From **Department of Microbiology, Tumor and Cell Biology**
Karolinska Institutet, Stockholm, Sweden

NK CELL RECOGNITION OF HERPESVIRUSES –
MECHANISMS OF VIRAL IMMUNE ESCAPE

Alexander Rölle

Karolinska Institutet

Stockholm 2008

Main Supervisor:
Professor Klas Kärre
Karolinska Institutet

Opponent:
Professor Andrew McMichael
University of Oxford

All previously published papers were reproduced with permission from the publisher.

Published by Karolinska Institutet. Printed by Larserics Digital Print AB, Sundbyberg

© Alexander Rölle, 2008
ISBN 978-91-7409-073-4

To those who are not privileged to have access to education

ABSTRACT

Herpesviruses are ubiquitous pathogens which after an often asymptomatic infection establish life-long latency in the host. Control of the viral infection depends on both innate and adaptive immune system. Clinical evidence and murine models have firmly established a decisive role of Natural Killer (NK) cells in the early control of herpesvirus infections. In order to escape adaptive T cell responses, herpesviruses target antigen presentation in the infected host cell. This usually leads to downregulation of MHC class I molecules which in turn should increase susceptibility to NK cells. However, a broad arsenal of viral gene products counteracts this danger and interferes with NK cell recognition on various levels. The studies in this thesis address three of these immunoevasins in human cytomegalovirus (HCMV) are discussed: UL16, UL18 and UL40.

In **paper I** we showed that productive HCMV infection induces ULBP1, ULBP2, ULBP3 and MICA, ligands for the NK cell activating receptor NKG2D. The upregulation of ULBP1 and ULBP2 was delayed on the cell surface in the presence of the viral protein UL16, known to bind to these two molecules. Using a HCMV mutant deficient for UL16, we observed higher levels of NKG2D ligands on infected target cells and this increase resulted in a higher susceptibility to NKG2D-mediated polyclonal NK cell responses. This is one of the first studies demonstrating the direct viral targeting of an NK cell activating pathway during productive infection.

In **paper II** we tried to investigate the molecular reasons for the previously known extraordinary high affinity of the viral UL18 protein, an MHC class I homolog, to the inhibitory cellular receptor LIR-1, that had been reported earlier. Based on a model of the UL18 structure, we introduced mutations in different sites of UL18 and assessed their influence on binding and on the functional consequences for the LIR-1/UL18 interaction. Substitutions of residues K42/A43 and Q202, located in the α_1- and α_3-domain respectively, reduced the binding affinity between UL18 and LIR-1 by about half. Disruption of an additional disulfide bridge, predicted by our model, completely abolished binding of UL18 to β_2m and the interaction with LIR-1. We propose that the high binding affinity of UL18 for LIR-1 is in part due to a more stable α_3-domain and a larger binding interface when compared to
HLA-A2/LIR-1

In **paper III** we extended the concept of herpesvirus interference with the NKG2D pathway to Herpes simplex virus (HSV) type I. We detected higher NKG2D levels on NK cells recovered from patients with acute blister formation. In vitro we could demonstrate that HSV downregulates the NKG2D ligand MICA by an as of yet undefined late viral gene. Surprisingly, the lysis of HSV infected target cells by polyclonal NK cells became more dependent on NKG2D, possibly indicating that other viral evasion mechanisms targeting NK cell recognition operate in parallel.

In **paper IV** we revisited the HCMV protein UL40, that has been described to upregulate HLA-E on infected cells and thereby transduce inhibitory signals via the NK cell receptor CD94/NKG2A.We hypothesized that UL40 may have additional functions and using an HCMV mutant deficient for UL40, observed decreased levels of cellular heat shock protein (Hsp) 60 and 70, when compared to cells infected with a wild type virus. Functional knockdown of these molecules resulted in higher susceptibility to NK cell lysis and apoptotic stimuli, suggesting that HCMV induces Hsps in order to prevent target cell death. Based on previous reports regarding the role of Hsp derived sequences in the context of CD94/NKG2A-HLA-E dependent recognition, we propose a model for UL40 action that can reconcile our findings with the existing literature.

The multiple recognition strategies of NK cells and the multiple immune evasion molecules of herpesviruses offer a fascinating system to learn more not only about viruses but also about the immune system and the co-evolution driven by host-virus interactions.

LIST OF PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following papers, which I will from now on refer to with their roman numerals

I. Alexander Rölle, Mehrdad Mousavi-Jazi, Michael Eriksson, Jenny Odeberg, Cecilia Söderberg-Nauclér, David Cosman, Klas Kärre and Cristina Cerboni. Effects of human cytomegalovirus infection on ligands for the activating NKG2D receptor of NK cells: up-regulation of UL16-binding protein (ULBP)1 and ULBP2 is counteracted by the viral UL16 protein. *Journal of Immunology, 2003, Jul 15;171(2):902-8*

II. Claudia Wagner, Alexander Rölle, David Cosman, Hans-Gustaf Ljunggren, Kurt Berndt and Adnane Achour
Structural elements underlying the high binding affinity of human cytomegalovirus UL18 to leukocyte immunoglobulin-like receptor-1.
Journal of Molecular Biology, 2007, Oct 26;373(3):695-705

III. Alexander Rölle*, Danika Schepis*, Mauro D'Amato, Marie Studahl, Tomas Bergström, Klas Kärre and Louise Berg
Herpes simplex virus infection downmodulates NKG2D ligand expression, yet increases NKG2D dependent NK cell recognition.
Submitted Manuscript

IV. Alexander Rölle, Johanna Platzek, Michael Uhlin and Klas Kärre
A novel function for human cytomegalovirus protein UL40 - induction of heatshock protein 60 confers protection to NK cell cytotoxicity
Submitted Manuscript

* both authors contributed equally

CONTENTS

1 Aims of this thesis ... 1
2 Introduction ... 3
  2.1 A short history of immunology ... 3
  2.2 The current view of the human immune system ... 4
  2.3 Natural Killer (NK) cells ... 5
    2.3.1 Discovery, early characterization and missing-self ... 5
    2.3.2 NK cell development and education ... 6
    2.3.3 Phenotype and heterogeneity of NK cells ... 7
    2.3.4 NK Cell activation, control and effector functions ... 8
  2.4 NK cells in health and disease ... 19
    2.4.1 NK cell deficiencies ... 19
    2.4.2 NK cells as therapeutic tools or targets ... 20
    2.4.3 NK cells in bacterial and parasitic infections ... 21
    2.4.4 NK cells in viral infections ... 22
  2.5 Herpesviruses (Herpesviridae) ... 25
    2.5.1 Classification, Clinical Aspects, Replication ... 25
    2.5.2 Virus - Host Interactions ... 26
3 Results and Discussion ... 30
  3.1 The role of MHC class I in HCMV infection ... 30
    3.1.1 Differences between viral strains ... 31
  3.2 UL18 – a viral high affinity ligand for an inhibitory receptor ... 31
    3.2.1 UL18 expression ... 32
    3.2.2 A structural approach to the enigmatic function of UL18 ... 32
    3.2.3 UL18 – which role does it play in productive infection? ... 35
  3.3 UL40 – Immune escape via CD94/NKG2A receptors ... 36
    3.3.1 Upregulation of HLA-E by UL40-derived leader sequences ... 36
    3.3.2 Heat shock proteins, stress and HLA-E – a dual role for UL40? ... 38
  3.4 UL16 – interference with an activating NK cell pathway ... 40
    3.4.1 Identification of UL16 ... 40
    3.4.2 The role of UL16 in productive HCMV infection ... 40
    3.4.3 The molecular mode of action of UL16 ... 41
    3.4.4 Viral microRNAs (miRNAs) interfering with host functions ... 43
  3.5 Herpes simplex virus and NK cell recognition ... 43
  3.6 The NKG2D receptor ligand system in other viral infections ... 44
  3.7 Other NK cell activating pathways targeted by HCMV infection ... 45
    3.7.1 DNAM-1/CD226 ... 45
    3.7.2 NKp30 ... 45
  3.8 Epstein-Barr virus and NK cell recognition ... 46
  3.9 Evolutionary Aspects ... 47
4 Concluding Remarks ... 49
5 Acknowledgements ... 51
6 References ... 51

LIST OF ABBREVIATIONS

ADCC	antibody-dependent cellular cytotoxicity
AIDS	acquired immunodeficiency syndrome
APC	antigen presenting cell
β2m	β_2- microglobulin
EBV	epstein-Barr virus
CD	cluster of differentiation
CMV	cytomegalovirus
SMAC	supramolecular activation cluster
CTLs	cytotoxic T cells
DAP	DNAX associated protein
DNAM	DNAX accessory molecule 1
EBV	Epstein-Barr virus
ER	endoplasmatic reticulum
GM-CSF	granulocyte monocyte colony stimulating factor
gp	glycoprotein
Grb2	growth factor receptor bound protein 2
HCMV	human cytomegalovirus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HSV	herpes simplex virus
IFN	interferon
IL	interleukin
IS	immune synapse
ICAM	intercellular adhesion molecule
ITAM	immunoreceptor tyrosine-based activation motif
ITIM	immunoreceptor tyrosine-based inhibitory motif
ITSM	immunoreceptor tyrosine-based switch motif
KIR	killer cell immunoglobuline-like receptor
LCMV	lymphochoriomeningitis virus
LIR/LILR	leukocyte immunoglobulin-like receptor
LPS	lipopolysaccharide
MCMV	mouse cytomegalovirus
MHC	major histocompatibility complex
MHV	mouse hepatitis virus
MIC	MHC class I chain-related protein
MIP1-α	macrophage inflammatory protein
MTOC	microtubule organizing center
NCR	natural cytotoxicity receptors
NK	natural killer
NKC	natural killer gene complex
ORF	open reading frame
PAMP	pathogen-associated molecular pattern
pDC	plasmacytoid dendritic cell

PI3K	phosphoinositide 3-kinase
PLC-γ2	phospholipase C-γ2
SCID	Severe combined immuno deficiency
SHIP	SH2-containing inositol polyphosphate 5-phosphatase
SHP	SH2-containing protein tyrosine phosphatase
siRNA	small-interfering RNA
STAT	signal-transducing activator of transcription
TCR	T cell receptor
TGF	transforming growth factor
TLR	Toll-like receptor
TNF	tumor necrosis factor
TREM	triggering receptor expressed by myeloid cells
ULBP	UL16-binding protein
XLP	X-linked proliferative disease

1 AIMS OF THIS THESIS

When I initiated my PhD studies, a role of Natural Killer (NK) cells in the defense against certain viral infections was generally accepted. Particularly strong evidence from murine models was available for their involvement in the immune defense against Herpesvirus infections. However, the molecular details of the interaction between NK cells and virally infected cells were unclear, partially owing to the lack of knowledge about the receptors and ligands governing NK cell control. Although their inhibition by receptors recognizing MHC class I molecules was known for more than a decade, the NK cell activating pathways had just started to be unravelled.

Viral infection is often accompanied by a pronounced downregulation of MHC class I molecules. In tumors this can lead to increased lysis by NK cells – “missing-self” recognition - but in virus infected cells, this increase in susceptibility is rarely seen. A key question, therefore, was to identify and characterize the molecules and mechanisms underlying NK cell evasion by Herpesviruses.

In human cytomegalovirus (HCMV), a member of the beta-herpesvirus family, some viral proteins had been suggested to mediate immune escape from NK cells. UL16 was proposed to interfere with some newly identified activating ligands, UL18 was suggested to compensate for the loss of MHC class I by delivering an inhibitory signal to NK cells and UL40 had been demonstrated to upregulate a non-classical MHC class I molecule on the surface of infected cells which could possibly signal via the inhibitory CD94/NKG2A receptor on NK cells. The general aim of my studies was to extend our knowledge about the role of these viral molecules, and since many of the previous functional studies were performed with transfection based overexpression systems, I wanted to study their role in virally infected cells whenever possible.

In **paper I** we set out to assess the role of UL16 in a productive infection by comparing a wild type strain with a mutant deficient for UL16. We studied surface levels as well as the intracellular localization of ligands for the activating receptor NKG2D in the presence and absence of UL16. Most importantly, we investigated functional aspects of the observed differences.

In **paper II** our aim was to understand the extraordinary high affinity of the HCMV protein UL18 for the inhibitory cellular receptor LIR-1, originally reported by other groups. With an approach combining molecular modelling with binding studies and functional assays we dissected the molecular determinants defining the interface between UL18 and its cellular counterpart.

In **paper III**, that also includes the analysis of clinical material, we extended the concept of viral targeting of the NKG2D pathway, to Herpes Simplex Type I (HSV-1), a representative of the alpha-herpesviruses.

In **paper IV**, we revisited the well established HCMV immunoevasin UL40, whose peptides can bind to HLA-E and thereby provide protection from NK cells. We were inspired by a previous study demonstrating that heat shock protein derived peptide sequences also can bind HLA-E molecules, but that such complexes are non-permissive for inhibitory recognition by CD94/NKG2A. Based on this finding, we proposed and took the first step to test a hypothesis based on an additional, novel mode of protection during productive HCMV infection, involving the induction of cellular heat shock proteins.

2 INTRODUCTION

2.1 A SHORT HISTORY OF IMMUNOLOGY

Infectious diseases have been part of human history from the very beginning. Already the oldest written records of ancient civilizations reaching back four millennia, such as the Babylonian epic of Gilgamesh or the annals of the early Egyptian dynasties bear witness to ravaging epidemics. The view to interpret these events as some sort of divine intervention or punishment seems archaic, yet it already implies a concept of what became later known as immunity, which protected some, seen in this case as favoured by the gods, but not others. The first explicit account of this observation is often ascribed to Thucydides who records the so called plague of Athens 430 B.C. in his famous History of the Peloponnesian war. People who had survived the disease seemed to be protected against subsequent reinfection and could take care of the sick and dying.

In the tenth century the Islamic physician Rhazes was amongst the first to cast this ancient knowledge into the framework of a theory of smallpox which is the oldest concept of acquired immunity that we know of. Interestingly, this disease would remain a red thread of immunology in centuries to come and connect hallmarks like the first immunizations by Jenner as well as the first eradication of a human disease, proclaimed by the WHO in December 1979, presumably one of the greatest triumphs in the history of medicine. About 500 years after Rhazes, the Italian physician Girolamo Fracastoro, building on other works, advanced the concept that all diseases were caused by germs (On Contagion, 1546). While theories of acquired immunity were modified, rejected and reformulated to take empirical evidence and clinical experiences into account, a common practice in many cultures around the world was variolation, a prophylactic measure that relied on the inoculation of crusts derived from benign small pox cases into healthy subjects.

In the beginning of the 18th century Voltaire observed this practice during his travels in England where inoculation became increasingly accepted, owing to an impressive reduction of mortality. In this cultural context, Edward Jenner conducted his acclaimed experiments that led to a safer and even more efficacious smallpox vaccine, published in 1798 and that enjoyed overwhelming global success within a few years. While Jenner is credited with opening the era of the science of immunology, he never seemed to have speculated on the mechanism underlying his successful vaccine. Possibly in the tradition of his teacher, John Hunter, who is recorded to have advised him by repeating William Harvey's famous advice, "Don't think, try.”

In the middle of the 19th century the rapid rise of modern microbiology, inextricably connected to the names of Louis Pasteur and Robert Koch, led to the identification of microorganisms as the causing agent of many important diseases and the systematic development of vaccines became a general approach to infectious diseases. The successful modification of the human immune response made the immune system itself a topic of intense research. 1884 Ilya Metchnikoff published his groundbreaking

cellular theory of immunity, which did not only introduce the concept of natural immunity as a first line of host defense. He also widened Darwin's theory of a "struggle for existence" within the same species to a struggle between different species, here the host versus microbial pathogens, as a driving force for evolution. This view of co-evolution of host and microbe proved to be exceptionally visionary and is nowadays more relevant than ever before: it is increasingly appreciated that millennia of co-existence between the immune system with countless pathogens has left traces in the genomes of microbes and humans alike.

Although my thesis deals with innate cellular immunity, it should be briefly mentioned that immunology in the last years of the 19th century was not limited to that area. In 1890 Behring and Kitasato demonstrated that a soluble blood constituent of immunized animals was protective against Diphteria and Tetanus which marked the identification of antibodies, a field quickly expanded, e.g. by Paul Ehrlich with his sidechain theory. In the first years of the 20th century hypersensitivity reactions, including allergy and transplantation, as well as immunopathology and inflammation in general became part of immunological research. The combination of these fields essentially defines the discipline of modern immunology.

2.2 THE CURRENT VIEW OF THE HUMAN IMMUNE SYSTEM

The current view of the human immune system continues to rely on the traditional division into an innate and an adaptive branch. Innate immunity represents the first line of defense encountered by a pathogen and is often concentrated, but not limited to, anatomically exposed locations such as epithelial and mucosal surfaces. It comprises a broad array of defense mechanisms, some of which not even restricted to immune cells such as antibacterial peptides, inhibitors that target and destabilize retroviral capsids or the complement system, that can either directy lyse pathogens or coats them to facilitate uptake by innate immune cells. Cellular constituents of the innate immune system are granulocytes, dendritic cells (DCs), monocytes and macrophages and finally Natural Killer (NK) cells, which will play the main role in this thesis.

A common characterics of most of these components is that they recognize molecular patterns that are highly conserved on pathogens (pathogen-associated molecular patterns, PAMPs) or associated with cellular injury and stress. Consequently most of the receptors mediating this recognition are referred to as PRRs (pattern recognition receptors). Prototypic examples of these germ-line encoded molecules are the toll-like receptors (TLRs), lectin-like receptors and RNA helicases. Their ligands comprise a wide variety of different molecules, ranging from viral nucleic acids to bacterial components such as flagellin, lipoteichoic acid or LPS. As diverse as these different substances are, they have in common that they are often highly conserved, repetitive structures that are widely expressed in a broad range of pathogens and usually associated with cellular injury or danger for the host. PAMP recognition by innate receptors initiates the expression of inflammatory cytokines, chemokines and adhesion molecules which synergize to recruit and activate immune effector cells. The innate

immune response is available almost instantly upon pathogenic challenge and if it is not sufficient to clear an infection, it can still serve to delay its progress until a more powerful adaptive immune response is mounted.

DCs are often referred to as sentinels of the immune system, representing a crucial link between innate and adaptive immunity. Upon the early recognition of PAMPs in peripheral tissues, DCs secrete cytokines such as IL-12 or type I interferons which in turn activate other cells of the innate immune system. In parallel, they undergo profound phenotypic and functional changes that include the upregulation of costimulatory molecules, a process called maturation. After their migration to secondary lymphoid organs, they present processed antigen, derived from either intracellular or extracellular sources, to T lymphocytes and thereby initiate and moderate the adaptive immune response. In parallel, a humoral response, either T cell-dependent or independent gives rise to large numbers of antibody producing B-cells. The adaptive immune system, consisting of T and B cells, relies on an incredible number of receptor specificities that is ensured by somatic recombination of genetic elements. While innate immune cells can proliferate during the course of an immune response, expansion of T cells and B cells is much more dramatic and usually restricted to a few highly specific clones. Successful T and B cell responses can, in contrast to the their innate counterparts, give rise to long-lasting protective memory, although one report also suggested the generation of memory in the course of an NK cell response (1). Another layer of complexity is added by specialized T cell subsets, e.g. T regulatory cells and so called Th17 cells, both of which are involved in the regulation and orchestration of many aspects of the innate and adaptive immune response.

However, the traditional view of innate and adaptive immunity as two separate entities is increasingly replaced by a more integrated picture that takes the multifaceted interdependence into account. General examples are the secretion of cytokines that have a biological effect on both innate and adaptive cells or the binding of innate F_c receptors to targets opsonized with antibodies. Beside being potent sources of different cytokines that can influence T cell responses (2, 3). NK cells have been shown to be involved in a complex cross-talk with DCs that can lead to very different outcomes – from mutual activation over DC maturation to the killing of immature DCs by NK cells, which might have far reaching consequences for subsequent adaptive immune responses.

2.3 NATURAL KILLER (NK) CELLS

2.3.1 Discovery, early characterization and missing-self

Natural Killer (NK) cells were the last major lymphocyte population to be identified. Their initial characterization dates back to 1975 and took place independently in two different laboratories (4-6). Their name refers to the capability to recognize and eliminate tumor cells in vitro without prior sensitization which was in contrast to the requirements of T cell mediated responses.

Early studies showed their involvement in vivo not only in the rejection of syngeneic tumors (7), but also in the clearance of several viral infections, that I will review in more detail later. In spite of these functional data, the specificity of NK cells, however, remained obscure. The finding that IFNs activated NK cells but at the same time protected certain susceptible targets only added to the mystery (8).

Amongst the molecules that were excluded to play a role in NK cell control were MHC class I molecules, since NK cells could readily lyse tumor cells lacking them (9). This conclusion emphasizes the dogma for immune recognition at the time and highlights the paradigmatic shift that was to take place in 1981: in his PhD thesis Klas Kärre speculated on the basis of "natural cytotoxicity" and postulated that NK cells would detect the absence of MHC class I molecules on potential target cells. Later the term "missing-self" was coined for this mode of recognition (10), which for the first time implied the existence of an inhibitory receptor for MHC class I molecules.

This possibility was of particular interest, as tumors and virus infected cells had already been shown to downregulate MHC class I. Experimental evidence for missing-self recognition became available when in vivo studies demonstrated that NK cells rejected tumor cells or bone marrow grafts displaying low amounts of MHC class I. A few years later, the first inhibitory receptors specific for MHC class I were identified in mice as well as in humans. Subsequent work of an increasing number of research groups led to the current paradigm that the activation of NK cells is governed by the integration of multiple signals received via both, activating and inhibitory receptors. The impressive number of NK cell receptors and ligands which I will review in more detail in future sections, allows for very different, complex interactions of NK cells with different target cells. Some of those interactions are dominated by missing-self recognition, others are largely dependent on activating signals that can override the presence of MHC class I molecules and many are a combination of several activating and inhibitory pathways operating in parallel. To take this into account the concept of "missing-self" recognition was in recent years complemented by the concept of "induced-self" or "stressed-self" recognition, not to forget the direct recognition of pathogen-encoded molecules by NK cells. Examples for these different modes of recognition will be discussed more extensively later.

2.3.2 NK cell development and education

NK cells are considered to be bone marrow derived, although an increasing body of evidence suggests that secondary lymphoid tissues are a major site of NK cell differentiation as well. Contemporary models (reviewed by (11)) propose four distinct stages of human NK cell development based on certain cell surface molecules. NK cell progenitors lose the haematopoietic stem cell marker CD34, and become pre-NK cells after the acquisition of c-kit (CD117) and the functional capacity to respond to IL-15. Further differentiation results in lineage-committed immature NK cells, that express some NK-associated receptors and antigens. In the final stage of development, NK cells

express all inhibitory and activating NK receptors and become competent for the secretion of IFN-γ and/or the release of cytotoxic granules.

One of the central processes in the generation of a functional NK cell repertoire is the acquisition of inhibitory receptors for MHC class I, which is one possible way of preventing autoreactivity. Each individual carries several genes for inhibitory receptors and their expression is essentially determined by a random, stochastic process on the single cell level. This can give rise to a proportion of cells that does not express any cognate receptor for the MHC class I molecules present in the respective individual (12). The intriguing question how self-tolerance is achieved during NK cell education is a central area of interest in the field and based on murine models currently three different models are proposed. The licensing model proposed by Yokoyama and colleagues views NK cell development as a process in which NK cells who successfully engage inhibitory ligands are actively induced - licensed - to fully mature. Raulet and coworkers advocate an alternative model, in which there is active anergy induction of NK cells that fail to receive inhibition - a process they termed disarming. There is substantial data in support of each model and an attempt to reconcile both perspectives has been recently presented by Höglund and coworkers in form of the "rheostat model". This model explains the available data on education as a tuning of NK cell activation thresholds along a continous scale, rather than as a binary switch ("on" vs. "off", "disarmed" vs. "licensed"). The tuning is implemented by quantitative differences in the interaction of MHC class I molecules with their respective receptors during NK cell education.

2.3.3 Phenotype and heterogeneity of NK cells

The phenotype of NK cells is usually based on the cell surface markers NK1.1 and DX5 in mice and $CD56^{+}/CD3^{-}$ in humans. Even though the inclusion of CD56 allows for the common subdivision of human NK cells in a small $CD56^{high}$ and a large $CD56^{low}$ population, the expression of CD56 is not restricted to NK cells alone (13), therefore a more recent definition suggests to use $NKp46^{+}/CD3^{-}$ for mature NK cells (14). This surface phenotype applies to 5-20% of lymphocytes in human peripheral blood where their cell turnover is around 2 weeks.

Two main subsets are currently discriminated: a majority of about 90% of peripheral blood and spleen NK cells are characterized by a $CD56^{dim}CD16^{+}$ phenotype and display cytotoxicity and some IFN-γ production when encountering an appropriate target cell. NK cells in lymph nodes and and tonsils are instead $CD56^{bright}CD16^{-}$ (15) and while they lack perforin and KIR receptors, they express higher levels of CD94/NKG2A and ILT-2, secrete large amounts of IFN-γ, TNF-α and GM-CSF in response to cytokines such as IL-12, IL-15 and IL-18. They also exhibit a high proliferative capacity, in part due to the constitutive expression of the high affinity receptor for IL-2 (16). Another difference between the two subsets is their respective repertoire of chemokine receptors. Homing of $CD56^{bright}$ cells to secondary lymphatic tissues has been suggested to be mainly controlled by the chemokine receptor CCR7

(17) and L-Selectin (18), while the recruitment of CD56^{dim} cells to inflammatory sites seems to rely on CXCR1 and CX3CR1 and ChemR. A recent report suggested that CD56^{bright} and CD56^{dim} NK cells are, in contrast to previous belief, actually not distinct lineages but rather sequential steps of differention where CD56^{bright} cells are a transitional stage before terminal differentiation of CD56^{bright} cells (19)

Certain cytokine environments and/or anatomical locations can give rise to other NK cell populations with a distinct phenotype. Of note in this context is a population of CD56^{bright}CD16^{-}KIR^{+} NK cells that represents the majority of immune cells in the human placenta in the early phases of gestation. They have been shown to overexpress a variety of receptors and molecules important for NK cell activation and effector functions, such as KIR2DL4, NKG2C, NGK2E, Ly49L and Granzyme A (14) and there is evidence that local secretion of TGF-β accounts for the distinct phenotype of decidual NK cells. An involvement in vascularization of the feto-maternal interface and for the maintenance of tolerance towards the fetus has been proposed (reviewed in (20-22)).

In the course of many infections, NK cells, and to a smaller extent γδ T cells and NKT cells, are one of the earliest potent sources of cytokines and chemokines which can have a decisive impact on the shaping of subsequent adaptive immune responses (2, 3). A related topic that I can only touch upon in the framework of this thesis is the emerging and rapidly expanding field of crosstalk between NK cells and other immune cells, most notably DCs. Initial evidence for direct activation of NK cell activity against tumors in a murine system (23) was corroborated in studies with human cells (24-29). Taken together, these studies demonstrate mutual activation of both cell types, including DC maturation. Under certain conditions the lysis of, largely immature, DCs by NK cells was observed. The rules determining the outcome of a DC-NK encounter are insufficiently understood. Generally most of the NK-DC crosstalk is considered to involve the activating NK cell receptor NKp30 (29) and is often executed by a CD94/NKG2A^{+} KIR- NK cell subset (30), even though NKG2D has been described to play a role as well. While the exact anatomical site of NK-DC crosstalk remains unclear, examples that the different forms of crosstalk have functional relevance are available: In mice proliferation of a crucial NK cell subset during MCMV infection is driven by spleen-resident DCs (31) and the donor NK cell-mediated killing of recipient DCs is assumed to contribute to the favourable outcome of haploidentical SCT (32).

2.3.4 NK Cell activation, control and effector functions

The large and diverse array of activating and inhibitory NK cell receptors, some of which can also be expressed by certain T cells and NKT cells, is a long list of molecules with different properties, regulation and genomic organization. While the current view of NK cell control as a balance between activating and inhibitory signals is conceptually appealing, the knowledge about how signals from multiple receptors are integrated on a molecular level is still limited. One key to understanding these complex

events is certainly the spatial distribution of receptors, adhesion molecules and adaptors during the interaction between NK cells and their targets.

Studies in T cells have shown a high degree of organization at the intercellular contact site. The interface, in analogy to neuronal synapses was termed immune or immunological synapse (IS) and has subsequently been studied in NK cells as well (33). The formation of the NK-IS is often divided into several stages (34): in a first step proteins are accumulated at the contact site. For HLA-C, the ligand for inhibitory KIR receptors on NK cells, up to 20% of overall cell surface protein has been shown to relocalize to the early synapse (35). In a second phase, segregation of recruited proteins takes place, leading to a specific patterning. In case of the inhibitory IS in human NK cells, adhesion molecules such as LFA-1 and ICAM-1 tend to cluster centrally, surrounded by KIR and HLA molecules. However, the clear division into a central supramolecular activation cluster (c-SMAC) and a peripheral supramolecular activation cluster (p-SMAC) that has been demonstrated in the T cell IS, is not always found in NK cells and seems to depend on the expression levels of the inhibitory ligand: low levels of HLA-C resulted in multifocal patterning at the IS while higher HLA-C levels allowed a clear separation of MHC class I from ICAM-1 and gave rise to a more homogenous, ring-shaped organization, as seen in T cells (36). The activating IS is characterized by CD2, LFA-1, Mac-1 and profound cytoskeletal rearrangements in the p-SMAC and the effector molecule perforin in the c-SMAC. After assembly of a mature synapse, bidirectional protein transfer occurs between NK cells and target cells, although the functional consequences of this phenomenon are not fully resolved (37, 38). Interestingly, the lack of cytotoxicity in decidual NK cells, that express normal levels of activating receptors and components of the lytic machinery, also manifests itself in an impairment of synapse formation (39).

Even though NK cells have been shown to release a broad variety of different cytokines and chemokines, their main effector function is usually considered to be either the release of cytotoxic granules into the IS or the secretion of IFN-γ. The composition of lytic granules in NK cells differs slightly from T cells. Among other components such as calreticulin and cathepsins, they contain the pore-forming molecules perforin and granulysin as well as serin proteases called granzymes that are non-redundant and activate several distinct pathways leading to target cell death by apoptosis (reviewed in (40)). Most intensely studied has been the role of granzyme B that triggers apoptosis by activating both caspase-3 dependent as well as caspase independent cascades ultimately leading to target cell death. An entirely different possibility for how NK cells can trigger cell death, though with a slower kinetics, is by the ligation of death receptors such as TRAIL or Fas with their respective ligands on target cells (reviewed in (41, 42)).

Inhibitory receptors on human NK cells recognize, with some exceptions, MHC class I molecules and comprise three major families: the killer cell immunoglobulin-like receptors (KIRs, CD158), the leukocyte immunoglobulin-like receptors (LILRs) and the heterodimeric CD94/NKG2A (CD159) receptor.

Name	Synonyms	Structure	Chromosome	Signaling	Ligand
2B4	CD244	Ig monomer	1q23.1	4 ITSM	CD48
KLRG1	Mafa	C-lectin homodimer	12p12-p13	1 ITIM	E-cadherin N-Cadherin R-Cadherin
CD94/NKG2A	CD159a	C-lectin heterodimer	12p13	1 ITIM	HLA-E
NKR-P1A	CD161	C-lectin homodimer	12p13	1 ITIM	LLT1 (OCIL, CLEC2D
LAIR-1	CD305	Ig monomer	19q13.4	2 ITIM	Collagen
LIR-1	ILT2, CD85j	Ig monomer	19q13.4	4 ITIM	HLA class I
KIR2DL3	CD158b2	Ig monomer	19q13.4	2 ITIM	HLA-C S77/N80
KIR2DL2	CD158b1	Ig monomer	19q13.4	2 ITIM	HLA-C S77/N80
KIR2DL1	CD158b	Ig monomer	19q13.4	2 ITIM	HLA-C S77/K80
KIR3DL1	CD158e1	Ig monomer	19q13.4	2 ITIM	HLA-Bw4
KIR2DL5	CD158f	Ig monomer	19q13.4	2 ITIM	?
KIR3DL2	CD158k	Ig homodimer	19q13.4	2 ITIM	HLA-A3, HLA-A11
CEACAM1	CD66a	Ig monomer	19q13.2	2 ITIM	CD66
SIGLEC7	CDw328, p75	Ig monomer	19q13.4	1 ITIM	α-2,8 disialic acid

Table 1: Overview of selected inhibitory human NK cell receptors (adapted from (43))

2.3.4.1 Inhibitory NK cell receptors in the immunoglobulin superfamily

KIRs belong to type I glycoproteins, are encoded on chromosome 19 and are part of the immunoglobulin superfamily. As such they comprise either three (KIR3D) or two (KIR2D) Ig-like domains in their extracellular part. Generally, KIRs with a long cytoplasmic tail (KIR3DL and KIR2DL) have an inhibitory function whereas receptors with a short tail in the intracellular part (KIR3DS and KIR2DS) transduce activating signals. A notable exception is KIR2DL4 that functions as an activating receptor, in spite of a long cytoplasmic tail. The number of KIR genes varies in different individuals and there is also allelic diversity described for many of the different genes.This sets the stage for large scale epidemiological studies assessing the impact of KIR and HLA polymorphism on human disease. Adding to the complexity, a given NK cell can express any given subset of these KIR genes. The process governing the acquisition of KIR receptors during NK cell development has been described as random and monoallelic. Genetic studies suggest that the KIR complex is evolving rapidly under the selective pressure of pathogens (reviewed in (44)). Associations of certain KIR alleles or KIR-HLA combinations have not only been found for infections but also for autoimmune diseases and preeclampsia.

One member of the LILR family, LIR-1 (also LILRB1, CD85j or ILT2) is a central molecule in **paper II** of this thesis. The original description of LIR-1, that consists of four domains (D1-D4) dates back to a screen for binding partners for the HCMV glycoprotein UL18 (45). Subsequently, LIR-1 was found to also recognize virtually all MHC class I molecules, the classical, HLA-A, B, C (45-47) as well as the non classical, HLA-E, F, G (45, 46, 48-50). Most intriguing is the finding that the affinity of LIR-1 to viral UL18 exceeds its' affinity to cellular MHC class I molecules by three orders of magnitude (47). In **paper II** we set out to identify the molecular reasons for this extraordinary high affinity. LIR-1 is expressed on monocytes and macrophages, DCs and B cells but also on subpopulation of NK cells (3-33% according to the initial report) and T cells. While other members of the LILRs can associate with activating adaptor proteins, LIR-1 posesses four ITIMs in its cytoplasmic tail (51).

2.3.4.2 Inhibitory receptors in the c- lectin like superfamily

CD94/NKG2A receptors are type II glycoproteins, encoded on chromosome 12. They display a C-type lectin-like fold and, in contrast to KIRs, have a homologue in mice. Initially their heterodimeric nature was not clear but after CD94 alone (52, 53) could not account for the complex results obtained in studies focusing on this receptor (54), it became clear that CD94 formed a complex with other proteins (55), that turned out to be members of the NKG2 family (56-59). While KIRs bind to classical MHC class I molecules, CD94/NKG2A binds to HLA-E in humans (60, 61) and Qa-1 in mice (62). HLA-E binds peptides derived from the leader sequence of classical MHC class I molecules. These leader peptides depend on TAP for loading into HLA-E molecules, which are not stabilized for transport unless they have bound peptide (63). The downregulation of either, MHC class I or TAP, thus results in a consecutive decrease in HLA-E levels. During the preparation of this thesis the crystal structure of CD94/NKG2A in complex with HLA-E was published independently by two groups (64, 65). The CD94 subunit dominated the binding area to the peptide bound by HLA-E and Kaiser et al. suggest that the evolution of CD94 is driven by pathogens ("purifying selection"), whereas NKG2A/C are maintained by positive selection that ensures the binding of the heterodimer to the invariant HLA-E molecule.

In case of CD94/NKG2A a particular aspect of HLA-E recognition by CD94/NKG2A recognition has been studied by Michaelsson et al. (66). As stated above, the levels of cell surface HLA-E can be seen as an indirect monitoring of MHC class I and TAP expression. Michaelsson et al. demonstrated that the heat shock protein 60 (hsp60) contain peptide sequences that can bind HLA-E. However, HLA-E in complex with hsp60 peptides did not transduce inhibitory signals to NK cells via CD94/NKG2A. Evidence was presented that under conditions of stress, in that particular study in form of increasing cell density, the leader sequence-derived peptides are presumably substituted by a heat shock protein (hsp) 60 derived sequence. This led to increased NK susceptibility without altered HLA-E levels, presumably because the HLA-E molecules

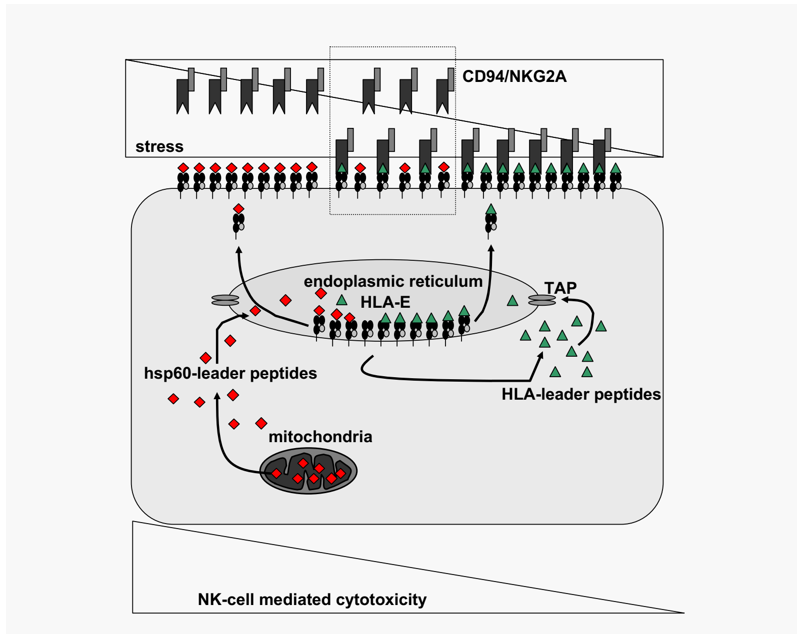

Fig. 1 Conceptual model of stress-induced peptide interference (SPI), adapted from Cristina Teixeira de Matos, PhD thesis Karolinska Institutet 2006

contain hsp60 derived peptides, nonpermissive for inhibitory recognition via CD94/NKG2A. For this phenomenon the term stress-induced peptide interference (SPI) was coined (Fig 1).

2.3.4.3 Peptide dependency of NK cell recognition

While peptide discrimination by NK cells seems conceptually surprising, there is actually growing evidence for this concept. The initial description of peptide dependency of NK cell recognition dates back to a report by Peruzzi and coworkers who showed that peptides on HLA-B*2705 influence recognition by KIRs (67). Similar findings were obtained for the Ly49 family in mice, where Franksson et al. demonstrated that the inhibition of murine NK cells by the receptor Ly49C was affected by the peptide presented by the cognate MHC class I ligand H-2K^b (68). A report by Hansasuta et al. (69) demonstrated that binding of HLA-A3 and -A11 tetramers to KIR3DL2 transfectants is peptide specific. In a similarly designed study HLA-A and HLA-B tetramers, refolded with different peptides bound differently to NK cells expressing the cognate receptor KIR3DL1 (70). The determination of the structure of HLA-E in complex with two different peptides revealed a molecular basis for peptide discrimination by CD94/NKG2 receptors (71).

While the extracellular domains of KIRs, CD94/NKG2A, LIR-1 or Ly49 receptors look distinctly different, they share a common signalling motif in their intracellular part. This motif is defined by the amino acid sequence (I/L/V/S)XYXX(L/V) and is called

immunoreceptor tyrosine-based inhibitory motif (ITIM), one in case of CD94/NKG2A and two in case of the KIRs. Ligand engagement of the receptor triggers the recruitment of lipid phosphatase SHIP-1 or the tyrosin phosphatases SHP-1 and SHP-2. These phosphatases suppress NK cell activation by dephosphorylating key molecules of activating NK cell pathways (43, 72).

2.3.4.4 Ligands for inhibitory NK cell receptors

The major histocompatibility complex (MHC) on chromosome 6 in humans represents the by far most polymorphic region in the human genome. It was originally described as a decisive determinant in the context of transplantation and when Zinkernagel and Doherty discovered its essential role for the T cell recognition of viral antigens, a finding that was acknowledged with the Nobel Prize in 1996, the MHC became a field of central interest for all immunologists, not just transplantation experts. MHC class I molecules present mainly endogenous peptides to $CD8^+$ T cells while MHC class II molecules are important for the presentation of exogenous antigens, processed by APCs to $CD4^+$ T cells. In humans, MHC class I molecules are also termed Human Leukocyte Antigen (HLA). The so called classical MHC class Ia molecules consist of HLA-A, -B and -C, while the non-classical MHC class Ib molecules are termed HLA-E, -G and -F. An MHC class Ib molecule that is actually not encoded within the MHC complex is CD1, which is involved in the presentation of lipids to a subset of T cells and NKT cells. MHC class molecules are formed by the non-covalent association of the 45 kD heavy chain, consisting of three immunoglobulin-like domains (α_{1-3}) with 12 kD β_2-microglobulin (β_2m). The α_1- and α_2-domain form a peptide binding groove between two α-helices that rest on a platform of β-sheets. KIR binding to MHC class I is distinctly different from the binding of the T cell receptor (TCR).

The crucial importance of antigen processing and presentation pathways for the resolution of viral infections by T cells makes them a prime target for immune evasion strategies by pathogens. Many of those evasion mechanism result in the reduction of MHC class I on the cell surface of infected cell.

For inhibitory receptors binding ligands other than MHC class I, see table 1 (include NKR-P1(b/D) (binding to Ocil), CD244 (also known as 2B4) binding to CD48, KLRG1 binding to E-Cadherin, N-Cadherin and R-Cadherin, CEACAM1 binding to CD66 and SIGLEC7 binding to α-2,8 disialic acid).

2.3.4.5 Activating receptors

Somatic recombination gives rise to an incredible number of specificities in T cells and B cells, but any given cell usually only expresses one single receptor for the recognition of antigen. NK cells pursue a different strategy by relying on combinations of various germline encoded receptors, most of them expressed simultaneously. Long et al. suggested to use the term “coactivating receptor” in order to highlight that, with

Name	Synonyms	Structure	Chromosome	Signaling	Ligand
2B4	CD244	Ig monomer	1q23.1	ITSM, SAP	CD48
CD94/NKG2C	CD159c	C-lectin heterodimer	12p13	DAP12	HLA-E
NKG2D	CD314	C-lectin homodimer	12p13	DAP10	MICA, MICB, ULBP1-4
NKp80	CLEC5C	C-lectin homodimer	12p13.2-p12.3	?	AICL
KIR2DS1	CD158h	Ig monomer	19q13.4	DAP12	HLA-C N77/K80
KIR2DS2	CD158j	Ig monomer	19q13.4	DAP12	?
KIR2DS4	CD158i	Ig monomer	19q13.4	DAP12	HLA-Cw4
KIR3DS1	CD158e2	Ig monomer	19q13.4	DAP12	?
KIR2DL4	CD158d	Ig monomer	19q13.4	F_cεRI-γ	HLA-G
NKp46	CD335	Ig monomer	19q13.4	F_cεRI-γ	Viral haema-glutinins and cellular heparansulfates?
NKp44	CD336	Ig monomer	6p21.1	DAP12	
NKp30	CD337	Ig monomer	6p21.3	F_cεRI-γ	
CD16		Ig monomer	1q23	F_cεRI-γ	Ig
DNAM-1	CD226	Ig monomer	18q22.3	Protein kinase C	CD112, CD155
CRACC	CD319, SLAMF7	Ig monomer	1q23.1-q24.1	ITSM, EAT2	CRACC
NTBA	SLAMF6	Ig monomer	1q23.2	ITSM, SAP, EAT2	NTB-a

Table 2: Overview of selected activating human NK cell receptors (adapted from (43))

exception of CD16, most activating NK cell receptor only trigger a response in synergy with other activating receptors (73). Under the influence of the missing-self hypothesis, research on NK cell receptors had initially focused on inhibitory receptors and only in the end of the 90s activating receptors, such as NKG2D or the Natural Cytotoxicity Receptors (NCRs) were identified (74-77). Interestingly, most of the activating receptors are not able to signal directly, as is the case for ITIM bearing inhibitory receptors. Instead, in the absence of a longer cytoplasmic domain, they require the association with adaptor molecules which harbour immunoreceptor tyrosine-based activation motifs (ITAMs), containing the sequence (D/E)XXYXX(L/I)X6-8YXX(L/I) in their cytoplasmic domain which is essential for triggering the full signaling cascade leading to cell activation. The adaptor molecules provide an additional possibility for controlling NK cell activation and thus represent another layer of regulation. Even though there are many activating receptors, their signalling pathways usually converge already on the level of the adaptor proteins. For the sake of clarity, I present here the ITAM-based activating receptors in three groups depending on which adaptor molecule they require for signalling and cell surface expression.

F_cεRI-γ and/or CD3ζ can form homo- or heterodimers and serve as adaptors for CD16 (F_cγRIIIA), that mediates antibody dependent cytotoxicity (ADCC), the activating KIR

receptor KIR2DL4, that binds to HLA-G, NKR-P1A and the Natural cytotoxicity receptors NKp46 and NKp30. NKp46 has been suggested to bind to influenza virus hemaglutinin (78). Cellular heparansulfates (79, 80) and vimentin (81) have been reported to be ligands for NKp46 and NKp30 as well, although this notion has been partially questioned by a different report (82). As described above, NKp30 has been implicated to play a role for the activation and the killing of immature DCs, possibly affecting adaptive immune responses. The ligands recognized by NKp30 so far include the CMV protein pp65 (83) and the nuclear protein BAT-3, that can relocalize to the cell membrane under certain circumstances (84). A recent presentation demonstrated a new B7-related molecule as another novel cellular ligand for NKp30 (85).

The second group comprises receptors associating with the adaptor protein DAP12, such as the activating KIRs with short intracytoplasmic tails (KIR2D-S), the third member of the natural cytotoxicity receptors, NKp44 as well as the CD94/NKG2C/E heterodimers. It has been proposed that KIR2DS recognizes viral peptides on MHC class I molecules, while NKp44 was reported to bind to viral haemaglutinins (86), just as NKp46 but also to the surface of Mycobacteria (87). Vieillard et al. also described the induction of a yet undefined NKp44 ligand on HIV-infected $CD4^+$ T cells by a peptide derived from the viral protein gp41 (88).
Taken together the described ligands for the NCRs so far are mainly pathogen-derived molecules. However, the NCRs clearly must bind to other, cellular ligands as well, given their important role in the lysis of tumor cells (89-91).

ITAM-based signalling downstream of the DAP12, $F_c\varepsilon RI$-γ and CD3ζ adaptors closely resembles pathways found in T and B cells. Phosphorylation of tyrosine residues in the ITAM motif by Src family kinases, leads to a recruitment of tyrosine kinases Syk and ZAP-70 by virtue of their SH2 domains. ZAP70 has been shown to be important in ADCC whereas Syk is involved in both ADCC as well as cytotoxicity. Downstream, phosphorylation of additional adaptors such as LAT, NTAL, 3BP2 and SLP-76 amplifies the signal and creates docking sites for downstream effectors, including PI3K, the Vav family, PLCγ1 and PLCγ2. Negative regulation can take place on several levels of this signalling cascade and is mediated e.g. by SH-2-containing 5'inositol phosphatase 1 (SHIP-1) or CD45 (43, 72).

A different group of ITAM-independent NK cell receptors are formed by three members of the signalling lymphocytic activating molecule (SLAM) family: CD244, formerly named 2B4, NTB-A and CRACC binding to CD48, NTB-A and CRACC respectively. The signalling motif utilized by these receptors is called immunoreceptor tyrosine-based switch motif (ITSMs) and binds to the adaptors SAP and ERT. The importance of this pathway is highlighted by patients suffering from X-linked lymphoproliferative disease (XLP). This condition is caused by a loss of function mutation in the SAP gene (92) and leads to the inability to control EBV-induced malignancies. The literature regarding the precise downstream signalling pathways, the differences between human and murine system as well as the fact that CD244 exhibits inhibitory functions under certain circumstances, is complex and controversial and beyond the scope of this thesis.

Studies on the role of TLRs in NK cells remain in their infancy, but a report by Sivori (93) demonstrated NK cell activation by dsRNA and polyI:C via TLR3 and TLR9. Another study suggested that agonists for TLR3 and TLR7 mainly enhance IFN-γ production but have no effect on NKG2D mediated cytotoxicity (94).

Adhesion molecules constitute a very diverse group of NK cell receptors that are involved in the initial phases of NK cells target contact and the formation of conjugates. Beyond simple adhesion, most of these molecules are also involved in signalling such as the $\alpha_1\beta_2$ integrin LFA-1 binding to ICAM-1 on target cells, ultimately resulting in Vav1 phosphorylation that is required for cytoskeletal rearrangements that promote adhesion and can even be sufficient to trigger cytotoxicity. This is emphasized by impaired cytotoxicity in NK cells recovered from patients with integrin deficiencies, although other studies suggest partial redundancy amongst the adhesion molecules with Mac-1 and β_1-integrin as possible substitutes.

More recent work has focused on another group of adhesion molecules expressed on human NK cells: DNAX accessory molecule 1 (DNAM-1 or CD226), which is physically associated with LFA-1, CD96 (also Tactile) and CRTAM, all of them binding to Nectins and Nectin-like molecules (Necl) (81). Ligand engagement increases adhesion and NK cell mediated cytotoxicity against tumor cells (82, 83) but also, in cooperation with NKp30, against both mature and immature DCs (84).

The activating NK cell receptor that has probably been most intensely studied in the past years is the homodimer NKG2D. Since it is of central importance for **paper I** and **III**, I would like to devote a separate section to this molecule.

2.3.4.6 The activating NK cell receptor NKG2D

NKG2D has been described together with its adaptor molecule DAP10 in 1999 (95, 96). The receptor is a c-type lectin-like molecule and is expressed by all NK cells, CD8αβ and γδ T cells, while expression on CD4 T cells so far has only been detected in the context of pathologic conditions (97, 98). In mice additional cell populations expressing NKG2D are the majority of NKT cells and the recently identified so called interferon-producing killer dendritic cells (IKDCs). NKG2D is upregulated by IL-15 and downmodulated by TGF-β or IL-21 (99-102).

NKG2D-mediated signalling is independent of ITAM-motifs and Syk but instead relies on a unique tyrosin-based motif (YINM) in the cytoplasmic domain of its adaptor protein DAP10 (103), that is mainly regulated by AP-1 transcription factors (104). This motif binds the regulatory subunit p85 of phosphoinositide 3-kinase (PI3K) , leading to Jak2, STAT5, ERK, MAP kinase and Akt activation (99). A parallel pathway, also dependent on DAP10, is linked to the adaptor growth factor receptor-bound protein 2 (Grb-2), that mediates the downstream activation of Vav-1, SLP-76 and PLCγ2 (94). Full NKG2D-mediated activation requires PI3K as well as Grb-2 and induces, depending on the triggering stimulus, cytotoxicity, proliferation as well as cytokine (IFN-γ, GM-CSF, TNF-α) and chemokine (MIP-1α/β) secretion (93). In line with the

concept of coactivating NK cell receptors, NKG2D can complement NCR or KIR2DS2 mediated NK cell reponses, while on T cells it has so far only been shown to be a co-stimulatory rather than a primary receptor.

Interestingly, in mice, a recent study showed that NKG2D is intricately linked with another crucial molecule for the control of NK cell functions. IL-15 was shown to phosphorylate DAP10, thereby priming the adaptor for NKG2D-dependent signalling Conversely, the targeting of DAP10 for ubiquitin-mediated degradation rendered NK cells unresponsive to IL-15 (105). An in vivo correlate of this NK cell priming might be the trans-presentation of IL-15 by CD11c^{high} DCs to NK cells in response to viral and bacterial infections (106). In mice NKG2D exists in two splice variants, one of which is able to bind to DAP12 as well (107, 108). This difference to humans complicates generalizations from the studies of NKG2D-driven responses in mice.

2.3.4.7 NKG2D ligands

In contrast to most other NK cell receptors, NKG2D binds to a surprisingly diverse set of ligands, all of them distant relatives of MHC class I molecules (Fig. 2). Currently seven ligands have been described in both humans and mice, although they do not seem to be orthologous. A common structural feature of all NKG2D ligands is the MHC class I-like $\alpha_1\alpha_2$ domain while the sequence, additional domains and the anchoring in the membrane can differ considerably. The currently known ligands in mice comprise the Rae1 family, H60 and MULT1 (108-111). In humans so far the MHC class I related chain A and B (MICA and MICB) encoded within the MHC complex on the short arm of chromosome 6 (95, 112-114) and the UL16-binding proteins (115, 116), encoded on the long arm of chromosome 6 and also known as RAET1 proteins (ULBP1-4 and Raet1G), have been identified. MICA and MICB possess an additional alpha3-domain and a transmembrane domain which makes them more closely resemble classical class I molecules, although they do not associate with β_2m, nor do they bind peptides. Another similarity is the high degree of polymorphism with more than 70 alleles known. Associations with several diseases have been reported but since they are located close to the HLA-B locus in the MHC it is possible that those associations are indirect. ULBP1-3, that were initially identified during a screen for binding partners of the HCMV protein UL16, are GPI-anchored, while ULBP4 and RAET1G also have a transmembrane domain. The GPI-anchored molecules where shown to cluster in domains rich in lipid rafts at the activating immunological synapse and although association of MICA with these microdomains was weaker, it was still recruited to the IS (117).

Several cocrystal structures of NKG2D bound to its ligands have been resolved (118-120) and while initial interpretations proposed an “induced-fit”mechanism (118) to allow binding of such a diverse set of ligands this has been called into question by thermodynamic analysis, suggesting a degenerated binding interface, relying on only few key tyrosine residues, a binding mode termed rigid adaptation. Binding itself occurs with affinities exceeding those observed for KIR-HLA or CD94/NKG2A-HLA-E interactions by an order of magnitude (121).

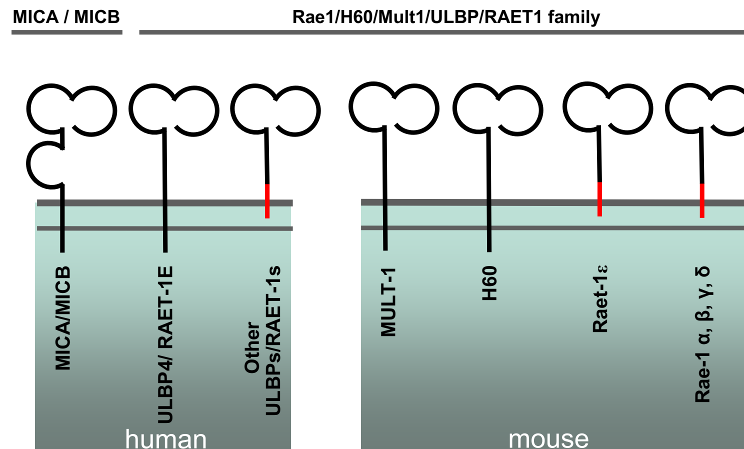

Fig. 2 Schematic representation of NKG2D ligands in humans and mice

NKG2D ligands are generally absent on healthy cells but upregulated by a variety of cellular stress stimuli, such as viral infection, tumor transformation or exposure to heat shock. A pioneering report by Gasser et al. demonstrated that an underlying principle of some of these stress factors is genotoxicity (122, 123). A cooperation of the enzymes ATM, detecting double-strand breaks and ATR, detecting stalled replication, leads to a phosphorylation of chk1 and chk2, ultimately resulting in cell cycle arrest and the activation of DNA damage repair systems or, if the genomic damage cannot be dealt with, apoptosis. It has been known previously that tumor cells often activate the DNA damage response and as expected, many tumor cell lines constitutively express NKG2D ligands (123). Another study reported the ATM/ATR-dependent expression of NKG2D ligands on T cells after antigenic stimulation (124). Control of tumors by NKG2D has first been suggested by a report showing increased rejection of tumors ectopically expressing NKG2D ligands in syngeneic mice (125). Investigating the early phase of tumor initiation, Smyth et al. demonstrated that neutralization of NKG2D in vivo leads to increased incidence of a chemically induced fibrosarcoma (126) and a recent study using NKG2D-deficient mice confirmed a role for NKG2D in tumor surveillance (127). In models for a spontaneous prostate carcinoma and a myc-driven lymphoma, NKG2D-deficient animals developed larger tumors or showed decreased survival. Interestingly, a number of reports demonstrate shedding of soluble NKG2D ligands (128-130) from tumors that can in turn lead to receptor downmodulation on NK cells and T cells after engaging those ligands, possibly representing a mechanism of immune escape by desensitizing NK cells in regard to NKG2D dependent activation. This was demonstrated in transgenic mice where constitutive expression of Rae-1epsilon led to downmodulated NKG2D surface expression, impaired NK cell cytotoxicity against Rae-1ε^{+} targets and increased susceptibility to chemically induced tumors in vivo. Administration of poly(I:C) administration partially restored functional NKG2D responses (125).

Much less is known about DNA damage in viral infection although it seems conceivable that for example the involvement of the nucleus in the replication cycle of several viruses would trigger similar responses. In fact, recent reports link infection with retrovirus Abelson murine leukaemia virus (AMLV) and HSV to the DNA damage response (131, 132). In **paper I** we show for the first time the induction of NKG2D ligands on the cell surface by a viral infection (here: HCMV) and several other groups extended this finding to other viruses, such as EBV (133), influenza (134), RSV and measles virus (135). Viral infection is almost certainly a powerful drive for the diversification of NKG2D ligands as numerous viruses deploy immune evasive gene products designed to interfere with the NKG2D receptor ligand system. Since this is a central topic in my thesis I will later devote a separate section to this exciting field of research.

Other alternative stimuli that have been reported to induce NKG2D ligands are TLR triggering (136) and the binding of the E.coli protein AfaE to CD55 (137).

2.4 NK CELLS IN HEALTH AND DISEASE

2.4.1 NK cell deficiencies

NK cell deficiencies are rare and it is tempting to see this as indication for the importance of NK cells in host survival. The most famous case report of an isolated NK cell deficiency was published by Biron and coworkers in 1989 (138). The patient suffered from repeated disseminated and life-threatening varicella infections, later succeeded by CMV pneumonitis and severe HSV lesions. Apart from the lack of CD56 or CD16 expressing lymphocytes no other immunological compartments was found to display abnormalities. Although the molecular reasons for this dramatic case were never established – it was not even clear whether it was caused by a genetic defect - susceptibility to herpesvirus infections became a hallmark of NK cell deficiencies and inspired a whole line of research within the NK cell field.

Another patient with NK cell deficiency developed bacterial infections at the age of 17 but died from disseminated varicella two years later. One report describes a 23-month-old girl that initially suffered from varicella encephalitis and succumbed to a recurrence half a year later (139). The most recent example was a study by Eidenschenk et al.(140) that describes four children in the same family that all suffer from an isolated NK cell defect that gave rise to EBV-related lymphoproliferation and frequent airway infections of viral origin. The scale of the study allowed to narrow down the defect to an area on chromosome 8, and will hopefully soon reveal a gene relevant for NK cell development and/or function.

Over the last years an increasing number of genetic syndromes have been associated with impaired NK cell function, and sometimes increased susceptibility to HSV and EBV. However, for many syndromes, parallel defects in T cell responses or other immune compartments make a conclusive analysis in terms of the critical role of NK

cells difficult. This limitation applies for example to the mutations found in severe combined immunodeficiency (SCID). Disruption of NK cell development is caused by mutations in the gene encoding the cytokine receptor common-gamma chain, Janus kinase-3 or adenosin deaminase (ADA). SCID patients with these mutations do suffer from increased susceptibility to viral infections, most often from members of the herpes family.

Other mutations result in the impairment of cytolytic effector mechanisms, as is the case in familial hemophagocytic lymphohistiocytosis (FHLH), Chediak-Higashi (CHS), Hermansky-Pudlak, Papillon-Lefevre and Griscelli syndrome. In these conditions, cellular components of lytic granules or in the machinery ensuring their trafficking are affected, again resulting in more frequent and severe infections by EBV and HSV but also Candida and Aspergillus. Defects in NK cell activating pathways include XLP, which I mentioned earlier, leukocyte adhesion deficiency (LAD), caused by a variety of mutations in integrins or Wiskott-Aldrich Syndrome, a result of mutations in the WASP gene, that is important for cytoskeletal rearrangements following cell activation. For a more in-depth coverage of NK cell deficiencies I refer to the reviews by Orange et al. (141, 142). In summary, in the majority of case reports NK cell defects are associated with an increased susceptibility to herpesvirus infections, which will be the main topic of a later section of this thesis.

2.4.2 NK cells as therapeutic tools or targets

One can probably consider the trials with “LAK” – lymphokine activated killer cells – as the first adoptive transfer of NK cells in a clinical setting (143). These are hard to interpret, given the heterogeneity of the infused effector populations and also the lack of knowledge about NK cell receptors and ligands at the time. During the last years the interest in using NK cells for therapeutic purposes has intensified. The treatment of acute myeloid leukaemia (AML) with stem cell transfer (SCT) has been conducted across HLA class I barriers in so called haploidentical transplantation (mother or father to child). In this protocol donor KIRs and recipient HLA haplotypes were retrospectively analysed, revealing that a mismatch translated into prolonged disease-free survival, interpreted as an NK cell mediated graft-versus-leukemia effect (32) as well as reduced graft-versus-host disease (GVHD). Trials conducted at some centers partially supported these initial findings whereas others, in particular studies of “matched (for HLA-A and B but not C) unrelated” donor recipient combinations, could not repeat the favourable clinical outcome (144-146). Comparisons between these studies are difficult, since the treatment protocols differ substantially which certainly accounts for some of the discrepancies. After SCT, patients harbour a chimeric immune system which then allows the transfer of donor derived effector cells, also called donor lymphocyte infusions (DLI). NK cell based IL-2 supported DLI was explored treating AML relapses (147). In 5 out of 19 patients with poor prognosis complete remission was achieved and probably again positively associated with a KIR-HLA mismatch. A possible future development of adoptive NK cell transfer could be the ex vivo

expansion of donor NK cell subsets displaying an optimal receptor repertoire for the respective recipient/tumor (148).

Another approach is to block inhibitory interactions of KIRs with HLA-C in vivo in the hope to activate NK cell mediated anti-tumor responses. A trial investigating this possibility is currently ongoing (149). Other possibilities include the direct stimulation of NK cells by TLR agonists, or the indirect activation via the generation of IL-12 producing DCs. The efforts to translate our rapidly expanding knowledge on NK cell activation and control into a clinical context have been mainly focused on cancer. The principles for how to manipulate NK cells in vivo should however be applicable to the treatment of infectious diseases as well, provided NK cells are a relevant cell population for the clearance of the infectious agent.

2.4.3 NK cells in bacterial and parasitic infections

While the overwhelming majority of studies on NK cells focuses on their role in cancer or viral infections, there are some reports regarding their relevance for immune responses against bacterial and parasitic pathogens. In most of these studies, cytokine secretion, primarily IFN-γ is the dominant effector function rather than cytotoxic activity. Different studies in mice with Listeria monocytogenes showed either a positive or a negative influence on disease progression, although the reasons for these discrepancies are currently unknown (150, 151). The same applies to NK cells in Shigella flexneri infection (152). Studies investigating NK cells in parasitic infections have been have mainly focused on Trypansoma cruzi (153, 154) and the malaria causing parasite Plasmodium falciparum.

In mouse models, a pivotal role of IFN-γ for the pathogenesis of malaria has been established (155) and there is substantial evidence for the involvement of NK cells (reviewed in (156)). In human systems, it is still controversial whether NK cells (157, 158) or γδ T cells are the main source of this inflammatory cytokine (159), although the activation of the latter could still be regulated by NK receptors. IFN-γ production by NK cells was reported to be dependent on contact with accessory cells but also on availability of cytokines IFN-α, IL-12 and IL-18 (160, 161). The receptors mediating the contact dependent interactions have not been identified. Based on a hybrid system with plasmodium falciparum infected human erythrocytes and murine NK cells, they are independent of ITAM motifs but dependent on MyD88 (160). Particularly interesting is the recognition of the blood stage of Plasmodium, given that erythrocytes neither express HLA class I nor other known inhibitory or activating ligands for NK cells. Since the induction of cellular ligands seems unlikely, NK cells most probably recognize a pathogen-derived structure. The Plasmodium falciparum Membrane Protein-1 (PfEMP-1) was a candidate molecule but experiments with knockout parasites rather demonstrated an inhibitory role on different immune cells, including NK cells (162, 163).

2.4.4 NK cells in viral infections

While this section does not aspire to give a comprehensive review of NK cells in viral infections, I would like to highlight a few examples before focusing on herpesviruses. Even though NK cell research had originated in tumor biology, soon a possible role in the innate reponse against viruses was investigated in parallel. The first studies implying a possible involvement of NK cells in the antiviral host defense was a report showing activation of NK cells by injection of type I IFNs into mice (8) and in vitro assays with human cells demonstrating preferential killing of virus-infected fibroblasts by IFN-activated NK cells (164). Further support was added by Lymphocytic choriomeningitis virus (LCMV) (165, 166) that induced NK cell blastogenesis and proliferation (167, 168) and MHV3 (169). First studies on human NK cells were focused on HSV-1 infection (170, 171). NK depletion experiments firmly established a role for NK cells in the antiviral immune response against mouse hepatitis virus (MHV), vaccinia virus and murine cytomegalovirus (MCMV) (172). NK cells accumulate in several organs during MCMV infection (173) and the relative contribution of different NK cell effector mechanisms can vary in an organ-dependent manner. While, both perforin and IFN-γ play a role in clearance (174) , perforin has been described to be the more important effector molecule in the spleen, while IFN-γ-mediated effects dominate in the liver (175).

Today, our knowledge about NK cell receptors and their respective ligands often allows a molecular dissection of the interaction between NK cells and infected target cells and helps to reveal immune evasion strategies employed by different viruses. While this is particularly true for the continous line of research dealing with NK cells in herpesvirus infections, I would like to quickly give an overview on research conducted on other viral pathogens.

A report investigating the role of NK cells in a murine model of Ebola virus demonstrated that immunization of mice with Ebola virus-like particles (VLPs) conferred protection against a lethal challenge of the virus. This effect was largely dependent on the presence of NK cells, since NK cell depletion or the use of NK cell or perforin-deficient mice almost abrogated protection induced by VLP immunization, whereas adoptive transfer of VLP-activated NK cells improved survival of naïve mice when challenged with Ebola (176). It is unclear whether in this system NK cells exert protection by directly lysing infected cells or indirectly via affecting the adaptive immune response.

Involvement of NK cells in the innate resistance against Coronaviruses was studied in rag-$1^{-/-}$ mice where the chemokine CXCL10 led to recruitment of NK cells to the cerebral site of infection, and their subsequent activation resulted in a marked reduction in viral titers (177). Targeting of the NK cell activating cytokine IL-18 has been described as an evasion mechanism for Poxviruses (178, 179), whose control is vivo seems to rely NKG2D (180). During the preparation of this thesis, the interference of an adenoviral gene product with the NKG2D ligands MICA and MICB was shown (181).

In mice deficient for the activating NK cell receptor NKp46, NK cells accumulated at the site of influenza virus infection, yet were unable to clear the virus (182) – a finding in line with the presumed viral ligand of the receptor, reported by the same group (78). There is also some evidence that human NK cells might contribute to the immune response against influenza (183).

One example where the NK cell response to infection is affected by a viral pathogen is Hepatitis C virus (HCV). In the same issue of JEM two studies (184, 185) showed independently that binding of the major viral envelope protein E2 to CD81, which is a tetraspanin expressed on almost all nucleated cells, inhibits CD16-induced cytokine release, cytotoxic granule release and proliferation of NK cells. HCV infection also highlights the importance of NK cell DC crosstalk. NK cells from HCV infected patients are impaired in their ability to activate DCs, in part due to higher CD94/NKG2A expression and secretion of TGF-β and IL-10 compared to NK cells recovered form healthy controls. Conversely, DCs from chronically infected patients did not upregulate MICA and MICB in response to IFN-α, due to impaired autocrine/paracrine IL-15 secretion (186, 187), thus failing to activate NK cells in an NKG2D dependent manner. It has also been observed that NK cells from chronically infected patients, display lower levels of NCRs and are functionally impaired against targets that require the engagement of these receptors (188).

In an increasing number of diseases a certain disease outcome is correlated with certain KIR receptors and/or HLA alleles. The first report linking an inhibitory KIR and its cognate ligand to the resolution of Hepatitis C Virus (HCV) (189) demonstrated a protective effect of the combination of KIR2DL3 and HLA-C1 and this effect was more pronounced in homozygous than in heterozygous individuals. However, the data also suggested that the positive effect of the association could be overcome by higher initial viral dose, as protection was not observed in individuals that were assumed to have received a larger inoculum, based on the route of transmission. This is reminiscent of Ly49H mediated clearance of MCMV, that I will review in more detail later. The authors hypothesized that the weak inhibition mediated by this particular KIR-HLA combination when compared to KIR2DL2-HLA-C1 interactions would favour NK cell activation. A protective effect of a certain KIR/HLA genotype combination has also been reported for papilloma virus (190), while for Herpes Simplex Type I (HSV-1) an association of recurring symptomatic infection with the presence of KIR2DL2 and KIR2DS2 has recently been suggested (191).

An exciting, complex and comparably new field of research is the role of NK cells in HIV infection. Studies investigating NK cell numbers, phenotype and function in HIV produced conflicting results. While some early reports described normal numbers but severe functional impairment of the NK cells (192), others reported a significant decrease of $CD56^{+}CD16^{+}$ NK cell numbers while a functionally impaired $CD16^{dim}CD56^{-}$ subset expanded (193). Newer data suggested that despite reduced NK cell numbers, the remaining cells were highly active during active HIV replication, as measured by cytokine release and degranulation, while the expanded anergic subset expressed low perforin and elevated SHIP-1 levels (194, 195).

First evidence that HIV avoids NK cell recognition came with the description of selective downmodulation of HLA-A and HLA-B but not HLA-C, arguably the most important inhibitory NK cell ligand, by the viral protein Nef (196). Another study demonstrated that the remaining HLA-C levels were sufficient for ensuring HLA-E surface expression. Together, HLA-C and –E protected HIV-infected HLA-transfectants from NK cells (197) and blocking these molecules resulted in NK lysis of HIV-infected primary T cell blasts (198). An important role for NK cells in HIV clearance was implied by a report that associated HIV-1 exposed but uninfected individuals with increased NK cell activity (199, 200). Likewise, NK cells recovered from viremic patients instead displayed reduced NCR levels while expression of inhibitory NK cell receptors, with exception of NKG2A, was largely maintained or slightly increased (201). This phenotype was reversible when viremia was suppressed by highly active antiretroviral therapy (HAART) (202). The precise mechanisms for possible NK cell control of HIV are not yet well understood. One report proposed the direct recognition of a ligand for the activating receptor NKp44 that is induced by the viral protein gp41 in infected $CD4^+$ T cells (88) and an earlier study suggested that the inhibition of viral replication was mediated by the secretion of CC chemokines by NK cells (203). The authors interpreted this as competitive blocking of chemokine receptors that are known co-receptors for viral entry. Alternatively, receptor-bound CC chemokines were recently reported to be ligands for NKp46 and could as such represent recognition structures on the infected cells (204).

A substantial part of the available literature on NK cells and HIV is focused on the impact of KIR- and HLA-alleles on HIV infection. In 2002 a report by Martin and coworkers (205), found an association between KIR3DS1 in combination with the HLA-Bw4 allele on one hand and delayed disease progression on the other hand. The presence of the activating KIR and its presumed cognate ligand appeared to slow down the depletion of $CD4^+$ T cells. A follow-up study extended the protective effect to opportunistic infections common in AIDS patients (206). The precise association remains controversial as other groups have only partially confirmed those results (207, 208), possibly owing to polymorphism of KIR3DS1 which was not addressed in the initial report. In vitro studies by Alter et al. (209) demonstrated that KIR3DS1+ NK cells inhibit HIV replication in co-culture with HLA-Bw4-$80I^+$ CD4 T cells in a contact dependent manner. Furthermore, the infected $CD4^+$ T cells were preferentially lysed compared to uninfected cells or infected cells carrying a haplotype other than HLA-Bw4-80I. A related article (210) suggests again a correlation between the viral load in viremic patients and the degree of NK cell activation and suggests that HIV-1 derived single-stranded RNA mediated this effect in the presence of accessory cells, e.g. plasmacytoid DCs or monocytes.

However, the molecular mechanism for a protection mediated by a direct interplay between KIR3DS1 and HLA-Bw4I80 remains entirely unclear since to my knowledge no evidence regarding direct binding is available to date. To the contrary, two independent studies failed to show binding of KIR3DS1-F_c fusion proteins to HLA-Bw4-80I expressing 721.221 cells (211) or of a HLA-Bw4 tetramer to KIR3DS1 expressing 293T cells (212). A recent study by Long et al. (213) showed a higher degree of NK cell activation in carriers of the KIR3DS1 allele as well as lower CD38

expression that is usually considered a marker of HIV progression, but did not detect any correlation with the joint expression of HLA-Bw4I80. Since the epistatic interactions of KIR and HLA are not unequivocally confirmed, one could imagine that both loci are involved in the protection against HIV, yet in two, largely independent pathways that remain to be defined.

Another large scale study reported additional associations for certain allelic combinations (214). The strongest protection was mediated by KIR3DL1*004 and HLA-B*57. This is particularly intriguing, given that KIR3DL1*004 is barely expressed at the cell surface (215). In this regard it resembles KIR3DS1 which, at least according to some studies, also does not seem to depend on the presence of its cognate HLA ligand for mediating protection. It is possible that both receptors have intracellular, currently unknown functions that confer partial resistance to the progression of HIV infection. Another possibility which has not been extensively addressed in the context of HIV is whether virus-derived peptides or peptides derived from proteins induced by viral infection are differentially recognized by KIRs and might hence represent an underlying reason for some of the observed associations. Peptide discrimination by KIR has been discussed in a previous section.

2.5 HERPESVIRUSES (HERPESVIRIDAE)

The viruses that have doubtlessly taught us most about NK cell biology are certainly the herpesviruses in general, and murine and human cytomegalovirus in particular. Herpesviruses are ubiquitous pathogens and have been identified in countless animal species with their growing number exceeding 200. The following overview is based on (216)

2.5.1 Classification, Clinical Aspects, Replication

The classification of herpesviruses is largely based on tissue tropism and distinguishes α-, β- and γ-herpesviruses. The α-herpesviruses, with HSV as the prototypical representative, are not species-restricted, have a comparably rapid replication cycle and persist primarily in sensory ganglia. In contrast, β-herpesviruses such as CMV, have a narrow host spectrum, replicate slowly and establish latency in secretory glands, lymphoreticular and other cells. The γ-herpesviruses like Epstein-Barr virus (EBV) are restricted to family or order of the natural host and usually infect B cells or T cells. Herpesviruses are enveloped viruses with an icosaedral capsid that harbours a large double-stranded DNA genome of 230 kb in case of CMV and 152 kB in case of HSV. Very simplified, the genome is organized in a unique long (UL) and a unique short (US) region, flanked by terminal and internal repeats respectively.

In industrialized countries the majority of the population is seropositive for CMV and HSV, but also EBV with even higher percentages in developing countries. Transmission of CMV usually occurs via saliva or breast milk. Placental transmission is also of great medical importance, as CMV is the most common viral cause of

congenital disorders and gives rise to neuronal impairments, growth retardation and jaundice in the fetus, provided the primary infection of the mother occurs within the first trimenon of the pregnancy.

Primary infections usually take place in early childhood and display a mild, often asymptomatic course. They usually lead to a state termed latency where the virus resides silently in certain cell types while no or only very few viral genes are transcribed at a very low level. In fact the name Herpesviruses roots back to the greek word "herpein" that means "creeping", referring to the dormant state of the virus after establishing latency. Reactivation of the virus can occur under certain conditions, although the precise mechanisms underlying this process are only incompletely understood. Clinically, reactivation is usually associated with immunosuppression, in which context it can be life-threatening. Typical patient groups suffering from excessive CMV disease are transplant recipients or AIDS patients, where the disease can cause CMV retinitis, hepatits, encephalitis or pneumonitis and severity correlates inversely with the CD4 T cell count. An association of cytomegalovirus with autoimmune diseases has been suggested on the basis of the detection of cross-reactive autoantibodies, but it is not clear whether they contribute to etiology or whether they are rather a consequence of a deviant immune response.

The viral replication cycle follows the same general scheme for all family members: Binding of viral glycoproteins to surface molecules on host cells leads to membrane fusion, subsequent internalization of the viral particle into the cytoplasm and the release of the nucleocapsid that then translocates to the nucleus where the viral genome circularizes and takes an episomal shape. Expression of the viral genome takes place in three phases, into which genes are grouped according to the kinetics of their expression: immediate early, early and late. Parallel to late gene expression, viral DNA is synthesized. Translated proteins either traffic to the cell membrane, where some of them elicit cellular immune responses, or localize to the nucleus where assembly of viral particles takes place under the assistance of scaffolding proteins and the inner nuclear membrane. Viral particles migrate via the ER and the Golgi apparatus to the cell membrane where they are released after having undergone further modification.

This replication cycle constitutes what is known as lytic replication and reflects the events taking place in infected cells that I used throughout my studies. However, out of the 100-200 genes carried by most herpesviruses, a substantial portion is dispensable for viral growth and replication but instead represents the potential arsenal of gene products available for subversion of the immune response. Viral immunoevasins comprise an exceptionally broad range of molecules, including homologs of host cytokines/chemokines and their respective receptors, F_c-receptors or proteins that interfere with intracellular signaling (217)

2.5.2 Virus - Host Interactions

Although herpesviruses elicit a broad immune response including NK cells and B cells producing neutralizing antibodies, they are ultimately controlled by $CD8^+$ T cell

responses that recognize immunodominant epitopes, derived from viral proteins, presented in the context of MHC class I molecules. It is astonishing that up to 5% of all T cells in the peripheral blood can be specific for CMV antigens. In the memory compartment their proportion can be as high as 10% (218). The T cell response differs widely between individuals and can be directed to the gene products of 5 to 70 ORFs with a tendency to oligoclonality in old age (218). There is however no indication that the large proportion of the T cell pool that is dedicated to CMV antigens, compromises the susceptibility to heterologous infections (219).

To counteract T cell recognition a common feature of herpesvirus immune evasion is the downmodulation of MHC molecules, a strategy, though, that is not limited to herpesviruses, as it is employed by other virus families, such as Adeno- or Lentiviruses as well (220). While the reduction of cell surface MHC could solve the challenge of T cell recognition, it should theoretically create a new problem, i.e. an increased susceptibility to NK cells due to less inhibitory signals or in other words: “missing-self recognition”. Indeed, the clinical presentation of NK cell deficient patients, that I have outlined earlier, confirms a role of NK cells in the control of herpes virus infections. Nevertheless, surveillance by NK cells does not remain unanswered by HCMV. It is estimated that up to 50 ORFs are dedicated to immune escape, some of them specifically targeting NK cell recognition systems.

Amongst the first studies investigating the role of NK cells in viral infections were reports indicating their importance in the innate phase of MCMV infection. MCMV can achieve MHC class I downmodulation by at least three viral genes: m04 encodes gp34 that associates with MHC class I molecules in the ER and remains bound to them even on the cell surface. Initially this was seen as a steric obstacle for the binding of TCRs, but more recent evidence suggests that in a complex interplay with other CMV genes, gp34 actually might promote antigen presentation (221, 222). The gene m06 encodes gp48 which targets assembled MHC class I complexes in the ER for lysosomal degradation (223). The gene m152 encodes the gp40 protein that is structurally an MHC class I homologue. It exerts a dual function by trapping MHC class I complexes in the cis-golgi compartment and downregulating Rae-1, one of the murine NKG2D ligands (224).

In HCMV, to date six different viral genes have been described to interfere with MHC class I antigen presentation: The tegument protein pp65 prevents processing of the viral immediate early antigen-1 (IE-1) for presentation (225). The US2 and US11 genes downregulate MHC class I molecules by rapidly translocating them to the cytosol where they get tagged for proteasomal degradation (226, 227). US2 and US11 differ in terms of allele specificity and the maturation stage in which they bind to MHC class I within the ER (228). Other targets of US2 have been identified as the MHC class II pathway and the non-classical MHC class I molecule HFE (229, 230). Other immuno evasive genes US3 retains MHC class I molecules and interferes with MHC class II (231-233) and US6 blocks the transporter associated with antigen processing (TAP)(231), thereby preventing the loading of MHC class I molecules with peptides (234), which is a mode of action that resembles the HSV-1 protein ICP47 (235). Less well studied is US10 which is possibly involved in delaying intracellular MHC class I

trafficking (236). UL82, encoding pp71 has recently been suggested to possess additional functions beside its known role in viral replication, namely to impair the transport of MHC class I between ER and cis-golgi (237).

As mentioned above γ-herpesviruses are highly species specific and there is no direct homology between human and murine CMV. However, MCMV is still a valuable model since the functional analogies between MCMV and HCMV, as exemplified by the gene products interfering with MHC class I antigen presentation, are striking and the pathology also follows a comparable course.

Consequently, MCMV is probably the most studied viral infection in regard to NK cells in the murine system. Initially NK cell depletions or adoptive transfer had indicated a role for NK cells for the control of MCMV infection (238, 239). Another early observation was the resistance of some mouse strains, such as C57BL/6 to infection (240). Genetic studies allowed to map the responsible gene to the NK gene complex (NKC) and identified the locus responsible for mediating protection as *cmv1*, which turned out to encode the NK cell receptor Ly49H, also known as Klra8 (241, 242). Ly49H is one of the few members of the Ly49 family that has an activating function. This depends on its association with the adaptor DAP12, which was shown to be decisive for the Cmv1/Ly49 mediated protection (243). Formal proof for Ly49H as the crucial determinant for susceptibility was the introduction of a Ly49H transgene into Balb/c mice, that rendered them resistant to infection (244). It was then shown that Ly49H recognizes the viral protein m157 (245, 246) and that the Ly49H$^+$ subset of NK cells undergoes selective expansion early in infection. The selective pressure by this NK cell subset has been shown to be sufficient to give rise to escape mutants after sequential infections in vivo (247, 248). The finding that m157 can also bind to the inhibitory receptor Ly49I in the susceptible 129/J mouse strain (245), implies that it can act as a decoy, resembling the mode of action of another immune evasin of MCMV, the MHC class I homolog m144 (249, 250) or the rat Clr-b (Ocil) that serves as ligand for the inhibitory NK cell receptor NKR-P1B in rat cytomegalovirus (RCMV) infection (251).

However, there are also Ly49H independent pathways for NK cell control of MCMV, such as in New Zealand White (NZW) mice (252), even though the responsible genes remain to be identified in this strain. In MA/My mice, resistance is mediated by the combination of a H-2^k haplotype and the activating NK cell receptor, Ly49P (253, 254). It is still unclear which precise ligand is recognized by the Ly49P$^+$ NK cells in this model but it it seems possible that Ly49P recognizes an H2^k-restricted peptide either derived from a viral gene product or from a cellular protein induced by the infection.

As I described above, MCMV (as well as HCMV) dedicates multiple genes to the targeting of the MHC class I presentation pathway, highlighting its importance for immune control. Judging from the genetic effort, the NKG2D receptor ligand system enjoys almost as high priority in terms of immune evasion. So far, four viral genes interfering with different ligands have been identified in MCMV: m138 (255), m145 (256, 257), m152/gp40 (224, 258, 259), m155 (258), that downmodulate Mult-1

(Krmpotic JEM 2005 and NJI 2002), H60 and Rae-1 (234, 235) by different mechanisms. For human herpesviruses the downregulation of NKG2D has not only been reported for HCMV but also for Kaposi's sarcoma-associated herpesvirus K5 (260), and HSV-1 **(paper III**). Evasion of NKG2D mediated immune responses will be discussed in more depth in relation to my own results.

3 RESULTS AND DISCUSSION

I will attempt to discuss the results of my PhD studies in an integrated review of all four papers, that combines the relevant literature with my own data. However, I will try to avoid extensive repetitions and for details of my experimental results I refer to the original papers I-IV.

When I set out, immune evasion by herpesviruses had mainly been described in terms of T cell recognition, as the downmodulation of MHC class I molecules on infected cells was well established. In contrast, NK cell evasion was not characterized on a molecular and hardly on a cellular level. It was well known from earlier studies in animal models, that I have described above, that the control of HSV and MCMV infection is dependent on NK cells but in vitro studies with HCMV yielded confusing results.

3.1 THE ROLE OF MHC CLASS I IN HCMV INFECTION

Almost unequivocally, different authors showed that MHC class I downregulation induced by HCMV was not correlated with susceptibility to NK cells which the missing-self paradigm would suggest (261-264). Only one study claims that susceptibility of HCMV infected cells is governed by MHC class I levels and the viral genes, US2-11, affecting them (265). The first reports trying to explain this, focused on the modulation of adhesion molecules ICAM-1 and LFA-3 by viral infection that had been described earlier (266-268). Fletcher et al. found that, depending on the viral strain used, infection induced changes of the adhesion molecule LFA-3. Upregulation of LFA-3 by strains AD169, C1F and R7 led to increased lysis by NK cells, while downregulation by strains Towne, Toledo, Davis and C1FE resulted in decreased lysis by NK cells (262). However, this correlation only held true for fibroblasts and not for other cell types in the same study. More direct evidence for a crucial impact of adhesion molecules for the NK cell recognition of infected cells was not provided since blocking experiments by monoclonal antibodies were lacking. Another limitation was the purity of the effector population, since cytokine-activated bulk PBMCs were used for the functional assays. A study from our own laboratory, that included AD169 but a panel of different clinical isolates could only detect an increase of ICAM-1, that was similar across all strains and in line with the finding by Leong et al. (263), whereas LFA-3 surface levels remained unchanged (263). The conclusion of this study by Cerboni et al. was that clinical strains reliably conferred protection of the infected cells from NK cell lysis, regardless of MHC class I levels, but that the widely used laboratory strain AD169 exhibited a more variable pattern (261).

3.1.1 Differences between viral strains

Extensive in vitro passage of HCMV in the absence of selective immune pressure can lead to the accumulation of genetic deletions, often affecting genes for immune evasion, e.g. homologues for a TNF receptor or CC chemokines (269, 270), tissue tropism (271). The attenuated virulence of these laboratory strains compared to more recent clinical isolates, which have undergone no or fewer passages in vitro, also led to their assessment as vaccine candidates. Best characterized is a loss of 15 kB in the widely used strain AD169 and a 13 kB deletion in the strain Toledo, that contain at least 23 and 19 genes respectively (272). Some of these were later shown to be directly involved in interference with NK cell recognition, which advises caution when working with laboratory strains, as they might basically be deletion mutants for genes of critical relevance for a particular question. In line with this consideration, clinical isolates have by different authors been shown to confer substantially more protection against NK cell mediated responses (261, 273, 274). Additionally there is also considerable sequence variability within immunomodulatory genes between different clinical isolates (275) and functional consequences of these variations have already been demonstrated (276)

The loss of MHC class I expression in Herpesvirus infected cells in combination with an unchanged or even decreased susceptibility to NK cells poses an intriguing conceptual problem. We chose to address this problem in the context of α-herpesviruses (HSV-1, **paper III**), β-herpesviruses (HCMV, **paper I, II** and **IV**) and γ-herpesviruses (EBV, unpublished data). Theoretically, viral infection can deal with the problem of MHC class I downregulation in regard to NK cells in two different ways: HCMV could either provide an alternative ligand for inhibitory NK cell receptors or it could interfere with activating pathways.

3.2 UL18 – A VIRAL HIGH AFFINITY LIGAND FOR AN INHIBITORY RECEPTOR

The first possibility was investigated primarily in the context of viral MHC class I homologues. Already in 1988 Beck and coworkers identified a MHC class I homologue (277) with 13 glycosylation sites in the HCMV genome that when coexpressed with β_2m could form complexes that were displayed at the cell surface (278). Since this protein, termed UL18, was dispensable for viral replication (279) but found in all clinical isolates analysed (280), it is considered to be a likely candidate for an immune evasin. Before the identification of the MHC class I downmodulating US genes, it was assumed that sequestration of β_2m by UL18 would disturb the assembly of cellular MHC class I complexes, accounting for their reduced surface expression after HCMV infection. However, when the deletion of US2 and US11 was shown to be sufficient to restore MHC class I levels on infected cells, it became clear that UL18 had to have another mode of action (281).

The stability of UL18 is, similar to MHC class I, dependent on the presence of peptides in the peptide binding groove and it has been shown that the peptides are similar in

terms of length and amino acids in the important anchoring positions 2 and 9 (282). Peptide binding was shown to be partially TAP-independent, since adenoviral expression of UL18 in TAP deficient fibroblasts still resulted in surface expression. This, perhaps convieniently for HCMV, made UL18 resistant to downmodulation by the US6 gene product (283) with whom it shares a slightly overlapping temporal expression pattern. Interestingly, in the same study the ICP47 gene of HSV-1, which also blocks TAP did downregulate cell surface expression of UL18.

3.2.1 UL18 expression

UL18 is not part of the HCMV virion (284), indicating that its function is required in the infected cells. However, mRNA levels are usually low and become detectable late in the infected cells (285, 286). Only few groups have managed to demonstrate protein expression in a productive HCMV infection (287), which might be partially attributable to the quality of available antibodies, since heavy glycosylation often negatively affects immunogenicity. One recent report addressed the regulation of cell surface expression of UL18 and concluded, that two motifs in cytoplasmic tail might account for intracellular retention in UL18 transfectants (288). Since HCMV infection of these transfectants resulted in detectable UL18 levels on the cell surface, the authors speculated that the retention motifs are somehow utilized by the virus for a tight control of UL18 cell surface expression. However, there is little doubt that UL18 is expressed during active infection in vivo, as mRNA can be detected in patients with HCMV viremia (285), UL18-specific T cell reponses have been observed (289) and LIR-1 expression in transplant patients is significantly increased several weeks before manifestation of HCMV disease (290).

For this reason most studies resort to the use of overexpression systems often relying on viral vectors such as vaccinia or adenoviruses. While an approach with viral expression vectors facilitates in vitro studies considerably, they might give rise to artefacts caused by overexpression or by effects of the vectors themselves on cellular metabolism and function. They also do not allow to assess if the mode of action of the viral gene of interest depends on the coexpression of other viral genes or on cellular genes, induced by viral infection. For this reason, we decided to use systems permitting productive infection (**paper I, III** and **IV**), whenever we set out to study the role of a certain viral immunoevasin.

3.2.2 A structural approach to the enigmatic function of UL18

Based on the finding that UL18 bound peptides similar to MHC class I. Fahnestock et al. suggested that UL18 instead of capturing β_2m would simply be a decoy that engages inhibitory NK cell receptors in the absence of cellular MHC class I molecules (282). Cosman et al. used a UL18-F_c fusion protein to screen for a binding partner and this search ultimately led to the immunoprecipitation and subsequent expression cloning of the LIR-1/ILT-2 receptor that I have introduced earlier. The same methodological

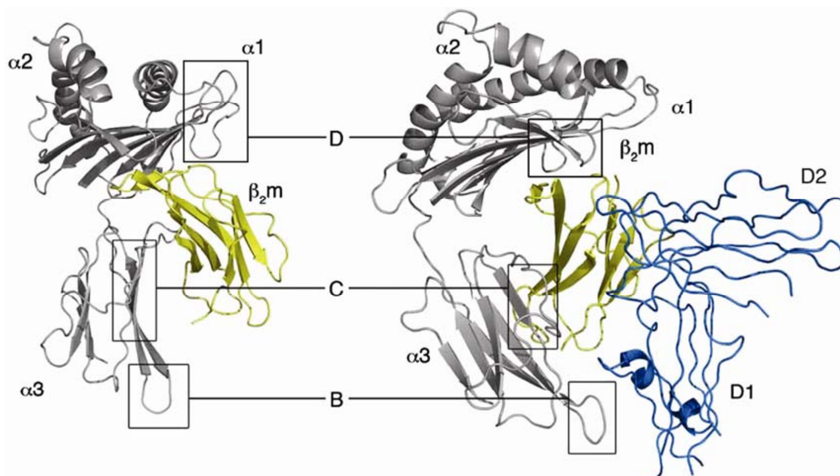

Fig. 3 Identification of three UL18 regions potentially important for binding to LIR-1

approach then identified a broad range of classical (HLA-A, B, C) (45, 46), and non-classical MHC class I molecules HLA-G and F (48, 49) as cellular counterstructures for LIR-1. The structural reasons for this promiscuous binding of a rather diverse set of ligands is that LIR-1 binds to the α_3-domain of the heavy chain (47), which is the least polymorphic in MHC class I and to the invariant light chain, β_2m (291). The probably most striking finding was reported by Chapman et al. (47), who observed in surface plasmon resonance studies, that the affinity of UL18 for LIR-1 exceeded that of cellular MHC class I molecules by the factor 1000 and this was corroborated by fusion protein stainings comparing LIR-1 binding to UL18- vs. HLA-B-transfected cells. The high affinity was not dependent on glycosylation since less and differently glycosylated UL18, produced in insect cells behaved similarly. However, extensive and complex glycosylation might be one of the reasons why UL18 has defied crystallization attempts by several groups for a decade.

In order to understand the structural features in UL18 that confer such extraordinary high affinity for LIR-1 we chose an approach that combined molecular modelling and site-directed mutagenesis with binding and functional assays (**paper II**). To this end, we compared the published structure of LIR-1 in complex with HLA-A2 (291) with a model of UL18 that was based on HLA-Cw4, the HLA allele displaying the highest sequence homology to UL18 (276). This allowed us to identify three key areas that differed distinctly between UL18 and HLA class I (Fig. 3B-D): firstly, two loops (residues 13-19 and 36-44) protruded further from the alpha1-domain than their counterparts in HLA-Cw4. Secondly, the loop connecting the two helices of the α_2-domain was significantly longer in UL18 (while the helix itself as well as the connection to the α_3-domain was shorter) stretching upwards from the peptide binding cleft. While this area is, based on the HLA-A2-LIR-1 co-crystal, usually not considered to be important for binding of LIR-1, it could still have important implications for

binding of TCR or inhibitory NK cell receptors like KIR. The most striking feature of our UL18 model was the prediction of a second disulfide bridge in the α_3-domain.

Chapman et al. established in domain swapping experiments that the domain D1 of LIR-1 interacts with the α_3-domain of both, UL18 as well as MHC class I (47). A follow-up study (292) comparing differences between LIR-1 and LIR-2, that does not bind UL18, allowed to identify key residues involved in the binding of UL18 to its cellular receptor. In 2003 the same group managed to crystallize the structure of HLA-A2 in complex with LIR-1, although the D3 and D4 domain were omitted from the LIR-1 construct (291). Two contact areas were revealed that contributed to binding. Firstly, the tip of LIR-1 interacted with 6 residues of the α_3-domain as had been proposed by earlier studies. Binding of UL18 to LIR-1 seems to take place in a similar manner, since a mutation of Q202, located in an exposed α_3-loop and predicted to form hydrogen bonds with two LIR-1 residues, decreased binding and function (**paper III**). Secondly, β_2m made an unusually high contribution to binding by forming almost 70% of the binding interface mainly interacting with the hinge region between D1 and D2 domain of LIR-1. The crucial role of β_2m for LIR-1 binding and inhibitory function had already been reported earlier in context of HLA-G-mediated NK cell inhibition (293) and HLA-B27 to LIR-1 transfectants (294). In our mutational study the stability of UL18 heavy chain association with β_2m was compromised by deletion of the loop formed by residues 36 to 44 in the α_1-domain. The consequence was a gradual loss of β_2m over time and a consecutive decrease of binding to LIR-1, although the addition of exogenous β_2m could restore the initial configuration and function. A contributing factor to decreased complex stability might be the N-glycosylation site at residue N38 that lacks in our mutant.

The disruption of an additional disulfide bridge in the α_3-domain of UL18 had the most dramatic impact. The single disulfide bridge that is found in classical MHC class I molecules has been shown to be important for the export of MHC molecule to the cell surface but its presence is not required for the binding of β_2m by MHC class I molecules. Based on our model of UL18 one could however hypothesize that a second disulfide bond in the α_3-domain confers additional stability and/or results in a slightly altered binding of β_2m which in turn could lead to an optimized interface between β_2m and LIR-1. In line with this speculation, we observed a total loss of β_2m binding to the mutated UL18 protein and no detectable binding to LIR-1.

A double mutation targeting residues K42 and A43 in the protruding loop in the α_3-domain of UL18 surprisingly reduced binding to LIR-1 as well, even though this area has previously not been suggested to affect binding of UL18 to LIR-1. While direct involvement in the binding interface is a possibility, the mutations might alternatively have an impact on the positioning of β_2m and influence binding to LIR-1 indirectly.

Taken together, our results give important clues about the distinct structural features that set UL18 apart from its closest known relative, HLA-A2. A stabilization of the α_3-domain by an additional disulfide bond and possibly an altered configuration of β_2m in the UL18 complex clearly are of importance for the binding of UL18 to LIR-1. The crucial importance of β_2m for stability of UL18 and binding to LIR-1 as well as a role

for the second disulfide bridge was supported by another study (295), which also suggested a contribution of the α_3-domain to the high affinity nature of the interaction. However, only the crystal structure will be able to conclusively explain the superior affinity of UL18 compared with cellular MHC class I molecules.

3.2.3 UL18 – which role does it play in productive infection?

But even if the structure is solved and provides a satisfactory molecular explanation for the high affinity of UL18 to LIR-1, a central question remains the role of UL18 in a productive HCMV infection. The early assumption that UL18-LIR-1 interactions would be the logical, straightforward answer to the mystery of NK cell inhibition in HCMV infection in spite of low MHC class I levels on the target cells, turned out to be hard to prove – until today the body of evidence for a NK cell inhibitory role in productive infection remains frustratingly small.

In MCMV the deletion of the viral MHC class I homolog m144 led to a marked decrease in viral replication in vivo and this effect was NK cell dependent (250). In HCMV however, controversial results were obtained regarding the analogous UL18 protein. Reyburn et al. reported a UL18 mediated protection of 721.221 transfectants (296), which was supported by a later report where retrovirally UL18-transduced swine endothelial cells became resistant to peripheral blood NK cells (297). In contrast, Leong et al. (263) described actually an increase in NK susceptibility when UL18 was present during productive infection comparing a wild type and a deletion mutant of HCMV and experiments in 293EBV transfectants confirmed these results. A later study also observed a slight increase in NK cell sensitivity of endothelial cells and macrophages when UL18 was present (298).

A weakness of the study by Reyburn is, that protection of the transfectants might theoretically be mediated by HLA-E rather than by UL18 because the transfected cells were selected on the basis of β_2m and not UL18 expression. The biological significance of the xenogeneic study, using swine endothelial cells could also be called into question. Critical activating NK cell ligands are presumably absent in that system, the protection was fairly weak and mainly seen at higher E:T ratios and untransduced cells were used for comparison instead of cells transduced with a relevant vector control.

More recently, a study (299) has finally taken into account that LIR-1 is only expressed on a subset of NK cells and re-evaluated the role of UL18 by analysing the response of NK cell subpopulations to UL18. The results presented by the authors suggested that the inhibitory function of UL18 is restricted to the LIR-1$^+$ NK cells while activation by an unknown mechanism is the predominant effect in the LIR-1$^-$ subpopulation. Depending on the LIR-1 distribution in a polyclonal NK cell culture, either the inhibitory or the activating role of UL18 could dominate. During the preparation of this thesis the crystal structure of CD94/NKG2A in complex with HLA-E was published (64, 65). Within one of the two study, the authors could show a weak binding of UL18

to the activating heterodimer CD94/NKG2C but not to its inhibitory counterpart CD94/NKG2A(64).

It is very possible that this marks the identification of an additional receptor for UL18, which caused headache for a field that was biased to try and explain all available data on UL18 exclusively in the framework of high affinity binding to LIR-1. It remains unclear however, how CD94/NKG2C could escape the substantial efforts in the mid-90s to find a cellular binding partner for UL18. If recognition of UL18 by CD94/NKG2C takes indeed place during a productive infection, it could possibly represent the reason for the expansion of CD94/NKG2C^{+} NK cells in response to CMV infected fibroblasts as demonstrated by Guma et al. (300). This would then resemble MCMV infection where the selective proliferation of an NK cell subset is driven by the recognition of a viral ligand, m157, by an activating receptor, Ly49H. However, Guma and coworkers suggest that the proliferation depends on the presence of the US2-US11 genes in the viral genome. The conclusion seems oversimplified since other mutants, including a UL18-deficient virus, used in the same study clearly affect the expansion of CD94/NKG2C^{+} NK cells as well.

Given that LIR-1 is expressed on other immune cells as well, the role of UL18 has also been investigated in CD8^{+}T cells, where LIR-1 dependent (287)and independent T cell activation was described (301) and DCs, which responded by phenotypic and functional changes to exposure with UL18 fusion proteins (302).

3.3 UL40 – IMMUNE ESCAPE VIA CD94/NKG2A RECEPTORS

3.3.1 Upregulation of HLA-E by UL40-derived leader sequences

The inhibitory CD94/NKG2A receptor has itself been implicated to play a role in NK cell evasion of HCMV infected cells. In 2000, a database search for sequences that could bind to HLA-E, the CD94/NKG2A ligand, revealed that a part of the ORF UL40 of HCMV was identical with the nonamers derived from the leadersequence of HLA-C (303), even though its relative position in the UL40 gene was slightly less N-terminal. As reviewed in the introductory part of this thesis, MHC class I leader sequences had previously been shown to bind to HLA-E, ensuring stable surface expression of this conserved non-classical MHC class I molecule (61, 63). In the context of CMV infection, UL40 offered obvious value for HCMV immune evasion, as downmodulation of classical MHC class I should also result in a reduction of cell surface levels of HLA-E. With a virally encoded HLA-E binding sequence, HCMV could theoretically compensate for the loss of cellular leader sequences during infection and stabilize or, as the authors speculated, even upregulate HLA-E expression ensuring NK cell inhibition. As predicted, the infection of fibroblasts with high titers of an adenoviral vector encoding for UL40 increased HLA-E cell surface levels. This effect was significantly enhanced by co-infection with a second vector carrying HLA-E and also took place in fibroblasts recovered from a TAP-deficient patient, implying that the known HCMV encoded TAP-inhibitor US6 would not interfere with the action of

UL40. The increase in HLA-E expression conferred protection from lysis by the NK cell line NKL and was almost entirely reversible by blocking antibodies against MHC class I or CD94. Productive infection with HCMV strains AD169, Toledo or 742 resulted in a subtle upregulation of HLA-E which was again augmented by a co-infection with an adenoviral HLA-E vector, even though a direct comparison is difficult given that the co-infection experiments were read out at a different time point (48 instead of 89 hours).

Almost at the same time of this publication, another group reported similar findings (304). The independent identification of UL40 as a source for a suitable HLA-E ligand was studied in K562 HLA-E transfected cells, that were protected from NKL lysis, when either pulsed with a canonical HLA-E binding peptide or co-transfected with UL40 and protection was again reversible by anti MHC class I or anti-CD94 antibodies. Using the HLA-E restricted T cell line TER-1 against UL40-HLA-E cotransfected, TAP-deficient RMA-S cells, the authors also concluded that UL40-mediated effects on HLA-E levels are TAP independent. However, a later report by the same group suggested that TAP activity, even in the presence of US6, is in general not the critical bottleneck for HLA-E expression, at least not in K562 transfectants (234). In another study, transfection of UL40 into the HLA-E- parental cell line K562 led to reliable protection, only when the cells were in addition exposed to IFN-γ (305).

Evidence that UL40 derived sequences are indeed presented on HLA-E was provided by studies with a $CD8^+$ HLA-E restricted T cell subset described as NK-CTLs (, due to a limited TCR repertoire and broad reactivity against classical NK cell targets) (306, 307). More direct evidence was provided by biochemical studies, showing that in a system devoid of classical MHC class I molecules, UL40 can be processed into peptides that are loaded to HLA-E on HEK-293T/$UL40^+$/$HLA\text{-}E^+$ transtectants (308).

Experiments using a UL40 deletion mutant essentially confirmed the initial reports by showing CD94/NKG2A-dependent protection of fibroblasts infected with AD169wt but not with the mutant deficient for UL40 (274). Notably, this study relied mainly on DEL, a new NK cell line, for functional assays, that due to its high CD94/NKG2A levels is particularly suitable to demonstrate effects involving this inhibitory receptor. In contrast to these results, a different report concluded that the presence or absence of UL40 neither had a major influence on HLA-E levels nor on the recognition of infected cells by either NKL or polyclonal NK cells (265). It seems difficult to reconcile these data with the earlier studies, given that NKL cells were also used in the initial description of UL40 while the infection itself was conducted in a comparable way. Strain differences between the UL40 gene of AD169 cultured in different laboratories or allelic differences in HLA-E are possible, although not very convincing explanations.

3.3.2 Heat shock proteins, stress and HLA-E – a dual role for UL40?

Our own data (**paper IV**) fall right in between these conflicting reports: while we could show a protective effect of UL40 when using polyclonal NK cells, we also failed to detect substantial differences in terms of HLA-E expression when comparing cells infected with AD169wt and AD169ΔUL40 viruses. We therefore decided to explore a different possibility of CD94/NKG2A mediated protection by UL40 during productive HCMV infection. Some years ago, Michaelsson and coworkers had presented a model, that was termed stress-induced peptide interference and that I have introduced earlier in this thesis (66). In the model of Michaelsson et al., cellular stress leads to the replacement of MHC class I derived leader sequences on HLA-E by heat shock protein 60 derived sequences. After this replacement CD94/NKG2A fails bind to HLA-E. The peptide dependency of CD94/NKG2A binding to HLA-E (309, 310) or the murine counterpart Qa-1 had been described before (311), but was not linked to cellular stress and its surveillance by NK cells. The recent publication of the crystal structure of the CD94/NKG2A-HLA-E co-crystal adds a molecular explanation for the ability of CD94/NKG2A to discriminate subtle differences even between different leader sequences binding to HLA-E (64, 65, 71).

We hypothesized along the SPI model, that the role of UL40 could be to prevent heat shock protein induction by infection, thereby avoiding peptide interference and consecutive activation of NK cells. This would represent a mode of action that is independent of HLA-E levels but still dependent on CD94/NKG2A – UL40 would have a qualitative rather than a quantitative impact on CD94/NKG2A-HLA-E mediated NK cell inhibition.

We thus compared cell infected with AD169wt and AD169ΔUL40. In line with our expectations, HCMV infection did induce heat shock protein 60 and 70 but surprisingly this induction was more pronounced in the presence of UL40, not less pronounced as we had hypothesized. Using siRNAs for functional knockdown of HLA-E, Hsp60 and Hsp70 we showed that Hsp60 has a protective effect in infected fibroblasts per se, since levels of this cellular chaperone correlated with susceptibility to polyclonal NK cells. While knockdown of Hsp70 resulted in a similar pattern, the targeting of HLA-E only had marginal effects on the NK sensitivity of uninfected and both infected cell populations. A possible explanation might be either the low surface levels of HLA-E on fibroblasts that simply cannot be reduced much further or alternatively, that the polyclonal effector populations used in our experiments are not as restricted by CD94/NKG2A as for example the DEL cell line used by Wang et al. (274).

While our initial idea on some role of Hsp60 in UL40 action seemed relevant, the exact hypothesis must be wrong, since UL40 enhanced rather than prevented Hsp expression. We speculated that it might be beneficial for the virus to induce Hsp60 since this may prevent apoptosis and thereby contribute to secure viral replication in the host cell. But elevated intracellular Hsp60 levels would lead to stress induced peptide interference and therefore we could envisage that this requires a further countermeasure in form of competing HLA-E binding peptides, able to confer inhibitory recognition by

NKG2A. The well-described leader sequence within the UL40 gene could represent this very countermeasure and by successfully outcompeting Hsp60-derived sequences for access to HLA-E, it would allow the antiapoptotic effect of Hsp60 to fully unfold. In this context it would be of crucial interest to elute and identify HLA-E peptide pools from cells infected with either AD169wt or AD169ΔUL40. According to our hypothesis, HLA-E of wt infected cells would mainly contain classical leader sequences whose origin could be either cellular or viral, both of which would inhibit NK cells via CD94/NKG2A. In contrast, HLA-E eluates from cells infected with the deletion mutant would contain hsp60-derived sequences which could account for the increased NK cell susceptibility in situations where killing is controlled mainly by CD94/NKG2A-HLA-E interactions. Unfortunately, given the low expression levels of HLA-E on HCMV-permissive cells, such an approach would face major logistical challenges in regard to cell numbers and amounts of virus that would be required. Transfected cell lines would presumably not be a promising approach either, since the induction of heatshock proteins depends on productive infection. Transfection of the UL40 gene into K562 cells of fibroblasts did not lead to elevated Hsp60 levels (unpublished data). An alternative for the detection of Hsp60 sequences on HLA-E molecules of AD169ΔUL40-infected cells could be the generation of HLA-E restricted T cells, specific for Hsp60.

Cytoprotection by Hsp60 and other members of the heatshock family has been described before but the literature is very ambiguous and it seems to be highly dependent on the choice of experimental system whether Hsp60 exerts and pro- or, like in our study, anti-apoptotic effects (312-318). Although it does not affect the interpretation of our in vitro experiments, it should be noted that immunoregulatory roles have been assigned to Hsp60 as well (319), implying that the induction of cellular Hsps could have other more indirect effects for the immune response during HCMV infection in vivo. Other examples for the exploitation of cellular heat shock responses by viruses are the findings that Hepatitis B replication depends on Hsp60 (320) or that HSV-2 encodes Hsp homologs (321, 322) and additionally induces Hsp70 (323), which had earlier also been reported for Adenoviruses and SV40 (324)

Other anti-apoptotic genes have been characterized in HCMV previously, such as UL36, UL37 and UL38 (325-327). UL38 was recently (328) found to prevent apoptosis by negatively regulating the tuberous sclerosis protein complex (TSC2, also known as tuberin) that is a component of the cellular stress reaction regulating growth via the mTOR pathway (reviewed in (329)). In MCMV the gene m41 encodes an anti-apoptotic golgi-resident protein that extends the lifespan of the infected cell (330). Although there are no sequence homologies, it is interesting that the cluster in which m41 is located in the MCMV genome, corresponds to the region that contains UL37, UL41 and UL40 in HCMV.

3.4 UL16 – INTERFERENCE WITH AN ACTIVATING NK CELL PATHWAY

When I started to work on my PhD, very little was known about the role of activating NK cell receptors in infections, with exception of early reports involving the $F_c\gamma$ receptor, mediating ADCC. In 2001 several significant findings had finally accelerated progress in this area. The importance of Ly49H for clearance of MCMV in certain mouse strains had started to become clear and drew a lot of attention. Likewise, the identification of murine NKG2D ligands Rae-1 (331) and H60 (111, 332) increased interest in this pathway, first in the context of tumors but soon also in regard to their role in infection, particularly in MCMV.

3.4.1 Identification of UL16

Groh and coworkers found that the human NKG2D ligand MIC, identified together with its receptor two years earlier (95, 96), was induced by HCMV infection of fibroblasts (89). Cosman and coworkers set out to identify cellular binding partners for the HCMV protein UL16, originally described in 1992 but with so far unknown function (116). They followed an approach that had already resulted in the successful identification of LIR-1 as the receptor for UL18 (45). Cells that bound a UL16-F_c fusion protein were used for expression cloning of their respective cDNA libraries and eventually revealed MICB as well as a novel protein, named UL16 binding protein 1 (ULBP1) and 2 (ULBP2) as cellular targets for UL16. Neither the related molecules ULBP3 and ULBP4 nor MICA interacted with UL16 (45, 115).

The generation of ULBP fusion proteins allowed to characterize NKG2D as the cellular receptor for this new class of GPI-linked cell surface molecules. Ligation of all three ULBPs to the NKG2D/DAP10 receptor complex induced cytokine and chemokine release by NK cells (45, 99) and also triggered cytotoxicity against MHC class I^+ Daudi cells, together with one earlier report (333), providing one of the first examples that activating signals can override the presence of inhibitory MHC class I. The demonstration that a HCMV-derived protein could bind NKG2D ligands obviously implied the possibility of immune evasion from NKG2D bearing immune cells. The first study addressing the mode of action of UL16 found that in C1R-MICB transfectants MICB was retained by physical interaction with UL16 in the Golgi compartment (334). This retention was almost exclusively mediated by a YQRL motif in the transmembrane domain of UL16 since the mutation of this sequence restored cell surface expression. As clear and convincing as the results in overexpressing systems were, studies of UL16 in a productive HCMV infection were required to conclusively establish a role for of this viral glycoprotein in immune evasion.

3.4.2 The role of UL16 in productive HCMV infection

A report by Welte et al. showed that infection of MRC-5 fibroblasts with AD169 induced mRNA for all NKG2D ligands within12 hours post infection (335). Although

mRNA levels of NKG2D ligands do not necessarily correspond to protein expression (45), cell surface stainings demonstrated upregulation of ULBP3 and MICA but downregulation of ULBP2 that was weakly expressed by uninfected cells. Transfection of UL16 into 293T cells, also constitutively expressing NKG2D ligands, led to intracellular retention and reduction of cell surface levels of ULBP1, ULBP2 and MICB but not of ULBP3 and MICA (335). In this system, NKL killing of UL16-transfected cells was virtually abrogated, in spite of residual MICA and ULBP3. Another report observed decreased staining of an NKG2D-F_c fusion protein on fibroblasts in the presence of UL16 (336).

In our own study (**paper I**) we could show that HCMV infection led to upregulation of all three ULBPs in human lung (HL) and MICA in human foreskin (HF) fibroblasts. However, the upregulation of ULBP1 and ULBP2 was delayed in the presence of UL16, particularly in four clinical isolates of HCMV. The delay in ULBP upregulation could be overcome by infection with AD169 at higher multiplicities of infection. Comparing HL fibroblasts infected either with AD169wt or with a mutant deficient in UL16 (AD169ΔUL16), we observed that the increased levels of NKG2D ligands on AD169ΔUL16 infected cells translated into higher susceptibility to NKG2D mediated NK cell responses. Preincubation of the effector cells with a blocking antibody against NKG2D reduced killing of the infected targets to comparable levels. This was the first time, that the targeting of an NK cell activating pathway by a viral immunoevasin has been demonstrated during a productive infection.

While our report was under review, another article also relying on a U16 deletion mutant, was published and proposed an entirely different mode of action for UL16 (337). While confirming a protective role of UL16 in HL fibroblasts, the data suggested a general resistance to recombinant lytic granules, such as granzyme B, perforin, porcine NK lysin or streptolysin O. While the two mechanisms might not be mutually exclusive, one may question whether the exposure of cells to lytic granules represents a relevant experimental system, given that these proteins are usually released in very confined, highly organized areas such as the immunological synapse, where local concentrations might differ significantly from the ones used by Odeberg et al..

3.4.3 The molecular mode of action of UL16

With the protective role of UL16 in productive HCMV infection firmly established, subsequent studies focused on the molecular mechanisms of intracellular NKG2D ligand retention, that had been observed previously (335, 338). Domain swapping experiments replacing either the transmembrane and cytoplasmic or the extracellular part of UL16 with corresponding parts of the mouse IL-4 receptor revealed that in EL-4 ULBP2 transfectants, the transmembrane and cytoplasmic portion of UL16 contributes to the intracellular retention of UL16 itself. The extracellular part participates in downmodulation of cell surface ULBP2 (339). The role of the transmembrane and cytoplasmic parts of UL16 as well as its intracellular localization became also the focus of two later studies that contributed incrementally to our understanding of UL16

biology (340, 341), but mainly reproduced previous studies. Noteworthy, they suggested that UL16 might traffic to the inner nuclear membrane in 293T transfectants, although it it is possible that this represents an artefact of overexpression.

A study with a similar methodological approach as Dunn et al. investigated an intriguing aspect of UL16 binding to NKG2D ligands: MICB and ULBP1/2 only share 23-27% of sequence homology, while MICB is very closely related to MICA (85% identiy), which is however not bound by UL16. The authors concluded that the alpha2-domain was the crucial determinant for the discrimination between MICB an MICA (342). By site-directed mutagenesis Spreu and coworkers identified residues in the second alpha-helix of MICB, presumably involved in direct interaction with UL16 whereas residues in the first alpha-helix contributed more indirectly, as their sidechains were not solvent exposed. Interestingly, the residues that had a strong impact on binding, were often found at corresponding positions in ULBP1 and ULBP2 but not ULBP3.

However, there were indications that MICA was not beyond the reach of HCMV immunoevasins either. A report by Zou et al. observed downregulation of MICA during productive infection of the astrocytoma cell line U373 but confirmed earlier reports describing an upregulation of MICA in fibroblast lines (343). The discrepancy was due to different MICA alleles, since the fibroblasts expressed a truncated version (*008) which was resistant to downmodulation by an unknown viral gene other than UL1-UL20.

In the same year a new MHC class I like homolog, UL142, was described and shown to inhibit some but not all polyclonal NK cell cultures tested (280). Out of one hundred individual NK cell clones derived from five donors, 23 were inhibited by UL142 expression alone. The authors speculated that UL142 could either engage inhibitory receptors, reminiscent of how UL18 is supposed to contribute to immune evasion, or interfere with NK cell activating ligands induced by infection. Support for the second alternative came in form of a report that showed downmodulation of MICA in EL-4 transfectants by a lentiviral vector construct encoding UL142, which also reduced susceptibility to polyclonal NK cells (344). However, it is not possible to connect the earlier study on MICA downmodulation in U373 with the descriptions of UL142, since Zou et al. used AD169 which is known to lack the UL142 gene. This in turn implies that clinical isolates or strains like Toledo and TB40/E must contain at least two immunoevasins targeting MICA. This, in concert with UL16, allows HCMV to target almost all known NKG2D ligands, in spite of their diversity. The remaining ULBP3 which has not been described in the context of immune evasion has the lowest affinity for NKG2D and HCMV might be able to afford not to devote genetic material to this ligand.

3.4.4 Viral microRNAs (miRNAs) interfering with host functions

The most recent addition to the immunoevasive arsenal of HCMV was the description of a virally encoded microRNA, miRNA-UL112, that binds 3' untranslated region of MICB mRNA and inhibits translation of the protein (345). This was only the second study demonstrating a direct manipulation of host genes by viral miRNAs, a fascinating concept that had been introduced half a year earlier for Herpes-Simplex Virus (346) where a miRNA exerted an antiapototic function. Together, these two reports are pointing towards a conceptually novel field of virus-host interactions and in combination with the impressive coding potential of herpesvirus genomes will probably become a focus of intense interest in years to come. The synergy of microRNA-based and protein-mediated regulation, e.g. miRNA112 and UL16 for MICB, might render the immune evasion mechanism significantly more fail proof in regard to countermeasures by the host.

3.5 HERPES SIMPLEX VIRUS AND NK CELL RECOGNITION

Prompted by the case report by Biron (138), in vitro studies started to address the question if and how NK cell recognize HSV-infected cells but yielded varying results. While some concluded that HSV inhibits the lysis of infected cells by LAK cells and that this inhibition was dependent on N-linked protein glycosylation (347), others reported an increased susceptibility of HSV-infected cells which was dependent on the presence of MHC class I molecules. Strangely enough, MHC levels were shown not to be different between uninfected and infected cells (348), so the role of MHC class I for triggering cytotoxicity remains unclear in this system. More recent studies describe an IL-15 induced enhanced NK cell activity after HSV infection (349) which is consistent with our own data on NKG2D upregulation during the acute phase of infection (**paper III**), confirming reports from the early days of NK cell research (171, 350, 351). Since HSV is not as host restricted as HCMV, the infection has been studied extensively in mouse models, although a review of the extensive corresponding literature is beyond the scope of this thesis.

When we studied HSV-1 infection (**paper III**) in regard to NK cell recognition of infected cells, we observed a loss of NKG2D tetramer binding after infection of HeLa cells and subsequent analysis of individual NKG2D ligands showed decreased MICA levels on the cell surface, a finding that could have different explanations. We considered MICA shedding, known from tumor cells, or epitope masking by a viral protein unlikely, given that overall amounts of cellular MICA remained unchanged and the downmodulation was observed with three different antibodies. Our results are most easily explained by intracellular retention or increased internalization, although formal proof is lacking at this stage. An interesting aspect that sets this HSV-1 evasion mechanism apart from UL142 in HCMV is that it is apparently also efficient against the truncated form of MICA, since HeLa cells express the *008 allele of MICA.

Although HSV-1 has successfully adapted to this common MICA allele, it was somewhat surprising that the killing of infected HeLa cells became more dependent on NKG2D, in spite of reduced ligand levels. At least two major explanations seemed conceivable: either the downmodulation of MHC class I upon infection increases the relative importance and possible impact of residual MICA, or any other activating ligand for that matter, on NK cell activation. Alternatively, another non-NKG2D ligand that plays a dominant role for the killing of uninfected HeLa cells by NK cells might be downregulated to an even greater extent compared to MICA. HeLa cell killing has previously been shown to partially depend on the newly identified NKp30 ligand BAT-3 (84) and experiments addressing the fate of this molecule during HSV infection are ongoing. Our work on HSV-1 extends the concept of NKG2D as a crucial receptor system the for recognition of infected cells to a member of the alpha-herpesviruses. But most of all it exemplifies the complexity of systems were a polyclonal NK cell population encounters a productive infection. The strength of this approach, that is probably more likely to mirror a physiological situation, is at the same time its' greatest weakness, since it is difficult to impossible to account for all relevant interactions between NK cells and their targets.

A recent report contrasting sharply with our data was recently presented by Chisholm et al. (352). The authors described an increased susceptibility of infected fibroblasts to NK cells and this increase was entirely caused by the induction of one or more NCR ligands of presumably cellular origin by the viral gene ICP0. While we used HeLa and U373 cells instead of fibroblasts, we could neither see a consistent increase of NK cell killing against infected cells, nor did we observe an involvement of ICP0, when comparing a HSV-1wt with a ΔICP0 mutant. However, in accordance with Chisholm et al, we found that ICP47-mediated downmodulation of MHC class I is not a determining factor for NK cell recognition of infected cells.

3.6 THE NKG2D RECEPTOR LIGAND SYSTEM IN OTHER VIRAL INFECTIONS

In Kaposi's sarcoma-associated herpesvirus (KSHV) the protein K5, one of the two gene products known to downmodulate MHC class I, was demonstrated to have a dual function by also targeting the NKG2D ligands MICA and MICB as well as AICL, the ligand for the activating receptor NKp80 (260). Although K5 leads to decreased surface expression of both molecules, they have different intracellular fates: while AICL is bound for endolysosomal degradation, MICA is ubiquitinylated and redistributed to an intracellular compartment, although the *008 allele is resistant to this mechanism. The downmodulation of activating ligands MICA and AICL rendered C1R or U937 cells resistant to primary NK cells from three different donors

The targeting of NKG2D ligands is not limited to herpesviruses but instead a reoccurring theme, particularly in viral infections, that could potentially be controlled by NK cells due to MHC class I downmodulation on the infected cell.

A very recent example is Adenovirus 5 that inhibits posttranslational maturation of MICA and MICB, including the *008 allele (181). The viral protein E3/19K mediating this effect colocalizes with MIC molecules in the ER although there is no proof yet for a direct interaction. Infection of fibroblasts with a mutant deficient for E3/19K led to elevated MICA/B levels and increased NK cells degranulation compared with cells, infected with the wild type.

The killing of HIV infected primary T cells by NK cells does not only involve NKp44, as described earlier, but is in part also dependent on NKG2D (353). The upregulation of NKG2D ligands in infected cells though seems to be limited by the HIV protein Nef. Nef has, along with other HIV proteins, been known to downregulate certain MHC class I alleles for some years, but it only became appreciated recently that it exerts an additional role by targeting NKG2D ligands (354) – a striking functional analogy to KSHV K5 or MCMV gp40 that both downmodulate MHC class I as well as NKG2D ligands.

3.7 OTHER NK CELL ACTIVATING PATHWAYS TARGETED BY HCMV INFECTION

3.7.1 DNAM-1/CD226

Activating pathways, other than NKG2D, have become the target of HCMV immune evasion mechanisms as well. In an elegant study, Tomasec and coworkers set out to identify the reasons for more efficient protection of infected cells conferred by low passage strains of HCMV, Toledo or TB40/E compared to laboratory strains, AD169 and Towne (273). During a screen of ORFs found in the so called UL/b'region that is lacking in AD169, resistance to a broad range of different NK cell effectors mapped to the gene UL141. Transfection of the UL141 gene into different cell lines ultimately revealed CD155 (also called poliovirus receptor or nectin-like molecule 5), a ligand for the NK cell activating receptor DNAM-1 (also CD226) as the molecular target for UL141. Biochemical experiments demonstrated that UL141 prevents maturation of CD155 and retains it in the ER. Although this bears superficial resemblance to the mode of action of UL16, there is no sequence similarity between the two molecules. An important difference is also that UL16 binds to stress-induced ligands whereas the ligand for UL141 is constitutively expressed on many healthy cells.

3.7.2 NKp30

A different study observed binding of an NKp30-F_c fusion protein to HCMV infected cells and immunoprecipitated the well characterized HCMV protein pp65, encoded by the ORF UL83 and known already for its involvement in immunomodulation from earlier work (225, 355-357). In a cell-free system direct interaction between NKp30 and pp65 was demonstrated and cells infected with a pp65 deletion mutant were more

susceptible to NKp30 mediated NK cell lysis than cells infected with a wild type (83). The preincubation of NK cells with pp65 but not with control proteins led to a general reduction of NKp30-dependent killing against a variety of different targets, including fibroblasts and immature dendritic cells and the authors suggested that the dissociation of the CD3ζ chain from the NKp30 receptor accounts for the decrease in NK cell activation. The downregulation of the CD3ζ chain and a consecutive functional impairment has also been reported in T cells isolated from individuals with cancer, chronic infections and autoimmune diseases (reviewed in (358)).

3.8 EPSTEIN-BARR VIRUS AND NK CELL RECOGNITION

After investigating HCMV and HSV-1 as representative examples of the beta- and alpha-herpesviruses, respectively, we were interested in characterizing the NK recognition of cells infected with EBV, a gamma-herpesvirus. Like in the other two families, the phenomenon of MHC class I downmodulation during lytic replication in EBV has been known for several years. Early reports suggested that cells latently infected with EBV, become more susceptible to NK cells, when the virus switches to lytic replication, triggered by chemical inducers such as *n*-butyrate. However, those studies were limited by the fact that bulk populations were used for all experiments, with only a fraction of cells switching into lytic replication of EBV (359).

In an experimental system that allowed for the clear separation of cells that switched into lytic replication, we found that for EBV the activating NK cell receptor NKp46 plays a dominant role, since blocking this molecule abrogated killing almost entirely (unpublished data). In contrast, blocking other NK cell receptors, such as NKG2D, 2B4, LIR-1 or CD94 had no or only marginal effects on target cell susceptibility. The finding that NKp46 is the major activating receptor for the recognition of Akata cells by NK cells is in sharp contrast to a recent study by Pappworth et al. (133) (and our own studies on HCMV and HSV), where recognition was clearly governed by NKG2D, yet only for the lytic population. One possible explanation is the use of different NK cell populations: while we used polyclonal NK cell lines, grown in IL-2, Pappworth et al.used either IFN-α activated NK cells or the NK cell lines NKL and DEL. While to our knowledge there are no fundamental differences between those effector populations in terms of NKG2D and NKp46 expression levels, their response to a particular target cell might be different nevertheless. It is an interesting thought that NK cells might respond differently to the same target, depending on the cytokine environment they were activated in.

Another possible explanation is that the Akata cells used in our study might represent an Akata subline, which displays high(er) levels of the yet unidentified NKp46 ligand. This could also explain the differences in regard to the levels of NKG2D ligands. While Pappworth et al. describe expression of ULBP1 and its' upregulation upon lytic replication of EBV, we could not detect ULBP1 in neither latent nor lytic cells. Instead we observed expression of ULBP3 and MICA on Akata cells which were both partially downmodulated when cells entered lytic replication. On a technical sidenote, different

antibodies were used for detection of NKG2D ligands in our study compared with Pappworth et al. which might possibly account for discrepancies in terms of NKG2D ligand cell surface stainings.

The differences of NK cell receptor usage in fairly similar systems emphasize the complexity of the interactions between NK cells and their target cells. The possibility to flexibly use different activating receptors against very similar targets might represent an evolutionary advantage: if one activating pathway is targeted by viral immune evasion, another one might be able compensate and allow for efficient NK cell activation anyhow.

3.9 EVOLUTIONARY ASPECTS

There is ample evidence for a long co-evolution of the human immune system with members of the herpesvirus family. The establishment of relatively peaceful, life-long latency that only rarely gets disturbed by phases of reactivation, indicates that herpesviruses and their host have reached a finely balanced equilibrium that minimizes damage to either side. Along this line of thought, an exciting report by Barton et al. (360) suggests that in mice latent herpesviruses, such as MCMV or murine gammaherpesvirus 68 even confer a competitive advantage for fighting off Listeria monocytogenes or Yersinia pestis infection. The effect was dependent on prolonged IFN-γ secretion and consecutive systemic activation of macrophages, although the mechanism of how latent infection translates into this general increase in higher innate immune activity remains elusive. However, if this report is confirmed in other species and extended to further pathogens, it might fundamentally change our view of herpesviruses from purely unwelcome pathogens to welcome symbionts.

The NKG2D receptor ligand system is maybe the most suitable example to beautifully illustrate the concept of co-evolution. While the receptor itself is highly conserved across species borders it is generally accepted that the main driving force for the remarkable ligand diversity is selective pressure by pathogens. I have already mentioned the example of m157 in MCMV infection where in vivo infection of the resistant B6 strain led to the rapid acquisition of mutations in m157 (247, 248) which allowed escape from Ly49H^{+} NK cells exerting the selective pressure. Targeting of NKG2D ligands by viral immuno evasins is usually limited to some but not all ligands, possibly indicating that the host responded to evasion mechanisms by gene duplications in order to restore adequate NKG2D mediated immune responses. In support of this view, it has been reported that the diversification of ULBPs was an evolutionary recent event (reviewed in (361)) which in other species gave rise to even more variants.

Polymorphisms are another advantage in favour of the immune system as they can carry certain host proteins beyond the reach of a given viral gene product. It is known that the MCMV proteins interfering with the MHC class I pathway bind some alleles worse than others (362) and for NKG2D ligands the truncated MICA allele *008, interestingly very common in human populations, is resistant to downmodulation by HCMV (343, 344).

4 CONCLUDING REMARKS

During my PhD studies I had the privilege to be part of an exceptionally dynamic field of research: the role of innate immunity in host defense. I started to study NK cells, a central component of the innate immune system at a timepoint when the knowledge regarding their delicate control by multiple receptors and ligands had reached a critical mass that allowed for a molecular dissection of NK cell target interactions. During the last five years an ever increasing number of reports investigated NK cells during the immune response against a broad repertoire of viruses, bacteria and parasites, resulting in major leaps for our molecular understanding of the host-pathogen relationship.

I chose to try and contribute to this development by studying NK cells in the context of different Herpesviruses. Herpesvirus infections usually manifest themselves as severe clinical problems only in comparably rare situations and could thus be considered of peripheral interest, given that the toll other infectious diseases, such as malaria, tuberculosis, HIV or HCV take on human societies is of a completely different scale. But nothing could be more wrong. Herpesviruses allowed for the generation of excellent model systems that are used to advance our understanding of immune recognition on a fundamental level. Their large genomes serve as a seemingly endless source of sophisticated proteins that interact with cellular counterparts and that teach us a lot not only about the viruses but also about their hosts - about the human body itself.

Human and murine cytomegalovirus were arguably one of the instrumental fields for the initial appreciation of the central role of NKG2D in host defense. Our study was amongst the first to show that this conserved receptor with its broad ligand specificity played a role in the NK cell response against a virally infected cell and subsequent studies, some of them using very powerful experimental systems, quickly widened the scope to other infections or tumor research. Within less than 10 years, this conserved receptor with its broad ligand specificity became recognized as one of the key molecules in the immune system and it was most instructive to follow and be part of this development. It remains to be seen whether UL40 will be known solely as a viral provider of HLA-E binding peptides or if our study took the first steps towards revealing another function of this molecule, relating to heat shock proteins and cellular stress. Although our study has provided some clues to explain the high affinity between UL18 and LIR-1, UL18 was and remains a mystery since its role in productive infection continues to be far from understood.

The choice to study NK cells in the context of a productive infection comes with many drawbacks. Expression levels of the molecules of interest are low, the variability is higher, conditions are often harder to reproduce and the results are inevitably more complex and difficult to interpret than “clean”, reductionistic systems. But eventually, this is what biology, what nature is all about – complex answers to simple questions.

In regard to complexity, I am convinced that the future will see more studies in the tradition of the pioneering study by Barton and coworkers, that I referred to in my brief remarks on evolution: the influence that different infections of all kinds have on each other in a complex host organism will certainly yield exciting and unexpected results. This applies not less to the emerging field of viral microRNA that, particularly in the context of herpesviruses reveals an entirely new level of regulation.

5 ACKNOWLEDGEMENTS

I always looked forward to write this part of my thesis. Not only because I knew that this section would be thoroughly read, but also because it is a unique opportunity to explicitely acknowledge all the people who have, in one way or another, contributed to this work. In particular I would like to thank:

Klas Kärre, who inspired me to study immunology, when I was an undergraduate exchange student. If I would have to choose only one personal and one scientific quality of yours that I admire and appreciate the most, the choice would be easy: firstly, your modesty and unpretentiousness which is a pleasure to experience. Secondly, your open mind to whichever experimental result comes your way. Unexpected, counterintuitive, complicated, even negative results are equally welcome and embraced as a part of biology and I sincerely respect that attitude towards science.

Cristina Cerboni, initially my main-supervisor – you were quite simply the best start one could possibly have into PhD studies. Enthusiastic, ambitious, empathic, dedicated to science and to life in general. I enjoyed every week that I worked with you as well as my two visits in Rome.

Adnane Achour and Claudia Wagner. Thank you for letting me join your UL18 project while I had a difficult time. The short and intense collaboration helped me get back on track and I am really grateful for that.

Louise Berg. Even though you never got formal credit for it, you were my inofficial co-supervisor and perhaps the person I most often discussed science and KI politics with – thank you!
Viktor Levitsky, my mentor, that I also knew from exchange student times. I miss our conversations about science, politics, history and everything else. Your personality was an enrichment for the IRIS center and the corridor and I hope our paths will cross again.
André Ortlieb – I finally did beat you in the running for "longest PhD studies ever" I very much look forward to see you back in Stockholm and revive our collaboration.
Danika Schepis for being a great office mate, collaborator and friend, that could always relate so easily to my thoughts and emotions. I am very happy that we shared and share an office and project.
The other colleagues in the office: **Michael Uhlin**-Zinkernagel, president Micke Melon, for your relaxed low-profile attitude and for being the living proof that following lab protocols is a waste of time. I will apply for postdoc in your lab after you become appointed professor in Borlänge**. Mantas Oka**s for spreading hectic happiness.
Jonas Sundbäck for all your help with small things over the years and in the last minutes.
Hanna Sjölin for making the start in the group so fun and enjoyable and for your support and encouragement during the final phase of my PhD.

All other current members of the lab in alphabetical order: **Hanna Brauner, Petter Brodin, Jens Gertow, Petter Höglund, Maria Johansson, Sofia Johansson, Susanne Johansson, Björn Önfeldt, Lakshmikanth Tadepally, Stina Wiklund.** Former colleagues: **Gustaf Vahle** for a great Keystone Meeting and one of my fondest memories during my time here: our sailing trip on the Swedish westcoast. **Katja Andersson** and **Marjet Elemans**, both for excellent travel company on Hawaii; **Michael Eriksson, Håkan Hall, Birgit Manno, Christina Matos, Jakob Michaelsson, Linda Öberg, Jelena Petrovic, Eleftheria Rosmaraki, Anna Sjöström-Douagi, Rutger van der Holst.** The members of the **Adnane Achour** and **Laszlo Szekely group,** especially **Emilie Flaberg.**

People outside the lab: **Anton Razuvaev** and your entire family for always making me feel so warmly welcome. For your subtle humor, your refreshing view on things and for keeping me rooted to culture and fine arts. **Bruno Vanherberghen** for sharing many aspects of life and work and together with **Melissa Norström** for taking care of the nooblet. **Magdalena and David Källström** for being friends for many years. **Manuela Hase** for taking the initiative to badminton matches, dinners, picknicks, exhibitions and insightful advice on many things. **Charlotta Nordkvist** for the good times we had together. **Benedict Chambers** for introducing me to the ways of the sword - fencing became one of the most constant sources of joy, pleasure and distraction. **Jorrit Boekel** for introducing me to the concept of fixed-gear bikes and illegal cycling races. **Ilaria Tassi** for being a great friend and a shining role model for how to make ones way in science.

The people remaining my link to Germany: the Marburg university crowd for keeping in touch through all these years and for a legendary visit, **Markus Grube, Marcus Hartmann, Wolfgang Jacob, Gunnar Kaufmann. Hauke Menning, Volker Helmert, Susanne Ebner, Kai Möller, Christian Tennie**, **Stephanie Loges and Christina Westhoff** for representing the bonds back home.

My sister, **Patricia Rölle** for just being an incredibly positive, easy-going person with an innate sense for problem-solving. My parents, **Margrit and Gerhard Rölle** for encouraging and supporting every single interest I developed when I grew up, amongst them the early wish "to become a natural scientist".

Åsa Hidmark for persistence, friendship and love. For being who you are and having become a part of my life. And for sharing the adventure of being parents to wonderful little Tristan.

Tristan, it will take some years until you might eventually and accidentally read these acknowledgements. You proved me wrong on almost all accounts and most importantly, you remind me constantly of what science should be all about: a fun, curious exploration of the world around us.

6 REFERENCES

1. O'Leary, J.G., M. Goodarzi, D.L. Drayton, and U.H. von Andrian. 2006. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. *Nature immunology* 7:507-516.
2. Martin-Fontecha, A., L.L. Thomsen, S. Brett, C. Gerard, M. Lipp, A. Lanzavecchia, and F. Sallusto. 2004. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nature immunology* 5:1260-1265.
3. Morandi, B., G. Bougras, W.A. Muller, G. Ferlazzo, and C. Munz. 2006. NK cells of human secondary lymphoid tissues enhance T cell polarization via IFN-gamma secretion. *European journal of immunology* 36:2394-2400.
4. Herberman, R.B., M.E. Nunn, H.T. Holden, and D.H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. II. Characterization of effector cells. *Int J Cancer* 16:230-239.
5. Kiessling, R., E. Klein, H. Pross, and H. Wigzell. 1975. "Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell. *European journal of immunology* 5:117-121.
6. Kiessling, R., E. Klein, and H. Wigzell. 1975. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. *European journal of immunology* 5:112-117.
7. Karre, K., G.O. Klein, R. Kiessling, G. Klein, and J.C. Roder. 1980. Low natural in vivo resistance to syngeneic leukaemias in natural killer-deficient mice. *Nature* 284:624-626.
8. Gidlund, M., A. Orn, H. Wigzell, A. Senik, and I. Gresser. 1978. Enhanced NK cell activity in mice injected with interferon and interferon inducers. *Nature* 273:759-761.
9. Stern, P., M. Gidlund, A. Orn, and H. Wigzell. 1980. Natural killer cells mediate lysis of embryonal carcinoma cells lacking MHC. *Nature* 285:341-342.
10. Ljunggren, H.G., and K. Karre. 1990. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today* 11:237-244.
11. Freud, A.G., and M.A. Caligiuri. 2006. Human natural killer cell development. *Immunological reviews* 214:56-72.
12. Anfossi, N., P. Andre, S. Guia, C.S. Falk, S. Roetynck, C.A. Stewart, V. Breso, C. Frassati, D. Reviron, D. Middleton, F. Romagne, S. Ugolini, and E. Vivier. 2006. Human NK cell education by inhibitory receptors for MHC class I. *Immunity* 25:331-342.
13. Lanier, L.L., A.M. Le, C.I. Civin, M.R. Loken, and J.H. Phillips. 1986. The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. *J Immunol* 136:4480-4486.
14. Walzer, T., S. Jaeger, J. Chaix, and E. Vivier. 2007. Natural killer cells: from CD3(-)NKp46(+) to post-genomics meta-analyses. *Current opinion in immunology* 19:365-372.
15. Fehniger, T.A., M.A. Cooper, G.J. Nuovo, M. Cella, F. Facchetti, M. Colonna, and M.A. Caligiuri. 2003. CD56bright natural killer cells are present in human lymph nodes and are activated by T cell-derived IL-2: a potential new link between adaptive and innate immunity. *Blood* 101:3052-3057.
16. Caligiuri, M.A., A. Zmuidzinas, T.J. Manley, H. Levine, K.A. Smith, and J. Ritz. 1990. Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors. *The Journal of experimental medicine* 171:1509-1526.

17. Campbell, J.J., S. Qin, D. Unutmaz, D. Soler, K.E. Murphy, M.R. Hodge, L. Wu, and E.C. Butcher. 2001. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. *J Immunol* 166:6477-6482.
18. Frey, M., N.B. Packianathan, T.A. Fehniger, M.E. Ross, W.C. Wang, C.C. Stewart, M.A. Caligiuri, and S.S. Evans. 1998. Differential expression and function of L-selectin on CD56bright and CD56dim natural killer cell subsets. *J Immunol* 161:400-408.
19. Romagnani, C., K. Juelke, M. Falco, B. Morandi, A. D'Agostino, R. Costa, G. Ratto, G. Forte, P. Carrega, G. Lui, R. Conte, T. Strowig, A. Moretta, C. Munz, A. Thiel, L. Moretta, and G. Ferlazzo. 2007. CD56brightCD16- killer Ig-like receptor- NK cells display longer telomeres and acquire features of CD56dim NK cells upon activation. *J Immunol* 178:4947-4955.
20. Tabiasco, J., M. Rabot, M. Aguerre-Girr, H. El Costa, A. Berrebi, O. Parant, G. Laskarin, K. Juretic, A. Bensussan, D. Rukavina, and P. Le Bouteiller. 2006. Human decidual NK cells: unique phenotype and functional properties -- a review. *Placenta* 27 Suppl A:S34-39.
21. Santoni, A., C. Carlino, and A. Gismondi. 2008. Uterine NK cell development, migration and function. *Reprod Biomed Online* 16:202-210.
22. Santoni, A., A. Zingoni, C. Cerboni, and A. Gismondi. 2007. Natural killer (NK) cells from killers to regulators: distinct features between peripheral blood and decidual NK cells. *Am J Reprod Immunol* 58:280-288.
23. 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* 5:405-411.
24. Osada, T., H. Nagawa, J. Kitayama, N.H. Tsuno, S. Ishihara, M. Takamizawa, and Y. Shibata. 2001. Peripheral blood dendritic cells, but not monocyte-derived dendritic cells, can augment human NK cell function. *Cell Immunol* 213:14-23.
25. Yu, Y., M. Hagihara, K. Ando, B. Gansuvd, H. Matsuzawa, T. Tsuchiya, Y. Ueda, H. Inoue, T. Hotta, and S. Kato. 2001. Enhancement of human cord blood CD34+ cell-derived NK cell cytotoxicity by dendritic cells. *J Immunol* 166:1590-1600.
26. Gerosa, F., B. Baldani-Guerra, C. Nisii, V. Marchesini, G. Carra, and G. Trinchieri. 2002. Reciprocal activating interaction between natural killer cells and dendritic cells. *The Journal of experimental medicine* 195:327-333.
27. Gerosa, F., A. Gobbi, P. Zorzi, S. Burg, F. Briere, G. Carra, and G. Trinchieri. 2005. The reciprocal interaction of NK cells with plasmacytoid or myeloid dendritic cells profoundly affects innate resistance functions. *J Immunol* 174:727-734.
28. Piccioli, D., S. Sbrana, E. Melandri, and N.M. Valiante. 2002. Contact-dependent stimulation and inhibition of dendritic cells by natural killer cells. *The Journal of experimental medicine* 195:335-341.
29. Ferlazzo, G., M.L. Tsang, L. Moretta, G. Melioli, R.M. Steinman, and C. Munz. 2002. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *The Journal of experimental medicine* 195:343-351.
30. Della Chiesa, M., M. Vitale, S. Carlomagno, G. Ferlazzo, L. Moretta, and A. Moretta. 2003. The natural killer cell-mediated killing of autologous dendritic cells is confined to a cell subset expressing CD94/NKG2A, but lacking inhibitory killer Ig-like receptors. *European journal of immunology* 33:1657-1666.
31. Andrews, D.M., A.A. Scalzo, W.M. Yokoyama, M.J. Smyth, and M.A. Degli-Esposti. 2003. Functional interactions between dendritic cells and NK cells during viral infection. *Nature immunology* 4:175-181.
32. Ruggeri, L., M. Capanni, E. Urbani, K. Perruccio, W.D. Shlomchik, A. Tosti, S. Posati, D. Rogaia, F. Frassoni, F. Aversa, M.F. Martelli, and A. Velardi. 2002.

Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science (New York, N.Y* 295:2097-2100.
33. Davis, D.M. 2002. Assembly of the immunological synapse for T cells and NK cells. *Trends in immunology* 23:356-363.
34. Wulfing, C., B. Purtic, J. Klem, and J.D. Schatzle. 2003. Stepwise cytoskeletal polarization as a series of checkpoints in innate but not adaptive cytolytic killing. *Proceedings of the National Academy of Sciences of the United States of America* 100:7767-7772.
35. Carlin, L.M., K. Eleme, F.E. McCann, and D.M. Davis. 2001. Intercellular transfer and supramolecular organization of human leukocyte antigen C at inhibitory natural killer cell immune synapses. *The Journal of experimental medicine* 194:1507-1517.
36. Almeida, C.R., and D.M. Davis. 2006. Segregation of HLA-C from ICAM-1 at NK cell immune synapses is controlled by its cell surface density. *J Immunol* 177:6904-6910.
37. Vanherberghen, B., K. Andersson, L.M. Carlin, E.N. Nolte-'t Hoen, G.S. Williams, P. Hoglund, and D.M. Davis. 2004. Human and murine inhibitory natural killer cell receptors transfer from natural killer cells to target cells. *Proceedings of the National Academy of Sciences of the United States of America* 101:16873-16878.
38. McCann, F.E., P. Eissmann, B. Onfelt, R. Leung, and D.M. Davis. 2007. The activating NKG2D ligand MHC class I-related chain A transfers from target cells to NK cells in a manner that allows functional consequences. *J Immunol* 178:3418-3426.
39. Kopcow, H.D., D.S. Allan, X. Chen, B. Rybalov, M.M. Andzelm, B. Ge, and J.L. Strominger. 2005. Human decidual NK cells form immature activating synapses and are not cytotoxic. *Proceedings of the National Academy of Sciences of the United States of America* 102:15563-15568.
40. Lieberman, J. 2003. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. *Nature reviews* 3:361-370.
41. Screpanti, V., R.P. Wallin, A. Grandien, and H.G. Ljunggren. 2005. Impact of FASL-induced apoptosis in the elimination of tumor cells by NK cells. *Mol Immunol* 42:495-499.
42. Smyth, M.J., K. Takeda, Y. Hayakawa, J.J. Peschon, M.R. van den Brink, and H. Yagita. 2003. Nature's TRAIL--on a path to cancer immunotherapy. *Immunity* 18:1-6.
43. Lanier, L.L. 2008. Up on the tightrope: natural killer cell activation and inhibition. *Nature immunology* 9:495-502.
44. Parham, P. 2005. MHC class I molecules and KIRs in human history, health and survival. *Nature reviews* 5:201-214.
45. Cosman, D., N. Fanger, L. Borges, M. Kubin, W. Chin, L. Peterson, and M.L. Hsu. 1997. A novel immunoglobulin superfamily receptor for cellular and viral MHC class I molecules. *Immunity* 7:273-282.
46. Colonna, M., F. Navarro, T. Bellon, M. Llano, P. Garcia, J. Samaridis, L. Angman, M. Cella, and M. Lopez-Botet. 1997. A common inhibitory receptor for major histocompatibility complex class I molecules on human lymphoid and myelomonocytic cells. *The Journal of experimental medicine* 186:1809-1818.
47. Chapman, T.L., A.P. Heikeman, and P.J. Bjorkman. 1999. The inhibitory receptor LIR-1 uses a common binding interaction to recognize class I MHC molecules and the viral homolog UL18. *Immunity* 11:603-613.
48. Navarro, F., M. Llano, T. Bellon, M. Colonna, D.E. Geraghty, and M. Lopez-Botet. 1999. The ILT2(LIR1) and CD94/NKG2A NK cell receptors respectively recognize HLA-G1 and HLA-E molecules co-expressed on target cells. *European journal of immunology* 29:277-283.
49. Lepin, E.J., J.M. Bastin, D.S. Allan, G. Roncador, V.M. Braud, D.Y. Mason, P.A. van der Merwe, A.J. McMichael, J.I. Bell, S.H. Powis, and C.A. O'Callaghan. 2000. Functional characterization of HLA-F and binding of HLA-

F tetramers to ILT2 and ILT4 receptors. *European journal of immunology* 30:3552-3561.

50. Shiroishi, M., K. Tsumoto, K. Amano, Y. Shirakihara, M. Colonna, V.M. Braud, D.S. Allan, A. Makadzange, S. Rowland-Jones, B. Willcox, E.Y. Jones, P.A. van der Merwe, I. Kumagai, and K. Maenaka. 2003. Human inhibitory receptors Ig-like transcript 2 (ILT2) and ILT4 compete with CD8 for MHC class I binding and bind preferentially to HLA-G. *Proceedings of the National Academy of Sciences of the United States of America* 100:8856-8861.
51. Ravetch, J.V., and L.L. Lanier. 2000. Immune inhibitory receptors. *Science (New York, N.Y* 290:84-89.
52. Aramburu, J., M.A. Balboa, M. Izquierdo, and M. Lopez-Botet. 1991. A novel functional cell surface dimer (Kp43) expressed by natural killer cells and gamma/delta TCR+ T lymphocytes. II. Modulation of natural killer cytotoxicity by anti-Kp43 monoclonal antibody. *J Immunol* 147:714-721.
53. Aramburu, J., M.A. Balboa, A. Ramirez, A. Silva, A. Acevedo, F. Sanchez-Madrid, M.O. De Landazuri, and M. Lopez-Botet. 1990. A novel functional cell surface dimer (Kp43) expressed by natural killer cells and T cell receptor-gamma/delta+ T lymphocytes. I. Inhibition of the IL-2-dependent proliferation by anti-Kp43 monoclonal antibody. *J Immunol* 144:3238-3247.
54. Perez-Villar, J.J., I. Melero, A. Rodriguez, M. Carretero, J. Aramburu, S. Sivori, A.M. Orengo, A. Moretta, and M. Lopez-Botet. 1995. Functional ambivalence of the Kp43 (CD94) NK cell-associated surface antigen. *J Immunol* 154:5779-5788.
55. Phillips, J.H., C. Chang, J. Mattson, J.E. Gumperz, P. Parham, and L.L. Lanier. 1996. CD94 and a novel associated protein (94AP) form a NK cell receptor involved in the recognition of HLA-A, HLA-B, and HLA-C allotypes. *Immunity* 5:163-172.
56. Brooks, A.G., P.E. Posch, C.J. Scorzelli, F. Borrego, and J.E. Coligan. 1997. NKG2A complexed with CD94 defines a novel inhibitory natural killer cell receptor. *The Journal of experimental medicine* 185:795-800.
57. Cantoni, C., R. Biassoni, D. Pende, S. Sivori, L. Accame, L. Pareti, G. Semenzato, L. Moretta, A. Moretta, and C. Bottino. 1998. The activating form of CD94 receptor complex: CD94 covalently associates with the Kp39 protein that represents the product of the NKG2-C gene. *European journal of immunology* 28:327-338.
58. Carretero, M., C. Cantoni, T. Bellon, C. Bottino, R. Biassoni, A. Rodriguez, J.J. Perez-Villar, L. Moretta, A. Moretta, and M. Lopez-Botet. 1997. The CD94 and NKG2-A C-type lectins covalently assemble to form a natural killer cell inhibitory receptor for HLA class I molecules. *European journal of immunology* 27:563-567.
59. Lazetic, S., C. Chang, J.P. Houchins, L.L. Lanier, and J.H. Phillips. 1996. Human natural killer cell receptors involved in MHC class I recognition are disulfide-linked heterodimers of CD94 and NKG2 subunits. *J Immunol* 157:4741-4745.
60. Borrego, F., M. Ulbrecht, E.H. Weiss, J.E. Coligan, and A.G. Brooks. 1998. Recognition of human histocompatibility leukocyte antigen (HLA)-E complexed with HLA class I signal sequence-derived peptides by CD94/NKG2 confers protection from natural killer cell-mediated lysis. *The Journal of experimental medicine* 187:813-818.
61. Braud, V.M., D.S. Allan, C.A. O'Callaghan, K. Soderstrom, A. D'Andrea, G.S. Ogg, S. Lazetic, N.T. Young, J.I. Bell, J.H. Phillips, L.L. Lanier, and A.J. McMichael. 1998. HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* 391:795-799.
62. Vance, R.E., J.R. Kraft, J.D. Altman, P.E. Jensen, and D.H. Raulet. 1998. Mouse CD94/NKG2A is a natural killer cell receptor for the nonclassical major histocompatibility complex (MHC) class I molecule Qa-1(b). *The Journal of experimental medicine* 188:1841-1848.

63. Braud, V., E.Y. Jones, and A. McMichael. 1997. The human major histocompatibility complex class Ib molecule HLA-E binds signal sequence-derived peptides with primary anchor residues at positions 2 and 9. *European journal of immunology* 27:1164-1169.
64. Kaiser, B.K., J.C. Pizarro, J. Kerns, and R.K. Strong. 2008. Structural basis for NKG2A/CD94 recognition of HLA-E. *Proceedings of the National Academy of Sciences of the United States of America* 105:6696-6701.
65. Petrie, E.J., C.S. Clements, J. Lin, L.C. Sullivan, D. Johnson, T. Huyton, A. Heroux, H.L. Hoare, T. Beddoe, H.H. Reid, M.C. Wilce, A.G. Brooks, and J. Rossjohn. 2008. CD94-NKG2A recognition of human leukocyte antigen (HLA)-E bound to an HLA class I leader sequence. *The Journal of experimental medicine* 205:725-735.
66. Michaelsson, J., C. Teixeira de Matos, A. Achour, L.L. Lanier, K. Karre, and K. Soderstrom. 2002. A signal peptide derived from hsp60 binds HLA-E and interferes with CD94/NKG2A recognition. *The Journal of experimental medicine* 196:1403-1414.
67. Peruzzi, M., N. Wagtmann, and E.O. Long. 1996. A p70 killer cell inhibitory receptor specific for several HLA-B allotypes discriminates among peptides bound to HLA-B*2705. *The Journal of experimental medicine* 184:1585-1590.
68. Franksson, L., J. Sundback, A. Achour, J. Bernlind, R. Glas, and K. Karre. 1999. Peptide dependency and selectivity of the NK cell inhibitory receptor Ly-49C. *European journal of immunology* 29:2748-2758.
69. Hansasuta, P., T. Dong, H. Thananchai, M. Weekes, C. Willberg, H. Aldemir, S. Rowland-Jones, and V.M. Braud. 2004. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. *European journal of immunology* 34:1673-1679.
70. Thananchai, H., G. Gillespie, M.P. Martin, A. Bashirova, N. Yawata, M. Yawata, P. Easterbrook, D.W. McVicar, K. Maenaka, P. Parham, M. Carrington, T. Dong, and S. Rowland-Jones. 2007. Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. *J Immunol* 178:33-37.
71. Hoare, H.L., L.C. Sullivan, C.S. Clements, L.K. Ely, T. Beddoe, K.N. Henderson, J. Lin, H.H. Reid, A.G. Brooks, and J. Rossjohn. 2008. Subtle changes in peptide conformation profoundly affect recognition of the non-classical MHC class I molecule HLA-E by the CD94-NKG2 natural killer cell receptors. *Journal of molecular biology* 377:1297-1303.
72. Tassi, I., J. Klesney-Tait, and M. Colonna. 2006. Dissecting natural killer cell activation pathways through analysis of genetic mutations in human and mouse. *Immunological reviews* 214:92-105.
73. Bryceson, Y.T., M.E. March, H.G. Ljunggren, and E.O. Long. 2006. Activation, coactivation, and costimulation of resting human natural killer cells. *Immunological reviews* 214:73-91.
74. Pessino, A., S. Sivori, C. Bottino, A. Malaspina, L. Morelli, L. Moretta, R. Biassoni, and A. Moretta. 1998. Molecular cloning of NKp46: a novel member of the immunoglobulin superfamily involved in triggering of natural cytotoxicity. *The Journal of experimental medicine* 188:953-960.
75. Vitale, M., C. Bottino, S. Sivori, L. Sanseverino, R. Castriconi, E. Marcenaro, R. Augugliaro, L. Moretta, and A. Moretta. 1998. NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis. *The Journal of experimental medicine* 187:2065-2072.
76. Pende, D., S. Parolini, A. Pessino, S. Sivori, R. Augugliaro, L. Morelli, E. Marcenaro, L. Accame, A. Malaspina, R. Biassoni, C. Bottino, L. Moretta, and A. Moretta. 1999. Identification and molecular characterization of NKp30, a novel triggering receptor involved in natural cytotoxicity mediated by human natural killer cells. *The Journal of experimental medicine* 190:1505-1516.
77. Vitale, M., M. Falco, R. Castriconi, S. Parolini, R. Zambello, G. Semenzato, R. Biassoni, C. Bottino, L. Moretta, and A. Moretta. 2001. Identification of

NKp80, a novel triggering molecule expressed by human NK cells. *European journal of immunology* 31:233-242.

78. Mandelboim, O., N. Lieberman, M. Lev, L. Paul, T.I. Arnon, Y. Bushkin, D.M. Davis, J.L. Strominger, J.W. Yewdell, and A. Porgador. 2001. Recognition of haemagglutinins on virus-infected cells by NKp46 activates lysis by human NK cells. *Nature* 409:1055-1060.
79. Bloushtain, N., U. Qimron, A. Bar-Ilan, O. Hershkovitz, R. Gazit, E. Fima, M. Korc, I. Vlodavsky, N.V. Bovin, and A. Porgador. 2004. Membrane-associated heparan sulfate proteoglycans are involved in the recognition of cellular targets by NKp30 and NKp46. *J Immunol* 173:2392-2401.
80. Zilka, A., G. Landau, O. Hershkovitz, N. Bloushtain, A. Bar-Ilan, F. Benchetrit, E. Fima, T.H. van Kuppevelt, J.T. Gallagher, S. Elgavish, and A. Porgador. 2005. Characterization of the heparin/heparan sulfate binding site of the natural cytotoxicity receptor NKp46. *Biochemistry* 44:14477-14485.
81. Garg, A., P.F. Barnes, A. Porgador, S. Roy, S. Wu, J.S. Nanda, D.E. Griffith, W.M. Girard, N. Rawal, S. Shetty, and R. Vankayalapati. 2006. Vimentin expressed on Mycobacterium tuberculosis-infected human monocytes is involved in binding to the NKp46 receptor. *J Immunol* 177:6192-6198.
82. Warren, H.S., A.L. Jones, C. Freeman, J. Bettadapura, and C.R. Parish. 2005. Evidence that the cellular ligand for the human NK cell activation receptor NKp30 is not a heparan sulfate glycosaminoglycan. *J Immunol* 175:207-212.
83. Arnon, T.I., H. Achdout, O. Levi, G. Markel, N. Saleh, G. Katz, R. Gazit, T. Gonen-Gross, J. Hanna, E. Nahari, A. Porgador, A. Honigman, B. Plachter, D. Mevorach, D.G. Wolf, and O. Mandelboim. 2005. Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. *Nature immunology* 6:515-523.
84. Pogge von Strandmann, E., V.R. Simhadri, B. von Tresckow, S. Sasse, K.S. Reiners, H.P. Hansen, A. Rothe, B. Boll, V.L. Simhadri, P. Borchmann, P.J. McKinnon, M. Hallek, and A. Engert. 2007. Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. *Immunity* 27:965-974.
85. Brandt, C.S., Kennedy, J., Fox, B., Gao Zeren, Levin, S.D., Eugene, C.Y., West, R., Xu, W. 2008. Identification and characterization of an activating Ligand for NKp30. In Keystone Symposium NK and NKT Cell Biology. Keystone Symposia, Keystone Resort, Colorado.
86. Arnon, T.I., M. Lev, G. Katz, Y. Chernobrov, A. Porgador, and O. Mandelboim. 2001. Recognition of viral hemagglutinins by NKp44 but not by NKp30. *European journal of immunology* 31:2680-2689.
87. Esin, S., G. Batoni, C. Counoupas, A. Stringaro, F.L. Brancatisano, M. Colone, G. Maisetta, W. Florio, G. Arancia, and M. Campa. 2008. Direct binding of human NK cell natural cytotoxicity receptor NKp44 to the surfaces of mycobacteria and other bacteria. *Infect Immun* 76:1719-1727.
88. Vieillard, V., J.L. Strominger, and P. Debre. 2005. NK cytotoxicity against CD4+ T cells during HIV-1 infection: a gp41 peptide induces the expression of an NKp44 ligand. *Proceedings of the National Academy of Sciences of the United States of America* 102:10981-10986.
89. Sivori, S., D. Pende, C. Bottino, E. Marcenaro, A. Pessino, R. Biassoni, L. Moretta, and A. Moretta. 1999. NKp46 is the major triggering receptor involved in the natural cytotoxicity of fresh or cultured human NK cells. Correlation between surface density of NKp46 and natural cytotoxicity against autologous, allogeneic or xenogeneic target cells. *European journal of immunology* 29:1656-1666.
90. Castriconi, R., A. Dondero, M.V. Corrias, E. Lanino, D. Pende, L. Moretta, C. Bottino, and A. Moretta. 2004. Natural killer cell-mediated killing of freshly isolated neuroblastoma cells: critical role of DNAX accessory molecule-1-poliovirus receptor interaction. *Cancer Res* 64:9180-9184.
91. El-Sherbiny, Y.M., J.L. Meade, T.D. Holmes, D. McGonagle, S.L. Mackie, A.W. Morgan, G. Cook, S. Feyler, S.J. Richards, F.E. Davies, G.J. Morgan, and

G.P. Cook. 2007. The requirement for DNAM-1, NKG2D, and NKp46 in the natural killer cell-mediated killing of myeloma cells. *Cancer Res* 67:8444-8449.

92. Pasquier, B., L. Yin, M.C. Fondaneche, F. Relouzat, C. Bloch-Queyrat, N. Lambert, A. Fischer, G. de Saint-Basile, and S. Latour. 2005. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *The Journal of experimental medicine* 201:695-701.
93. Sivori, S., M. Falco, M. Della Chiesa, S. Carlomagno, M. Vitale, L. Moretta, and A. Moretta. 2004. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors and dendritic cells. *Proceedings of the National Academy of Sciences of the United States of America* 101:10116-10121.
94. Girart, M.V., M.B. Fuertes, C.I. Domaica, L.E. Rossi, and N.W. Zwirner. 2007. Engagement of TLR3, TLR7, and NKG2D regulate IFN-gamma secretion but not NKG2D-mediated cytotoxicity by human NK cells stimulated with suboptimal doses of IL-12. *J Immunol* 179:3472-3479.
95. Bauer, S., V. Groh, J. Wu, A. Steinle, J.H. Phillips, L.L. Lanier, and T. Spies. 1999. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science (New York, N.Y* 285:727-729.
96. Wu, J., Y. Song, A.B. Bakker, S. Bauer, T. Spies, L.L. Lanier, and J.H. Phillips. 1999. An activating immunoreceptor complex formed by NKG2D and DAP10. *Science (New York, N.Y* 285:730-732.
97. Groh, V., A. Bruhl, H. El-Gabalawy, J.L. Nelson, and T. Spies. 2003. Stimulation of T cell autoreactivity by anomalous expression of NKG2D and its MIC ligands in rheumatoid arthritis. *Proceedings of the National Academy of Sciences of the United States of America* 100:9452-9457.
98. Allez, M., V. Tieng, A. Nakazawa, X. Treton, V. Pacault, N. Dulphy, S. Caillat-Zucman, P. Paul, J.M. Gornet, C. Douay, S. Ravet, R. Tamouza, D. Charron, M. Lemann, L. Mayer, and A. Toubert. 2007. CD4+NKG2D+ T cells in Crohn's disease mediate inflammatory and cytotoxic responses through MICA interactions. *Gastroenterology* 132:2346-2358.
99. Sutherland, C.L., N.J. Chalupny, K. Schooley, T. VandenBos, M. Kubin, and D. Cosman. 2002. UL16-binding proteins, novel MHC class I-related proteins, bind to NKG2D and activate multiple signaling pathways in primary NK cells. *J Immunol* 168:671-679.
100. Roberts, A.I., L. Lee, E. Schwarz, V. Groh, T. Spies, E.C. Ebert, and B. Jabri. 2001. NKG2D receptors induced by IL-15 costimulate CD28-negative effector CTL in the tissue microenvironment. *J Immunol* 167:5527-5530.
101. Lee, J.C., K.M. Lee, D.W. Kim, and D.S. Heo. 2004. Elevated TGF-beta1 secretion and down-modulation of NKG2D underlies impaired NK cytotoxicity in cancer patients. *J Immunol* 172:7335-7340.
102. Burgess, S.J., A.I. Marusina, I. Pathmanathan, F. Borrego, and J.E. Coligan. 2006. IL-21 down-regulates NKG2D/DAP10 expression on human NK and CD8+ T cells. *J Immunol* 176:1490-1497.
103. Billadeau, D.D., J.L. Upshaw, R.A. Schoon, C.J. Dick, and P.J. Leibson. 2003. NKG2D-DAP10 triggers human NK cell-mediated killing via a Syk-independent regulatory pathway. *Nature immunology* 4:557-564.
104. Marusina, A.I., S.J. Burgess, I. Pathmanathan, F. Borrego, and J.E. Coligan. 2008. Regulation of human DAP10 gene expression in NK and T cells by Ap-1 transcription factors. *J Immunol* 180:409-417.
105. Horng, T., J.S. Bezbradica, and R. Medzhitov. 2007. NKG2D signaling is coupled to the interleukin 15 receptor signaling pathway. *Nature immunology* 8:1345-1352.
106. Lucas, M., W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach. 2007. Dendritic cells prime natural killer cells by trans-presenting interleukin 15. *Immunity* 26:503-517.
107. Diefenbach, A., E. Tomasello, M. Lucas, A.M. Jamieson, J.K. Hsia, E. Vivier, and D.H. Raulet. 2002. Selective associations with signaling proteins determine

stimulatory versus costimulatory activity of NKG2D. *Nature immunology* 3:1142-1149.
108. Gilfillan, S., E.L. Ho, M. Cella, W.M. Yokoyama, and M. Colonna. 2002. NKG2D recruits two distinct adapters to trigger NK cell activation and costimulation. *Nature immunology* 3:1150-1155.
109. Diefenbach, A., J.K. Hsia, M.Y. Hsiung, and D.H. Raulet. 2003. A novel ligand for the NKG2D receptor activates NK cells and macrophages and induces tumor immunity. *European journal of immunology* 33:381-391.
110. Carayannopoulos, L.N., O.V. Naidenko, D.H. Fremont, and W.M. Yokoyama. 2002. Cutting edge: murine UL16-binding protein-like transcript 1: a newly described transcript encoding a high-affinity ligand for murine NKG2D. *J Immunol* 169:4079-4083.
111. Diefenbach, A., E.R. Jensen, A.M. Jamieson, and D.H. Raulet. 2001. Rae1 and H60 ligands of the NKG2D receptor stimulate tumour immunity. *Nature* 413:165-171.
112. Bahram, S., M. Bresnahan, D.E. Geraghty, and T. Spies. 1994. A second lineage of mammalian major histocompatibility complex class I genes. *Proceedings of the National Academy of Sciences of the United States of America* 91:6259-6263.
113. Bahram, S., N. Mizuki, H. Inoko, and T. Spies. 1996. Nucleotide sequence of the human MHC class I MICA gene. *Immunogenetics* 44:80-81.
114. Bahram, S., and T. Spies. 1996. Nucleotide sequence of a human MHC class I MICB cDNA. *Immunogenetics* 43:230-233.
115. Chalupny, N.J., C.L. Sutherland, W.A. Lawrence, A. Rein-Weston, and D. Cosman. 2003. ULBP4 is a novel ligand for human NKG2D. *Biochemical and biophysical research communications* 305:129-135.
116. Cosman, D., J. Mullberg, C.L. Sutherland, W. Chin, R. Armitage, W. Fanslow, M. Kubin, and N.J. Chalupny. 2001. ULBPs, novel MHC class I-related molecules, bind to CMV glycoprotein UL16 and stimulate NK cytotoxicity through the NKG2D receptor. *Immunity* 14:123-133.
117. Eleme, K., S.B. Taner, B. Onfelt, L.M. Collinson, F.E. McCann, N.J. Chalupny, D. Cosman, C. Hopkins, A.I. Magee, and D.M. Davis. 2004. Cell surface organization of stress-inducible proteins ULBP and MICA that stimulate human NK cells and T cells via NKG2D. *The Journal of experimental medicine* 199:1005-1010.
118. Radaev, S., B. Rostro, A.G. Brooks, M. Colonna, and P.D. Sun. 2001. Conformational plasticity revealed by the cocrystal structure of NKG2D and its class I MHC-like ligand ULBP3. *Immunity* 15:1039-1049.
119. Li, P., G. McDermott, and R.K. Strong. 2002. Crystal structures of RAE-1beta and its complex with the activating immunoreceptor NKG2D. *Immunity* 16:77-86.
120. Li, P., D.L. Morris, B.E. Willcox, A. Steinle, T. Spies, and R.K. Strong. 2001. Complex structure of the activating immunoreceptor NKG2D and its MHC class I-like ligand MICA. *Nature immunology* 2:443-451.
121. McFarland, B.J., and R.K. Strong. 2003. Thermodynamic analysis of degenerate recognition by the NKG2D immunoreceptor: not induced fit but rigid adaptation. *Immunity* 19:803-812.
122. Gasser, S., S. Orsulic, E.J. Brown, and D.H. Raulet. 2005. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. *Nature* 436:1186-1190.
123. Gasser, S., and D. Raulet. 2006. The DNA damage response, immunity and cancer. *Semin Cancer Biol* 16:344-347.
124. Cerboni, C., A. Zingoni, M. Cippitelli, M. Piccoli, L. Frati, and A. Santoni. 2007. Antigen-activated human T lymphocytes express cell-surface NKG2D ligands via an ATM/ATR-dependent mechanism and become susceptible to autologous NK- cell lysis. *Blood* 110:606-615.
125. Cerwenka, A., J.L. Baron, and L.L. Lanier. 2001. Ectopic expression of retinoic acid early inducible-1 gene (RAE-1) permits natural killer cell-mediated

rejection of a MHC class I-bearing tumor in vivo. *Proceedings of the National Academy of Sciences of the United States of America* 98:11521-11526.
126. Smyth, M.J., J. Swann, E. Cretney, N. Zerafa, W.M. Yokoyama, and Y. Hayakawa. 2005. NKG2D function protects the host from tumor initiation. *The Journal of experimental medicine* 202:583-588.
127. Guerra, N., Y.X. Tan, N.T. Joncker, A. Choy, F. Gallardo, N. Xiong, S. Knoblaugh, D. Cado, N.R. Greenberg, and D.H. Raulet. 2008. NKG2D-deficient mice are defective in tumor surveillance in models of spontaneous malignancy. *Immunity* 28:571-580.
128. Groh, V., J. Wu, C. Yee, and T. Spies. 2002. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. *Nature* 419:734-738.
129. Song, H., J. Kim, D. Cosman, and I. Choi. 2006. Soluble ULBP suppresses natural killer cell activity via down-regulating NKG2D expression. *Cell Immunol* 239:22-30.
130. Waldhauer, I., and A. Steinle. 2006. Proteolytic release of soluble UL16-binding protein 2 from tumor cells. *Cancer Res* 66:2520-2526.
131. Lilley, C.E., C.T. Carson, A.R. Muotri, F.H. Gage, and M.D. Weitzman. 2005. DNA repair proteins affect the lifecycle of herpes simplex virus 1. *Proceedings of the National Academy of Sciences of the United States of America* 102:5844-5849.
132. Wilkinson, D.E., and S.K. Weller. 2004. Recruitment of cellular recombination and repair proteins to sites of herpes simplex virus type 1 DNA replication is dependent on the composition of viral proteins within prereplicative sites and correlates with the induction of the DNA damage response. *Journal of virology* 78:4783-4796.
133. Pappworth, I.Y., E.C. Wang, and M. Rowe. 2007. The switch from latent to productive infection in epstein-barr virus-infected B cells is associated with sensitization to NK cell killing. *Journal of virology* 81:474-482.
134. Draghi, M., A. Pashine, B. Sanjanwala, K. Gendzekhadze, C. Cantoni, D. Cosman, A. Moretta, N.M. Valiante, and P. Parham. 2007. NKp46 and NKG2D recognition of infected dendritic cells is necessary for NK cell activation in the human response to influenza infection. *J Immunol* 178:2688-2698.
135. Ebihara, T., H. Masuda, T. Akazawa, M. Shingai, H. Kikuta, T. Ariga, M. Matsumoto, and T. Seya. 2007. Induction of NKG2D ligands on human dendritic cells by TLR ligand stimulation and RNA virus infection. *Int Immunol* 19:1145-1155.
136. Hamerman, J.A., K. Ogasawara, and L.L. Lanier. 2004. Cutting edge: Toll-like receptor signaling in macrophages induces ligands for the NKG2D receptor. *J Immunol* 172:2001-2005.
137. Tieng, V., C. Le Bouguenec, L. du Merle, P. Bertheau, P. Desreumaux, A. Janin, D. Charron, and A. Toubert. 2002. Binding of Escherichia coli adhesin AfaE to CD55 triggers cell-surface expression of the MHC class I-related molecule MICA. *Proceedings of the National Academy of Sciences of the United States of America* 99:2977-2982.
138. 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* 320:1731-1735.
139. Etzioni, A., C. Eidenschenk, R. Katz, R. Beck, J.L. Casanova, and S. Pollack. 2005. Fatal varicella associated with selective natural killer cell deficiency. *J Pediatr* 146:423-425.
140. Eidenschenk, C., J. Dunne, E. Jouanguy, C. Fourlinnie, L. Gineau, D. Bacq, C. McMahon, O. Smith, J.L. Casanova, L. Abel, and C. Feighery. 2006. A novel primary immunodeficiency with specific natural-killer cell deficiency maps to the centromeric region of chromosome 8. *Am J Hum Genet* 78:721-727.
141. Orange, J.S. 2002. Human natural killer cell deficiencies and susceptibility to infection. *Microbes Infect* 4:1545-1558.
142. Orange, J.S. 2006. Human natural killer cell deficiencies. *Curr Opin Allergy Clin Immunol* 6:399-409.

143. Rosenberg, S.A., M.T. Lotze, L.M. Muul, S. Leitman, A.E. Chang, S.E. Ettinghausen, Y.L. Matory, J.M. Skibber, E. Shiloni, J.T. Vetto, and et al. 1985. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *The New England journal of medicine* 313:1485-1492.
144. Mullighan, C.G., and E.W. Petersdorf. 2006. Genomic polymorphism and allogeneic hematopoietic transplantation outcome. *Biol Blood Marrow Transplant* 12:19-27.
145. Petersdorf, E.W. 2007. Risk assessment in haematopoietic stem cell transplantation: histocompatibility. *Best practice & research* 20:155-170.
146. Verheyden, S., and C. Demanet. 2008. NK cell receptors and their ligands in leukemia. *Leukemia* 22:249-257.
147. Miller, J.S., Y. Soignier, A. Panoskaltsis-Mortari, S.A. McNearney, G.H. Yun, S.K. Fautsch, D. McKenna, C. Le, T.E. Defor, L.J. Burns, P.J. Orchard, B.R. Blazar, J.E. Wagner, A. Slungaard, D.J. Weisdorf, I.J. Okazaki, and P.B. McGlave. 2005. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood* 105:3051-3057.
148. Ljunggren, H.G., and K.J. Malmberg. 2007. Prospects for the use of NK cells in immunotherapy of human cancer. *Nature reviews* 7:329-339.
149. Sheridan, C. 2006. First-in-class cancer therapeutic to stimulate natural killer cells. *Nat Biotechnol* 24:597.
150. Dunn, P.L., and R.J. North. 1991. Early gamma interferon production by natural killer cells is important in defense against murine listeriosis. *Infect Immun* 59:2892-2900.
151. Teixeira, H.C., and S.H. Kaufmann. 1994. Role of NK1.1+ cells in experimental listeriosis. NK1+ cells are early IFN-gamma producers but impair resistance to Listeria monocytogenes infection. *J Immunol* 152:1873-1882.
152. Le-Barillec, K., J.G. Magalhaes, E. Corcuff, A. Thuizat, P.J. Sansonetti, A. Phalipon, and J.P. Di Santo. 2005. Roles for T and NK cells in the innate immune response to Shigella flexneri. *J Immunol* 175:1735-1740.
153. Cardillo, F., J.C. Voltarelli, S.G. Reed, and J.S. Silva. 1996. Regulation of Trypanosoma cruzi infection in mice by gamma interferon and interleukin 10: role of NK cells. *Infect Immun* 64:128-134.
154. Lieke, T., S.E. Graefe, U. Klauenberg, B. Fleischer, and T. Jacobs. 2004. NK cells contribute to the control of Trypanosoma cruzi infection by killing free parasites by perforin-independent mechanisms. *Infect Immun* 72:6817-6825.
155. Grau, G.E., P.F. Piguet, P. Vassalli, and P.H. Lambert. 1989. Tumor-necrosis factor and other cytokines in cerebral malaria: experimental and clinical data. *Immunological reviews* 112:49-70.
156. Korbel, D.S., O.C. Finney, and E.M. Riley. 2004. Natural killer cells and innate immunity to protozoan pathogens. *Int J Parasitol* 34:1517-1528.
157. Artavanis-Tsakonas, K., and E.M. Riley. 2002. Innate immune response to malaria: rapid induction of IFN-gamma from human NK cells by live Plasmodium falciparum-infected erythrocytes. *J Immunol* 169:2956-2963.
158. Korbel, D.S., K.C. Newman, C.R. Almeida, D.M. Davis, and E.M. Riley. 2005. Heterogeneous human NK cell responses to Plasmodium falciparum-infected erythrocytes. *J Immunol* 175:7466-7473.
159. D'Ombrain, M.C., D.S. Hansen, K.M. Simpson, and L. Schofield. 2007. gammadelta-T cells expressing NK receptors predominate over NK cells and conventional T cells in the innate IFN-gamma response to Plasmodium falciparum malaria. *European journal of immunology* 37:1864-1873.
160. Baratin, M., S. Roetynck, C. Lepolard, C. Falk, S. Sawadogo, S. Uematsu, S. Akira, B. Ryffel, J.G. Tiraby, L. Alexopoulou, C.J. Kirschning, J. Gysin, E. Vivier, and S. Ugolini. 2005. Natural killer cell and macrophage cooperation in MyD88-dependent innate responses to Plasmodium falciparum. *Proceedings of the National Academy of Sciences of the United States of America* 102:14747-14752.

161. Newman, K.C., D.S. Korbel, J.C. Hafalla, and E.M. Riley. 2006. Cross-talk with myeloid accessory cells regulates human natural killer cell interferon-gamma responses to malaria. *PLoS Pathog* 2:e118.
162. Baratin, M., S. Roetynck, B. Pouvelle, C. Lemmers, N.K. Viebig, S. Johansson, P. Bierling, A. Scherf, J. Gysin, E. Vivier, and S. Ugolini. 2007. Dissection of the role of PfEMP1 and ICAM-1 in the sensing of plasmodium falciparum-infected erythrocytes by natural killer cells. *PLoS ONE* 2:e228.
163. D'Ombrain, M.C., T.S. Voss, A.G. Maier, J.A. Pearce, D.S. Hansen, A.F. Cowman, and L. Schofield. 2007. Plasmodium falciparum erythrocyte membrane protein-1 specifically suppresses early production of host interferon-gamma. *Cell Host Microbe* 2:130-138.
164. Santoli, D., G. Trinchieri, and H. Koprowski. 1978. Cell-mediated cytotoxicity against virus-infected target cells in humans. II. Interferon induction and activation of natural killer cells. *J Immunol* 121:532-538.
165. Welsh, R.M., Jr. 1978. Mouse natural killer cells: induction specificity, and function. *J Immunol* 121:1631-1635.
166. Welsh, R.M., Jr., R.M. Zinkernagel, and L.A. Hallenbeck. 1979. Cytotoxic cells induced during lymphocytic choriomeningitis virus infection of mice. II. "Specificities" of the natural killer cells. *J Immunol* 122:475-481.
167. Biron, C.A., L.R. Turgiss, and R.M. Welsh. 1983. Increase in NK cell number and turnover rate during acute viral infection. *J Immunol* 131:1539-1545.
168. Biron, C.A., and R.M. Welsh. 1982. Blastogenesis of natural killer cells during viral infection in vivo. *J Immunol* 129:2788-2795.
169. Schindler, L., H. Engler, and H. Kirchner. 1982. Activation of natural killer cells and induction of interferon after injection of mouse hepatitis virus type 3 in mice. *Infect Immun* 35:869-873.
170. Lopez, C. 1981. Resistance to herpes simplex virus - type 1 (HSV-1). *Curr Top Microbiol Immunol* 92:15-24.
171. Lopez, C., D. Kirkpatrick, S.E. Read, P.A. Fitzgerald, J. Pitt, S. Pahwa, C.Y. Ching, and E.M. Smithwick. 1983. Correlation between low natural killing of fibroblasts infected with herpes simplex virus type 1 and susceptibility to herpesvirus infections. *The Journal of infectious diseases* 147:1030-1035.
172. Bukowski, J.F., B.A. Woda, S. Habu, K. Okumura, and R.M. Welsh. 1983. Natural killer cell depletion enhances virus synthesis and virus-induced hepatitis in vivo. *J Immunol* 131:1531-1538.
173. Andrews, D.M., H.E. Farrell, E.H. Densley, A.A. Scalzo, G.R. Shellam, and M.A. Degli-Esposti. 2001. NK1.1+ cells and murine cytomegalovirus infection: what happens in situ? *J Immunol* 166:1796-1802.
174. Loh, J., D.T. Chu, A.K. O'Guin, W.M. Yokoyama, and H.W.t. Virgin. 2005. Natural killer cells utilize both perforin and gamma interferon to regulate murine cytomegalovirus infection in the spleen and liver. *Journal of virology* 79:661-667.
175. Tay, C.H., and R.M. Welsh. 1997. Distinct organ-dependent mechanisms for the control of murine cytomegalovirus infection by natural killer cells. *Journal of virology* 71:267-275.
176. Warfield, K.L., J.G. Perkins, D.L. Swenson, E.M. Deal, C.M. Bosio, M.J. Aman, W.M. Yokoyama, H.A. Young, and S. Bavari. 2004. Role of natural killer cells in innate protection against lethal ebola virus infection. *The Journal of experimental medicine* 200:169-179.
177. Trifilo, M.J., C. Montalto-Morrison, L.N. Stiles, K.R. Hurst, J.L. Hardison, J.E. Manning, P.S. Masters, and T.E. Lane. 2004. CXC chemokine ligand 10 controls viral infection in the central nervous system: evidence for a role in innate immune response through recruitment and activation of natural killer cells. *Journal of virology* 78:585-594.
178. Born, T.L., L.A. Morrison, D.J. Esteban, T. VandenBos, L.G. Thebeau, N. Chen, M.K. Spriggs, J.E. Sims, and R.M. Buller. 2000. A poxvirus protein that binds to and inactivates IL-18, and inhibits NK cell response. *J Immunol* 164:3246-3254.

179. Xiang, Y., and B. Moss. 1999. IL-18 binding and inhibition of interferon gamma induction by human poxvirus-encoded proteins. *Proceedings of the National Academy of Sciences of the United States of America* 96:11537-11542.
180. Fang, M., L.L. Lanier, and L.J. Sigal. 2008. A role for NKG2D in NK cell-mediated resistance to poxvirus disease. *PLoS Pathog* 4:e30.
181. McSharry, B.P., H.G. Burgert, D.P. Owen, R.J. Stanton, V. Prod'homme, M. Sester, K. Koebernick, V. Groh, T. Spies, S. Cox, A.M. Little, E.C. Wang, P. Tomasec, and G.W. Wilkinson. 2008. Adenovirus E3/19K promotes evasion of NK cell recognition by intracellular sequestration of the NKG2D ligands major histocompatibility complex class I chain-related proteins A and B. *Journal of virology* 82:4585-4594.
182. Gazit, R., R. Gruda, M. Elboim, T.I. Arnon, G. Katz, H. Achdout, J. Hanna, U. Qimron, G. Landau, E. Greenbaum, Z. Zakay-Rones, A. Porgador, and O. Mandelboim. 2006. Lethal influenza infection in the absence of the natural killer cell receptor gene Ncr1. *Nature immunology* 7:517-523.
183. Siren, J., T. Sareneva, J. Pirhonen, M. Strengell, V. Veckman, I. Julkunen, and S. Matikainen. 2004. Cytokine and contact-dependent activation of natural killer cells by influenza A or Sendai virus-infected macrophages. *The Journal of general virology* 85:2357-2364.
184. Crotta, S., A. Stilla, A. Wack, A. D'Andrea, S. Nuti, U. D'Oro, M. Mosca, F. Filliponi, R.M. Brunetto, F. Bonino, S. Abrignani, and N.M. Valiante. 2002. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *The Journal of experimental medicine* 195:35-41.
185. Tseng, C.T., and G.R. Klimpel. 2002. Binding of the hepatitis C virus envelope protein E2 to CD81 inhibits natural killer cell functions. *The Journal of experimental medicine* 195:43-49.
186. Jinushi, M., T. Takehara, T. Tatsumi, T. Kanto, V. Groh, T. Spies, T. Suzuki, T. Miyagi, and N. Hayashi. 2003. Autocrine/paracrine IL-15 that is required for type I IFN-mediated dendritic cell expression of MHC class I-related chain A and B is impaired in hepatitis C virus infection. *J Immunol* 171:5423-5429.
187. Jinushi, M., T. Takehara, T. Tatsumi, T. Kanto, T. Miyagi, T. Suzuki, Y. Kanazawa, N. Hiramatsu, and N. Hayashi. 2004. Negative regulation of NK cell activities by inhibitory receptor CD94/NKG2A leads to altered NK cell-induced modulation of dendritic cell functions in chronic hepatitis C virus infection. *J Immunol* 173:6072-6081.
188. Nattermann, J., G. Feldmann, G. Ahlenstiel, B. Langhans, T. Sauerbruch, and U. Spengler. 2006. Surface expression and cytolytic function of natural killer cell receptors is altered in chronic hepatitis C. *Gut* 55:869-877.
189. Khakoo, S.I., C.L. Thio, M.P. Martin, C.R. Brooks, X. Gao, J. Astemborski, J. Cheng, J.J. Goedert, D. Vlahov, M. Hilgartner, S. Cox, A.M. Little, G.J. Alexander, M.E. Cramp, S.J. O'Brien, W.M. Rosenberg, D.L. Thomas, and M. Carrington. 2004. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science (New York, N.Y* 305:872-874.
190. Carrington, M., S. Wang, M.P. Martin, X. Gao, M. Schiffman, J. Cheng, R. Herrero, A.C. Rodriguez, R. Kurman, R. Mortel, P. Schwartz, A. Glass, and A. Hildesheim. 2005. Hierarchy of resistance to cervical neoplasia mediated by combinations of killer immunoglobulin-like receptor and human leukocyte antigen loci. *The Journal of experimental medicine* 201:1069-1075.
191. Estefania, E., N. Gomez-Lozano, F. Portero, R. de Pablo, R. Solis, S. Sepulveda, M. Vaquero, M.A. Gonzalez, E. Suarez, G. Roustan, and C. Vilches. 2007. Influence of KIR gene diversity on the course of HSV-1 infection: resistance to the disease is associated with the absence of KIR2DL2 and KIR2DS2. *Tissue Antigens* 70:34-41.
192. Fontana, L., M.C. Sirianni, G. de Sanctis, M. Carbonari, B. Ensoli, and F. Aiuti. 1986. Deficiency of natural killer activity, but not of natural killer binding, in patients with lymphoadenopathy syndrome positive for antibodies to HTLV-III. *Immunobiology* 171:425-435.

193. Hu, P.F., L.E. Hultin, P. Hultin, M.A. Hausner, K. Hirji, A. Jewett, B. Bonavida, R. Detels, and J.V. Giorgi. 1995. Natural killer cell immunodeficiency in HIV disease is manifest by profoundly decreased numbers of CD16+CD56+ cells and expansion of a population of CD16dimCD56- cells with low lytic activity. *J Acquir Immune Defic Syndr Hum Retrovirol* 10:331-340.
194. Alter, G., J.M. Malenfant, R.M. Delabre, N.C. Burgett, X.G. Yu, M. Lichterfeld, J. Zaunders, and M. Altfeld. 2004. Increased natural killer cell activity in viremic HIV-1 infection. *J Immunol* 173:5305-5311.
195. Alter, G., T.J. Suscovich, M. Kleyman, N. Teigen, H. Streeck, M.T. Zaman, A. Meier, and M. Altfeld. 2006. Low perforin and elevated SHIP-1 expression is associated with functional anergy of natural killer cells in chronic HIV-1 infection. *Aids* 20:1549-1551.
196. Le Gall, S., L. Erdtmann, S. Benichou, C. Berlioz-Torrent, L. Liu, R. Benarous, J.M. Heard, and O. Schwartz. 1998. Nef interacts with the mu subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. *Immunity* 8:483-495.
197. Cohen, G.B., R.T. Gandhi, D.M. Davis, O. Mandelboim, B.K. Chen, J.L. Strominger, and D. Baltimore. 1999. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity* 10:661-671.
198. Bonaparte, M.I., and E. Barker. 2004. Killing of human immunodeficiency virus-infected primary T-cell blasts by autologous natural killer cells is dependent on the ability of the virus to alter the expression of major histocompatibility complex class I molecules. *Blood* 104:2087-2094.
199. Montoya, C.J., P.A. Velilla, C. Chougnet, A.L. Landay, and M.T. Rugeles. 2006. Increased IFN-gamma production by NK and CD3+/CD56+ cells in sexually HIV-1-exposed but uninfected individuals. *Clin Immunol* 120:138-146.
200. Scott-Algara, D., L.X. Truong, P. Versmisse, A. David, T.T. Luong, N.V. Nguyen, I. Theodorou, F. Barre-Sinoussi, and G. Pancino. 2003. Cutting edge: increased NK cell activity in HIV-1-exposed but uninfected Vietnamese intravascular drug users. *J Immunol* 171:5663-5667.
201. De Maria, A., M. Fogli, P. Costa, G. Murdaca, F. Puppo, D. Mavilio, A. Moretta, and L. Moretta. 2003. The impaired NK cell cytolytic function in viremic HIV-1 infection is associated with a reduced surface expression of natural cytotoxicity receptors (NKp46, NKp30 and NKp44). *European journal of immunology* 33:2410-2418.
202. Mavilio, D., J. Benjamin, M. Daucher, G. Lombardo, S. Kottilil, M.A. Planta, E. Marcenaro, C. Bottino, L. Moretta, A. Moretta, and A.S. Fauci. 2003. Natural killer cells in HIV-1 infection: dichotomous effects of viremia on inhibitory and activating receptors and their functional correlates. *Proceedings of the National Academy of Sciences of the United States of America* 100:15011-15016.
203. Kottilil, S., T.W. Chun, S. Moir, S. Liu, M. McLaughlin, C.W. Hallahan, F. Maldarelli, L. Corey, and A.S. Fauci. 2003. Innate immunity in human immunodeficiency virus infection: effect of viremia on natural killer cell function. *The Journal of infectious diseases* 187:1038-1045.
204. Christianson, D., Black, C., Sun, P. 2008. Ligand Recognition by NK Receptors. In Keystone Symposium NK and NKT Cell Biology. Keystone Symposia, Keystone Resort.
205. Martin, M.P., X. Gao, J.H. Lee, G.W. Nelson, R. Detels, J.J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, J. Trowsdale, M. Wilson, S.J. O'Brien, and M. Carrington. 2002. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. *Nat Genet* 31:429-434.
206. Qi, Y., M.P. Martin, X. Gao, L. Jacobson, J.J. Goedert, S. Buchbinder, G.D. Kirk, S.J. O'Brien, J. Trowsdale, and M. Carrington. 2006. KIR/HLA

pleiotropism: protection against both HIV and opportunistic infections. *PLoS Pathog* 2:e79.
207. Barbour, J.D., U. Sriram, S.J. Caillier, J.A. Levy, F.M. Hecht, and J.R. Oksenberg. 2007. Synergy or independence? Deciphering the interaction of HLA Class I and NK cell KIR alleles in early HIV-1 disease progression. *PLoS Pathog* 3:e43.
208. Gaudieri, S., D. DeSantis, E. McKinnon, C. Moore, D. Nolan, C.S. Witt, S.A. Mallal, and F.T. Christiansen. 2005. Killer immunoglobulin-like receptors and HLA act both independently and synergistically to modify HIV disease progression. *Genes Immun* 6:683-690.
209. Alter, G., M.P. Martin, N. Teigen, W.H. Carr, T.J. Suscovich, A. Schneidewind, H. Streeck, M. Waring, A. Meier, C. Brander, J.D. Lifson, T.M. Allen, M. Carrington, and M. Altfeld. 2007. Differential natural killer cell-mediated inhibition of HIV-1 replication based on distinct KIR/HLA subtypes. *The Journal of experimental medicine* 204:3027-3036.
210. Alter, G., T.J. Suscovich, N. Teigen, A. Meier, H. Streeck, C. Brander, and M. Altfeld. 2007. Single-stranded RNA derived from HIV-1 serves as a potent activator of NK cells. *J Immunol* 178:7658-7666.
211. Carr, W.H., D.B. Rosen, H. Arase, D.F. Nixon, J. Michaelsson, and L.L. Lanier. 2007. Cutting Edge: KIR3DS1, a gene implicated in resistance to progression to AIDS, encodes a DAP12-associated receptor expressed on NK cells that triggers NK cell activation. *J Immunol* 178:647-651.
212. Gillespie, G.M., A. Bashirova, T. Dong, D.W. McVicar, S.L. Rowland-Jones, and M. Carrington. 2007. Lack of KIR3DS1 binding to MHC class I Bw4 tetramers in complex with CD8+ T cell epitopes. *AIDS Res Hum Retroviruses* 23:451-455.
213. Long, B.R., L.C. Ndhlovu, J.R. Oksenberg, L.L. Lanier, F.M. Hecht, D.F. Nixon, and J.D. Barbour. 2008. Conferral of enhanced natural killer cell function by KIR3DS1 in early human immunodeficiency virus type 1 infection. *Journal of virology* 82:4785-4792.
214. Martin, M.P., Y. Qi, X. Gao, E. Yamada, J.N. Martin, F. Pereyra, S. Colombo, E.E. Brown, W.L. Shupert, J. Phair, J.J. Goedert, S. Buchbinder, G.D. Kirk, A. Telenti, M. Connors, S.J. O'Brien, B.D. Walker, P. Parham, S.G. Deeks, D.W. McVicar, and M. Carrington. 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat Genet* 39:733-740.
215. Pando, M.J., C.M. Gardiner, M. Gleimer, K.L. McQueen, and P. Parham. 2003. The protein made from a common allele of KIR3DL1 (3DL1*004) is poorly expressed at cell surfaces due to substitution at positions 86 in Ig domain 0 and 182 in Ig domain 1. *J Immunol* 171:6640-6649.
216. Pellet, E.P.R., Bernhard. 2007. The Family Herpesviridae: A Brief Introduction. In Fields Virology. D.M.H. Knipe, P.M., editor Lippincott, Williams&Wilkins, 2479-2499.
217. Lodoen, M.B., and L.L. Lanier. 2005. Viral modulation of NK cell immunity. *Nat Rev Microbiol* 3:59-69.
218. Sylwester, A.W., B.L. Mitchell, J.B. Edgar, C. Taormina, C. Pelte, F. Ruchti, P.R. Sleath, K.H. Grabstein, N.A. Hosken, F. Kern, J.A. Nelson, and L.J. Picker. 2005. Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. *The Journal of experimental medicine* 202:673-685.
219. Sester, M., U. Sester, B. Gartner, B. Kubuschok, M. Girndt, A. Meyerhans, and H. Kohler. 2002. Sustained high frequencies of specific CD4 T cells restricted to a single persistent virus. *Journal of virology* 76:3748-3755.
220. Yewdell, J.W., and A.B. Hill. 2002. Viral interference with antigen presentation. *Nature immunology* 3:1019-1025.
221. Kleijnen, M.F., J.B. Huppa, P. Lucin, S. Mukherjee, H. Farrell, A.E. Campbell, U.H. Koszinowski, A.B. Hill, and H.L. Ploegh. 1997. A mouse cytomegalovirus glycoprotein, gp34, forms a complex with folded class I MHC

molecules in the ER which is not retained but is transported to the cell surface. *The EMBO journal* 16:685-694.
222. Holtappels, R., D. Gillert-Marien, D. Thomas, J. Podlech, P. Deegen, S. Herter, S.A. Oehrlein-Karpi, D. Strand, M. Wagner, and M.J. Reddehase. 2006. Cytomegalovirus encodes a positive regulator of antigen presentation. *Journal of virology* 80:7613-7624.
223. Reusch, U., W. Muranyi, P. Lucin, H.G. Burgert, H. Hengel, and U.H. Koszinowski. 1999. A cytomegalovirus glycoprotein re-routes MHC class I complexes to lysosomes for degradation. *The EMBO journal* 18:1081-1091.
224. Krmpotic, A., D.H. Busch, I. Bubic, F. Gebhardt, H. Hengel, M. Hasan, A.A. Scalzo, U.H. Koszinowski, and S. Jonjic. 2002. MCMV glycoprotein gp40 confers virus resistance to CD8+ T cells and NK cells in vivo. *Nature immunology* 3:529-535.
225. Gilbert, M.J., S.R. Riddell, B. Plachter, and P.D. Greenberg. 1996. Cytomegalovirus selectively blocks antigen processing and presentation of its immediate-early gene product. *Nature* 383:720-722.
226. Wiertz, E.J., T.R. Jones, L. Sun, M. Bogyo, H.J. Geuze, and H.L. Ploegh. 1996. The human cytomegalovirus US11 gene product dislocates MHC class I heavy chains from the endoplasmic reticulum to the cytosol. *Cell* 84:769-779.
227. Wiertz, E.J., D. Tortorella, M. Bogyo, J. Yu, W. Mothes, T.R. Jones, T.A. Rapoport, and H.L. Ploegh. 1996. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. *Nature* 384:432-438.
228. Machold, R.P., E.J. Wiertz, T.R. Jones, and H.L. Ploegh. 1997. The HCMV gene products US11 and US2 differ in their ability to attack allelic forms of murine major histocompatibility complex (MHC) class I heavy chains. *The Journal of experimental medicine* 185:363-366.
229. 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* 5:1039-1043.
230. Ben-Arieh, S.V., B. Zimerman, N.I. Smorodinsky, M. Yaacubovicz, C. Schechter, I. Bacik, J. Gibbs, J.R. Bennink, J.W. Yewdell, J.E. Coligan, H. Firat, F. Lemonnier, and R. Ehrlich. 2001. Human cytomegalovirus protein US2 interferes with the expression of human HFE, a nonclassical class I major histocompatibility complex molecule that regulates iron homeostasis. *Journal of virology* 75:10557-10562.
231. Ahn, K., A. Gruhler, B. Galocha, T.R. Jones, E.J. Wiertz, H.L. Ploegh, P.A. Peterson, Y. Yang, and K. Fruh. 1997. The ER-luminal domain of the HCMV glycoprotein US6 inhibits peptide translocation by TAP. *Immunity* 6:613-621.
232. Jones, T.R., E.J. Wiertz, L. Sun, K.N. Fish, J.A. Nelson, and H.L. Ploegh. 1996. Human cytomegalovirus US3 impairs transport and maturation of major histocompatibility complex class I heavy chains. *Proceedings of the National Academy of Sciences of the United States of America* 93:11327-11333.
233. 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. *Journal of virology* 76:10929-10941.
234. Ulbrecht, M., V. Hofmeister, G. Yuksekdag, J.W. Ellwart, H. Hengel, F. Momburg, S. Martinozzi, M. Reboul, M. Pla, and E.H. Weiss. 2003. HCMV glycoprotein US6 mediated inhibition of TAP does not affect HLA-E dependent protection of K-562 cells from NK cell lysis. *Hum Immunol* 64:231-237.
235. Fruh, K., K. Ahn, H. Djaballah, P. Sempe, P.M. van Endert, R. Tampe, P.A. Peterson, and Y. Yang. 1995. A viral inhibitor of peptide transporters for antigen presentation. *Nature* 375:415-418.
236. Furman, M.H., N. Dey, D. Tortorella, and H.L. Ploegh. 2002. The human cytomegalovirus US10 gene product delays trafficking of major

histocompatibility complex class I molecules. *Journal of virology* 76:11753-11756.

237. Trgovcich, J., C. Cebulla, P. Zimmerman, and D.D. Sedmak. 2006. Human cytomegalovirus protein pp71 disrupts major histocompatibility complex class I cell surface expression. *Journal of virology* 80:951-963.
238. Bukowski, J.F., and R.M. Welsh. 1985. Inability of interferon to protect virus-infected cells against lysis by natural killer (NK) cells correlates with NK cell-mediated antiviral effects in vivo. *J Immunol* 135:3537-3541.
239. Bukowski, J.F., B.A. Woda, and R.M. Welsh. 1984. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *Journal of virology* 52:119-128.
240. Scalzo, A.A., N.A. Fitzgerald, A. Simmons, A.B. La Vista, and G.R. Shellam. 1990. Cmv-1, a genetic locus that controls murine cytomegalovirus replication in the spleen. *The Journal of experimental medicine* 171:1469-1483.
241. Daniels, K.A., G. Devora, W.C. Lai, C.L. O'Donnell, M. Bennett, and R.M. Welsh. 2001. Murine cytomegalovirus is regulated by a discrete subset of natural killer cells reactive with monoclonal antibody to Ly49H. *The Journal of experimental medicine* 194:29-44.
242. Lee, S.H., S. Girard, D. Macina, M. Busa, A. Zafer, A. Belouchi, P. Gros, and S.M. Vidal. 2001. Susceptibility to mouse cytomegalovirus is associated with deletion of an activating natural killer cell receptor of the C-type lectin superfamily. *Nat Genet* 28:42-45.
243. Sjolin, H., E. Tomasello, M. Mousavi-Jazi, A. Bartolazzi, K. Karre, E. Vivier, and C. Cerboni. 2002. Pivotal role of KARAP/DAP12 adaptor molecule in the natural killer cell-mediated resistance to murine cytomegalovirus infection. *The Journal of experimental medicine* 195:825-834.
244. Lee, S.H., A. Zafer, Y. de Repentigny, R. Kothary, M.L. Tremblay, P. Gros, P. Duplay, J.R. Webb, and S.M. Vidal. 2003. Transgenic expression of the activating natural killer receptor Ly49H confers resistance to cytomegalovirus in genetically susceptible mice. *The Journal of experimental medicine* 197:515-526.
245. Arase, H., E.S. Mocarski, A.E. Campbell, A.B. Hill, and L.L. Lanier. 2002. Direct recognition of cytomegalovirus by activating and inhibitory NK cell receptors. *Science (New York, N.Y* 296:1323-1326.
246. Smith, H.R., J.W. Heusel, I.K. Mehta, S. Kim, B.G. Dorner, O.V. Naidenko, K. Iizuka, H. Furukawa, D.L. Beckman, J.T. Pingel, A.A. Scalzo, D.H. Fremont, and W.M. Yokoyama. 2002. Recognition of a virus-encoded ligand by a natural killer cell activation receptor. *Proceedings of the National Academy of Sciences of the United States of America* 99:8826-8831.
247. French, A.R., J.T. Pingel, M. Wagner, I. Bubic, L. Yang, S. Kim, U. Koszinowski, S. Jonjic, and W.M. Yokoyama. 2004. Escape of mutant double-stranded DNA virus from innate immune control. *Immunity* 20:747-756.
248. Voigt, V., C.A. Forbes, J.N. Tonkin, M.A. Degli-Esposti, H.R. Smith, W.M. Yokoyama, and A.A. Scalzo. 2003. Murine cytomegalovirus m157 mutation and variation leads to immune evasion of natural killer cells. *Proceedings of the National Academy of Sciences of the United States of America* 100:13483-13488.
249. Cretney, E., M.A. Degli-Esposti, E.H. Densley, H.E. Farrell, N.J. Davis-Poynter, and M.J. Smyth. 1999. m144, a murine cytomegalovirus (MCMV)-encoded major histocompatibility complex class I homologue, confers tumor resistance to natural killer cell-mediated rejection. *The Journal of experimental medicine* 190:435-444.
250. Farrell, H.E., H. Vally, D.M. Lynch, P. Fleming, G.R. Shellam, A.A. Scalzo, and N.J. Davis-Poynter. 1997. Inhibition of natural killer cells by a cytomegalovirus MHC class I homologue in vivo. *Nature* 386:510-514.
251. Voigt, S., A. Mesci, J. Ettinger, J.H. Fine, P. Chen, W. Chou, and J.R. Carlyle. 2007. Cytomegalovirus evasion of innate immunity by subversion of the NKR-P1B:Clr-b missing-self axis. *Immunity* 26:617-627.

252. Rodriguez, M., P. Sabastian, P. Clark, and M.G. Brown. 2004. Cmv1-independent antiviral role of NK cells revealed in murine cytomegalovirus-infected New Zealand White mice. *J Immunol* 173:6312-6318.
253. Desrosiers, M.P., A. Kielczewska, J.C. Loredo-Osti, S.G. Adam, A.P. Makrigiannis, S. Lemieux, T. Pham, M.B. Lodoen, K. Morgan, L.L. Lanier, and S.M. Vidal. 2005. Epistasis between mouse Klra and major histocompatibility complex class I loci is associated with a new mechanism of natural killer cell-mediated innate resistance to cytomegalovirus infection. *Nat Genet* 37:593-599.
254. Dighe, A., M. Rodriguez, P. Sabastian, X. Xie, M. McVoy, and M.G. Brown. 2005. Requisite H2k role in NK cell-mediated resistance in acute murine cytomegalovirus-infected MA/My mice. *J Immunol* 175:6820-6828.
255. Lenac, T., M. Budt, J. Arapovic, M. Hasan, A. Zimmermann, H. Simic, A. Krmpotic, M. Messerle, Z. Ruzsics, U.H. Koszinowski, H. Hengel, and S. Jonjic. 2006. The herpesviral Fc receptor fcr-1 down-regulates the NKG2D ligands MULT-1 and H60. *The Journal of experimental medicine* 203:1843-1850.
256. Hasan, M., A. Krmpotic, Z. Ruzsics, I. Bubic, T. Lenac, A. Halenius, A. Loewendorf, M. Messerle, H. Hengel, S. Jonjic, and U.H. Koszinowski. 2005. Selective down-regulation of the NKG2D ligand H60 by mouse cytomegalovirus m155 glycoprotein. *Journal of virology* 79:2920-2930.
257. Krmpotic, A., M. Hasan, A. Loewendorf, T. Saulig, A. Halenius, T. Lenac, B. Polic, I. Bubic, A. Kriegeskorte, E. Pernjak-Pugel, M. Messerle, H. Hengel, D.H. Busch, U.H. Koszinowski, and S. Jonjic. 2005. NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145. *The Journal of experimental medicine* 201:211-220.
258. Lodoen, M.B., G. Abenes, S. Umamoto, J.P. Houchins, F. Liu, and L.L. Lanier. 2004. The cytomegalovirus m155 gene product subverts natural killer cell antiviral protection by disruption of H60-NKG2D interactions. *The Journal of experimental medicine* 200:1075-1081.
259. Lodoen, M., K. Ogasawara, J.A. Hamerman, H. Arase, J.P. Houchins, E.S. Mocarski, and L.L. Lanier. 2003. NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. *The Journal of experimental medicine* 197:1245-1253.
260. Thomas, M., J.M. Boname, S. Field, S. Nejentsev, M. Salio, V. Cerundolo, M. Wills, and P.J. Lehner. 2008. Down-regulation of NKG2D and NKp80 ligands by Kaposi's sarcoma-associated herpesvirus K5 protects against NK cell cytotoxicity. *Proceedings of the National Academy of Sciences of the United States of America* 105:1656-1661.
261. 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* 164:4775-4782.
262. Fletcher, J.M., H.G. Prentice, and J.E. Grundy. 1998. Natural killer cell lysis of cytomegalovirus (CMV)-infected cells correlates with virally induced changes in cell surface lymphocyte function-associated antigen-3 (LFA-3) expression and not with the CMV-induced down-regulation of cell surface class I HLA. *J Immunol* 161:2365-2374.
263. Leong, C.C., T.L. Chapman, P.J. Bjorkman, D. Formankova, E.S. Mocarski, J.H. Phillips, and L.L. Lanier. 1998. Modulation of natural killer cell cytotoxicity in human cytomegalovirus infection: the role of endogenous class I major histocompatibility complex and a viral class I homolog. *The Journal of experimental medicine* 187:1681-1687.
264. Carr, W.H., A.M. Little, E. Mocarski, and P. Parham. 2002. NK cell-mediated lysis of autologous HCMV-infected skin fibroblasts is highly variable among NK cell clones and polyclonal NK cell lines. *Clin Immunol* 105:126-140.

265. Falk, C.S., M. Mach, D.J. Schendel, E.H. Weiss, I. Hilgert, and G. Hahn. 2002. NK cell activity during human cytomegalovirus infection is dominated by US2-11-mediated HLA class I down-regulation. *J Immunol* 169:3257-3266.
266. Craigen, J.L., and J.E. Grundy. 1996. Cytomegalovirus induced up-regulation of LFA-3 (CD58) and ICAM-1 (CD54) is a direct viral effect that is not prevented by ganciclovir or foscarnet treatment. *Transplantation* 62:1102-1108.
267. Duncombe, A.S., J.E. Grundy, P. Oblakowski, H.G. Prentice, D.J. Gottlieb, D.M. Roy, J.E. Reittie, C. Bello-Fernandez, A.V. Hoffbrand, and M.K. Brenner. 1992. Bone marrow transplant recipients have defective MHC-unrestricted cytotoxic responses against cytomegalovirus in comparison with Epstein-Barr virus: the importance of target cell expression of lymphocyte function-associated antigen 1 (LFA1). *Blood* 79:3059-3066.
268. Grundy, J.E., and K.L. Downes. 1993. Up-regulation of LFA-3 and ICAM-1 on the surface of fibroblasts infected with cytomegalovirus. *Immunology* 78:405-412.
269. 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* 162:6967-6970.
270. 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. *Proceedings of the National Academy of Sciences of the United States of America* 96:9839-9844.
271. 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. *The Journal of general virology* 80 (Pt 11):2867-2877.
272. Cha, T.A., E. Tom, G.W. Kemble, G.M. Duke, E.S. Mocarski, and R.R. Spaete. 1996. Human cytomegalovirus clinical isolates carry at least 19 genes not found in laboratory strains. *Journal of virology* 70:78-83.
273. Tomasec, P., E.C. Wang, A.J. Davison, B. Vojtesek, M. Armstrong, C. Griffin, B.P. McSharry, R.J. Morris, S. Llewellyn-Lacey, C. Rickards, A. Nomoto, C. Sinzger, and G.W. Wilkinson. 2005. Downregulation of natural killer cell-activating ligand CD155 by human cytomegalovirus UL141. *Nature immunology* 6:181-188.
274. Wang, E.C., B. McSharry, C. Retiere, P. Tomasec, S. Williams, L.K. Borysiewicz, V.M. Braud, and G.W. Wilkinson. 2002. UL40-mediated NK evasion during productive infection with human cytomegalovirus. *Proceedings of the National Academy of Sciences of the United States of America* 99:7570-7575.
275. Garrigue, I., M.F. Corte, N. Magnin, L. Couzi, S. Capdepont, C. Rio, P. Merville, J. Dechanet-Merville, H. Fleury, and M.E. Lafon. 2007. Variability of UL18, UL40, UL111a and US3 immunomodulatory genes among human cytomegalovirus clinical isolates from renal transplant recipients. *J Clin Virol* 40:120-128.
276. Cerboni, C., A. Achour, A. Warnmark, M. Mousavi-Jazi, T. Sandalova, M.L. Hsu, D. Cosman, K. Karre, and E. Carbone. 2006. Spontaneous mutations in the human CMV HLA class I homologue UL18 affect its binding to the inhibitory receptor LIR-1/ILT2/CD85j. *European journal of immunology* 36:732-741.
277. Beck, S., and B.G. Barrell. 1988. Human cytomegalovirus encodes a glycoprotein homologous to MHC class-I antigens. *Nature* 331:269-272.
278. Browne, H., G. Smith, S. Beck, and T. Minson. 1990. A complex between the MHC class I homologue encoded by human cytomegalovirus and beta 2 microglobulin. *Nature* 347:770-772.
279. Browne, H., M. Churcher, and T. Minson. 1992. Construction and characterization of a human cytomegalovirus mutant with the UL18 (class I homolog) gene deleted. *Journal of virology* 66:6784-6787.

280. Wills, M.R., O. Ashiru, M.B. Reeves, G. Okecha, J. Trowsdale, P. Tomasec, G.W. Wilkinson, J. Sinclair, and J.G. Sissons. 2005. Human cytomegalovirus encodes an MHC class I-like molecule (UL142) that functions to inhibit NK cell lysis. *J Immunol* 175:7457-7465.
281. Jones, T.R., L.K. Hanson, L. Sun, J.S. Slater, R.M. Stenberg, and A.E. Campbell. 1995. Multiple independent loci within the human cytomegalovirus unique short region down-regulate expression of major histocompatibility complex class I heavy chains. *Journal of virology* 69:4830-4841.
282. Fahnestock, M.L., J.L. Johnson, R.M. Feldman, J.M. Neveu, W.S. Lane, and P.J. Bjorkman. 1995. The MHC class I homolog encoded by human cytomegalovirus binds endogenous peptides. *Immunity* 3:583-590.
283. Park, B., H. Oh, S. Lee, Y. Song, J. Shin, Y.C. Sung, S.Y. Hwang, and K. Ahn. 2002. The MHC class I homolog of human cytomegalovirus is resistant to down-regulation mediated by the unique short region protein (US)2, US3, US6, and US11 gene products. *J Immunol* 168:3464-3469.
284. Varnum, S.M., D.N. Streblow, M.E. Monroe, P. Smith, K.J. Auberry, L. Pasa-Tolic, D. Wang, D.G. Camp, 2nd, K. Rodland, S. Wiley, W. Britt, T. Shenk, R.D. Smith, and J.A. Nelson. 2004. Identification of proteins in human cytomegalovirus (HCMV) particles: the HCMV proteome. *Journal of virology* 78:10960-10966.
285. Hassan-Walker, A.F., A.V. Cope, P.D. Griffiths, and V.C. Emery. 1998. Transcription of the human cytomegalovirus natural killer decoy gene, UL18, in vitro and in vivo. *The Journal of general virology* 79 (Pt 9):2113-2116.
286. Hassan-Walker, A.F., F.M. Mattes, P.D. Griffiths, and V.C. Emery. 2001. Quantity of cytomegalovirus DNA in different leukocyte populations during active infection in vivo and the presence of gB and UL18 transcripts. *J Med Virol* 64:283-289.
287. Saverino, D., F. Ghiotto, A. Merlo, S. Bruno, L. Battini, M. Occhino, M. Maffei, C. Tenca, S. Pileri, L. Baldi, M. Fabbi, A. Bachi, A. De Santanna, C.E. Grossi, and E. Ciccone. 2004. Specific recognition of the viral protein UL18 by CD85j/LIR-1/ILT2 on CD8+ T cells mediates the non-MHC-restricted lysis of human cytomegalovirus-infected cells. *J Immunol* 172:5629-5637.
288. Maffei, M., F. Ghiotto, M. Occhino, M. Bono, A. De Santanna, L. Battini, G.L. Gusella, F. Fais, S. Bruno, and E. Ciccone. 2008. Human cytomegalovirus regulates surface expression of the viral protein UL18 by means of two motifs present in the cytoplasmic tail. *J Immunol* 180:969-979.
289. Elkington, R., S. Walker, T. Crough, M. Menzies, J. Tellam, M. Bharadwaj, and R. Khanna. 2003. Ex vivo profiling of CD8+-T-cell responses to human cytomegalovirus reveals broad and multispecific reactivities in healthy virus carriers. *Journal of virology* 77:5226-5240.
290. Berg, L., G.C. Riise, D. Cosman, T. Bergstrom, S. Olofsson, K. Karre, and E. Carbone. 2003. LIR-1 expression on lymphocytes, and cytomegalovirus disease in lung-transplant recipients. *Lancet* 361:1099-1101.
291. Willcox, B.E., L.M. Thomas, and P.J. Bjorkman. 2003. Crystal structure of HLA-A2 bound to LIR-1, a host and viral major histocompatibility complex receptor. *Nature immunology* 4:913-919.
292. Chapman, T.L., A.P. Heikema, A.P. West, Jr., and P.J. Bjorkman. 2000. Crystal structure and ligand binding properties of the D1D2 region of the inhibitory receptor LIR-1 (ILT2). *Immunity* 13:727-736.
293. Gonen-Gross, T., H. Achdout, T.I. Arnon, R. Gazit, N. Stern, V. Horejsi, D. Goldman-Wohl, S. Yagel, and O. Mandelboim. 2005. The CD85J/leukocyte inhibitory receptor-1 distinguishes between conformed and beta 2-microglobulin-free HLA-G molecules. *J Immunol* 175:4866-4874.
294. Allen, R.L., T. Raine, A. Haude, J. Trowsdale, and M.J. Wilson. 2001. Leukocyte receptor complex-encoded immunomodulatory receptors show differing specificity for alternative HLA-B27 structures. *J Immunol* 167:5543-5547.

295. Occhino, M., F. Ghiotto, S. Soro, M. Mortarino, S. Bosi, M. Maffei, S. Bruno, M. Nardini, M. Figini, A. Tramontano, and E. Ciccone. 2008. Dissecting the structural determinants of the interaction between the human cytomegalovirus UL18 protein and the CD85j immune receptor. *J Immunol* 180:957-968.
296. 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* 386:514-517.
297. Kim, J.S., S.E. Choi, I.H. Yun, J.Y. Kim, C. Ahn, S.J. Kim, J. Ha, E.S. Hwang, C.Y. Cha, S. Miyagawa, and C.G. Park. 2004. Human cytomegalovirus UL18 alleviated human NK-mediated swine endothelial cell lysis. *Biochemical and biophysical research communications* 315:144-150.
298. 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* 55:149-161.
299. Prod'homme, V., C. Griffin, R.J. Aicheler, E.C. Wang, B.P. McSharry, C.R. Rickards, R.J. Stanton, L.K. Borysiewicz, M. Lopez-Botet, G.W. Wilkinson, and P. Tomasec. 2007. The human cytomegalovirus MHC class I homolog UL18 inhibits LIR-1+ but activates LIR-1- NK cells. *J Immunol* 178:4473-4481.
300. Guma, M., M. Budt, A. Saez, T. Brckalo, H. Hengel, A. Angulo, and M. Lopez-Botet. 2006. Expansion of CD94/NKG2C+ NK cells in response to human cytomegalovirus-infected fibroblasts. *Blood* 107:3624-3631.
301. Wagner, C.S., G.C. Riise, T. Bergstrom, K. Karre, E. Carbone, and L. Berg. 2007. Increased expression of leukocyte Ig-like receptor-1 and activating role of UL18 in the response to cytomegalovirus infection. *J Immunol* 178:3536-3543.
302. Wagner, C.S., L. Walther-Jallow, E. Buentke, H.G. Ljunggren, A. Achour, and B.J. Chambers. 2008. Human cytomegalovirus-derived protein UL18 alters the phenotype and function of monocyte-derived dendritic cells. *J Leukoc Biol* 83:56-63.
303. 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 (New York, N.Y* 287:1031.
304. 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* 164:5019-5022.
305. Cerboni, C., M. Mousavi-Jazi, H. Wakiguchi, E. Carbone, K. Karre, and K. Soderstrom. 2001. Synergistic effect of IFN-gamma and human cytomegalovirus protein UL40 in the HLA-E-dependent protection from NK cell-mediated cytotoxicity. *European journal of immunology* 31:2926-2935.
306. Romagnani, C., G. Pietra, M. Falco, P. Mazzarino, L. Moretta, and M.C. Mingari. 2004. HLA-E-restricted recognition of human cytomegalovirus by a subset of cytolytic T lymphocytes. *Hum Immunol* 65:437-445.
307. Pietra, G., C. Romagnani, P. Mazzarino, M. Falco, E. Millo, A. Moretta, L. Moretta, and M.C. Mingari. 2003. HLA-E-restricted recognition of cytomegalovirus-derived peptides by human CD8+ cytolytic T lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 100:10896-10901.
308. Millo, E., G. Pietra, A. Armirotti, P. Vacca, M.C. Mingari, L. Moretta, and G. Damonte. 2007. Purification and HPLC-MS analysis of a naturally processed HCMV-derived peptide isolated from the HEK-293T/HLA-E+/Ul40+ cell transfectants and presented at the cell surface in the context of HLA-E. *J Immunol Methods* 322:128-136.

309. Brooks, A.G., F. Borrego, P.E. Posch, A. Patamawenu, C.J. Scorzelli, M. Ulbrecht, E.H. Weiss, and J.E. Coligan. 1999. Specific recognition of HLA-E, but not classical, HLA class I molecules by soluble CD94/NKG2A and NK cells. *J Immunol* 162:305-313.
310. Vales-Gomez, M., H.T. Reyburn, R.A. Erskine, M. Lopez-Botet, and J.L. Strominger. 1999. Kinetics and peptide dependency of the binding of the inhibitory NK receptor CD94/NKG2-A and the activating receptor CD94/NKG2-C to HLA-E. *The EMBO journal* 18:4250-4260.
311. Kraft, J.R., R.E. Vance, J. Pohl, A.M. Martin, D.H. Raulet, and P.E. Jensen. 2000. Analysis of Qa-1(b) peptide binding specificity and the capacity of CD94/NKG2A to discriminate between Qa-1-peptide complexes. *The Journal of experimental medicine* 192:613-624.
312. Chandra, D., G. Choy, and D.G. Tang. 2007. Cytosolic accumulation of HSP60 during apoptosis with or without apparent mitochondrial release: evidence that its pro-apoptotic or pro-survival functions involve differential interactions with caspase-3. *The Journal of biological chemistry* 282:31289-31301.
313. Kirchhoff, S.R., S. Gupta, and A.A. Knowlton. 2002. Cytosolic heat shock protein 60, apoptosis, and myocardial injury. *Circulation* 105:2899-2904.
314. Lin, K.M., B. Lin, I.Y. Lian, R. Mestril, I.E. Scheffler, and W.H. Dillmann. 2001. Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. *Circulation* 103:1787-1792.
315. Samali, A., J. Cai, B. Zhivotovsky, D.P. Jones, and S. Orrenius. 1999. Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of jurkat cells. *The EMBO journal* 18:2040-2048.
316. Xanthoudakis, S., and D.W. Nicholson. 2000. Heat-shock proteins as death determinants. *Nat Cell Biol* 2:E163-165.
317. Xanthoudakis, S., S. Roy, D. Rasper, T. Hennessey, Y. Aubin, R. Cassady, P. Tawa, R. Ruel, A. Rosen, and D.W. Nicholson. 1999. Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. *The EMBO journal* 18:2049-2056.
318. Ghosh, J.C., T. Dohi, B.H. Kang, and D.C. Altieri. 2008. Hsp60 regulation of tumor cell apoptosis. *The Journal of biological chemistry* 283:5188-5194.
319. Zanin-Zhorov, A., L. Cahalon, G. Tal, R. Margalit, O. Lider, and I.R. Cohen. 2006. Heat shock protein 60 enhances CD4+ CD25+ regulatory T cell function via innate TLR2 signaling. *J Clin Invest* 116:2022-2032.
320. Park, S.G., S.M. Lee, and G. Jung. 2003. Antisense oligodeoxynucleotides targeted against molecular chaperonin Hsp60 block human hepatitis B virus replication. *The Journal of biological chemistry* 278:39851-39857.
321. Chabaud, S., H. Lambert, A.M. Sasseville, H. Lavoie, C. Guilbault, B. Massie, J. Landry, and Y. Langelier. 2003. The R1 subunit of herpes simplex virus ribonucleotide reductase has chaperone-like activity similar to Hsp27. *FEBS Lett* 545:213-218.
322. Yamauchi, Y., K. Wada, F. Goshima, T. Daikoku, K. Ohtsuka, and Y. Nishiyama. 2002. Herpes simplex virus type 2 UL14 gene product has heat shock protein (HSP)-like functions. *J Cell Sci* 115:2517-2527.
323. Kobayashi, K., E. Ohgitani, Y. Tanaka, M. Kita, and J. Imanishi. 1994. Herpes simplex virus-induced expression of 70 kDa heat shock protein (HSP70) requires early protein synthesis but not viral DNA replication. *Microbiol Immunol* 38:321-325.
324. Sainis, I., C. Angelidis, G. Pagoulatos, and I. Lazaridis. 1994. The hsc70 gene which is slightly induced by heat is the main virus inducible member of the hsp70 gene family. *FEBS Lett* 355:282-286.
325. Goldmacher, V.S., L.M. Bartle, A. Skaletskaya, C.A. Dionne, N.L. Kedersha, C.A. Vater, J.W. Han, R.J. Lutz, S. Watanabe, E.D. Cahir McFarland, E.D. Kieff, E.S. Mocarski, and T. Chittenden. 1999. A cytomegalovirus-encoded mitochondria-localized inhibitor of apoptosis structurally unrelated to Bcl-2.

Proceedings of the National Academy of Sciences of the United States of America 96:12536-12541.
326. Skaletskaya, A., L.M. Bartle, T. Chittenden, A.L. McCormick, E.S. Mocarski, and V.S. Goldmacher. 2001. A cytomegalovirus-encoded inhibitor of apoptosis that suppresses caspase-8 activation. *Proceedings of the National Academy of Sciences of the United States of America* 98:7829-7834.
327. Terhune, S., E. Torigoi, N. Moorman, M. Silva, Z. Qian, T. Shenk, and D. Yu. 2007. Human cytomegalovirus UL38 protein blocks apoptosis. *Journal of virology* 81:3109-3123.
328. Moorman, N.J., I.M. Cristea, S.S. Terhune, M.P. Rout, B.T. Chait, and T. Shenk. 2008. Human cytomegalovirus protein UL38 inhibits host cell stress responses by antagonizing the tuberous sclerosis protein complex. *Cell Host Microbe* 3:253-262.
329. Sarbassov, D.D., S.M. Ali, and D.M. Sabatini. 2005. Growing roles for the mTOR pathway. *Curr Opin Cell Biol* 17:596-603.
330. Brune, W., M. Nevels, and T. Shenk. 2003. Murine cytomegalovirus m41 open reading frame encodes a Golgi-localized antiapoptotic protein. *Journal of virology* 77:11633-11643.
331. Cerwenka, A., A.B. Bakker, T. McClanahan, J. Wagner, J. Wu, J.H. Phillips, and L.L. Lanier. 2000. Retinoic acid early inducible genes define a ligand family for the activating NKG2D receptor in mice. *Immunity* 12:721-727.
332. Diefenbach, A., A.M. Jamieson, S.D. Liu, N. Shastri, and D.H. Raulet. 2000. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. *Nature immunology* 1:119-126.
333. Litwin, V., J. Gumperz, P. Parham, J.H. Phillips, and L.L. Lanier. 1993. Specificity of HLA class I antigen recognition by human NK clones: evidence for clonal heterogeneity, protection by self and non-self alleles, and influence of the target cell type. *The Journal of experimental medicine* 178:1321-1336.
334. Wu, J., N.J. Chalupny, T.J. Manley, S.R. Riddell, D. Cosman, and T. Spies. 2003. Intracellular retention of the MHC class I-related chain B ligand of NKG2D by the human cytomegalovirus UL16 glycoprotein. *J Immunol* 170:4196-4200.
335. Welte, S.A., C. Sinzger, S.Z. Lutz, H. Singh-Jasuja, K.L. Sampaio, U. Eknigk, H.G. Rammensee, and A. Steinle. 2003. Selective intracellular retention of virally induced NKG2D ligands by the human cytomegalovirus UL16 glycoprotein. *European journal of immunology* 33:194-203.
336. Vales-Gomez, M., H. Browne, and H.T. Reyburn. 2003. Expression of the UL16 glycoprotein of Human Cytomegalovirus protects the virus-infected cell from attack by natural killer cells. *BMC immunology* 4:4.
337. 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. *Journal of virology* 77:4539-4545.
338. Rolle, A., M. Mousavi-Jazi, M. Eriksson, J. Odeberg, C. Soderberg-Naucler, D. Cosman, K. Karre, and C. Cerboni. 2003. Effects of human cytomegalovirus infection on ligands for the activating NKG2D receptor of NK cells: up-regulation of UL16-binding protein (ULBP)1 and ULBP2 is counteracted by the viral UL16 protein. *J Immunol* 171:902-908.
339. 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. *The Journal of experimental medicine* 197:1427-1439.
340. Vales-Gomez, M., A. Winterhalter, P. Roda-Navarro, A. Zimmermann, L. Boyle, H. Hengel, A. Brooks, and H.T. Reyburn. 2006. The human cytomegalovirus glycoprotein UL16 traffics through the plasma membrane and the nuclear envelope. *Cellular microbiology* 8:581-590.
341. Vales-Gomez, M., and H.T. Reyburn. 2006. Intracellular trafficking of the HCMV immunoevasin UL16 depends on elements present in both its

cytoplasmic and transmembrane domains. *Journal of molecular biology* 363:908-917.

342. Spreu, J., T. Stehle, and A. Steinle. 2006. Human cytomegalovirus-encoded UL16 discriminates MIC molecules by their alpha2 domains. *J Immunol* 177:3143-3149.
343. Zou, Y., W. Bresnahan, R.T. Taylor, and P. Stastny. 2005. Effect of human cytomegalovirus on expression of MHC class I-related chains A. *J Immunol* 174:3098-3104.
344. Chalupny, N.J., A. Rein-Weston, S. Dosch, and D. Cosman. 2006. Down-regulation of the NKG2D ligand MICA by the human cytomegalovirus glycoprotein UL142. *Biochemical and biophysical research communications* 346:175-181.
345. Stern-Ginossar, N., N. Elefant, A. Zimmermann, D.G. Wolf, N. Saleh, M. Biton, E. Horwitz, Z. Prokocimer, M. Prichard, G. Hahn, D. Goldman-Wohl, C. Greenfield, S. Yagel, H. Hengel, Y. Altuvia, H. Margalit, and O. Mandelboim. 2007. Host immune system gene targeting by a viral miRNA. *Science (New York, N.Y* 317:376-381.
346. Gupta, A., J.J. Gartner, P. Sethupathy, A.G. Hatzigeorgiou, and N.W. Fraser. 2006. Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. *Nature* 442:82-85.
347. Confer, D.L., G.M. Vercellotti, D. Kotasek, J.L. Goodman, A. Ochoa, and H.S. Jacob. 1990. Herpes simplex virus-infected cells disarm killer lymphocytes. *Proceedings of the National Academy of Sciences of the United States of America* 87:3609-3613.
348. Kaufman, D.S., R.A. Schoon, and P.J. Leibson. 1992. Role for major histocompatibility complex class I in regulating natural killer cell-mediated killing of virus-infected cells. *Proceedings of the National Academy of Sciences of the United States of America* 89:8337-8341.
349. Ahmad, A., E. Sharif-Askari, L. Fawaz, and J. Menezes. 2000. Innate immune response of the human host to exposure with herpes simplex virus type 1: in vitro control of the virus infection by enhanced natural killer activity via interleukin-15 induction. *Journal of virology* 74:7196-7203.
350. Djeu, J.Y., N. Stocks, K. Zoon, G.J. Stanton, T. Timonen, and R.B. Herberman. 1982. Positive self regulation of cytotoxicity in human natural killer cells by production of interferon upon exposure to influenza and herpes viruses. *The Journal of experimental medicine* 156:1222-1234.
351. Fitzgerald, P.A., M. Mendelsohn, and C. Lopez. 1985. Human natural killer cells limit replication of herpes simplex virus type 1 in vitro. *J Immunol* 134:2666-2672.
352. Chisholm, S.E., K. Howard, M.V. Gomez, and H.T. Reyburn. 2007. Expression of ICP0 is sufficient to trigger natural killer cell recognition of herpes simplex virus-infected cells by natural cytotoxicity receptors. *The Journal of infectious diseases* 195:1160-1168.
353. Ward, J., M. Bonaparte, J. Sacks, J. Guterman, M. Fogli, D. Mavilio, and E. Barker. 2007. HIV modulates the expression of ligands important in triggering natural killer cell cytotoxic responses on infected primary T-cell blasts. *Blood* 110:1207-1214.
354. Cerboni, C., F. Neri, N. Casartelli, A. Zingoni, D. Cosman, P. Rossi, A. Santoni, and M. Doria. 2007. Human immunodeficiency virus 1 Nef protein downmodulates the ligands of the activating receptor NKG2D and inhibits natural killer cell-mediated cytotoxicity. *The Journal of general virology* 88:242-250.
355. 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. *Journal of virology* 78:10995-11006.
356. Browne, E.P., and T. Shenk. 2003. Human cytomegalovirus UL83-coded pp65 virion protein inhibits antiviral gene expression in infected cells. *Proceedings of*

the National Academy of Sciences of the United States of America 100:11439-11444.
357. 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* 101:4870-4877.
358. Baniyash, M. 2004. TCR zeta-chain downregulation: curtailing an excessive inflammatory immune response. *Nature reviews* 4:675-687.
359. Keating, S., S. Prince, M. Jones, and M. Rowe. 2002. The lytic cycle of Epstein-Barr virus is associated with decreased expression of cell surface major histocompatibility complex class I and class II molecules. *Journal of virology* 76:8179-8188.
360. Barton, E.S., D.W. White, J.S. Cathelyn, K.A. Brett-McClellan, M. Engle, M.S. Diamond, V.L. Miller, and H.W.t. Virgin. 2007. Herpesvirus latency confers symbiotic protection from bacterial infection. *Nature* 447:326-329.
361. Radosavljevic, M., and S. Bahram. 2003. In vivo immunogenetics: from MIC to RAET1 loci. *Immunogenetics* 55:1-9.
362. Wagner, M., A. Gutermann, J. Podlech, M.J. Reddehase, and U.H. Koszinowski. 2002. Major histocompatibility complex class I allele-specific cooperative and competitive interactions between immune evasion proteins of cytomegalovirus. *The Journal of experimental medicine* 196:805-816.