, whereas laboratory-adapted HCMV strains that have been passaged multiple times in fibroblasts appear not to have this property (47, 377). The viral genes UL131-128 have been identified as the genetic determinants of the DC-tropism in HCMV clinical isolates and endotheliotropic strains (46). The interaction between virus and MDC appears to be mediated by the binding of the viral envelope glycoprotein gB to the DC membrane protein DC-SIGN, which facilitates viral entry (24). Immature and mature monocyte-derived DC exhibit a different susceptibility to HCMV infection, and even if complete viral replication has been detected in both these cell populations, infected immature DC release large amounts of infectious virions in the supernatant, while mature DC appear to be relatively resistant to HCMV infection and hardly release any virus in culture (368, 378). Conversely, Langerhans cells display a different susceptibility to HCMV infection. In fact, immature Langerhans DC are difficult to infect with HCMV, but become permissive to HCMV after maturation (379).

3.2.1 HCMV inhibits MDC differentiation and maturation

MDC are one of the target cells of HCMV infection and the virus can affect the DC differentiation and maturation pathway as well as a wide range of other specific functions of these cells, as shown *in vitro*. Data provided by our group suggest that HCMV may impair the generation of DC, since HCMV infection of monocytes blocks cytokine-induced differentiation into monocyte-derived DC. These cells also exhibit signs of severely depressed immunological functions (203).

HCMV may also impair the immunophenotype and function of immature monocyte-derived DC. Immature DC infected with HCMV have reduced expression of MHC class I and class II molecules on the cell surface as well as an altered profile of cytokine secretion, a suppressed allogenic immunostimulatory capacity and a reduced ability to stimulate a cytotoxic T cell response to influenza virus (368, 380).

Furthermore, HCMV inhibits the maturation of monocyte-derived DC, such that there

is no up-regulation of MHC class I and II, CD83 and costimulatory molecules upon maturation. In addition, the virus causes reduced secretion of IL-6, IL-10, IL-12 and TNF-α upon maturation, and impairs the ability of mature DC to induce T cell proliferation (368, 381). Productive HCMV infection is also associated with downmodulation of CD80, CD83, CD86 and MHC class I and class II molecules in mature Langerhans DC. These cells also exhibit a reduced ability for allostimulation (379). The presence of a soluble component has been suggested to act to impair alloreactivity of mature MDC upon HCMV infection (381). Recently, this factor has been identified as soluble CD83 that is released into the culture supernatant of HCMV-infected mature MDC (378). Other investigators have found an additional mechanism to explain the suppressed T cell response in presence of HCMV-infected mature MDC. Upregulation of the expression of apoptosis-inducing molecules such as CD95 (FasL) and TRAIL have been observed on the surface of infected DC, and these molecules would render infected MDC able to delete activated lymphocytes in allogenic mixed leukocyte reactions (MLR) (382).

The effect of MCMV infection on MDC has also been examined *in vivo* and *in vitro*. These studies suggest that infection of DC with HCMV results in an inhibition of T cell activation, with reduced IL-2 and IL-12 secretion, along with impaired antigen uptake (383). When immature DC are infected with MCMV, two phases of phenotypic changes are observed; at early stages after infection, the expression levels of MHC class I and II and accessory molecules increase to levels similar to those observed after LPS stimulation, but at later times the expression levels of all markers examined is reduced (383, 384). Therefore, MCMV-infected murine DC would initially efficiently activate T cells, but would then be expected to lose this ability, a phenomenon which differs from the continuous and general ability to suppress the immunostimulatory properties of DC that is observed during *in vitro* infection of human DC.

All these findings strongly suggest that both HCMV and MCMV alter the immunostimulatory properties of MDC. HCMV-infected MDC are therefore less capable of developing into professional APC and this may lead to impaired immune responses not only against HCMV, but most likely also against other invading microbes.

3.2.2 The viral encoded IL-10 subverts MDC function

It has recently been suggested that the virus may be assisted by the virally encoded cmvIL-10 in blunting the anti-viral properties of DC. cmvIL-10 is produced and released in the culture supernatant of infected cells at late time points during viral replication and exhibits an ability to suppress MDC functions (385). For example, cmvIL-10 impairs the secretion of pro-inflammatory cytokines, such as IL-6, IL-12 and TNF-α upon maturation stimuli, with a long-term effect lasting on re-stimulated DC (385). This virokine also blocks LPS-induced upregulation of MHC class I and class II, costimulatory and adhesion molecules, with an impaired capacity of cmvIL-10 exposed MDC to stimulate autologous and allogenic T cell proliferation (386). In addition, cmvIL-10 induces increased apoptosis of DC upon LPS stimulation. Finally, this viral-encoded cytokine could enhance HCMV infection by up-regulating the virus entry receptor DC-SIGN (386). These observations indicate that the secretion of cmvIL-10 during HCMV infection may assist the virus to infect MDC and to cause long-term inhibition of MDC functionality.

3.2.3 HCMV hampers MDC trafficking properties (paper III)

To fulfill their immunologic function as antigen-presenting cells that potently stimulate T-cell responses, DC must migrate from the circulation to the sites of inflammation and infection, and then to the lymphoid organs (see Chapter 2). These peculiar DC trafficking properties are key events in the early host response to pathogens and could be a crucial target for the virus to hamper DC functionality. It has been recently shown that HCMV impairs the migration of monocyte-derived DC matured by LPS, TNF-α and IFN-γ toward lymphoid chemokines *in vitro* and that the DC migration is inhibited by reduced expression of the lymphoid chemokine receptor CCR7 on the surface of infected mature DC (387). To further elucidate the effect of HCMV infection on MDC migration ability, we examined if the virus could affect an earlier step of the DC trafficking pathway, i.e. the ability of immature MDC to migrate in response to inflammatory stimuli (**paper III**).

We demonstrated that the endotheliotropic HCMV strain TB40/E strongly inhibited the migration of immature monocyte-derived DC in response to the inflammatory chemokines CCL3/MIP-1α and CCL5/RANTES 1 day after infection. We also found that intact viral particles and viral replication were required to inhibit MDC migration and that IE or E viral genes were involved in this process. It is known that migration of DC toward inflammatory chemokines requires the expression of CCR1, CCR2 and CCR5 (339). Therefore, we analyzed chemokine receptor expression on the surface of mock infected and TB40/E-infected immature MDC and found that HCMV consistently downregulated the expression of the inflammatory chemokine receptors CCR1 and CCR5, while there was no effect on the expression of CCR2 (inflammatory receptor) or CCR7 (lymphoid receptor). Fluorescence-activated cell sorting (FACS) analysis of total and cell-surface expression of CCR1 and CCR5 in immature DC and confocal microscopy showed that the receptors were redistributed from the cell surface to an intracellular compartment during HCMV infection. Thus, HCMV reduces surface expression of CCR1 and CCR5 in MDC by internalization of these receptors.

Since chemokine secretion may be responsible for auto-desensitization and internalization of cognate receptors and this mechanism would be advantageous for DC to regulate their own migratory abilities (see Chapter 2), we determined whether the downregulation of CCR1 and CCR5 on HCMV-infected DC was mediated by secretion of CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES. We found that DC started to secrete these three inflammatory chemokines after contact with the virus. Furthermore, by blocking the activity of CCL3/MIP-1α, CCL4/MIP-1β and CCL5/RANTES with neutralizing antibodies in infected cultures we could almost completely reverse the effect of the virus on chemokine receptor expression and on MDC migration.

We therefore propose that HCMV, through the secretion of inflammatory chemokines, causes internalization of CCR1 and CCR5 in immature MDC, leading to impaired ability of these cells to migrate in response to inflammatory chemokines. As an alternative explanation, upregulation of chemokine secretion by infected DC may be favorable for HCMV to attract more immature DC and other inflammatory cells to the site of infection, in order to increase viral targets and to promote viral spread. If this is the case, CCR1 and CCR5 downregulation *via* homologous desensitization and internalization may represent a mere side effect, which would be subordinate to a viral strategy of cell recruitment. Nevertheless and as previously mentioned, HCMV also inhibits the ability of matured MDC to upregulate CCR7 and to migrate in response to lymphoid chemokines (387). Taken altogether, these data suggest that HCMV affects the ability of MDC to migrate at different stages of their maturation leading to multifaceted paralysis of these cells in response to different chemotactic stimuli (**Figure 6**).

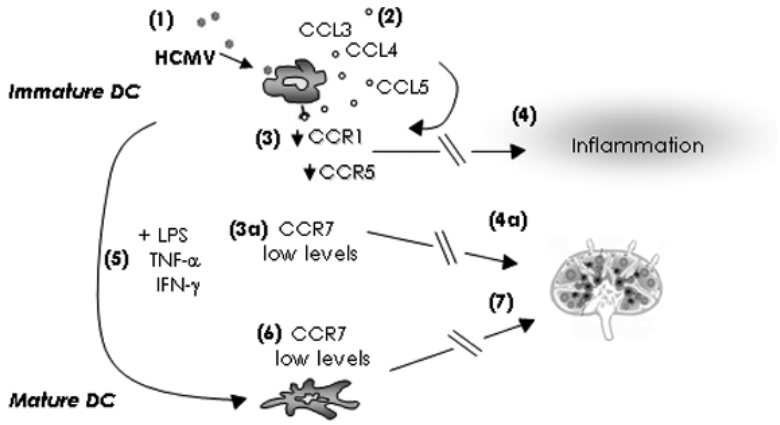

Figure 6. Proposed model by which HCMV influences DC migration. HCMV infects immature DC (1), resulting in the secretion of inflammatory chemokines (2), which bind to the cognate receptors CCR1 and CCR5, leading to internalization and a reduced surface expression (3). Downregulation of CCR1 and CCR5 expression is responsible for impaired DC migration to the sites of inflammation (4). Since HCMV does not affect the surface expression level of CCR7 in immature DC (3a), HCMV-infected immature DC do not migrate in response to the lymphoid chemokine CCL19 produced in lymph nodes (4a). HCMV-infected DC that encounter maturation stimuli (5) do not upregulate CCR7 (6) and therefore do not migrate to lymph nodes (7).

The impairment of MDC migration during HCMV infection *in vitro* may have important clinical implications, since it would interfere with the normal trafficking of these cells during infection in the natural host. In fact, the virus may infect immature MDC circulating in the blood or residing in peripheral tissues, which would not be able to migrate to inflamed/infected tissues where antigen uptake should take place. In addition, HCMV-infected immature DC would not upregulate CCR7, not even in the presence of maturation stimuli. Therefore, these infected cells would not be able to migrate to lymph nodes, which would interfere with the priming of T cell responses. Both these effects that have been observed in *in vitro* experimental systems may contribute to the immunosuppression that is often seen in HCMV-infected individuals.

3.2.4. HCMV subverts immunophenotype and function of MDC in the natural host (paper II and IV)

HCMV can subvert MDC functions by several mechanisms upon *in vitro* infection, as summarized in **Figure 7**. All mentioned studies examining the HCMV-MDC interactions have been performed using *in vitro* models of infection, while the effect of HCMV on MDC functions in humans had not been investigated at all. Therefore, we focused our attention on the behavior of MDC during HCMV infection in the natural host. We examined the DC immunophenotype and relevant functions in HT undergoing active HCMV infection as determined by a positive antigenemia test and in transplant patients who had never experienced active HCMV infection at the time of blood collection (**paper II**).

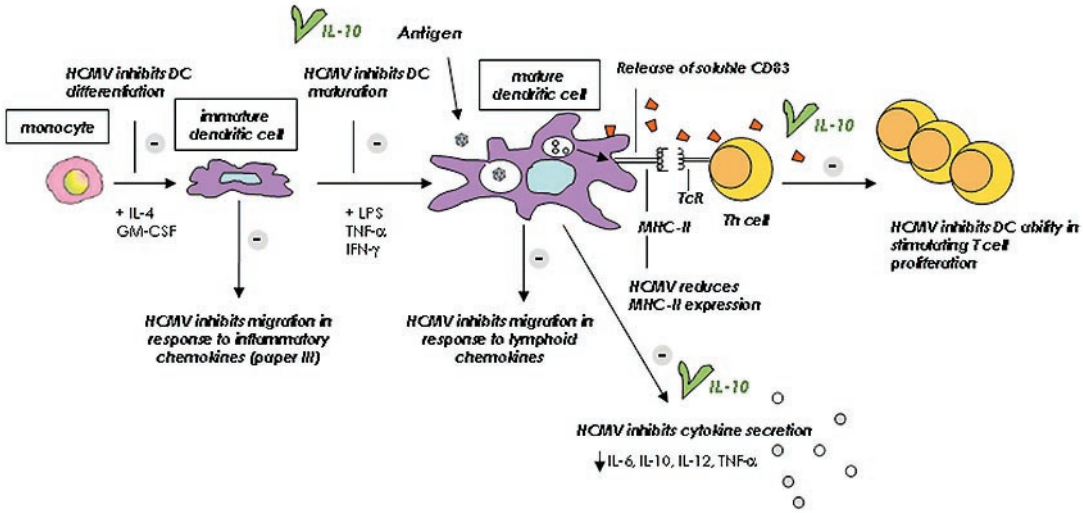

Figure 7. Diverse mechanisms by which HCMV inhibits MDC differentiation and maturation pathways and subverts MDC functions *in vitro*.

We detected viral RNA and antigens in both circulating DC and monocyte-derived DC from transplant patients undergoing an active HCMV infection, which suggests that, as in other viral infections (388, 389), circulating DC may act as carriers and mediate viral spread in the host. Furthermore, we found an impaired immunophenotype in immature monocyte-derived DC from patients undergoing active HCMV infection, with reduced expression of the classical DC marker CD1a and downregulation of MHC class I and II molecules. Immature DC obtained from infected patients did also exhibit an increased ability to secrete IL-10. In addition, while the immunophenotype of mature MDC was similar in infected and uninfected patients, the stimulatory potential of mature DC from HCMV-infected individuals was attenuated against alloantigens.

We could therefore conclude that HCMV subverts the MDC immunophenotype and function during infection in solid organ transplant recipients, and this impairment may contribute to the immunosuppression often observed in these patients.

Transplant recipients are ideal subjects to study the effect of HCMV on DC function because they often suffer from severe HCMV infections with a high viral load and systemic viral dissemination. On the other hand, these patients are constantly under immunosuppressive therapy with drugs, such as cyclosporine A and corticosteroids that have been demonstrated to impair MDC functions *in vitro* (390-392). In our study, we detected a general low level of LPS responsiveness in MDC from patients under immunosuppressive treatment as compared to healthy volunteers. In order to determine whether HCMV affected MDC functions in the absence of interfering factors caused by pharmacological immunosuppression, we decided to analyze monocyte-derived DC obtained from immunocompetent individuals undergoing symptomatic HCMV infection (i.e. HCMV mononucleosis) and compared the results with data obtained on DC mobilized from healthy blood donors (**paper IV**). All patients examined in this study were young and previously healthy. However,

since acquired HCMV infections in the normal host are generally asymptomatic (59), we cannot exclude that we involuntarily selected a group of individuals with high susceptibility to HCMV disease.

Diagnosis of HCMV-induced mononucleosis was established by serological criteria and all patients showed IgM positivity against HCMV. PCR and virus isolation were not performed as routine diagnostic methods at our hospitals for immunocompetent patients undergoing mononucleosis and these analyses were therefore not carried out for the patients in our study group. The lack of virological data is a weak point of this study, even though other common causes of mononucleosis were always excluded. In fact, in all the patients examined there was no serological evidence of acute EBV, adenovirus and HIV infection as revealed by the absence of specific serological markers. Immature and mature MDC obtained from patients undergoing HCMV mononucleosis expressed significantly lower levels of MHC class II molecules as compared to healthy controls. In addition, mature MDC from immunocompetent patients suffering HCMV infection resulted in inefficient stimulation of allogenic T cells and this finding is similar to what we observed in HCMV-infected transplant patients. This observation suggests that HCMV acts independently from immunosuppressive treatment in impairing the allostimulatory ability of MDC.

We also analyzed the pattern of cytokines and chemokines secreted by MDC obtained from patients undergoing HCMV mononucleosis. We found a pro-inflammatory secretion profile in DC from infected patients, with increased production of IL-1β, TNF-α, CCL2/MCP-1 and CCL3/MIP-1α upon LPS stimulation and reduced secretion of the anti-inflammatory cytokine IL-10 in both immature and mature MDC, as compared to results obtained from healthy volunteers. This is in contrast to our findings in transplant recipients (**paper II**), where immature MDC obtained from HCMV-infected patients exhibited increased secretion of IL-10. It is known that cyclosporine A increases the secretion of IL-10 by DC *in vitro* (393). We therefore suggest that HCMV may potentiate the effect of immunosuppressive drugs on IL-10 production by MDC in transplant patients, whereas in immunocompetent subjects, that are not under any pharmacological immunosuppression, the viral infection alone may skew the cytokine profile towards a pro-inflammatory pattern.

The production of inflammatory cytokines, such as TNF-α, in response to HCMV in immunocompetent subjects would be expected to help in combating the virus infection. However, TNF-α secretion by HCMV-infected monocytes has been previously shown to inhibit T cell proliferation through arachidonic acid and PGE2 release (202). Furthermore, an association has been reported between high TNF-α plasma levels and the development of HCMV disease in transplant patients (394, 395), and an inverse correlation has also been demonstrated between high IL-10/TNF-α ratio values in plasma and the development of symptomatic HCMV infection (396). Thus, the amplified pro-inflammatory cytokine profile with high TNF-α levels that we detected in mature MDC from patients undergoing HCMV mononucleosis may play a role in the antiviral response, but may also take part in the development of symptoms (i.e. fever, asthenia) and may contribute to the viral induced immunomodulation observed in infected patients.

In conclusion, the altered immunophenotype and the subversion of MDC immunostimulatory activity that we observed in immunocompetent and immunodepressed HCMV-infected patients imply importantly that HCMV-induced impairment of MDC functions is not only an *in vitro* phenomenon.

3.2.5 HCMV-PDC interaction causes PDC activation and opposite downstream effects on B cell and T cell subsets (paper V)

We became intrigued by the idea of investigating the effect of HCMV infection on PDC for the two following reasons: 1) a number of studies had reported that PDC exposed to HSV, MCMV, influenza virus and HIV secreted high amounts of IFN-α, became activated and acquired the ability to present antigens and expand allogenic T cells (145, 147, 272, 317). However, the effect of HCMV infection on PDC functionality had never been reported; 2) nonspecific polyclonal B cell activation caused by HCMV had been observed *in vitro* (245) and serological abnormalities, such as hypergammaglobulinemia, cryoglobulinemia and autoantibody production were often detected in HCMV-infected patients (233, 234, 236-238). Since PDC play a crucial role in the development of humoral immune responses (146, 246), we wondered if PDC could contribute to HCMV-induced hyperactivation of B cells.

We therefore assessed the effect of HCMV infection on human purified PDC *in vitro* and we analyzed the consequences of the infection on downstream B and T cell responses (**paper V**). We found that, in contrast to infection of MDC, endotheliotropic strains of HCMV (TB40/E and VR1814) infected PDC with low efficiency. In fact, only 1-3% of PDC were IE-positive at 1 day post infection, and the infection appeared to be mainly nonpermissive as no L viral antigens were detected in the infected cells. However, this nonpermissive infection resulted in clear phenotypic and functional changes of PDC.

HCMV led to partial maturation of PDC with up-regulation of MHC class II and CD83 molecules, but no increase of the costimulatory molecules CD80 and CD86. We also observed that HCMV induced IFN–α secretion by PDC and that, as for other herpesviruses (355, 356, 370), the production of this antiviral cytokine required engagement of the TLR9 pathway, as virus provoked IFN–α secretion was reduced in a dose-dependent fashion by addition of an inhibitory CpG DNA that is known to block the TLR9 pathway in a competitive way ((293, 294) and Chapter 2.3.1).

Our experiments showed that TB40/E-stimulated PDC secreted IFN–α and other cytokines, such as IL-6 and IL-10 are critical for B cell activation and differentiation (146, 246, 247), which led us to investigate whether soluble factors produced by HCMV-infected PDC could induce B cell activation *in vitro*. We found that in the presence of BCR stimulation, soluble factors produced by HCMV-infected PDC triggered B cell activation and proliferation. We then set up a co-culture model that involved costimulation of B cells with PDC, anti-Ig and HCMV and we observed that, through PDC stimulation, HCMV prompted B cell activation, but not plasma cell differentiation in a T-cell-independent way. Conversely, CpG-C, a known TLR9 activator (292), was capable of inducing plasma cell differentiation in our as well as previously described culture models (246). A plausible explanation for our findings may be that HCMV acts as a type A stimulatory CpG, which means that it is a strong inducer of type I IFN secretion by PDC but a poor activator of B cells (290). Conversely, the positive control used in our experiments, type C CpG, is capable of activating both PDC and B cells (292) and induces synergistically direct and indirect stimulation of B cells, as described in Chapter 2.3.1. We observed activation of the B cell compartment caused by HCMV *via* PDC at 3 and 7 days of co-culture which may contribute to the previously described findings of B cell hyperactivation by HCMV *in vitro* (245) and to the generation of the abnormal humoral response observed in HCMV-infected patients (233, 234, 236-238).

In order to study whether HCMV could affect the ability of PDC to serve as T cell stimulators, we examined the stimulatory activity of uninfected and HCMV-infected PDC in an allogenic MLR. Surprisingly and in contrast to the stimulatory effect caused by other

viruses (145, 147, 272, 317), we found that HCMV-exposed PDC were even less efficient than mock infected PDC in inducing allogenic T cell proliferation despite the increased expression levels of MHC class II molecules. We observed that the reduced immunostimulatory capacity of HCMV-infected PDC affected both $CD4^+$ and $CD8^+$ T cell subsets. These observations may be explained by a lack of enhanced expression of costimulatory molecules on PDC after HCMV infection. In fact, the failure of T cells to receive costimulation following antigen presentation may render them anergic and functionally incapable of proliferating (397, 398).

In **paper II** and **IV**, we described that HCMV impairs the stimulatory potential of MDC against alloantigens in both transplant and immunocompetent patients, and similar results have been reported using *in vitro* models of infection (368, 380, 381). The ability of HCMV to simultaneously suppress the immunostimulatory properties of the MDC and PDC subsets may represent an effective mechanism to evade the early host immune response, and may significantly contribute to the immunodepression detected in HCMV-infected patients.

In conclusion, the data obtained in the last study described in this thesis suggest that the contact between HCMV and PDC resulted in an activation of these cells *via* TLR9 and also resulted in opposite consequences for the two arms of the adaptive immunity. On one hand, HCMV-activated PDC secreted soluble factors that stimulated B cell activation and proliferation. On the other hand, HCMV inhibited the allostimulatory ability of these professional APC, leading to reduced proliferation of $CD4^+$ and $CD8^+$ T cells and hampered T cell responses. Our results represent the first evidence for a dual and divergent control of humoral and T cells responses *via* HCMV-activated PDC.

3.3 DC and HCMV: a double edged sword (paper II, IV, V)

Apart from the possibility that HCMV might use infected DC as vehicles to transport the virus to different tissues (as we suggest in **paper II**), the interactions between DC and HCMV plays a dual role in the generation of immunity. On one hand, DC are essential for the induction of an HCMV-specific immune response, due to their potent antigen presentation properties and their unique capacity to stimulate naïve T cells. On the other hand, in order for the virus to evade the host immune response, DC are themselves targets for HCMV and the virus is capable of blocking the differentiation and maturation of MDC and blunting the immunostimulatory ability of both MDC and PDC subsets ((203, 368) and **paper II**, **IV** and **V**).

Despite the functional debilitation of infected DC, large numbers of specific effector and memory $CD4^+$ and $CD8^+$ T cells ultimately develop in the host undergoing acute HCMV infection and such cells help in the clearance of a productive infection. Thus, the infected host develops countermeasures to combat the viral offensive. The most effective host counterattack to balance the viral subversion of DC would be cross-presentation. Cross-presentation is defined as exogenous acquisition of antigen from the tissues through macropinocytosis or phagocytosis and direct presentation of the antigen to $CD8^+$ T cells without requiring endogenous processing. Immature MDC maintained in co-culture with HCMV-infected fibroblasts acquire viral antigens for cross-presentation to specific CTL and a mechanism other than apoptosis is suggested for the initiation of cross-presentation. In addition, early HCMV-infected fibroblasts provide a maturation signal for immature DC during co-culture, which is a prerequisite for efficient stimulation of $CD8^+$ T cells (399, 400). Cross-presentation of HCMV antigens would enable noninfected well-

functioning DC to stimulate and promote $CD8^+$ T cell responses to HCMV and this mechanism most probably plays a crucial part in the generation of T cell responses to HCMV infection in the natural host (401). However, the effective role of cross-presentation in inducing an efficient cellular immunity to HCMV *in vivo* has not yet been assessed.

4. SUMMARY OF THE STUDIES INCLUDED IN THIS THESIS AND GENERAL CONCLUSIONS

In the course of acute HCMV infection, patients often suffer from immunological dysfunctions. Immunosuppression caused by the virus is transitory but significant, and leads to an increased frequency of invasive fungal and bacterial infections in transplant patients (87-89). Autoimmune phenomena are also often observed in HCMV-infected patients, and autoantibodies may contribute to the development of GVHD in infected BMT and graft rejection in solid organ recipients (233, 235, 236).

The central purpose of this PhD project has been to analyze the immunological dysfunctions observed in HCMV-infected patients and to better understand the mechanisms underlying these immunological abnormalities. In the first study of this thesis, we retrospectively analyzed sera obtained from LT with or without active HCMV infection. We observed the presence of anti-EC, anti-smooth muscle cell and anti-nuclear autoantibodies in the sera of 43% LT undergoing acute HCMV infection and we found a correlation between the appearance of autoantibodies and the presence of a high amount of virus in the blood.

The mechanisms by which the virus subverts the immune system of the host at a cellular level drew my attention and I searched for an appropriate cell model to investigate this matter. During this time, I became interested in the possible effects of HCMV on DC. These cells are the sentinels of the immune system, and play a strategic role in the initiation and coordination of adaptive immune response to infections. Recently, with the discovery of the type I IFN secreting-PDC and with the finding that the innate germ-line encoded TLR are differentially expressed in different DC subsets, these APC have also been demonstrated to be fundamental in triggering the innate response to infections and to link innate and adaptive immunity. Such cells would be an ideal target for a virus that is able to potently subvert the host immune response and this has been the general hypothesis underlying the subsequent studies included in this thesis. I therefore decided to examine the effect of HCMV on DC functions both in *in vitro* models of infection and during infection in the natural host.

Little was known about how the virus influenced MDC during HCMV infection *in vivo*. We compared blood DC and monocyte-derived DC from HT undergoing acute HCMV infection with DC obtained from HCMV-negative transplant patients. Blood DC and monocyte-derived DC of HCMV actively infected patients expressed IE antigens of HCMV and, as in other viral infections, these cells may serve as carriers and transport the virus to different tissues. In addition, we found an impaired immunophenotype in immature DC from HCMV-infected patients, with decreased expression of the classical DC marker CD1a, and reduced expression levels of MHC class I and class II molecules. Finally, we detected altered IL-10 secretion in immature DC and reduced ability of mature DC obtained from patients undergoing active HCMV infection to stimulate allogenic T cells.

In order to examine the significance of HCMV on DC function in the absence of the interfering factor caused by immunosuppressive therapy, we also analyzed monocyte-derived DC obtained from immunocompetent individuals undergoing symptomatic HCMV-infection (HCMV mononucleosis). We found that immature and mature DC obtained from HCMV-infected patients exibited an altered immunophenoype, with significantly lower expression levels of MHC class II molecules as compared to healthy controls. Similar to the finding in HCMV-infected transplant recipients, mature DC obtained from patients with

HCMV mononucleosis were less efficient in stimulating proliferation of allogenic lymphocytes than mature DC obtained from healthy controls. The pattern of cyto-chemokines secreted by DC obtained from HCMV-infected patients was characterized by a pro-inflammatory profile with increased production of IL-1β, TNF–α, CCL2 and CCL3 and reduced secretion of IL-10 upon maturation stimuli. We could therefore conclude that HCMV alters DC immunophenotype and function during infection in both immunocompetent and immunosuppressed hosts. These observations imply a viral strategy to interfere with early functions of the host immune system which would most likely contribute to the generation of immunosuppression in infected individuals.

The peculiar trafficking properties of DC are key events in the early host response to pathogens. We hypothesized that subverting the migratory abilities of DC may be a good strategy for HCMV to block the initiation of the host immune response. We therefore assessed *in vitro* DC migration assays in response to inflammatory chemokines upon HCMV infection. The HCMV endothelial-adapted strain TB40/E inhibited the migration of immature monocyte-derived DC in response to inflammatory chemokines by 95% at 1 day after infection. HCMV infection significantly reduced the cell-surface expression of CCR1 and CCR5, but left the expression of the lymphoid chemokine receptor CCR7 unchanged. HCMV infection also induced secretion of the inflammatory chemokines CCL3, CCL4 and CCL5 and neutralizing antibodies for these chemokines reduced the effects of HCMV on chemokine receptor expression and on DC migration. We suggest that HCMV may downregulate chemokine receptor expression on DC by inducing the secretion of inflammatory chemokines and thus hamper the migratory abilities of DC. The interference of HCMV with the migratory ability of DC may alter homeostatic DC trafficking and would most likely contribute to viral mediated immunosuppression in the infected natural host.

Lately, a newly discovered DC subset caught my attention. PDC represent a central cell type in innate and adaptive immunity and, *via* the production of type I IFN, these cells exert a dual role in antiviral responses by directly inhibiting viral replication and by determining the initiation and regulation of downstream B and T cell responses. Such a crucial cell type could be utilized as an interesting model to study *in vitro* the effect of HCMV infection on innate immune responses. In addition, PDC play a fundamental role in the development of humoral immune responses, and our question was: could these cells be involved in the generation of the autoimmune phenomena that we found in transplant patients? We therefore assessed the effect of HCMV infection on human PDC *in vitro*. Despite the low efficiency of HCMV infection in these cells, contact of the virus with PDC resulted in partial maturation of PDC. The virus-treated PDC upregulated MHC class II and CD83 molecule expression, but did not increase the expression of the co-stimulatory molecules CD80 and CD86. We also found that HCMV-infected PDC secreted high amounts of IFN-α by the engagement of TLR9.

Interestingly, the interaction between PDC and HCMV resulted in opposite consequences for B and T cell downstream responses. HCMV-activated PDC secreted soluble factors that stimulated B cell activation and proliferation. Conversely, HCMV inhibited the allostimulatory ability of PDC, leading to depressed proliferation of $CD4^+$ and $CD8^+$ lymphocytes and hampered T cell responses. In paper II, IV and V, we observed that HCMV exhibited a strategic capacity to simultaneously undermine the immunostimulatory properties of both MDC and PDC populations. Subverting the function of the two most potent APC subsets may give considerable support to a condition of immunosuppression in infected individuals. In addition, B cell hyperactivation induced by the virus through PDC stimulation could play a role in the generation of the abnormal humoral features that are

often observed in HCMV-infected patients, such as the autoantibody production that we described in the first study of this thesis.

In conclusion, attacking cells such as DC that play a key role in initiation and control of both innate and adaptive immune responses may be an effective immune evasion strategy for the virus. As a side effect, an HCMV-induced subversion of DC function may blunt the immune response to other pathogens, which would have important clinical implications in the acute phase of infection in immunocompromised patients, and may also contribute to the generation of autoimmune phenomena (**Figure 8**). Hyperactivation of humoral immunity may also impede the development of specific B cell responses and may represent another mechanism of viral immune evasion. B cell hyperactivation may however also be a simple epiphenomenon that doesn't affect the viral efficiency, but that clearly has important clinical implications for infected patients. In fact, autoantibodies have been suggested to contribute to the development of GVHD in infected BMT and to graft rejection in solid organ recipients.

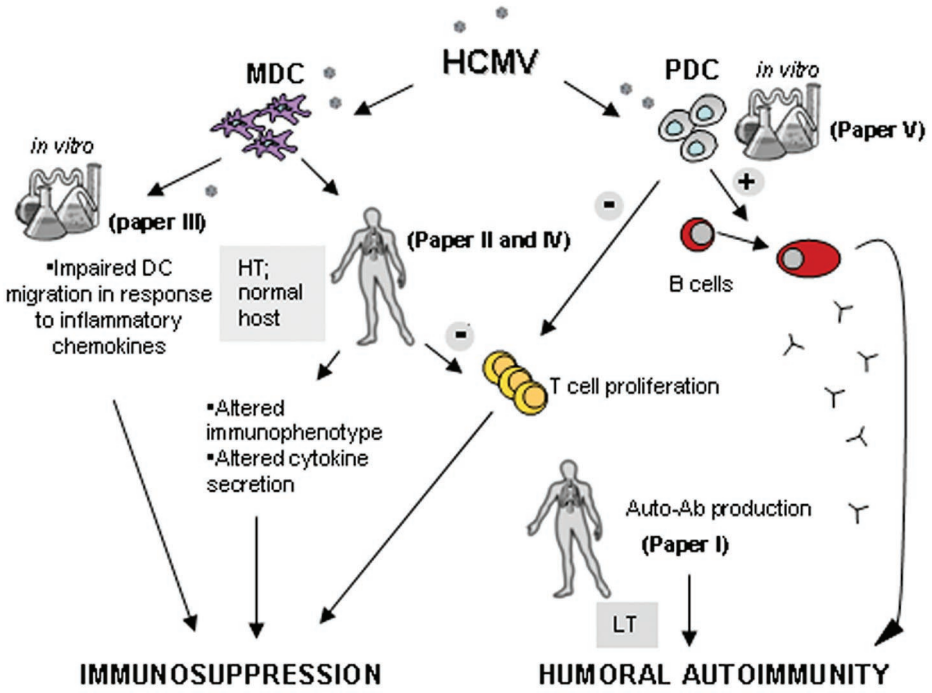

Figure 8. Schematic representation of the projects included in this thesis. HT, heart transplant patients; LT, liver transplant patients, Auto-Ab, autoantibody.

5. FUTURE PROSPECTIVES

One can only wonder what a virus that affects the core of the host immunity in such a powerful way may provoke during co-infection with another potent immunomodulating pathogen. During my post-graduate research training I attended two courses on tropical infections in Africa and I developed an interest in parasitic infections. In particular, I became interested in *Plasmodium falciparum* malaria that is causing a massive burden of disease and death in small children and pregnant women in Sub-Saharan Africa. This parasite also drew my attention because of its ability to profoundly subvert the host immune system by inducing immunosuppression (402, 403), triggering autoimmune phenomena (404) and causing severe immune-mediated syndromes (405). In addition, *Plasmodium falciparum* strongly affects both MDC and PDC functions (406-409). For these reasons, I would like to understand whether HCMV and *Plasmodium falciparum* can infect the same host and if these pathogens may interact with each other and what the result of this interaction could be.

HCMV is estimated to latently infect nearly the entire population in Sub-Saharan Africa. Pregnancy malaria is a common and severe infection in countries where malaria is endemic and it is associated with placental parasite infection and a local strong inflammatory response, including elevated pro-inflammatory cytokine (TNF–α, IFN-γ) levels and monocyte infiltration into the placental intervillous spaces (410). The physiological immunotolerance induced by pregnancy, together with immunosuppression caused by malaria infection and the high levels of TNF–α found in the placentas of pregnant women with malaria may act in synergy to induce local reactivation of latent HCMV. The outcome of an acute HCMV infection in the placenta of women with pregnancy malaria and the consequences for the offspring are completely unknown.

A consensus is recently developing that many diseases can be aetiologically linked to infection by more than one pathogen and that such polymicrobial diseases may have common underlying mechanisms regardless of whether the coinfecting pathogens are viral, bacterial, or parasitic (411). An example of polymicrobial disease is the endemic form of Burkitt's lymphoma, for which two human pathogens appear to be responsible; EBV and *Plasmodium falciparum* (412). EBV reactivation with elevated viral load has recently been observed in the blood of children living in holoendemic malaria areas as compared to children from districts where malaria transmission is sporadic or absent, suggesting a role for malaria infection in affecting the manteinance of EBV latency (413, 414). Similarly, HCMV may also reactivate in the presence of favorable conditions, such as immunosuppression and inflammation that can be induced by *Plasmodium falciparum* infection. HCMV reactivation can therefore occur during malaria infection and play its own role in the polymicrobial interactions that lead to the development of the endemic form of Burkitt's lymphoma. In this setting, the suppression of T cell responses and the hyperactivation of humoral responses induced by HCMV may contribute to the generation of malignant memory B cell clones. A possible role for HCMV in the development of endemic Burkitt's lymphoma has never been taken into consideration.

Looking for answers to these and additional issues related to polymicrobial diseases and their effect on the host immune system will be the topic of my future research.

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7. REFERENCES

1. Pass, R. 2001. Cytomegalovirus. In Fields Virology, Vol. 2. D. Knipe, and P. Howley, eds. Lippincott Williams and Wilkins, Philadelphia, p. 2675.
2. Roizman, B., and P. E. Pellet. 2001. The family Herpesviridae: a brief introduction. In Fields Virology, Vol. 2. D. Knipe, and P. Howley, eds. Lippincott Williams and Wilkins, Philadelphia, p. 2381.
3. Minton, E. J., C. Tysoe, J. H. Sinclair, and J. G. Sissons. 1994. Human cytomegalovirus infection of the monocyte/macrophage lineage in bone marrow. J Virol 68:4017.
4. Mocarski, E. S. 2001. Cytomegalovirus and their replication. In Fields Virology, Vol. 2. D. Knipe, and P. Howley, eds. Lippincott Williams and Wilkins, Philadelphia, p. 2629.
5. Murphy, E., D. Yu, J. Grimwood, J. Schmutz, M. Dickson, M. A. Jarvis, G. Hahn, J. A. Nelson, R. M. Myers, and T. E. Shenk. 2003. Coding potential of laboratory and clinical strains of human cytomegalovirus. Proc Natl Acad Sci U S A 100:14976.
6. Britt, W. J., and S. Boppana. 2004. Human cytomegalovirus virion proteins. Hum Immunol 65:395.
7. Giugni, T. D., C. Soderberg, D. J. Ham, R. M. Bautista, K. O. Hedlund, E. Moller, and J. A. Zaia. 1996. Neutralization of human cytomegalovirus by human CD13-specific antibodies. J Infect Dis 173:1062.
8. Grundy, J. E., J. A. McKeating, and P. D. Griffiths. 1987. Cytomegalovirus strain AD169 binds beta 2 microglobulin *in vitro* after release from cells. J Gen Virol 68 (Pt 3):777.
9. Wright, J. F., A. Kurosky, E. L. Pryzdial, and S. Wasi. 1995. Host cellular annexin II is associated with cytomegalovirus particles isolated from cultured human fibroblasts. J Virol 69:4784.
10. Benson, J. D., and E. S. Huang. 1990. Human cytomegalovirus induces expression of cellular topoisomerase II. J Virol 64:9.
11. Spear, G. T., N. S. Lurain, C. J. Parker, M. Ghassemi, G. H. Payne, and M. Saifuddin. 1995. Host cell-derived complement control proteins CD55 and CD59 are incorporated into the virions of two unrelated enveloped viruses. Human T cell leukemia/lymphoma virus type I (HTLV-I) and human cytomegalovirus (HCMV). J Immunol 155:4376.
12. Britt, W. J. 1984. Neutralizing antibodies detect a disulfide-linked glycoprotein complex within the envelope of human cytomegalovirus. Virology 135:369.
13. Navarro, D., P. Paz, S. Tugizov, K. Topp, J. La Vail, and L. Pereira. 1993. Glycoprotein B of human cytomegalovirus promotes virion penetration into cells, transmission of infection from cell to cell, and fusion of infected cells. Virology 197:143.
14. Mach, M., B. Kropff, M. Kryzaniak, and W. Britt. 2005. Complex formation by glycoproteins M and N of human cytomegalovirus: structural and functional aspects. J Virol 79:2160.
15. Li, L., J. A. Nelson, and W. J. Britt. 1997. Glycoprotein H-related complexes of human cytomegalovirus: identification of a third protein in the gCIII complex. J Virol 71:3090.
16. Spaete, R. R., K. Perot, P. I. Scott, J. A. Nelson, M. F. Stinski, and C. Pachl. 1993. Coexpression of truncated human cytomegalovirus gH with the UL115

gene product or the truncated human fibroblast growth factor receptor results in transport of gH to the cell surface. Virology 193:853.

17. Keay, S., and B. Baldwin. 1991. Anti-idiotype antibodies that mimic gp86 of human cytomegalovirus inhibit viral fusion but not attachment. J Virol 65:5124.
18. Kinzler, E. R., and T. Compton. 2005. Characterization of human cytomegalovirus glycoprotein-induced cell-cell fusion. J Virol 79:7827.
19. Compton, T., R. R. Nepomuceno, and D. M. Nowlin. 1992. Human cytomegalovirus penetrates host cells by pH-independent fusion at the cell surface. Virology 191:387.
20. Compton, T., D. M. Nowlin, and N. R. Cooper. 1993. Initiation of human cytomegalovirus infection requires initial interaction with cell surface heparan sulfate. Virology 193:834.
21. Keay, S., T. C. Merigan, and L. Rasmussen. 1989. Identification of cell surface receptors for the 86-kilodalton glycoprotein of human cytomegalovirus. Proc Natl Acad Sci U S A 86:10100.
22. Soderberg, C., T. D. Giugni, J. A. Zaia, S. Larsson, J. M. Wahlberg, and E. Moller. 1993. CD13 (human aminopeptidase N) mediates human cytomegalovirus infection. J Virol 67:6576.
23. Wang, X., S. M. Huong, M. L. Chiu, N. Raab-Traub, and E. S. Huang. 2003. Epidermal growth factor receptor is a cellular receptor for human cytomegalovirus. Nature 424:456.
24. Halary, F., A. Amara, H. Lortat-Jacob, M. Messerle, T. Delaunay, C. Houles, F. Fieschi, F. Arenzana-Seisdedos, J. F. Moreau, and J. Dechanet-Merville. 2002. Human cytomegalovirus binding to DC-SIGN is required for dendritic cell infection and target cell trans-infection. Immunity 17:653.
25. Pietropaolo, R., and T. Compton. 1999. Interference with annexin II has no effect on entry of human cytomegalovirus into fibroblast cells. J Gen Virol 80 (Pt 7):1807.
26. Gerna, G., D. Zipeto, E. Percivalle, M. Parea, M. G. Revello, R. Maccario, G. Peri, and G. Milanesi. 1992. Human cytomegalovirus infection of the major leukocyte subpopulations and evidence for initial viral replication in polymorphonuclear leukocytes from viremic patients. J Infect Dis 166:1236.
27. Gilbert, G. L., K. Hayes, I. L. Hudson, and J. James. 1989. Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leucocytes. Neonatal Cytomegalovirus Infection Study Group. Lancet 1:1228.
28. Saederup, N., S. A. Aguirre, T. E. Sparer, D. M. Bouley, and E. S. Mocarski. 2001. Murine cytomegalovirus CC chemokine homolog MCK-2 (m131-129) is a determinant of dissemination that increases inflammation at initial sites of infection. J Virol 75:9966.
29. Percivalle, E., M. G. Revello, L. Vago, F. Morini, and G. Gerna. 1993. Circulating endothelial giant cells permissive for human cytomegalovirus (HCMV) are detected in disseminated HCMV infections with organ involvement. J Clin Invest 92:663.
30. Gerna, G., F. Baldanti, and M. G. Revello. 2004. Pathogenesis of human cytomegalovirus infection and cellular targets. Hum Immunol 65:381.
31. Gerna, G., E. Percivalle, F. Baldanti, S. Sozzani, P. Lanzarini, E. Genini, D. Lilleri, and M. G. Revello. 2000. Human cytomegalovirus replicates abortively in polymorphonuclear leukocytes after transfer from infected endothelial cells via transient microfusion events. J Virol 74:5629.
32. Sinzger, C., and G. Jahn. 1996. Human cytomegalovirus cell tropism and pathogenesis. Intervirology 39:302.
33. Rice, G. P., R. D. Schrier, and M. B.

Oldstone. 1984. Cytomegalovirus infects human lymphocytes and monocytes: virus expression is restricted to immediate-early gene products. Proc Natl Acad Sci U S A 81:6134.

34. Sinzger, C., B. Plachter, A. Grefte, T. H. The, and G. Jahn. 1996. Tissue macrophages are infected by human cytomegalovirus *in vivo*. J Infect Dis 173:240.
35. Ibanez, C. E., R. Schrier, P. Ghazal, C. Wiley, and J. A. Nelson. 1991. Human cytomegalovirus productively infects primary differentiated macrophages. J Virol 65:6581.
36. Weinshenker, B. G., S. Wilton, and G. P. Rice. 1988. Phorbol ester-induced differentiation permits productive human cytomegalovirus infection in a monocytic cell line. J Immunol 140:1625.
37. Gonczol, E., P. W. Andrews, and S. A. Plotkin. 1984. Cytomegalovirus replicates in differentiated but not in undifferentiated human embryonal carcinoma cells. Science 224:159.
38. Reeves, M. B., P. A. MacAry, P. J. Lehner, J. G. Sissons, and J. H. Sinclair. 2005. Latency, chromatin remodeling, and reactivation of human cytomegalovirus in the dendritic cells of healthy carriers. Proc Natl Acad Sci U S A 102:4140.
39. Gerna, G., E. Percivalle, A. Sarasini, F. Baldanti, G. Campanini, and M. G. Revello. 2003. Rescue of human cytomegalovirus strain AD169 tropism for both leukocytes and human endothelial cells. J Gen Virol 84:1431.
40. MacCormac, L. P., and J. E. Grundy. 1999. Two clinical isolates and the Toledo strain of cytomegalovirus contain endothelial cell tropic variants that are not present in the AD169, Towne, or Davis strains. J Med Virol 57:298.
41. Gerna, G., E. Percivalle, A. Sarasini, and M. G. Revello. 2002. Human cytomegalovirus and human umbilical vein endothelial cells: restriction of primary isolation to blood samples and susceptibilities of clinical isolates from other sources to adaptation. J Clin Microbiol 40:233.
42. Revello, M. G., E. Percivalle, E. Arbustini, R. Pardi, S. Sozzani, and G. Gerna. 1998. *In vitro* generation of human cytomegalovirus pp65 antigenemia, viremia, and leukoDNAemia. J Clin Invest 101:2686.
43. Sinzger, C., K. Schmidt, J. Knapp, M. Kahl, R. Beck, J. Waldman, H. Hebart, H. Einsele, and G. Jahn. 1999. Modification of human cytomegalovirus tropism through propagation *in vitro* is associated with changes in the viral genome. J Gen Virol 80 (Pt 11):2867.
44. Hahn, G., M. G. Revello, M. Patrone, E. Percivalle, G. Campanini, A. Sarasini, M. Wagner, A. Gallina, G. Milanesi, U. Koszinowski, F. Baldanti, and G. Gerna. 2004. Human cytomegalovirus UL131-128 genes are indispensable for virus growth in endothelial cells and virus transfer to leukocytes. J Virol 78:10023.
45. Dolan, A., C. Cunningham, R. D. Hector, A. F. Hassan-Walker, L. Lee, C. Addison, D. J. Dargan, D. J. McGeoch, D. Gatherer, V. C. Emery, P. D. Griffiths, C. Sinzger, B. P. McSharry, G. W. Wilkinson, and A. J. Davison. 2004. Genetic content of wild-type human cytomegalovirus. J Gen Virol 85:1301.
46. Gerna, G., E. Percivalle, D. Lilleri, L. Lozza, C. Fornara, G. Hahn, F. Baldanti, and M. G. Revello. 2005. Dendritic-cell infection by human cytomegalovirus is restricted to strains carrying functional UL131-128 genes and mediates efficient viral antigen presentation to $CD8^+$ T cells. J Gen Virol 86:275.
47. Riegler, S., H. Hebart, H. Einsele, P. Brossart, G. Jahn, and C. Sinzger. 2000. Monocyte-derived dendritic cells are permissive to the complete replicative cycle of human cytomegalovirus. J Gen Virol 81:393.

48. Pass, R. F. 1985. Epidemiology and transmission of cytomegalovirus. J Infect Dis 152:243.

49. Bello, C., and H. Whittle. 1991. Cytomegalovirus infection in Gambian mothers and their babies. J Clin Pathol 44:366.

50. Rodier, M. H., J. Berthonneau, A. Bourgoin, G. Giraudeau, G. Agius, C. Burucoa, A. Hekpazo, and J. L. Jacquemin. 1995. Seroprevalences of Toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou, Republic of Benin. Acta Trop 59:271.

51. Minamishima, I., K. Ueda, T. Minematsu, Y. Minamishima, M. Umemoto, H. Take, and K. Kuraya. 1994. Role of breast milk in acquisition of cytomegalovirus infection. Microbiol Immunol 38:549.

52. Waner, J. L., D. R. Hopkins, T. H. Weller, and E. N. Allred. 1977. Cervical excretion cytomegalovirus: correlation with secretory and humoral antibody. J Infect Dis 136:805.

53. Abulafia, O., B. DuBeshter, A. E. Dawson, and D. M. Sherer. 1993. Presence of cytomegalovirus inclusion bodies in a recurrent ulcerative vaginal lesion. Am J Obstet Gynecol 169:1179.

54. Barbara, J. A., and G. E. Tegtmeier. 1987. Cytomegalovirus and blood transfusion. Blood Rev 1:207.

55. Onorato, I. M., D. M. Morens, W. J. Martone, and S. K. Stansfield. 1985. Epidemiology of cytomegaloviral infections: recommendations for prevention and control. Rev Infect Dis 7:479.

56. Pass, R. F., S. C. Hutto, D. W. Reynolds, and R. B. Polhill. 1984. Increased frequency of cytomegalovirus infection in children in group day care. Pediatrics 74:121.

57. Handsfield, H. H., S. H. Chandler, V. A. Caine, J. D. Meyers, L. Corey, E. Medeiros, and J. K. McDougall. 1985. Cytomegalovirus infection in sex partners: evidence for sexual transmission. J Infect Dis 151:344.

58. Sohn, Y. M., M. K. Oh, K. B. Balcarek, G. A. Cloud, and R. F. Pass. 1991. Cytomegalovirus infection in sexually active adolescents. J Infect Dis 163:460.

59. Zanghellini, F., S. B. Boppana, V. C. Emery, P. D. Griffiths, and R. F. Pass. 1999. Asymptomatic primary cytomegalovirus infection: virologic and immunologic features. J Infect Dis 180:702.

60. Taylor-Wiedeman, J., J. G. Sissons, L. K. Borysiewicz, and J. H. Sinclair. 1991. Monocytes are a major site of persistence of human cytomegalovirus in peripheral blood mononuclear cells. J Gen Virol 72 (Pt 9):2059.

61. Soderberg-Naucler, C., K. N. Fish, and J. A. Nelson. 1997. Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. Cell 91:119.

62. Soderberg-Naucler, C., K. N. Fish, and J. A. Nelson. 1997. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. J Clin Invest 100:3154.

63. Docke, W. D., C. Kiessling, M. Worm, M. Friedrich, A. Pruss, M. Weitz, S. Prosch, F. Kern, H. D. Volk, W. Sterry, and K. Asadullah. 2003. Subclinical activation of latent cytomegalovirus (CMV) infection and anti-CMV immune response in patients with atopic dermatitis. Br J Dermatol 148:954.

64. Docke, W. D., S. Prosch, E. Fietze, V. Kimel, H. Zuckermann, C. Klug, U. Syrbe, D. H. Kruger, R. von Baehr, and H. D. Volk. 1994. Cytomegalovirus reactivation and tumour necrosis factor. Lancet 343:268.

65. Kutza, A. S., E. Muhl, H. Hackstein, H. Kirchner, and G. Bein. 1998. High incidence of active cytomegalovirus infection among septic patients. Clin Infect Dis 26:1076.

66. Guyotat, D., R. Gibert, J. Chomel, E. Archimbaud, S. Bossard, J. Maupas, D. Fiere, and M. Aymard. 1987. Incidence and prognosis of cytomegalovirus infections following allogenic bone marrow transplantation. J Med Virol 23:393.

67. Koskinen, P. K., E. A. Kallio, J. M. Tikkanen, R. K. Sihvola, P. J. Hayry, and K. B. Lemstrom. 1999. Cytomegalovirus infection and cardiac allograft vasculopathy. Transpl Infect Dis 1:115.

68. Lautenschlager, I., K. Hockerstedt, E. Taskinen, and E. von Willebrand. 1996. Expression of adhesion molecules and their ligands in liver allografts during cytomegalovirus (CMV) infection and acute rejection. Transpl Int 9 Suppl 1:S213.

69. Ricart, M. J., J. Malaise, A. Moreno, M. Crespo, and L. Fernandez-Cruz. 2005. Cytomegalovirus: occurrence, severity, and effect on graft survival in simultaneous pancreas-kidney transplantation. Nephrol Dial Transplant 20 Suppl 2:ii25.

70. Harkins, L., A. L. Volk, M. Samanta, I. Mikolaenko, W. J. Britt, K. I. Bland, and C. S. Cobbs. 2002. Specific localisation of human cytomegalovirus nucleic acids and proteins in human colorectal cancer. Lancet 360:1557.

71. Samanta, M., L. Harkins, K. Klemm, W. J. Britt, and C. S. Cobbs. 2003. High prevalence of human cytomegalovirus in prostatic intraepithelial neoplasia and prostatic carcinoma. J Urol 170:998.

72. Cobbs, C. S., L. Harkins, M. Samanta, G. Y. Gillespie, S. Bharara, P. H. King, L. B. Nabors, C. G. Cobbs, and W. J. Britt. 2002. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res 62:3347.

73. Prosch, S., C. E. Wendt, P. Reinke, C. Priemer, M. Oppert, D. H. Kruger, H. D. Volk, and W. D. Docke. 2000. A novel link between stress and human cytomegalovirus (HCMV) infection: sympathetic hyperactivity stimulates HCMV activation. Virology 272:357.

74. Fowler, K. B., S. Stagno, R. F. Pass, W. J. Britt, T. J. Boll, and C. A. Alford. 1992. The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med 326:663.

75. Lazzarotto, T., S. Varani, B. Guerra, A. Nicolosi, M. Lanari, and M. P. Landini. 2000. Prenatal indicators of congenital cytomegalovirus infection. J Pediatr 137:90.

76. Boppana, S. B., K. B. Fowler, W. J. Britt, S. Stagno, and R. F. Pass. 1999. Symptomatic congenital cytomegalovirus infection in infants born to mothers with preexisting immunity to cytomegalovirus. Pediatrics 104:55.

77. Gaytant, M. A., G. I. Rours, E. A. Steegers, J. M. Galama, and B. A. Semmekrot. 2003. Congenital cytomegalovirus infection after recurrent infection: case reports and review of the literature. Eur J Pediatr 162:248.

78. Klemola, E., R. Von Essen, G. Henle, and W. Henle. 1970. Infectious-mononucleosis-like disease with negative heterophil agglutination test. Clinical features in relation to Epstein-Barr virus and cytomegalovirus antibodies. J Infect Dis 121:608.

79. Jordan, M. C., W. Rousseau, J. A. Stewart, G. R. Noble, and T. D. Chin. 1973. Spontaneous cytomegalovirus mononucleosis. Clinical and laboratory observations in nine cases. Ann Intern Med 79:153.

80. Revello, M. G., M. Zavattoni, A. Sarasini, E. Percivalle, L. Simoncini, and G. Gerna. 1998. Human cytomegalovirus in blood of immunocompetent persons during primary infection: prognostic implications for pregnancy. J Infect Dis 177:1170.

81. Shepp, D. H., J. E. Moses, and M. H. Kaplan. 1996. Seroepidemiology of cytomegalovirus in patients with advanced HIV disease: influence on disease expression and survival. J Acquir

Immune Defic Syndr Hum Retrovirol 11:460.

82. Springer, K. L., and A. Weinberg. 2004. Cytomegalovirus infection in the era of HAART: fewer reactivations and more immunity. J Antimicrob Chemother 54:582.

83. Varani, S., P. Spezzacatena, R. Manfredi, F. Chiodo, A. Mastroianni, P. Ballarini, A. Boschini, T. Lazzarotto, and M. P. Landini. 2000. The incidence of cytomegalovirus (CMV) antigenemia and CMV disease is reduced by highly active antiretroviral therapy. Eur J Epidemiol 16:433.

84. Kestelyn, P. G., and E. T. Cunningham, Jr. 2001. HIV/AIDS and blindness. Bull World Health Organ 79:208.

85. Ng, W. T., and P. Versace. 2005. Ocular association of HIV infection in the era of highly active antiretroviral therapy and the global perspective. Clin Experiment Ophthalmol 33:317.

86. Pereyra, F., and R. H. Rubin. 2004. Prevention and treatment of cytomegalovirus infection in solid organ transplant recipients. Curr Opin Infect Dis 17:357.

87. Nichols, W. G., L. Corey, T. Gooley, C. Davis, and M. Boeckh. 2002. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. J Infect Dis 185:273.

88. George, M. J., D. R. Snydman, B. G. Werner, J. Griffith, M. E. Falagas, N. N. Dougherty, and R. H. Rubin. 1997. The independent role of cytomegalovirus as a risk factor for invasive fungal disease in orthotopic liver transplant recipients. Boston Center for Liver Transplantation CMVIG-Study Group. Cytogam, MedImmune, Inc. Gaithersburg, Maryland. Am J Med 103:106.

89. Wagner, J. A., H. Ross, S. Hunt, P. Gamberg, H. Valantine, T. C. Merigan, and E. B. Stinson. 1995. Prophylactic ganciclovir treatment reduces fungal as well as cytomegalovirus infections after heart transplantation. Transplantation 60:1473.

90. Grattan, M. T., C. E. Moreno-Cabral, V. A. Starnes, P. E. Oyer, E. B. Stinson, and N. E. Shumway. 1989. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. Jama 261:3561.

91. McDonald, K., T. S. Rector, E. A. Braulin, S. H. Kubo, and M. T. Olivari. 1989. Association of coronary artery disease in cardiac transplant recipients with cytomegalovirus infection. Am J Cardiol 64:359.

92. von Willebrand, E., E. Pettersson, J. Ahonen, and P. Hayry. 1986. CMV infection, class II antigen expression, and human kidney allograft rejection. Transplantation 42:364.

93. Lonnqvist, B., O. Ringden, B. Wahren, G. Gahrton, and G. Lundgren. 1984. Cytomegalovirus infection associated with and preceding chronic graft-versus-host disease. Transplantation 38:465.

94. Matthes-Martin, S., S. W. Aberle, C. Peters, W. Holter, T. Popow-Kraupp, U. Potschger, G. Fritsch, R. Ladenstein, A. Rosenmayer, K. Dieckmann, and H. Gadner. 1998. CMV-viraemia during allogenic bone marrow transplantation in paediatric patients: association with survival and graft-versus-host disease. Bone Marrow Transplant 21 Suppl 2:S53.

95. Soderberg, C., S. Larsson, B. L. Rozell, S. Sumitran-Karuppan, P. Ljungman, and E. Moller. 1996. Cytomegalovirus-induced CD13-specific autoimmunity--a possible cause of chronic graft-vs-host disease. Transplantation 61:600.

96. Hebart, H., L. Kanz, G. Jahn, and H. Einsele. 1998. Management of cytomegalovirus infection after solid-organ or stem-cell transplantation. Current guidelines and future prospects. Drugs 55:59.

97. Singh, N. 2001. Preemptive therapy versus universal prophylaxis with ganciclovir for cytomegalovirus in solid organ transplant recipients. Clin Infect Dis 32:742.

98. Rahbar, A., L. Bostrom, U. Lagerstedt, I. Magnusson, C. Soderberg-Naucler, and V. A. Sundqvist. 2003. Evidence of active cytomegalovirus infection and increased production of IL-6 in tissue specimens obtained from patients with inflammatory bowel diseases. Inflamm Bowel Dis 9:154.

99. Kishore, J., U. Ghoshal, U. C. Ghoshal, N. Krishnani, S. Kumar, M. Singh, and A. Ayyagari. 2004. Infection with cytomegalovirus in patients with inflammatory bowel disease: prevalence, clinical significance and outcome. J Med Microbiol 53:1155.

100. Takahashi, Y., and T. Tange. 2004. Prevalence of cytomegalovirus infection in inflammatory bowel disease patients. Dis Colon Rectum 47:722.

101. Asadullah, K., S. Prosch, H. Audring, I. Buttnerova, H. D. Volk, W. Sterry, and W. D. Docke. 1999. A high prevalence of cytomegalovirus antigenaemia in patients with moderate to severe chronic plaque psoriasis: an association with systemic tumour necrosis factor alpha overexpression. Br J Dermatol 141:94.

102. Einsele, H., M. Steidle, C. A. Muller, P. Fritz, J. Zacher, H. Schmidt, and J. G. Saal. 1992. Demonstration of cytomegalovirus (CMV) DNA and anti-CMV response in the synovial membrane and serum of patients with rheumatoid arthritis. J Rheumatol 19:677.

103. Hamerman, D., I. Gresser, and C. Smith. 1982. Isolation of cytomegalovirus from synovial cells of a patient with rheumatoid arthritis. J Rheumatol 9:658.

104. Mehraein, Y., C. Lennerz, S. Ehlhardt, K. Remberger, A. Ojak, and K. D. Zang. 2004. Latent Epstein-Barr virus (EBV) infection and cytomegalovirus (CMV) infection in synovial tissue of autoimmune chronic arthritis determined by RNA- and DNA-in situ hybridization. Mod Pathol 17:781.

105. Murayama, T., F. Jisaki, M. Ayata, D. Sakamuro, T. Hironaka, K. Hirai, N. Tsuchiya, K. Ito, and T. Furukawa. 1992. Cytomegalovirus genomes demonstrated by polymerase chain reaction in synovial fluid from rheumatoid arthritis patients. Clin Exp Rheumatol 10:161.

106. Zhang, C., K. Shen, Z. Jiang, and X. He. 2001. Early diagnosis and monitoring of active HCMV infection in children with systemic lupus erythematosus. Chin Med J (Engl) 114:1309.

107. Nawata, M., N. Seta, M. Yamada, I. Sekigawa, N. Lida, and H. Hashimoto. 2001. Possible triggering effect of cytomegalovirus infection on systemic lupus erythematosus. Scand J Rheumatol 30:360.

108. Hayashi, T., S. Lee, H. Ogasawara, I. Sekigawa, N. Iida, Y. Tomino, H. Hashimoto, and S. Hirose. 1998. Exacerbation of systemic lupus erythematosus related to cytomegalovirus infection. Lupus 7:561.

109. Vasquez, V., R. A. Barzaga, and B. A. Cunha. 1992. Cytomegalovirus-induced flare of systemic lupus erythematosus. Heart Lung 21:407.

110. Stratta, P., L. Colla, S. Santi, A. Grill, L. Besso, L. Godio, L. Davico-Bonino, G. Mazzucco, V. Ghisetti, A. Barbui, and C. Canavese. 2002. IgM antibodies against cytomegalovirus in SLE nephritis: viral infection or aspecific autoantibody? J Nephrol 15:88.

111. Lunardi, C., C. Bason, R. Navone, E. Millo, G. Damonte, R. Corrocher, and A. Puccetti. 2000. Systemic sclerosis immunoglobulin G autoantibodies bind the human cytomegalovirus late protein UL94 and induce apoptosis in human endothelial cells. Nat Med 6:1183.

112. Namboodiri, A. M., K. M. Rocca, and J. P. Pandey. 2004. IgG antibodies to human cytomegalovirus late protein UL94 in patients with systemic sclerosis. Autoimmunity 37:241.

113. Horwitz, C. A., W. Henle, G. Henle, H. Polesky, H. H. Balfour, Jr., R. A. Siem, S. Borken, and P. C. Ward. 1977. Heterophil-negative infectious mononucleosis and mononucleosis-like illnesses. Laboratory confirmation of 43 cases. Am J Med 63:947.

114. Horwitz, C. A., W. Henle, and G. Henle. 1979. Diagnostic aspects of the cytomegalovirus mononucleosis syndrome in previously healthy persons. Postgrad Med 66:153.

115. Taylor, G. H. 2003. Cytomegalovirus. Am Fam Physician 67:519.

116. Just-Nubling, G., S. Korn, B. Ludwig, C. Stephan, H. W. Doerr, and W. Preiser. 2003. Primary cytomegalovirus infection in an outpatient setting--laboratory markers and clinical aspects. Infection 31:318.

117. Wreghitt, T. G., E. L. Teare, O. Sule, R. Devi, and P. Rice. 2003. Cytomegalovirus infection in immunocompetent patients. Clin Infect Dis 37:1603.

118. Gandhi, M. K., and R. Khanna. 2004. Human cytomegalovirus: clinical aspects, immune regulation, and emerging treatments. Lancet Infect Dis 4:725.

119. Maine, G. T., R. Stricker, M. Schuler, J. Spesard, S. Brojanac, B. Iriarte, K. Herwig, T. Gramins, B. Combs, J. Wise, H. Simmons, T. Gram, J. Lonze, D. Ruzicki, B. Byrne, J. D. Clifton, L. E. Chovan, D. Wachta, C. Holas, D. Wang, T. Wilson, S. Tomazic-Allen, M. A. Clements, G. L. Wright, Jr., T. Lazzarotto, A. Ripalti, and M. P. Landini. 2000. Development and clinical evaluation of a recombinant-antigen-based cytomegalovirus immunoglobulin M automated immunoassay using the Abbott AxSYM analyzer. J Clin Microbiol 38:1476.

120. Basson, J., J. C. Tardy, and M. Aymard. 1989. Pattern of anti-cytomegalovirus IgM antibodies determined by immunoblotting. A study of kidney graft recipients developing a primary or recurrent CMV infection. Arch Virol 108:259.

121. Nielsen, S. L., I. Sorensen, and H. K. Andersen. 1988. Kinetics of specific immunoglobulins M, E, A, and G in congenital, primary, and secondary cytomegalovirus infection studied by antibody-capture enzyme-linked immunosorbent assay. J Clin Microbiol 26:654.

122. Lazzarotto, T., P. Spezzacatena, S. Varani, L. Gabrielli, P. Pradelli, B. Guerra, and M. P. Landini. 1999. Anticytomegalovirus (anti-CMV) immunoglobulin G avidity in identification of pregnant women at risk of transmitting congenital CMV infection. Clin Diagn Lab Immunol 6:127.

123. Lazzarotto, T., P. Spezzacatena, P. Pradelli, D. A. Abate, S. Varani, and M. P. Landini. 1997. Avidity of immunoglobulin G directed against human cytomegalovirus during primary and secondary infections in immunocompetent and immunocompromised subjects. Clin Diagn Lab Immunol 4:469.

124. Gerna, G., E. Percivalle, F. Baldanti, A. Sarasini, M. Zavattoni, M. Furione, M. Torsellini, and M. G. Revello. 1998. Diagnostic significance and clinical impact of quantitative assays for diagnosis of human cytomegalovirus infection/disease in immunocompromised patients. New Microbiol 21:293.

125. Einsele, H., G. Ehninger, H. Hebart, K. M. Wittkowski, U. Schuler, G. Jahn, P. Mackes, M. Herter, T. Klingebiel, J. Loffler, S. Wagner, and C. A. Muller. 1995. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood 86:2815.

126. Fox, J. C., I. M. Kidd, P. D. Griffiths, P. Sweny, and V. C. Emery. 1995. Longitudinal analysis of cytomegalovirus load in renal transplant recipients using a quantitative polymerase chain reaction: correlation with disease. J Gen Virol 76 (Pt 2):309.

127. Francisci, D., A. Tosti, F. Baldelli, G. Stagni, and S. Pauluzzi. 1997. The pp65 antigenaemia test as a predictor of cytomegalovirus-induced end-organ disease in patients with AIDS. Aids 11:1341.

128. Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, P. Grossi, and G. Milanesi. 1991. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia, and DNAemia. J Infect Dis 164:488.

129. van der Bij, W., R. Torensma, W. J. van Son, J. Anema, J. Schirm, A. M. Tegzess, and T. H. The. 1988. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. J Med Virol 25:179.

130. Grazia Revello, M., M. Zavattoni, E. Percivalle, P. Grossi, and G. Gerna. 1989. Correlation between immunofluorescent detection of human cytomegalovirus immediate early antigens in polymorphonuclear leukocytes and viremia. J Infect Dis 160:159.

131. Diviacco, S., P. Norio, L. Zentilin, S. Menzo, M. Clementi, G. Biamonti, S. Riva, A. Falaschi, and M. Giacca. 1992. A novel procedure for quantitative polymerase chain reaction by coamplification of competitive templates. Gene 122:313.

132. Fox, J. C., P. D. Griffiths, and V. C. Emery. 1992. Quantification of human cytomegalovirus DNA using the polymerase chain reaction. J Gen Virol 73 (Pt 9):2405.

133. Zipeto, D., F. Baldanti, D. Zella, M. Furione, A. Cavicchini, G. Milanesi, and G. Gerna. 1993. Quantification of human cytomegalovirus DNA in peripheral blood polymorphonuclear leukocytes of immunocompromised patients by the polymerase chain reaction. J Virol Methods 44:45.

134. Gerna, G., M. Furione, F. Baldanti, E. Percivalle, P. Comoli, and F. Locatelli. 1995. Quantitation of human cytomegalovirus DNA in bone marrow transplant recipients. Br J Haematol 91:674.

135. Vivarelli, M., N. De Ruvo, T. Lazzarotto, R. Bellusci, M. P. Landini, S. Varani, and A. Cavallari. 2000. Abstension from treatment of low-level pp65 cytomegalovirus antigenemia after liver transplantation: a prospective study. Transplantation 70:1183.

136. Janeway, C. A., Jr., and R. Medzhitov. 2002. Innate immune recognition. Annu Rev Immunol 20:197.

137. Medzhitov, R., and C. A. Janeway, Jr. 1998. Innate immune recognition and control of adaptive immune responses. Semin Immunol 10:351.

138. Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17:189.

139. Harrison, C. J., and J. L. Waner. 1985. Natural killer cell activity in infants and children excreting cytomegalovirus. J Infect Dis 151:301.

140. Borysiewicz, L. K., B. Rodgers, S. Morris, S. Graham, and J. G. Sissons. 1985. Lysis of human cytomegalovirus infected fibroblasts by natural killer cells: demonstration of an interferon-independent component requiring expression of early viral proteins and characterization of effector cells. J Immunol 134:2695.

141. Starr, S. E., L. Smiley, C. Wlodaver, H. M. Friedman, S. A. Plotkin, and C. Barker. 1984. Natural killing of

cytomegalovirus-infected targets in renal transplant recipients. Transplantation 37:161.

142. Oh, S. H., S. Bandyopadhyay, D. S. Miller, and S. E. Starr. 1987. Cooperation between CD16(Leu-11b)+ NK cells and HLA-DR+ cells in natural killing of herpesvirus-infected fibroblasts. J Immunol 139:2799.
143. Biron, C. A., K. S. Byron, and J. L. Sullivan. 1989. Severe herpesvirus infections in an adolescent without natural killer cells. N Engl J Med 320:1731.
144. Biron, C. A. 2001. Interferons alpha and beta as immune regulators--a new look. Immunity 14:661.
145. Dalod, M., T. Hamilton, R. Salomon, T. P. Salazar-Mather, S. C. Henry, J. D. Hamilton, and C. A. Biron. 2003. Dendritic cell responses to early murine cytomegalovirus infection: subset functional specialization and differential regulation by interferon alpha/beta. J Exp Med 197:885.
146. Jego, G., A. K. Palucka, J. P. Blanck, C. Chalouni, V. Pascual, and J. Banchereau. 2003. Plasmacytoid dendritic cells induce plasma cell differentiation through type I interferon and interleukin 6. Immunity 19:225.
147. Kadowaki, N., S. Antonenko, J. Y. Lau, and Y. J. Liu. 2000. Natural interferon alpha/beta-producing cells link innate and adaptive immunity. J Exp Med 192:219.
148. Katze, M. G., Y. He, and M. Gale, Jr. 2002. Viruses and interferon: a fight for supremacy. Nat Rev Immunol 2:675.
149. Agnello, D., C. S. Lankford, J. Bream, A. Morinobu, M. Gadina, J. J. O'Shea, and D. M. Frucht. 2003. Cytokines and transcription factors that regulate T helper cell differentiation: new players and new insights. J Clin Immunol 23:147.
150. Landini, M. P., E. Rossier, and H. Schmitz. 1988. Antibodies to human cytomegalovirus structural polypeptides during primary infection. J Virol Methods 22:309.
151. Porath, A., N. Hanuka, A. Keynan, and I. Sarov. 1987. Virus-specific serum IgG, IgM, and IgA antibodies in cytomegalovirus mononucleosis patients as determined by immunoblotting technique. J Med Virol 22:223.
152. Landini, M. P., and M. La Placa. 1991. Humoral immune response to human cytomegalovirus proteins: a brief review. Comp Immunol Microbiol Infect Dis 14:97.
153. Britt, W. J., L. Vugler, E. J. Butfiloski, and E. B. Stephens. 1990. Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus-infected cells in analysis of the human neutralizing antibody response. J Virol 64:1079.
154. Mach, M., B. Kropff, P. Dal Monte, and W. Britt. 2000. Complex formation by human cytomegalovirus glycoproteins M (gpUL100) and N (gpUL73). J Virol 74:11881.
155. Urban, M., M. Klein, W. J. Britt, E. Hassfurther, and M. Mach. 1996. Glycoprotein H of human cytomegalovirus is a major antigen for the neutralizing humoral immune response. J Gen Virol 77 (Pt 7):1537.
156. Borysiewicz, L. K., J. K. Hickling, S. Graham, J. Sinclair, M. P. Cranage, G. L. Smith, and J. G. Sissons. 1988. Human cytomegalovirus-specific cytotoxic T cells. Relative frequency of stage-specific CTL recognizing the 72-kD immediate early protein and glycoprotein B expressed by recombinant vaccinia viruses. J Exp Med 168:919.
157. Borysiewicz, L. K., S. Morris, J. D. Page, and J. G. Sissons. 1983. Human cytomegalovirus-specific cytotoxic T lymphocytes: requirements for *in vitro* generation and specificity. Eur J Immunol 13:804.
158. Quinnan, G. V., Jr., W. H. Burns, N. Kirmani, A. H. Rook, J. Manischewitz, L. Jackson, G. W. Santos, and R. Saral. 1984. HLA-restricted cytotoxic T lymphocytes are an early immune response

and important defense mechanism in cytomegalovirus infections. Rev Infect Dis 6:156.

159. Reusser, P., S. R. Riddell, J. D. Meyers, and P. D. Greenberg. 1991. Cytotoxic T-lymphocyte response to cytomegalovirus after human allogeneic bone marrow transplantation: pattern of recovery and correlation with cytomegalovirus infection and disease. Blood 78:1373.

160. Couriel, D., J. Canosa, H. Engler, A. Collins, C. Dunbar, and A. J. Barrett. 1996. Early reactivation of cytomegalovirus and high risk of interstitial pneumonitis following T-depleted BMT for adults with hematological malignancies. Bone Marrow Transplant 18:347.

161. Przepiorka, D., C. Ippoliti, A. Panina, J. Goodrich, S. Giralt, K. van Besien, R. Mehra, A. B. Deisseroth, B. Andersson, M. Luna, and et al. 1994. Ganciclovir three times per week is not adequate to prevent cytomegalovirus reactivation after T cell-depleted marrow transplantation. Bone Marrow Transplant 13:461.

162. Gillespie, G. M., M. R. Wills, V. Appay, C. O'Callaghan, M. Murphy, N. Smith, P. Sissons, S. Rowland-Jones, J. I. Bell, and P. A. Moss. 2000. Functional heterogeneity and high frequencies of cytomegalovirus-specific CD8(+) T lymphocytes in healthy seropositive donors. J Virol 74:8140.

163. Weekes, M. P., M. R. Wills, K. Mynard, A. J. Carmichael, and J. G. Sissons. 1999. The memory cytotoxic T-lymphocyte (CTL) response to human cytomegalovirus infection contains individual peptide-specific CTL clones that have undergone extensive expansion *in vivo*. J Virol 73:2099.

164. Ouyang, Q., W. M. Wagner, A. Wikby, S. Walter, G. Aubert, A. I. Dodi, P. Travers, and G. Pawelec. 2003. Large numbers of dysfunctional CD8+ T lymphocytes bearing receptors for a single dominant CMV epitope in the very old. J Clin Immunol 23:247.

165. Vescovini, R., A. Telera, F. F. Fagnoni, C. Biasini, M. C. Medici, P. Valcavi, P. di Pede, G. Lucchini, L. Zanlari, G. Passeri, F. Zanni, C. Chezzi, C. Franceschi, and P. Sansoni. 2004. Different contribution of EBV and CMV infections in very long-term carriers to age-related alterations of CD8+ T cells. Exp Gerontol 39:1233.

166. Chen, S. F., W. W. Tu, M. A. Sharp, E. C. Tongson, X. S. He, H. B. Greenberg, T. H. Holmes, Z. Wang, G. Kemble, A. M. Manganello, S. P. Adler, C. L. Dekker, D. B. Lewis, and A. M. Arvin. 2004. Antiviral CD8 T cells in the control of primary human cytomegalovirus infection in early childhood. J Infect Dis 189:1619.

167. Tu, W., S. Chen, M. Sharp, C. Dekker, A. M. Manganello, E. C. Tongson, H. T. Maecker, T. H. Holmes, Z. Wang, G. Kemble, S. Adler, A. Arvin, and D. B. Lewis. 2004. Persistent and selective deficiency of CD4+ T cell immunity to cytomegalovirus in immunocompetent young children. J Immunol 172:3260.

168. Gamadia, L. E., E. B. Remmerswaal, J. F. Weel, F. Bemelman, R. A. van Lier, and I. J. Ten Berge. 2003. Primary immune responses to human CMV: a critical role for IFN-gamma-producing CD4+ T cells in protection against CMV disease. Blood 101:2686.

169. Gamadia, L. E., R. J. Rentenaar, R. A. van Lier, and I. J. ten Berge. 2004. Properties of CD4(+) T cells in human cytomegalovirus infection. Hum Immunol 65:486.

170. Jonjic, S., I. Pavic, P. Lucin, D. Rukavina, and U. H. Koszinowski. 1990. Efficacious control of cytomegalovirus infection after long-term depletion of CD8+ T lymphocytes. J Virol 64:5457.

171. Davignon, J. L., P. Castanie, J. A. Yorke, N. Gautier, D. Clement, and C. Davrinche. 1996. Anti-human cytomegalovirus activity of cytokines produced by CD4+ T-cell clones specif-

ically activated by IE1 peptides *in vitro* J Virol 70:2162.

172. Davignon, J. L., D. Clement, J. Alriquet, S. Michelson, and C. Davrinche. 1995. Analysis of the proliferative T cell response to human cytomegalovirus major immediate-early protein (IE1): phenotype, frequency and variability. Scand J Immunol 41:247.
173. McLaughlin-Taylor, E., H. Pande, S. J. Forman, B. Tanamachi, C. R. Li, J. A. Zaia, P. D. Greenberg, and S. R. Riddell. 1994. Identification of the major late human cytomegalovirus matrix protein pp65 as a target antigen for CD8+ virus-specific cytotoxic T lymphocytes. J Med Virol 43:103.
174. van Zanten, J., M. C. Harmsen, P. van der Meer, W. van der Bij, W. J. van Son, M. van der Giessen, J. Prop, L. de Leij, and T. H. The. 1995. Proliferative T cell responses to four human cytomegalovirus-specific proteins in healthy subjects and solid organ transplant recipients. J Infect Dis 172:879.
175. He, H., C. R. Rinaldo, Jr., and P. A. Morel. 1995. T cell proliferative responses to five human cytomegalovirus proteins in healthy seropositive individuals: implications for vaccine development. J Gen Virol 76 (Pt 7):1603.
176. Bunde, T., A. Kirchner, B. Hoffmeister, D. Habedank, R. Hetzer, G. Cherepnev, S. Proesch, P. Reinke, H. D. Volk, H. Lehmkuhl, and F. Kern. 2005. Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells. J Exp Med 201:1031.
177. Sacre, K., G. Carcelain, N. Cassoux, A. M. Fillet, D. Costagliola, D. Vittecoq, D. Salmon, Z. Amoura, C. Katlama, and B. Autran. 2005. Repertoire, diversity, and differentiation of specific CD8 T cells are associated with immune protection against human cytomegalovirus disease. J Exp Med 201:1999.
178. Cerboni, C., M. Mousavi-Jazi, A. Linde, K. Soderstrom, M. Brytting, B. Wahren, K. Karre, and E. Carbone. 2000. Human cytomegalovirus strain-dependent changes in NK cell recognition of infected fibroblasts. J Immunol 164:4775.
179. Odeberg, J., H. Browne, S. Metkar, C. J. Froelich, L. Branden, D. Cosman, and C. Soderberg-Naucler. 2003. The human cytomegalovirus protein UL16 mediates increased resistance to natural killer cell cytotoxicity through resistance to cytolytic proteins. J Virol 77:4539.
180. Odeberg, J., C. Cerboni, H. Browne, K. Karre, E. Moller, E. Carbone, and C. Soderberg-Naucler. 2002. Human cytomegalovirus (HCMV)-infected endothelial cells and macrophages are less susceptible to natural killer lysis independent of the downregulation of classical HLA class I molecules or expression of the HCMV class I homologue, UL18. Scand J Immunol 55:149.
181. Reyburn, H. T., O. Mandelboim, M. Vales-Gomez, D. M. Davis, L. Pazmany, and J. L. Strominger. 1997. The class I MHC homologue of human cytomegalovirus inhibits attack by natural killer cells. Nature 386:514.
182. Tomasec, P., V. M. Braud, C. Rickards, M. B. Powell, B. P. McSharry, S. Gadola, V. Cerundolo, L. K. Borysiewicz, A. J. McMichael, and G. W. Wilkinson. 2000. Surface expression of HLA-E, an inhibitor of natural killer cells, enhanced by human cytomegalovirus gpUL40. Science 287:1031.
183. Ulbrecht, M., S. Martinozzi, M. Grzeschik, H. Hengel, J. W. Ellwart, M. Pla, and E. H. Weiss. 2000. Cutting edge: the human cytomegalovirus UL40 gene product contains a ligand for HLA-E and prevents NK cell-mediated lysis. J Immunol 164:5019.
184. Dunn, C., N. J. Chalupny, C. L. Sutherland, S. Dosch, P. V. Sivakumar, D. C. Johnson, and D. Cosman. 2003. Human cytomegalovirus glycoprotein UL16

causes intracellular sequestration of NKG2D ligands, protecting against natural killer cell cytotoxicity. J Exp Med 197:1427.

185. Kubin, M., L. Cassiano, J. Chalupny, W. Chin, D. Cosman, W. Fanslow, J. Mullberg, A. M. Rousseau, D. Ulrich, and R. Armitage. 2001. ULBP1, 2, 3: novel MHC class I-related molecules that bind to human cytomegalovirus glycoprotein UL16, activate NK cells. Eur J Immunol 31:1428.
186. Taylor, R. T., and W. A. Bresnahan. 2005. Human cytomegalovirus immediate-early 2 gene expression blocks virus-induced beta interferon production. J Virol 79:3873.
187. Abate, D. A., S. Watanabe, and E. S. Mocarski. 2004. Major human cytomegalovirus structural protein pp65 (ppUL83) prevents interferon response factor 3 activation in the interferon response. J Virol 78:10995.
188. Browne, E. P., and T. Shenk. 2003. Human cytomegalovirus UL83-coded pp65 virion protein inhibits antiviral gene expression in infected cells. Proc Natl Acad Sci U S A 100:11439.
189. Loenen, W. A., C. A. Bruggeman, and E. J. Wiertz. 2001. Immune evasion by human cytomegalovirus: lessons in immunology and cell biology. Semin Immunol 13:41.
190. Hegde, N. R., R. A. Tomazin, T. W. Wisner, C. Dunn, J. M. Boname, D. M. Lewinsohn, and D. C. Johnson. 2002. Inhibition of HLA-DR assembly, transport, and loading by human cytomegalovirus glycoprotein US3: a novel mechanism for evading major histocompatibility complex class II antigen presentation. J Virol 76:10929.
191. Tomazin, R., J. Boname, N. R. Hegde, D. M. Lewinsohn, Y. Altschuler, T. R. Jones, P. Cresswell, J. A. Nelson, S. R. Riddell, and D. C. Johnson. 1999. Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by CD4+ T cells. Nat Med 5:1039.
192. Odeberg, J., B. Plachter, L. Branden, and C. Soderberg-Naucler. 2003. Human cytomegalovirus protein pp65 mediates accumulation of HLA-DR in lysosomes and destruction of the HLA-DR alpha-chain. Blood 101:4870.
193. Miller, D. M., B. M. Rahill, J. M. Boss, M. D. Lairmore, J. E. Durbin, J. W. Waldman, and D. D. Sedmak. 1998. Human cytomegalovirus inhibits major histocompatibility complex class II expression by disruption of the Jak/Stat pathway. J Exp Med 187:675.
194. Lilley, B. N., H. L. Ploegh, and R. S. Tirabassi. 2001. Human cytomegalovirus open reading frame TRL11/IRL11 encodes an immunoglobulin G Fc-binding protein. J Virol 75:11218.
195. Atalay, R., A. Zimmermann, M. Wagner, E. Borst, C. Benz, M. Messerle, and H. Hengel. 2002. Identification and expression of human cytomegalovirus transcription units coding for two distinct Fcgamma receptor homologs. J Virol 76:8596.
196. Spiller, O. B., B. P. Morgan, F. Tufaro, and D. V. Devine. 1996. Altered expression of host-encoded complement regulators on human cytomegalovirus-infected cells. Eur J Immunol 26:1532.
197. Almeida, G. D., C. D. Porada, S. St Jeor, and J. L. Ascensao. 1994. Human cytomegalovirus alters interleukin-6 production by endothelial cells. Blood 83:370.
198. Michelson, S., P. Dal Monte, D. Zipeto, B. Bodaghi, L. Laurent, E. Oberlin, F. Arenzana-Seisdedos, J. L. Virelizier, and M. P. Landini. 1997. Modulation of RANTES production by human cytomegalovirus infection of fibroblasts. J Virol 71:6495.
199. Moses, A. V., and H. M. Garnett. 1990. The effect of human cytomegalovirus on the production and biologic action of interleukin-1. J Infect Dis 162:381.
200. Smith, P. D., S. S. Saini, M. Raffeld, J.

F. Manischewitz, and S. M. Wahl. 1992. Cytomegalovirus induction of tumor necrosis factor-alpha by human monocytes and mucosal macrophages. J Clin Invest 90:1642.

201. Turtinen, L. W., A. Assimacopoulos, and A. T. Haase. 1989. Increased monokines in cytomegalovirus infected myelomonocytic cell cultures. Microb Pathog 7:135.
202. Nokta, M. A., M. I. Hassan, K. Loesch, and R. B. Pollard. 1996. Human cytomegalovirus-induced immunosuppression. Relationship to tumor necrosis factor-dependent release of arachidonic acid and prostaglandin E2 in human monocytes. J Clin Invest 97:2635.
203. Gredmark, S., and C. Soderberg-Naucler. 2003. Human cytomegalovirus inhibits differentiation of monocytes into dendritic cells with the consequence of depressed immunological functions. J Virol 77:10943.
204. Cheeran, M. C., S. Hu, W. S. Sheng, P. K. Peterson, and J. R. Lokensgard. 2003. CXCL10 production from cytomegalovirus-stimulated microglia is regulated by both human and viral interleukin-10. J Virol 77:4502.
205. Cheeran, M. C., S. Hu, S. L. Yager, G. Gekker, P. K. Peterson, and J. R. Lokensgard. 2001. Cytomegalovirus induces cytokine and chemokine production differentially in microglia and astrocytes: antiviral implications. J Neurovirol 7:135.
206. Michelson, S., J. Alcami, S. J. Kim, D. Danielpour, F. Bachelerie, L. Picard, C. Bessia, C. Paya, and J. L. Virelizier. 1994. Human cytomegalovirus infection induces transcription and secretion of transforming growth factor beta 1. J Virol 68:5730.
207. Redman, T. K., W. J. Britt, C. M. Wilcox, M. F. Graham, and P. D. Smith. 2002. Human cytomegalovirus enhances chemokine production by lipopolysaccharide-stimulated lamina propria macrophages. J Infect Dis 185:584.
208. Kotenko, S. V., S. Saccani, L. S. Izotova, O. V. Mirochnitchenko, and S. Pestka. 2000. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A 97:1695.
209. Spencer, J. V., K. M. Lockridge, P. A. Barry, G. Lin, M. Tsang, M. E. Penfold, and T. J. Schall. 2002. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. J Virol 76:1285.
210. Jenkins, C., A. Abendroth, and B. Slobedman. 2004. A novel viral transcript with homology to human interleukin-10 is expressed during latent human cytomegalovirus infection. J Virol 78:1440.
211. Penfold, M. E., D. J. Dairaghi, G. M. Duke, N. Saederup, E. S. Mocarski, G. W. Kemble, and T. J. Schall. 1999. Cytomegalovirus encodes a potent alpha chemokine. Proc Natl Acad Sci U S A 96:9839.
212. Baillie, J., D. A. Sahlender, and J. H. Sinclair. 2003. Human cytomegalovirus infection inhibits tumor necrosis factor alpha (TNF-alpha) signaling by targeting the 55-kilodalton TNF-alpha receptor. J Virol 77:7007.
213. Benedict, C. A., K. D. Butrovich, N. S. Lurain, J. Corbeil, I. Rooney, P. Schneider, J. Tschopp, and C. F. Ware. 1999. Cutting edge: a novel viral TNF receptor superfamily member in virulent strains of human cytomegalovirus. J Immunol 162:6967.
214. Gao, J. L., and P. M. Murphy. 1994. Human cytomegalovirus open reading frame US28 encodes a functional beta chemokine receptor. J Biol Chem 269:28539.
215. Margulies, B. J., H. Browne, and W. Gibson. 1996. Identification of the human cytomegalovirus G protein-coupled receptor homologue encoded by UL33 in infected cells and enveloped

virus particles. Virology 225:111.

216. Billstrom, M. A., G. L. Johnson, N. J. Avdi, and G. S. Worthen. 1998. Intracellular signaling by the chemokine receptor US28 during human cytomegalovirus infection. J Virol 72:5535.
217. Bodaghi, B., T. R. Jones, D. Zipeto, C. Vita, L. Sun, L. Laurent, F. Arenzana-Seisdedos, J. L. Virelizier, and S. Michelson. 1998. Chemokine sequestration by viral chemoreceptors as a novel viral escape strategy: withdrawal of chemokines from the environment of cytomegalovirus-infected cells. J Exp Med 188:855.
218. Vieira, J., T. J. Schall, L. Corey, and A. P. Geballe. 1998. Functional analysis of the human cytomegalovirus US28 gene by insertion mutagenesis with the green fluorescent protein gene. J Virol 72:8158.
219. Zipeto, D., B. Bodaghi, L. Laurent, J. L. Virelizier, and S. Michelson. 1999. Kinetics of transcription of human cytomegalovirus chemokine receptor US28 in different cell types. J Gen Virol 80 (Pt 3):543.
220. Beisser, P. S., L. Laurent, J. L. Virelizier, and S. Michelson. 2001. Human cytomegalovirus chemokine receptor gene US28 is transcribed in latently infected THP-1 monocytes. J Virol 75:5949.
221. Haskell, C. A., M. D. Cleary, and I. F. Charo. 2000. Unique role of the chemokine domain of fractalkine in cell capture. Kinetics of receptor dissociation correlate with cell adhesion. J Biol Chem 275:34183.
222. Beisser, P. S., C. S. Goh, F. E. Cohen, and S. Michelson. 2002. Viral chemokine receptors and chemokines in human cytomegalovirus trafficking and interaction with the immune system. CMV chemokine receptors. Curr Top Microbiol Immunol 269:203.
223. Naniche, D., and M. B. Oldstone. 2000. Generalized immunosuppression: how viruses undermine the immune response. Cell Mol Life Sci 57:1399.
224. Griffin, D. 1996. Potential mechanisms of immune suppression. In Viral Pathogenesis. N. Nathanson, ed. Lippincott-Raven, Philadelphia, p. 207.
225. Rinaldo, C. R., Jr., W. P. Carney, B. S. Richter, P. H. Black, and M. S. Hirsch. 1980. Mechanisms of immunosuppression in cytomegaloviral mononucleosis. J Infect Dis 141:488.
226. Levin, M. J., C. R. Rinaldo, Jr., P. L. Leary, J. A. Zaia, and M. S. Hirsch. 1979. Immune response to herpesvirus antigens in adults with acute cytomegaloviral mononucleosis. J Infect Dis 140:851.
227. Reynolds, D. W., P. H. Dean, R. F. Pass, and C. A. Alford. 1979. Specific cell-mediated immunity in children with congenital and neonatal cytomegalovirus infection and their mothers. J Infect Dis 140:493.
228. Fiorilli, M., M. C. Sirianni, P. Iannetti, A. Pana, M. Divizia, and F. Aiuti. 1982. Cell-mediated immunity in human cytomegalovirus infection. Infect Immun 35:1162.
229. Schrier, R. D., G. P. Rice, and M. B. Oldstone. 1986. Suppression of natural killer cell activity and T cell proliferation by fresh isolates of human cytomegalovirus. J Infect Dis 153:1084.
230. Schrier, R. D., and M. B. Oldstone. 1986. Recent clinical isolates of cytomegalovirus suppress human cytomegalovirus-specific human leukocyte antigen-restricted cytotoxic T-lymphocyte activity. J Virol 59:127.
231. Simmons, P., K. Kaushansky, and B. Torok-Storb. 1990. Mechanisms of cytomegalovirus-mediated myelosuppression: perturbation of stromal cell function versus direct infection of myeloid cells. Proc Natl Acad Sci U S A 87:1386.
232. Torok-Storb, B., P. Simmons, D. Khaira, D. Stachel, and D. Myerson. 1992. Cytomegalovirus and marrow

function. Ann Hematol 64 Suppl:A128.

233. Soderberg, C., S. Sumitran-Karuppan, P. Ljungman, and E. Moller. 1996. CD13-specific autoimmunity in cytomegalovirus-infected immunocompromised patients. Transplantation 61:594.
234. Hebart, H., H. Einsele, R. Klein, I. Fischer, S. Buhler, K. Dietz, G. Jahn, P. A. Berg, L. Kanz, and C. A. Muller. 1996. CMV infection after allogeneic bone marrow transplantation is associated with the occurrence of various autoantibodies and monoclonal gammopathies. Br J Haematol 95:138.
235. Fredrich, R., M. Toyoda, L. S. Czer, K. Galfayan, O. Galera, A. Trento, D. Freimark, S. Young, and S. C. Jordan. 1999. The clinical significance of antibodies to human vascular endothelial cells after cardiac transplantation. Transplantation 67:385.
236. Toyoda, M., K. Galfayan, O. A. Galera, A. Petrosian, L. S. Czer, and S. C. Jordan. 1997. Cytomegalovirus infection induces anti-endothelial cell antibodies in cardiac and renal allograft recipients. Transpl Immunol 5:104.
237. Kantor, G. L., L. S. Goldberg, B. L. Johnson, Jr., M. M. Derechin, and E. V. Barnett. 1970. Immunologic abnormalities induced by postperfusion cytomegalovirus infection. Ann Intern Med 73:553.
238. Wager, O., J. A. Rasanen, A. Hagman, and E. Klemola. 1968. Mixed cryoimmunoglobulinaemia in infectious mononucleois and Cytomegalovirus mononucleosis. Int Arch Allergy Appl Immunol 34:345.
239. Horwitz, M. S., and N. Sarvetnick. 1999. Viruses, host responses, and autoimmunity. Immunol Rev 169:241.
240. Benoist, C., and D. Mathis. 2001. Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? Nat Immunol 2:797.
241. Lunardi, C., C. Bason, R. Corrocher, and A. Puccetti. 2005. Induction of endothelial cell damage by hCMV molecular mimicry. Trends Immunol 26:19.
242. Barnaba, V. 1996. Viruses, hidden self-epitopes and autoimmunity. Immunol Rev 152:47.
243. Grundy, J. E., K. M. Lawson, L. P. MacCormac, J. M. Fletcher, and K. L. Yong. 1998. Cytomegalovirus-infected endothelial cells recruit neutrophils by the secretion of C-X-C chemokines and transmit virus by direct neutrophil-endothelial cell contact and during neutrophil transendothelial migration. J Infect Dis 177:1465.
244. Naucler, C. S., S. Larsson, and E. Moller. 1996. A novel mechanism for virus-induced autoimmunity in humans. Immunol Rev 152:175.
245. Hutt-Fletcher, L. M., N. Balachandran, and M. H. Elkins. 1983. B cell activation by cytomegalovirus. J Exp Med 158:2171.
246. Poeck, H., M. Wagner, J. Battiany, S. Rothenfusser, D. Wellisch, V. Hornung, B. Jahrsdorfer, T. Giese, S. Endres, and G. Hartmann. 2004. Plasmacytoid dendritic cells, antigen, and CpG-C license human B cells for plasma cell differentiation and immunoglobulin production in the absence of T-cell help. Blood 103:3058.
247. Jego, G., V. Pascual, A. K. Palucka, and J. Banchereau. 2005. Dendritic cells control B cell growth and differentiation. Curr Dir Autoimmun 8:124.
248. Banchereau, J., F. Briere, C. Caux, J. Davoust, S. Lebecque, Y. J. Liu, B. Pulendran, and K. Palucka. 2000. Immunobiology of dendritic cells. Annu Rev Immunol 18:767.
249. Banchereau, J., S. Paczesny, P. Blanco, L. Bennett, V. Pascual, J. Fay, and A. K. Palucka. 2003. Dendritic cells: controllers of the immune system and a new promise for immunotherapy. Ann N Y Acad Sci 987:180.
250. Fernandez, N. C., A. Lozier, C. Flament, P. Ricciardi-Castagnoli, D. Bellet, M. Suter, M. Perricaudet, T. Tursz,

E. Maraskovsky, and L. Zitvogel. 1999. Dendritic cells directly trigger NK cell functions: cross-talk relevant in innate anti-tumor immune responses *in vivo*. Nat Med 5:405.

251. Rescigno, M., F. Granucci, and P. Ricciardi-Castagnoli. 1999. Dendritic cells at the end of the millennium. Immunol Cell Biol 77:404.
252. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, I. Durand, M. Cella, A. Lanzavecchia, and J. Banchereau. 1997. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor alpha: II. Functional analysis. Blood 90:1458.
253. Caux, C., B. Vanbervliet, C. Massacrier, C. Dezutter-Dambuyant, B. de Saint-Vis, C. Jacquet, K. Yoneda, S. Imamura, D. Schmitt, and J. Banchereau. 1996. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. J Exp Med 184:695.
254. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. J Exp Med 176:1693.
255. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. J Exp Med 180:83.
256. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. J Exp Med 179:1109.
257. Langenkamp, A., M. Messi, A. Lanzavecchia, and F. Sallusto. 2000. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. Nat Immunol 1:311.
258. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. Immunol Today 20:561.
259. Lanzavecchia, A., and F. Sallusto. 2001. The instructive role of dendritic cells on T cell responses: lineages, plasticity and kinetics. Curr Opin Immunol 13:291.
260. Moser, M., and K. M. Murphy. 2000. Dendritic cell regulation of TH1-TH2 development. Nat Immunol 1:199.
261. Fayette, J., I. Durand, J. M. Bridon, C. Arpin, B. Dubois, C. Caux, Y. J. Liu, J. Banchereau, and F. Briere. 1998. Dendritic cells enhance the differentiation of naive B cells into plasma cells *in vitro*. Scand J Immunol 48:563.
262. Dubois, B., C. Massacrier, B. Vanbervliet, J. Fayette, F. Briere, J. Banchereau, and C. Caux. 1998. Critical role of IL-12 in dendritic cell-induced differentiation of naive B lymphocytes. J Immunol 161:2223.
263. Grouard, G., M. C. Rissoan, L. Filgueira, I. Durand, J. Banchereau, and Y. J. Liu. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J Exp Med 185:1101.
264. O'Doherty, U., M. Peng, S. Gezelter, W. J. Swiggard, M. Betjes, N. Bhardwaj, and R. M. Steinman. 1994. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology 82:487.
265. Chicha, L., D. Jarrossay, and M. G. Manz. 2004. Clonal type I interferon-producing and dendritic cell precursors are contained in both human lymphoid and myeloid progenitor populations. J Exp Med 200:1519.
266. Colonna, M., G. Trinchieri, and Y. J.

Liu. 2004. Plasmacytoid dendritic cells in immunity. Nat Immunol 5:1219.

267. Barchet, W., M. Cella, and M. Colonna. 2005. Plasmacytoid dendritic cells-virus experts of innate immunity. Semin Immunol 17:253.

268. McKenna, K., A. S. Beignon, and N. Bhardwaj. 2005. Plasmacytoid dendritic cells: linking innate and adaptive immunity. J Virol 79:17.

269. Liu, Y. J. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 23:275.

270. Asselin-Paturel, C., A. Boonstra, M. Dalod, I. Durand, N. Yessaad, C. Dezutter-Dambuyant, A. Vicari, A. O'Garra, C. Biron, F. Briere, and G. Trinchieri. 2001. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. Nat Immunol 2:1144.

271. Cella, M., F. Facchetti, A. Lanzavecchia, and M. Colonna. 2000. Plasmacytoid dendritic cells activated by influenza virus and CD40L drive a potent TH1 polarization. Nat Immunol 1:305.

272. Fonteneau, J. F., M. Gilliet, M. Larsson, I. Dasilva, C. Munz, Y. J. Liu, and N. Bhardwaj. 2003. Activation of influenza virus-specific CD4+ and CD8+ T cells: a new role for plasmacytoid dendritic cells in adaptive immunity. Blood 101:3520.

273. Ito, T., Y. J. Liu, and N. Kadowaki. 2005. Functional diversity and plasticity of human dendritic cell subsets. Int J Hematol 81:188.

274. Iwasaki, A., and R. Medzhitov. 2004. Toll-like receptor control of the adaptive immune responses. Nat Immunol 5:987.

275. Hoshino, K., O. Takeuchi, T. Kawai, H. Sanjo, T. Ogawa, Y. Takeda, K. Takeda, and S. Akira. 1999. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. J Immunol 162:3749.

276. Takeuchi, O., T. Kawai, P. F. Muhlradt, M. Morr, J. D. Radolf, A. Zychlinsky, K. Takeda, and S. Akira. 2001. Discrimination of bacterial lipoproteins by Toll-like receptor 6. Int Immunol 13:933.

277. Heil, F., H. Hemmi, H. Hochrein, F. Ampenberger, C. Kirschning, S. Akira, G. Lipford, H. Wagner, and S. Bauer. 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303:1526.

278. Hemmi, H., O. Takeuchi, T. Kawai, T. Kaisho, S. Sato, H. Sanjo, M. Matsumoto, K. Hoshino, H. Wagner, K. Takeda, and S. Akira. 2000. A Toll-like receptor recognizes bacterial DNA. Nature 408:740.

279. Matsumoto, M., K. Funami, M. Tanabe, H. Oshiumi, M. Shingai, Y. Seto, A. Yamamoto, and T. Seya. 2003. Subcellular localization of Toll-like receptor 3 in human dendritic cells. J Immunol 171:3154.

280. Leadbetter, E. A., I. R. Rifkin, A. M. Hohlbaum, B. C. Beaudette, M. J. Shlomchik, and A. Marshak-Rothstein. 2002. Chromatin-IgG complexes activate B cells by dual engagement of IgM and Toll-like receptors. Nature 416:603.

281. Rassa, J. C., and S. R. Ross. 2003. Viruses and Toll-like receptors. Microbes Infect 5:961.

282. Tabeta, K., P. Georgel, E. Janssen, X. Du, K. Hoebe, K. Crozat, S. Mudd, L. Shamel, S. Sovath, J. Goode, L. Alexopoulou, R. A. Flavell, and B. Beutler. 2004. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. Proc Natl Acad Sci U S A 101:3516.

283. Compton, T., E. A. Kurt-Jones, K. W. Boehme, J. Belko, E. Latz, D. T. Golenbock, and R. W. Finberg. 2003. Human cytomegalovirus activates inflammatory cytokine responses via CD14 and Toll-

like receptor 2. J Virol 77:4588.

284. Bieback, K., E. Lien, I. M. Klagge, E. Avota, J. Schneider-Schaulies, W. P. Duprex, H. Wagner, C. J. Kirschning, V. Ter Meulen, and S. Schneider-Schaulies. 2002. Hemagglutinin protein of wild-type measles virus activates toll-like receptor 2 signaling. J Virol 76:8729.

285. Kadowaki, N., S. Ho, S. Antonenko, R. W. Malefyt, R. A. Kastelein, F. Bazan, and Y. J. Liu. 2001. Subsets of human dendritic cell precursors express different toll-like receptors and respond to different microbial antigens. J Exp Med 194:863.

286. Coban, C., K. J. Ishii, T. Kawai, H. Hemmi, S. Sato, S. Uematsu, M. Yamamoto, O. Takeuchi, S. Itagaki, N. Kumar, T. Horii, and S. Akira. 2005. Toll-like receptor 9 mediates innate immune activation by the malaria pigment hemozoin. J Exp Med 201:19.

287. Krieg, A. M. 1999. Mechanisms and applications of immune stimulatory CpG oligodeoxynucleotides. Biochim Biophys Acta 1489:107.

288. Lundberg, P., P. Welander, X. Han, and E. Cantin. 2003. Herpes simplex virus type 1 DNA is immunostimulatory *in vitro* and *in vivo*. J Virol 77:11158.

289. Krieg, A. M., A. K. Yi, S. Matson, T. J. Waldschmidt, G. A. Bishop, R. Teasdale, G. A. Koretzky, and D. M. Klinman. 1995. CpG motifs in bacterial DNA trigger direct B-cell activation. Nature 374:546.

290. Krug, A., S. Rothenfusser, V. Hornung, B. Jahrsdorfer, S. Blackwell, Z. K. Ballas, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. Eur J Immunol 31:2154.

291. Hartmann, G., and A. M. Krieg. 2000. Mechanism and function of a newly identified CpG DNA motif in human primary B cells. J Immunol 164:944.

292. Hartmann, G., J. Battiany, H. Poeck, M. Wagner, M. Kerkmann, N. Lubenow, S. Rothenfusser, and S. Endres. 2003. Rational design of new CpG oligonucleotides that combine B cell activation with high IFN-alpha induction in plasmacytoid dendritic cells. Eur J Immunol 33:1633.

293. Duramad, O., K. L. Fearon, B. Chang, J. H. Chan, J. Gregorio, R. L. Coffman, and F. J. Barrat. 2005. Inhibitors of TLR-9 act on multiple cell subsets in mouse and man *in vitro* and prevent death *in vivo* from systemic inflammation. J Immunol 174:5193.

294. Stunz, L. L., P. Lenert, D. Peckham, A. K. Yi, S. Haxhinasto, M. Chang, A. M. Krieg, and R. F. Ashman. 2002. Inhibitory oligonucleotides specifically block effects of stimulatory CpG oligonucleotides in B cells. Eur J Immunol 32:1212.

295. de Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y. J. Liu, S. Lebecque, and C. Caux. 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. J Immunol 160:1666.

296. Nagorsen, D., F. M. Marincola, and M. C. Panelli. 2004. Cytokine and chemokine expression profiles of maturing dendritic cells using multiprotein platform arrays. Cytokine 25:31.

297. Greenbaum, L. A., J. B. Horowitz, A. Woods, T. Pasqualini, E. P. Reich, and K. Bottomly. 1988. Autocrine growth of CD4+ T cells. Differential effects of IL-1 on helper and inflammatory T cells. J Immunol 140:1555.

298. Satoskar, A. R., M. Okano, S. Connaughton, A. Raisanen-Sokolwski, J. R. David, and M. Labow. 1998. Enhanced Th2-like responses in IL-1 type 1 receptor-deficient mice. Eur J Immunol 28:2066.

299. Chomarat, P., C. Dantin, L. Bennett,

J. Banchereau, and A. K. Palucka. 2003. TNF skews monocyte differentiation from macrophages to dendritic cells. J Immunol 171:2262.

300. Diehl, S., J. Anguita, A. Hoffmeyer, T. Zapton, J. N. Ihle, E. Fikrig, and M. Rincon. 2000. Inhibition of Th1 differentiation by IL-6 is mediated by SOCS1. Immunity 13:805.
301. Rincon, M., J. Anguita, T. Nakamura, E. Fikrig, and R. A. Flavell. 1997. Interleukin (IL)-6 directs the differentiation of IL-4-producing CD4+ T cells. J Exp Med 185:461.
302. Muraguchi, A., T. Hirano, B. Tang, T. Matsuda, Y. Horii, K. Nakajima, and T. Kishimoto. 1988. The essential role of B cell stimulatory factor 2 (BSF-2/IL-6) for the terminal differentiation of B cells. J Exp Med 167:332.
303. Chomarat, P., J. Banchereau, J. Davoust, and A. K. Palucka. 2000. IL-6 switches the differentiation of monocytes from dendritic cells to macrophages. Nat Immunol 1:510.
304. Trinchieri, G., S. Pflanz, and R. A. Kastelein. 2003. The IL-12 family of heterodimeric cytokines: new players in the regulation of T cell responses. Immunity 19:641.
305. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive CD4+ T cells. J Immunol 154:5071.
306. Lanzavecchia, A., and F. Sallusto. 2001. Regulation of T cell immunity by dendritic cells. Cell 106:263.
307. Trinchieri, G. 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3:133.
308. Adam, C., S. King, T. Allgeier, H. Braumuller, C. Luking, J. Mysliwietz, A. Kriegeskorte, D. H. Busch, M. Rocken, and R. Mocikat. 2005. DC-NK cell cross talk as a novel CD4+ T-cell-independent pathway for antitumor CTL induction. Blood 106:338.
309. Marcenaro, E., M. Della Chiesa, F. Bellora, S. Parolini, R. Millo, L. Moretta, and A. Moretta. 2005. IL-12 or IL-4 prime human NK cells to mediate functionally divergent interactions with dendritic cells or tumors. J Immunol 174:3992.
310. Ferlazzo, G., M. Pack, D. Thomas, C. Paludan, D. Schmid, T. Strowig, G. Bougras, W. A. Muller, L. Moretta, and C. Munz. 2004. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. Proc Natl Acad Sci U S A 101:16606.
311. Caux, C., C. Massacrier, B. Vanbervliet, C. Barthelemy, Y. J. Liu, and J. Banchereau. 1994. Interleukin 10 inhibits T cell alloreaction induced by human dendritic cells. Int Immunol 6:1177.
312. Corinti, S., C. Albanesi, A. la Sala, S. Pastore, and G. Girolomoni. 2001. Regulatory activity of autocrine IL-10 on dendritic cell functions. J Immunol 166:4312.
313. Groux, H., M. Bigler, J. E. de Vries, and M. G. Roncarolo. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. J Exp Med 184:19.
314. Steinbrink, K., M. Wolfl, H. Jonuleit, J. Knop, and A. H. Enk. 1997. Induction of tolerance by IL-10-treated dendritic cells. J Immunol 159:4772.
315. Trinchieri, G., and D. Santoli. 1978. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells. Enhancement of human natural killer cell activity by interferon and antagonistic inhibition of susceptibility of target cells to lysis. J Exp Med 147:1314.
316. Blanco, P., A. K. Palucka, M. Gill, V. Pascual, and J. Banchereau. 2001. Induction of dendritic cell differentiation by IFN-alpha in systemic lupus ery-

thematosus. Science 294:1540.

317. Fonteneau, J. F., M. Larsson, A. S. Beignon, K. McKenna, I. Dasilva, A. Amara, Y. J. Liu, J. D. Lifson, D. R. Littman, and N. Bhardwaj. 2004. Human immunodeficiency virus type 1 activates plasmacytoid dendritic cells and concomitantly induces the bystander maturation of myeloid dendritic cells. J Virol 78:5223.
318. Ito, T., R. Amakawa, M. Inaba, S. Ikehara, K. Inaba, and S. Fukuhara. 2001. Differential regulation of human blood dendritic cell subsets by IFNs. J Immunol 166:2961.
319. Luft, T., K. C. Pang, E. Thomas, P. Hertzog, D. N. Hart, J. Trapani, and J. Cebon. 1998. Type I IFNs enhance the terminal differentiation of dendritic cells. J Immunol 161:1947.
320. Kapsenberg, M. L. 2003. Dendritic-cell control of pathogen-driven T-cell polarization. Nat Rev Immunol 3:984.
321. Liu, Y. J., H. Kanzler, V. Soumelis, and M. Gilliet. 2001. Dendritic cell lineage, plasticity and cross-regulation. Nat Immunol 2:585.
322. Liu, Y. J., and B. Blom. 2000. Introduction: TH2-inducing DC2 for immunotherapy. Blood 95:2482.
323. Arpinati, M., C. L. Green, S. Heimfeld, J. E. Heuser, and C. Anasetti. 2000. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. Blood 95:2484.
324. Rissoan, M. C., V. Soumelis, N. Kadowaki, G. Grouard, F. Briere, R. de Waal Malefyt, and Y. J. Liu. 1999. Reciprocal control of T helper cell and dendritic cell differentiation. Science 283:1183.
325. Iwasaki, A., and B. L. Kelsall. 2001. Unique functions of CD11b$^+$, CD8 alpha$^+$, and double-negative Peyer's patch dendritic cells. J Immunol 166:4884.
326. Boonstra, A., C. Asselin-Paturel, M. Gilliet, C. Crain, G. Trinchieri, Y. J. Liu, and A. O'Garra. 2003. Flexibility of mouse classical and plasmacytoid-derived dendritic cells in directing T helper type 1 and 2 cell development: dependency on antigen dose and differential toll-like receptor ligation. J Exp Med 197:101.
327. de Jong, E. C., P. L. Vieira, P. Kalinski, J. H. Schuitemaker, Y. Tanaka, E. A. Wierenga, M. Yazdanbakhsh, and M. L. Kapsenberg. 2002. Microbial compounds selectively induce Th1 cell-promoting or Th2 cell-promoting dendritic cells *in vitro* with diverse th cell-polarizing signals. J Immunol 168:1704.
328. Ito, T., R. Amakawa, M. Inaba, T. Hori, M. Ota, K. Nakamura, M. Takebayashi, M. Miyaji, T. Yoshimura, K. Inaba, and S. Fukuhara. 2004. Plasmacytoid dendritic cells regulate Th cell responses through OX40 ligand and type I IFNs. J Immunol 172:4253.
329. de Jong, E. C., H. H. Smits, and M. L. Kapsenberg. 2005. Dendritic cell-mediated T cell polarization. Springer Semin Immunopathol 26:289.
330. Smits, H. H., E. C. de Jong, J. H. Schuitemaker, T. B. Geijtenbeek, Y. van Kooyk, M. L. Kapsenberg, and E. A. Wierenga. 2002. Intercellular adhesion molecule-1/LFA-1 ligation favors human Th1 development. J Immunol 168:1710.
331. Ohshima, Y., L. P. Yang, T. Uchiyama, Y. Tanaka, P. Baum, M. Sergerie, P. Hermann, and G. Delespesse. 1998. OX40 costimulation enhances interleukin-4 (IL-4) expression at priming and promotes the differentiation of naive human CD4(+) T cells into high IL-4-producing effectors. Blood 92:3338.
332. Flynn, S., K. M. Toellner, C. Raykundalia, M. Goodall, and P. Lane. 1998. CD4 T cell cytokine differentiation: the B cell activation molecule, OX40 ligand, instructs CD4 T cells to express interleukin 4 and upregulates expression of the chemokine receptor, Blr-1. J Exp Med 188:297.

333. Boring, L., J. Gosling, S. W. Chensue, S. L. Kunkel, R. V. Farese, Jr., H. E. Broxmeyer, and I. F. Charo. 1997. Impaired monocyte migration and reduced type 1 (Th1) cytokine responses in C-C chemokine receptor 2 knockout mice. J Clin Invest 100:2552.

334. Levings, M. K., R. Sangregorio, F. Galbiati, S. Squadrone, R. de Waal Malefyt, and M. G. Roncarolo. 2001. IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. J Immunol 166:5530.

335. Reis e Sousa, C., G. Yap, O. Schulz, N. Rogers, M. Schito, J. Aliberti, S. Hieny, and A. Sher. 1999. Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. Immunity 11:637.

336. Vieira, P. L., E. C. de Jong, E. A. Wierenga, M. L. Kapsenberg, and P. Kalinski. 2000. Development of Th1-inducing capacity in myeloid dendritic cells requires environmental instruction. J Immunol 164:4507.

337. Sallusto, F., P. Schaerli, P. Loetscher, C. Schaniel, D. Lenig, C. R. Mackay, S. Qin, and A. Lanzavecchia. 1998. Rapid and coordinated switch in chemokine receptor expression during dendritic cell maturation. Eur J Immunol 28:2760.

338. Sozzani, S., P. Allavena, G. D'Amico, W. Luini, G. Bianchi, M. Kataura, T. Imai, O. Yoshie, R. Bonecchi, and A. Mantovani. 1998. Differential regulation of chemokine receptors during dendritic cell maturation: a model for their trafficking properties. J Immunol 161:1083.

339. Sozzani, S., P. Allavena, A. Vecchi, and A. Mantovani. 1999. The role of chemokines in the regulation of dendritic cell trafficking. J Leukoc Biol 66:1.

340. Lin, C. L., R. M. Suri, R. A. Rahdon, J. M. Austyn, and J. A. Roake. 1998. Dendritic cell chemotaxis and transendothelial migration are induced by distinct chemokines and are regulated on maturation. Eur J Immunol 28:4114.

341. Penna, G., M. Vulcano, S. Sozzani, and L. Adorini. 2002. Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells. Hum Immunol 63:1164.

342. Jahnsen, F. L., F. Lund-Johansen, J. F. Dunne, L. Farkas, R. Haye, and P. Brandtzaeg. 2000. Experimentally induced recruitment of plasmacytoid (CD123high) dendritic cells in human nasal allergy. J Immunol 165:4062.

343. Farkas, L., K. Beiske, F. Lund-Johansen, P. Brandtzaeg, and F. L. Jahnsen. 2001. Plasmacytoid dendritic cells (natural interferon- alpha/beta-producing cells) accumulate in cutaneous lupus erythematosus lesions. Am J Pathol 159:237.

344. Penna, G., S. Sozzani, and L. Adorini. 2001. Cutting edge: selective usage of chemokine receptors by plasmacytoid dendritic cells. J Immunol 167:1862.

345. Muller, A., B. Homey, H. Soto, N. Ge, D. Catron, M. E. Buchanan, T. McClanahan, E. Murphy, W. Yuan, S. N. Wagner, J. L. Barrera, A. Mohar, E. Verastegui, and A. Zlotnik. 2001. Involvement of chemokine receptors in breast cancer metastasis. Nature 410:50.

346. Sallusto, F., B. Palermo, D. Lenig, M. Miettinen, S. Matikainen, I. Julkunen, R. Forster, R. Burgstahler, M. Lipp, and A. Lanzavecchia. 1999. Distinct patterns and kinetics of chemokine production regulate dendritic cell function. Eur J Immunol 29:1617.

347. Sallusto, F., C. R. Mackay, and A. Lanzavecchia. 2000. The role of chemokine receptors in primary, effector, and memory immune responses. Annu Rev Immunol 18:593.

348. Burkly, L., C. Hession, L. Ogata, C. Reilly, L. A. Marconi, D. Olson, R. Tizard, R. Cate, and D. Lo. 1995. Expression of relB is required for the development of thymic medulla and dendritic cells. Nature 373:531.

349. Penna, G., M. Vulcano, A. Roncari, F. Facchetti, S. Sozzani, and L. Adorini. 2002. Cutting edge: differential chemokine production by myeloid and plasmacytoid dendritic cells. J Immunol 169:6673.

350. Megjugorac, N. J., H. A. Young, S. B. Amrute, S. L. Olshalsky, and P. Fitzgerald-Bocarsly. 2004. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. J Leukoc Biol 75:504.

351. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. Eur J Immunol 31:3026.

352. Klagge, I. M., and S. Schneider-Schaulies. 1999. Virus interactions with dendritic cells. J Gen Virol 80 (Pt 4):823.

353. Larsson, M., A. S. Beignon, and N. Bhardwaj. 2004. DC-virus interplay: a double edged sword. Semin Immunol 16:147.

354. Turville, S. G., P. U. Cameron, A. Handley, G. Lin, S. Pohlmann, R. W. Doms, and A. L. Cunningham. 2002. Diversity of receptors binding HIV on dendritic cell subsets. Nat Immunol 3:975.

355. Krug, A., G. D. Luker, W. Barchet, D. A. Leib, S. Akira, and M. Colonna. 2004. Herpes simplex virus type 1 activates murine natural interferon-producing cells through toll-like receptor 9. Blood 103:1433.

356. Lund, J., A. Sato, S. Akira, R. Medzhitov, and A. Iwasaki. 2003. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. J Exp Med 198:513.

357. Diebold, S. S., T. Kaisho, H. Hemmi, S. Akira, and C. Reis e Sousa. 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science 303:1529.

358. Haynes, L. M., D. D. Moore, E. A. Kurt-Jones, R. W. Finberg, L. J. Anderson, and R. A. Tripp. 2001. Involvement of toll-like receptor 4 in innate immunity to respiratory syncytial virus. J Virol 75:10730.

359. Tassaneetrithep, B., T. H. Burgess, A. Granelli-Piperno, C. Trumpfheller, J. Finke, W. Sun, M. A. Eller, K. Pattanapanyasat, S. Sarasombath, D. L. Birx, R. M. Steinman, S. Schlesinger, and M. A. Marovich. 2003. DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. J Exp Med 197:823.

360. Alvarez, C. P., F. Lasala, J. Carrillo, O. Muniz, A. L. Corbi, and R. Delgado. 2002. C-type lectins DC-SIGN and L-SIGN mediate cellular entry by Ebola virus in cis and in trans. J Virol 76:6841.

361. Wang, Q. C., Z. H. Feng, Q. H. Nie, and Y. X. Zhou. 2004. DC-SIGN: binding receptors for hepatitis C virus. Chin Med J (Engl) 117:1395.

362. Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, and Y. van Kooyk. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell 100:587.

363. Libraty, D. H., S. Pichyangkul, C. Ajariyakhajorn, T. P. Endy, and F. A. Ennis. 2001. Human dendritic cells are activated by dengue virus infection: enhancement by gamma interferon and implications for disease pathogenesis. J Virol 75:3501.

364. Klagge, I. M., V. ter Meulen, and S. Schneider-Schaulies. 2000. Measles virus-induced promotion of dendritic cell maturation by soluble mediators does not overcome the immunosuppressive activity of viral glycoproteins on the cell surface. Eur J Immunol 30:2741.

365. Cella, M., M. Salio, Y. Sakakibara, H. Langen, I. Julkunen, and A. Lanzavecchia. 1999. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. J Exp Med 189:821.

366. Smith, A. P., C. Paolucci, G. Di Lullo, S. E. Burastero, F. Santoro, and P. Lusso. 2005. Viral replication-independent blockade of dendritic cell maturation and interleukin-12 production by human herpesvirus 6. J Virol 79:2807.

367. Engelmayer, J., M. Larsson, M. Subklewe, A. Chahroudi, W. I. Cox, R. M. Steinman, and N. Bhardwaj. 1999. Vaccinia virus inhibits the maturation of human dendritic cells: a novel mechanism of immune evasion. J Immunol 163:6762.

368. Moutaftsi, M., A. M. Mehl, L. K. Borysiewicz, and Z. Tabi. 2002. Human cytomegalovirus inhibits maturation and impairs function of monocyte-derived dendritic cells. Blood 99:2913.

369. Salio, M., M. Cella, M. Suter, and A. Lanzavecchia. 1999. Inhibition of dendritic cell maturation by herpes simplex virus. Eur J Immunol 29:3245.

370. Krug, A., A. R. French, W. Barchet, J. A. Fischer, A. Dzionek, J. T. Pingel, M. M. Orihuela, S. Akira, W. M. Yokoyama, and M. Colonna. 2004. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. Immunity 21:107.

371. Yonezawa, A., R. Morita, A. Takaori-Kondo, N. Kadowaki, T. Kitawaki, T. Hori, and T. Uchiyama. 2003. Natural alpha interferon-producing cells respond to human immunodeficiency virus type 1 with alpha interferon production and maturation into dendritic cells. J Virol 77:3777.

372. Pollara, G., K. Speidel, L. Samady, M. Rajpopat, Y. McGrath, J. Ledermann, R. S. Coffin, D. R. Katz, and B. Chain. 2003. Herpes simplex virus infection of dendritic cells: balance among activation, inhibition, and immunity. J Infect Dis 187:165.

373. Morrow, G., B. Slobedman, A. L. Cunningham, and A. Abendroth. 2003. Varicella-zoster virus productively infects mature dendritic cells and alters their immune function. J Virol 77:4950.

374. Schneider-Schaulies, S., I. M. Klagge, and V. ter Meulen. 2003. Dendritic cells and measles virus infection. Curr Top Microbiol Immunol 276:77.

375. Kawamura, T., H. Gatanaga, D. L. Borris, M. Connors, H. Mitsuya, and A. Blauvelt. 2003. Decreased stimulation of CD4+ T cell proliferation and IL-2 production by highly enriched populations of HIV-infected dendritic cells. J Immunol 170:4260.

376. Smed-Sorensen, A., K. Lore, L. Walther-Jallow, J. Andersson, and A. L. Spetz. 2004. HIV-1-infected dendritic cells up-regulate cell surface markers but fail to produce IL-12 p70 in response to CD40 ligand stimulation. Blood 104:2810.

377. Jahn, G., S. Stenglein, S. Riegler, H. Einsele, and C. Sinzger. 1999. Human cytomegalovirus infection of immature dendritic cells and macrophages. Intervirology 42:365.

378. Senechal, B., A. M. Boruchov, J. L. Reagan, D. N. Hart, and J. W. Young. 2004. Infection of mature monocyte-derived dendritic cells with human cytomegalovirus inhibits stimulation of T-cell proliferation via the release of soluble CD83. Blood 103:4207.

379. Hertel, L., V. G. Lacaille, H. Strobl, E. D. Mellins, and E. S. Mocarski. 2003. Susceptibility of immature and mature Langerhans cell-type dendritic cells to infection and immunomodulation by human cytomegalovirus. J Virol 77:7563.

380. Grigoleit, U., S. Riegler, H. Einsele, K. Laib Sampaio, G. Jahn, H. Hebart, P. Brossart, F. Frank, and C. Sinzger. 2002. Human cytomegalovirus induces

a direct inhibitory effect on antigen presentation by monocyte-derived immature dendritic cells. Br J Haematol 119:189.

381. Beck, K., U. Meyer-Konig, M. Weidmann, C. Nern, and F. T. Hufert. 2003. Human cytomegalovirus impairs dendritic cell function: a novel mechanism of human cytomegalovirus immune escape. Eur J Immunol 33:1528.
382. Raftery, M. J., M. Schwab, S. M. Eibert, Y. Samstag, H. Walczak, and G. Schonrich. 2001. Targeting the function of mature dendritic cells by human cytomegalovirus: a multilayered viral defense strategy. Immunity 15:997.
383. Andrews, D. M., C. E. Andoniou, F. Granucci, P. Ricciardi-Castagnoli, and M. A. Degli-Esposti. 2001. Infection of dendritic cells by murine cytomegalovirus induces functional paralysis. Nat Immunol 2:1077.
384. Mathys, S., T. Schroeder, J. Ellwart, U. H. Koszinowski, M. Messerle, and U. Just. 2003. Dendritic cells under influence of mouse cytomegalovirus have a physiologic dual role: to initiate and to restrict T cell activation. J Infect Dis 187:988.
385. Chang, W. L., N. Baumgarth, D. Yu, and P. A. Barry. 2004. Human cytomegalovirus-encoded interleukin-10 homolog inhibits maturation of dendritic cells and alters their functionality. J Virol 78:8720.
386. Raftery, M. J., D. Wieland, S. Gronewald, A. A. Kraus, T. Giese, and G. Schonrich. 2004. Shaping phenotype, function, and survival of dendritic cells by cytomegalovirus-encoded IL-10. J Immunol 173:3383.
387. Moutaftsi, M., P. Brennan, S. A. Spector, and Z. Tabi. 2004. Impaired lymphoid chemokine-mediated migration due to a block on the chemokine receptor switch in human cytomegalovirus-infected dendritic cells. J Virol 78:3046.
388. Cameron, P. U., P. S. Freudenthal, J. M. Barker, S. Gezelter, K. Inaba, and R. M. Steinman. 1992. Dendritic cells exposed to human immunodeficiency virus type-1 transmit a vigorous cytopathic infection to CD4+ T cells. Science 257:383.
389. Spira, A. I., P. A. Marx, B. K. Patterson, J. Mahoney, R. A. Koup, S. M. Wolinsky, and D. D. Ho. 1996. Cellular targets of infection and route of viral dissemination after an intravaginal inoculation of simian immunodeficiency virus into rhesus macaques. J Exp Med 183:215.
390. Duperrier, K., A. Farre, J. Bienvenu, N. Bleyzac, J. Bernaud, L. Gebuhrer, D. Rigal, and A. Eljaafari. 2002. Cyclosporin A inhibits dendritic cell maturation promoted by TNF-alpha or LPS but not by double-stranded RNA or CD40L. J Leukoc Biol 72:953.
391. Matyszak, M. K., S. Citterio, M. Rescigno, and P. Ricciardi-Castagnoli. 2000. Differential effects of corticosteroids during different stages of dendritic cell maturation. Eur J Immunol 30:1233.
392. Szabo, G., C. Gavala, and P. Mandrekar. 2001. Tacrolimus and cyclosporine A inhibit allostimulatory capacity and cytokine production of human myeloid dendritic cells. J Investig Med 49:442.
393. Tajima, K., R. Amakawa, T. Ito, M. Miyaji, M. Takebayashi, and S. Fukuhara. 2003. Immunomodulatory effects of cyclosporin A on human peripheral blood dendritic cell subsets. Immunology 108:321.
394. Humar, A., P. St Louis, T. Mazzulli, A. McGeer, J. Lipton, H. Messner, and K. S. MacDonald. 1999. Elevated serum cytokines are associated with cytomegalovirus infection and disease in bone marrow transplant recipients. J Infect Dis 179:484.
395. Tong, C. Y., A. Bakran, H. Williams, L. E. Cuevas, J. S. Peiris, and C. A. Hart. 2001. Association of tumour necrosis factor alpha and interleukin 6 levels with

cytomegalovirus DNA detection and disease after renal transplantation. J Med Virol 64:29.

396. Nordoy, I., F. Muller, K. P. Nordal, H. Rollag, E. Lien, P. Aukrust, and S. S. Froland. 2000. The role of the tumor necrosis factor system and interleukin-10 during cytomegalovirus infection in renal transplant recipients. J Infect Dis 181:51.
397. Schultze, J., L. M. Nadler, and J. G. Gribben. 1996. B7-mediated costimulation and the immune response. Blood Rev 10:111.
398. Schwartz, R. H. 1990. A cell culture model for T lymphocyte clonal anergy. Science 248:1349.
399. Tabi, Z., M. Moutaftsi, and L. K. Borysiewicz. 2001. Human cytomegalovirus pp65- and immediate early 1 antigen-specific HLA class I-restricted cytotoxic T cell responses induced by cross-presentation of viral antigens. J Immunol 166:5695.
400. Arrode, G., C. Boccaccio, J. P. Abastado, and C. Davrinche. 2002. Cross-presentation of human cytomegalovirus pp65 (UL83) to CD8+ T cells is regulated by virus-induced, soluble-mediator-dependent maturation of dendritic cells. J Virol 76:142.
401. Arrode, G., and C. Davrinche. 2003. Dendritic cells and HCMV cross-presentation. Curr Top Microbiol Immunol 276:277.
402. Walsh, D. S., S. Looareesuwan, S. Vaniganonta, C. Viravan, and H. K. Webster. 1995. Cutaneous delayed-type hypersensitivity responsiveness in patients during and after Plasmodium falciparum and Plasmodium vivax infections. Clin Immunol Immunopathol 77:89.
403. Williamson, W. A., and B. M. Greenwood. 1978. Impairment of the immune response to vaccination after acute malaria. Lancet 1:1328.
404. Daniel-Ribeiro, C. T., and G. Zanini. 2000. Autoimmunity and malaria: what are they doing together? Acta Trop 76:205.
405. Artavanis-Tsakonas, K., J. E. Tongren, and E. M. Riley. 2003. The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. Clin Exp Immunol 133:145.
406. Pichyangkul, S., K. Yongvanitchit, U. Kum-arb, H. Hemmi, S. Akira, A. M. Krieg, D. G. Heppner, V. A. Stewart, H. Hasegawa, S. Looareesuwan, G. D. Shanks, and R. S. Miller. 2004. Malaria blood stage parasites activate human plasmacytoid dendritic cells and murine dendritic cells through a Toll-like receptor 9-dependent pathway. J Immunol 172:4926.
407. Urban, B. C., D. J. Ferguson, A. Pain, N. Willcox, M. Plebanski, J. M. Austyn, and D. J. Roberts. 1999. Plasmodium falciparum-infected erythrocytes modulate the maturation of dendritic cells. Nature 400:73.
408. Urban, B. C., T. Mwangi, A. Ross, S. Kinyanjui, M. Mosobo, O. Kai, B. Lowe, K. Marsh, and D. J. Roberts. 2001. Peripheral blood dendritic cells in children with acute Plasmodium falciparum malaria. Blood 98:2859.
409. Urban, B. C., and D. J. Roberts. 2002. Malaria, monocytes, macrophages and myeloid dendritic cells: sticking of infected erythrocytes switches off host cells. Curr Opin Immunol 14:458.
410. Abrams, E. T., H. Brown, S. W. Chensue, G. D. Turner, E. Tadesse, V. M. Lema, M. E. Molyneux, R. Rochford, S. R. Meshnick, and S. J. Rogerson. 2003. Host response to malaria during pregnancy: placental monocyte recruitment is associated with elevated beta chemokine expression. J Immunol 170:2759.
411. Brodgen, K. 2002. Polymicrobial diseases. B. K. G. J, ed. ASM Press, Washington DC, p. 3.

412. Rochford, R., M. J. Cannon, and A. M. Moormann. 2005. Endemic Burkitt's lymphoma: a polymicrobial disease? Nat Rev Microbiol 3:182.

413. Moormann, A. M., K. Chelimo, O. P. Sumba, M. L. Lutzke, R. Ploutz-Snyder, D. Newton, J. Kazura, and R. Rochford. 2005. Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. J Infect Dis 191:1233.

414. Rasti, N., K. I. Falk, D. Donati, B. A. Gyan, B. Q. Goka, M. Troye-Blomberg, B. D. Akanmori, J. A. Kurtzhals, D. Dodoo, R. Consolini, A. Linde, M. Wahlgren, and M. T. Bejarano. 2005. Circulating epstein-barr virus in children living in malaria-endemic areas. Scand J Immunol 61:461.