

Center for Infectious Medicine
Department of Medicine
Karolinska Institutet, Stockholm, Sweden

**NK CELL AND DENDRITIC CELL INTERACTIONS IN
INNATE IMMUNE RESPONSES**

Catrine M. Persson



**Karolinska
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To my family

ABSTRACT

Natural Killer (NK) cells are cytotoxic cells of the innate immune system. They have been found to be critical in the defense against infections and also against some tumors. Recent studies have shown that NK cells require signals from accessory cells to induce their recruitment and activation at the site of infection or tumor outgrowth. One group of these accessory cells is the family of dendritic cells (DC). DC are antigen presenting cells, acting as sensors of the immune system with the capacity to activate the adaptive immune response. Thus, DC have been called nature's adjuvants. In the last fifteen years, another feature of DC has been under investigation, namely their interaction with NK cells, which can influence the outcome of the adaptive immune response. In this thesis I have investigated different aspects of the interactions between NK cells and DC, including killing of DC and DC-induced activation of NK cells.

Immature resting DC are killed by activated NK cells. However, when toll-like receptors (TLR) are stimulated on DC, they become functionally mature and more resistant to NK cell mediated killing. In my first study, I investigated the role of the non-classical MHC class I molecule Qa1 in the reduced susceptibility of mature DC to NK cell lysis. We found that the interaction between Qa1 on mature DC with its inhibitory receptor NKG2A/CD94 on NK cells was crucial in protecting mature DC from NK cell-mediated killing both in vitro and in vivo, even in the absence of classical MHC class I molecules. However, mature DC were only protected from NK cells expressing NKG2A inhibitory receptor as NK cells lacking this molecule also displayed cytotoxicity against mature DC.

In addition to the elimination of DC by NK cells, another consequence of NK-DC interaction was investigated in this thesis. Stimulated DC's ability to activate NK cells. DC are no longer considered to be a homogenous cell type, instead several subtypes have been described both in mice and humans. Bone-marrow derived DC grown in GM-CSF have been mostly used in reported studies. In paper II we explored another DC subtype, the plasmacytoid DC (pDC), that we suggest may be more potent in recruiting and activating NK cells in peripheral tissue. CpG-activated pDC injected i.p. in mice induced strong recruitment of NK cells to the peritoneal cavity, which was in part dependent on CXCR3 and CD62L. The recruited NK cells were also activated in terms of cytotoxicity against the classical NK cell target YAC-1 and were able to produce IFN γ after restimulation ex vivo. The costimulatory molecules CD28-CD80/86 were involved in the activation of NK cells induced by stimulated pDC.

Finally, an infectious model consisting of *Toxoplasma gondii* (*T. gondii*) was used to investigate NK cells interaction with parasite-infected DC. *T. gondii*-infected DC were extremely sensitive to NK cell mediated lysis, which required infection with live parasites. After lysis of infected DC, parasites rapidly re-infected effector NK cells. In vivo, NK cells were found to be readily infected with *T. gondii* following inoculation of *T. gondii*-infected DC or free parasites, which was significantly reduced in mice lacking killing machinery. We speculate that NK cells kill infected DC that leads to re-infection of the NK cells and that this actually may be beneficial for the parasite to induce chronicity in its host.

In summary, this thesis provides some new insights in events that take place during NK cells interaction with DC. Hopefully, the work presented here may be beneficial in the attempts to improve therapies targeting these cells in the future.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals

- I. **Persson CM**, Assarsson E, Vahlne G., Brodin P, Chambers BJ
Critical role of Qa1^b in the protection of mature dendritic cells from NK cell mediated killing. *Scand Journal of Immunology*. 2008, 67(1), 30-36
- II. **Persson CM**, Chambers BJ. Plasmacytoid dendritic cell-induced migration and activation of NK cells in vivo. *Manuscript submitted*
- III. **Persson CM***, Lambert H*, Vutova P, Nederby J, Dellacasa I, Yagita H, Ljunggren HG, Grandien A, Barragan A, Chambers BJ. Transmission of *Toxoplasma gondii* from dendritic cells to NK cells. *Infection and Immunity* 2009, 77(3), 970-976

* contributed equally

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LIST OF ABBREVIATIONS

| | |
|---------------|---|
| Ab | Antibody |
| ADCC | Antibody-dependent cell-mediated cytotoxicity |
| B6 | C57BL/6 |
| CCR | Chemokine receptor |
| CD | Cluster of differentiation |
| cDC | Conventional dendritic cells |
| CTL | Cytotoxic T lymphocyte |
| DC | Dendritic cell |
| EAE | Experimental autoimmune encephalomyelitis |
| Fc γ R | Fc gamma receptor |
| Flt3L | Fms-like tyrosine kinase 3 ligand |
| GM-SCF | Granulocyte-macrophage colony stimulating factor |
| IFN | Interferon |
| Ig | Immunoglobulin |
| IL | Interleukin |
| iNOS | Inducible nitric oxide synthase |
| KIR | Killer cell Ig-like receptor |
| LN | Lymph node |
| LPS | Lipopolysaccharide |
| mDC | Myeloid dendritic cells |
| MHC | Major histocompatibility complex |
| MIP | Macrophage inflammatory protein |
| Naip | Neuronal apoptosis inhibitory rotein |
| NK | Natural Killer |
| NLR | Nod-like receptors |
| NOD | Nucleotide-binding oligomerization domain |
| PAMP | Pathogen-associated molecular patterns |
| PDC | Plasmacytoid dendritic cells |
| PLGF | Placental growth factor |
| Qdm | Qa-1 determinant modifier |
| RAG | Recombinant activating gene |
| RANTES | regulated on activation, normal T cell expressed and secreted |

| | |
|-------|---------------------------------------|
| TLR | Toll-like receptor |
| TNF | Tumor necrosis factor |
| TRAIL | TNF-related apoptosis-inducing ligand |
| VEGF | Vascular endothelial growth factor |
| wt | Wildtype |

1 AIMS OF THIS THESIS

The general aim of this thesis was to get more insights into NK cell-DC interactions both in vitro and in vivo. The specific aims were:

- To investigate the mechanisms behind why TLR-stimulated or IFN γ -matured DC are more resistant to NK cell mediated killing. **(paper I)**
- To examine the mechanisms by which NK cells become activated and recruited to tissues in vivo by plasmacytoid DC. **(paper II)**
- To study the role of NK cell-dendritic cell interactions in the early stage of infection with the parasite *Toxoplasma gondii*. **(paper III)**

2 INTRODUCTION

2.1 OVERVIEW OF THE IMMUNE SYSTEM

The immune system is a fascinating creation. Always awake and ready to respond, constantly battling with pathogens but also with the difficult task of balancing immunity and self-tolerance. In this introduction I attempt to give a brief overview of the immune system and specifically the cells I have been studying during my thesis work.

Immunity comes from the latin word *immunis*, which means exempt, as in exemption from military service, tax payments or other public services. The probably earliest reference to the phenomenon of immunity can be traced back to Thucydides, a great historian of the Peloponnesian war, when he in 430 BC described the plague in Athens. He wrote that only those who had recovered from the plague could nurse the sick because they would not contract the disease a second time (1). One of the first pioneers in immunology was Edward Jenner who in 1796 introduced the term vaccination (from the Latin *vacca*, cow) to describe his discovery that cowpox could induce protection against human smallpox (2), a term that is still used today. Vaccinology was further developed by Louis Pasteur, who demonstrated that it is possible to attenuate, or weaken, a pathogen and administer it as a vaccine. Later, in the late 19th century, Robert Koch proved that infectious diseases are caused by microorganisms. After that, many excellent researchers have followed to provide the amazing knowledge existing today about the complex science of immunology.

The immune system consists of many components and is generally divided into two divisions, namely the innate and adaptive immune response. For a more detailed description of the immune system I refer to the textbook “Immunobiology – The immune system in health and disease” by Charles Janeway and co-authors (3).

2.1.1 Innate immunity

This part of the immune system is the first line of defense and can respond immediately to intruders of the body without prior stimulus. It consists of physical barriers, such as skin and epithelia lining the respiratory, intestinal and urogenital tract. Here, antimicrobial substances, for example defensins, are secreted to fight infections. If a pathogen crosses the epithelia it will face different cell types like dendritic cells (DC) and

natural killer (NK) cells (which will be further discussed), macrophages and different granulocytes. These cells have different tasks, but one thing common for cells of the immune system is that almost no cell work alone. There is a constant communication between cells and tissues in terms of cytokines, chemokines and cell-to cell contact. One important task of the innate immune system is to differ self from non-self. One instrument to do so is through pattern recognition molecules, which includes Toll-like receptors (TLR) and the more newly defined family of NOD-like receptors (NLR). These receptors recognize so-called PAMPs (pathogen-associated molecular patterns) that are structures typical for microorganisms, often essential and not present in the host. TLR detect ligands, such as LPS (gram-negative bacteria), double-stranded RNA (viruses), CpG DNA motifs, lipoteichoic acid (Gram-positive bacteria), extracellularly or in the lumen of endocytic vesicles. NLR (including for example Nod1, Nod2 and Ipaf), on the other hand, are intracellular proteins responsible for detecting microbes in the cytosol. Another type of innate immune defense mechanisms is the complement system that upon activation results in clearance or lysis of the pathogen that has been attacked by the components of the complement system. If a pathogen enters the host, an early immune response is induced but does not lead to protective immunity unless the infectious agent is able to breach the barriers of the innate immune system. This is where the adaptive immunity comes in.

2.1.2 Adaptive immunity

If the keywords for the innate immune system are unspecific and fast, this part of the immune system is characterized by high specificity, memory and the ability to proliferate and differentiate on demand. Adaptive immunity is required to fight long-lasting infections and to create an immunological memory that leads to protective immunity on a second encounter with the same pathogen. It consists basically of two different groups of lymphocytes, B cells and T cells. B cells derive from the bone marrow and upon activation transform into antibody-secreting plasma cells. T cells also originate from the bone marrow but mature in the thymus, thence the name. T cells can be further divided into cytotoxic T cells ($CD8^+$ T cells) and T helper cells ($CD4^+$ T cells). The function of $CD8^+$ T cells is to kill cells that may have an intracellular infection e.g. virus infection or transformed cells. $CD8^+$ T cells recognise peptides from foreign or transformed proteins expressed on MHC class I molecules. The T helper (T_H) cells can be further divided into several subsets based upon their cytokine production, which can activate or suppress other immune cells or immune functions. T_H1 cells that produce for example $IFN\gamma$ are very important for clearing intracellular infections. T_H2 cells that produce

for example IL-4 and IL-13 are more involved in inducing Ab responses crucial for the clearing of parasites and extracellular pathogens. T_H17 cells, which produce IL-17, have been suggested to be important for fighting extracellular pathogens, such as fungi and bacteria. There are also regulatory T cells (Treg) that play a crucial role in regulating immune responses and maintaining tolerance by producing TGFβ. One important feature of lymphocytes is the constant recirculation through blood, tissues and lymphoid organ, which enables them to encounter antigens carried from infected sites by macrophages and DC.

2.2 NATURAL KILLER CELLS

Natural Killer cells were discovered first in 1975 by Kiessling *et al.* (4, 5) and Herberman *et al.* (6, 7). They discovered a lymphoid cell type able to lyse tumor cells without prior stimuli that was T-cell independent. Since then, the role of NK cells in the immune system has been continuously growing, from the beginning being recognized for their ability to kill tumors and virally infected cells to being one of the key players in bridging innate and adaptive immune functions via cytokines and interactions with other cell types. More recently, the role of NK cells in immune homeostasis and autoimmunity is being put under the microscope.

NK cells represent a lymphoid population that has innate immune functions. Unlike T-cells, NK cells do not express a diverse set of antigen-specific receptors. Instead they display a heterogenous array of cell surface receptors enabling them to respond to cytokines, pathogens and to recognize the difference between stressed/transformed/infected cells and normal cells.

In humans, NK cells can be divided into two functionally distinct subsets based on the expression of CD56. The CD56^{bright} NK cells have poor cytolytic capacity, but produce a lot of cytokines, especially IFNγ. The CD56^{dim} NK cells, on the other hand, are the main killer population, but are poorer at producing cytokines (8). Also, it has been demonstrated that CD56^{bright} NK cells express different levels of chemokine receptors and tend to accumulate within in inflammatory sites. A CD56 homologue is not expressed in mouse and for long no functionally distinct subsets had been described in the mouse system. In 2006, Hayakawa and colleagues reported that the mature Mac-1^{high} NK cells could be further divided into 2 distinct subsets with functional differences based on their expression of CD27 (9). The CD27^{high} NK cells dominated in lymph nodes and were better producers of IFNγ in response to IL12/IL-18 and responded much

greater to activating ligand expressed on tumor cells compared to CD27^{low} subset. Also these populations differed in their expression inhibitory Ly49 receptors, where CD27^{low} NK cells express more inhibitory receptors, which may be correlated to their reduced ability to kill target cells. Throughout this thesis I will focus on mouse system unless mentioned otherwise.

A recent topic regarding NK cells is that they also might possess memory function, a feature that has always been attributed T cells and B cells. Indeed, a study by O'Leary and colleagues suggested the existence of NK cell memory in a model of hapten-induced contact hypersensitivity in mice lacking B cells and T cells (10). More recently, Sun and co-workers demonstrated long-lived NK cells in mice after MCMV infection that rapidly degranulated and produced cytokines on reactivation. Adoptive transfer of these NK cells to naive mice resulted in secondary expansion of these cells and protective immunity, suggesting that NK cells possess immunological memory (11).

2.2.1 NK cell distribution

NK cells develop primary in the bone marrow in adults (12) and their differentiation is dependent on IL-15 produced by stromal cells (13). LN and thymus have also recently been suggested to be alternative sources of NK cells (14). NK cells are widely distributed in the body but the largest populations can be found in spleen, lung, liver, bone marrow and peripheral blood. NK cells make up for about 2 % of lymphocytes in a mouse spleen and 2 to 18% of lymphocytes in human blood. They have a turnover rate at about 2 weeks in human blood (15), which is consistent with data from the mouse (16, 17).

One problem in studying distribution of NK cells in tissues has been the lack of appropriate markers expressed only on NK cells. Many of the antibodies used are not NK-specific (NK1.1, CD49b) or not expressed by all NK cells (Ly49G2, CD49b). In 2007, Walzer and co-workers described the NK cell activating receptor NKp46 to be the best NK cell marker across mammalian species (17). Using antibodies to this molecule or using transgenic mice where GFP has been put under the control of the NKp46 promoter has now allowed us to begin to identify the location of NK cells under different conditions *in vivo*.

2.2.2 NK cell regulation

Although NK cells were originally discovered for their ability to kill tumor cells without prior stimulation, this was probably due to circumstance since animal facilities in the early 1970s were usually not pathogen-free. Indeed, I have noticed little or no NK cell activity in the spleens in control mice at our specific pathogen-free animal facility. Studies have similarly shown that human resting peripheral blood NK cells are not that potent effector cells (18). In addition, it has been demonstrated that murine, resting splenic NK cells have reduced expression of granzyme B and perforin, resulting in poor cytotoxic potential which could be induced upon stimulation with cytokines or infection with MCMV (19). Thus it has been suggested that NK cells, like T cells, require priming for activation, a process that involves cytokines such as IFN α , IL-15 (20) and IL-18 (21) in mice.

NK cells are regulated by different cytokines/chemokines produced by cells in the surrounding (reviewed in (22)). IL-2 is a classical NK cell-activating cytokine widely used to culture NK cells in vitro. IL-12 promotes IFN γ production and enhances cytotoxicity by NK cells, while type I interferons especially promote cytotoxic functions during viral infections. Another important cytokine is IL-15, which is important not only for NK cell survival but also differentiation both in vitro and in vivo (23, 24). In vivo, IL-15 produced by DC has been demonstrated to activate NK cells (20). IL-18 can synergize with IL-12, IL-15 or type I IFNs to amplify IFN γ production (25), proliferation (26) and cytotoxicity (27) during infection. TGF β , on the other hand, can inhibit NK cell functions, such as IFN γ production and cytotoxicity (28, 29).

Upon inflammation, NK cells can migrate to various tissues. NK cells express a variety of chemokine receptors that can vary depending on subset or maturation state. Four receptors seem to play a key role in the recruitment of NK cells to sites of inflammation: CCR2, CCR5, CXCR3 and CX₃CR1 (reviewed in (30)). The ligands for these receptors are many, such as MIP1 α , RANTES and IP-10, enabling NK cells to have a broad responsiveness to inflammatory stimulus. For example CCR2 and CCR5 are required for NK cell recruitment to the liver in mice infected with mouse cytomegalovirus (MCMV). CXCR3 have been shown to be important for recruitment to inflamed lymph nodes (31). More recently, it has been shown that S1P₅ is important for NK cell trafficking in vivo (32). However, few studies have examined the trafficking of NK cells during non-pathological conditions, thus, it is unclear what signals are required for daily trafficking of NK cells.

2.2.3 NK cell cytotoxicity

NK cell-mediated killing of tumor cells and infected cells is a very important function (33). NK cells can kill their target cells by two major pathways, both requiring close contact between the NK cells and target cells. The first pathway involves perforin and granzyme that is stored in large granules inside NK cells and get released by exocytosis. Perforin is a membrane disrupting protein that is very important for NK cell cytotoxicity. This protein has been found to play important roles in NK cells ability to suppress tumors and also in some infection models (34, 35). Granzymes trigger apoptotic cell death of the target cells. There are 11 known granzymes (A-H, K, N and M) with various substrates (36). Ten of these (A-G, H, M and N) are expressed in mice and 5 (A, B, H, K, and M) in humans (37). This granule-exocytosis pathway can induce cell death by activating apoptotic caspases but also in the absence of these molecules (38).

The second pathway is by expression of FasL and TRAIL (TNF-related apoptosis-inducing ligand), which can interact with death receptors on target cells leading to caspase-dependent apoptosis. TRAIL is a type II transmembrane protein belonging to the TNF superfamily. There are 5 known receptors in humans (TRAIL-R1-5) and 3 in mice (TRAIL-R2 and 2 decoy receptor), but only TRAIL-R1 and R2 are capable of transducing apoptotic signals (39). TRAIL is expressed on most NK cells after stimulation with IL-2, IL-15 or IFNs and has shown to be important for elimination of DC in vivo and also in suppression of TRAIL sensitive tumor cells (34, 40, 41). FASL expression by NK cells has been mostly shown to contribute to tumor suppression (42) and it has been demonstrated that NK cells by the production of IFN γ , can induce FAS expression on tumor cells and kill them in a FAS-dependent manner (43).

2.2.4 Target cell recognition by NK cells

How NK cells could discriminate between target cells or cells to be spared was initially a mystery. In 1981, Klas Kärre postulated in his thesis a model for this phenomenon, which he called “The missing-self hypothesis”. The basis for this hypothesis was that NK cells could detect the absence or reduced expression of MHC class I molecules on normal cells, making these cells susceptible to NK cell mediated lysis (**Figure 1**). This was at the time quit controversial since it was totally opposite to how T cells sensed danger. The theory has it origin in the observation of rejection of allogeneic lymphoma and bone marrow grafts (H-2^{a/a} rejects H-2^{b/b}) and the phenomenon of F1 hybrid resistance, where F1 host (H-2^{a/b}) rejects a graft of parental origin that is H-2^{a/a} or H-2^{b/b}. In these cases

the graft fails to express at least one H-2 class I allele present in the host and Kärre *et al.* proposed that this was enough to be eliminated by NK cells. This theory was validated when it was demonstrated that H-2 class I deficient cell lines were shown to be less malignant than wildtype after low-dose inoculation in vivo and that this phenomenon was abolished when NK cells were depleted (44, 45). After that, many studies followed investigating the relationship between MHC class I expression and NK cell susceptibility.

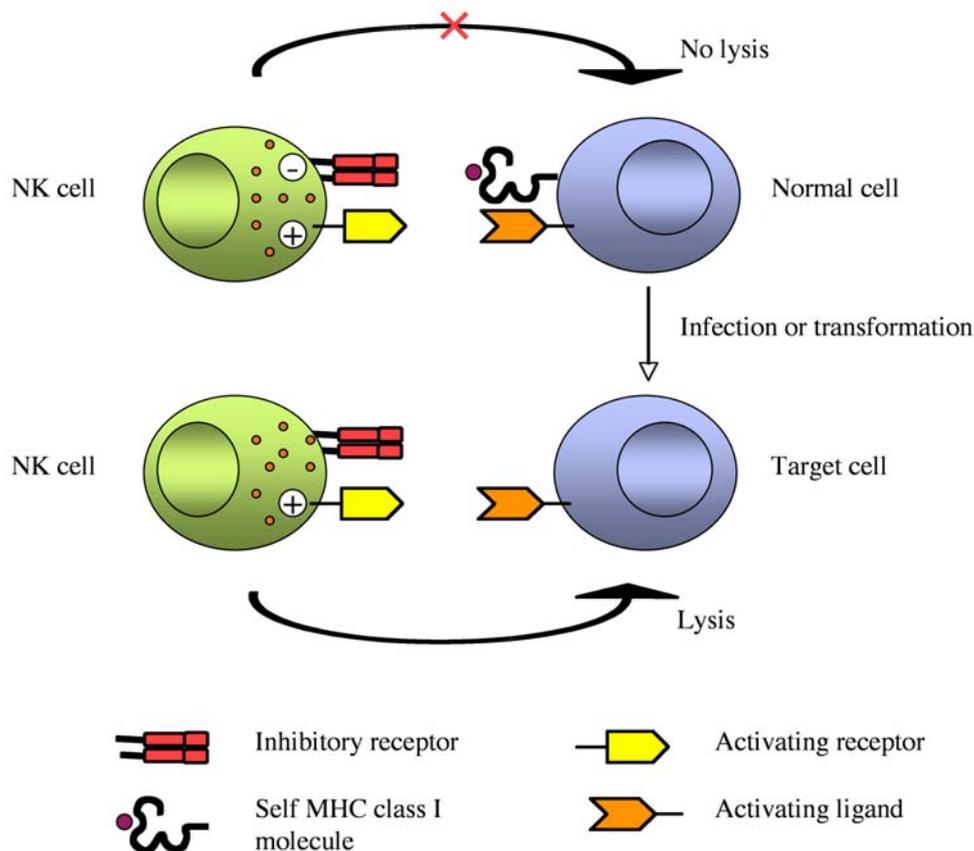


Figure 1. NK cell activation is regulated by a balance between activating and inhibitory receptors. A normal cell might or might not express activating ligands. However, because a normal cell expresses self-MHC class I molecules, lysis will not appear. Upon transformation or infection, expression of self-MHC class I ligands are often reduced or lost, shifting the balance towards activation which leads to lysis of the target cell (missing-self recognition).

Within the frame of the missing-self theory it is stated that NK cells can lyse a target cell if self MHC class I is absent or reduced, provided that an activating receptor is engaged on the NK cell. Thus, the activation of NK

cells is depending on the balance between activating and inhibitory signals received via receptors on the NK cell surface. The activating signal for NK cells is switched on whenever NK cells encounter a possible target cell and the inactivation process is a fail-self mechanism to prevent killing of the normal self-MHC class I expressing cells. Loss or reduced expression of MHC class I molecules are common events in virally infected cells or tumor cells enabling these cells to escape from T cell recognition. Instead they become targets for NK cells. Over the years this model has been redefined and remodeled such that the concept of missing-self recognition has been extended. Thus, missing self would describe autologous cells that have reduced self-MHC class I molecules due to transformation or infection, which can be detected by NK cells resulting in target cell lysis. Non-self recognition applies to an allogeneic transplant setting where NK cells face foreign, non-self MHC class I molecules. These molecules are not able to engage the inhibitory receptors on the surface of NK cells leading to lysis of the allogeneic cell. Under some circumstances, such as transformation or infection, stimulatory ligands on the target cell may be induced to such extent so that inhibitory signals of the MHC class I molecules are overcome, resulting in lysis of the target, so-called induced-self.

Below follows a brief summary of some of the receptors involved in target cell recognition by NK cells.

2.2.4.1 Inhibitory receptors

The first inhibitory receptor on NK cells recognizing MHC class I was discovered by W. M. Yokoyama and was named Ly49 (46). Since then, this family of receptors has grown extensively. There are currently three main families of inhibitory receptors; Ly-49 in rodents, CD94/NKG2A both in humans and rodents and KIR (killer cell immunoglobulin-like receptors) which seems to be functional only in humans and not in rodents. The inhibitory receptors can be structurally very different, but they share a common feature in that they carry an immunoreceptor tyrosine-based inhibitory motif (ITIM) in their cytoplasmic domain. These receptors are very polymorphic and expressed in different patterns to create a very heterogenous NK population in every individual.

Ly49 receptors belong to the C-type lectin superfamily. They are expressed as disulphide-linked homodimers on NK cells, T cells and NKT cells. There are both inhibitory and activating (discussed later) Ly49 receptors. In the mouse, the inhibitory isoforms include Ly49A, C, G and I which recognize classical MHC class I molecules (reviewed in (47)). Humans have one Ly49 gene, which is likely a pseudogene.

CD94/NKG2A receptor is a member of the C-type lectin family of receptors. They are disulfide-linked, dimeric type 2 integral membrane proteins. CD94/NKG2A is expressed on NK cells and on some CD8⁺ T cells and NKT cells. The ligand is the non-classical MHC class I molecule HLA-E in humans (48) and Qa1 in mice (49). Qa1 is expressed on most normal cells and predominantly bind the Qdm (Qa-1 determinant modifier) peptide, sequence AMAPRTLTL, derived from the leader sequence of H-2D/L molecules (50). The sequence of the peptide bound to Qa1 is very important for the recognition by NKG2A (51). Thus, the inhibitory signal could be relieved if Qdm dissociates or is replaced by another peptide. It has been shown that other peptides can bind Qa1, such as peptides from the Salmonella GroEL and the mammalian Hsp-60 molecule (52). CD94/NKG2A is the first inhibitory receptor expressed during development and nearly 90 % of fetal NK cells express high levels of this receptor. This expression allows NK cells to distinguish between class I high and low targets and may induce self-tolerance through the recognition of Qa1/Qdm complex (53). There is also a splice variant of NKG2A named NKG2B (54).

KIR stands for Killer cell Immunoglobulin-like Receptors and are monomeric type I integral membrane proteins belonging to the Ig superfamily. These receptors are very polymorphic and each receptor is expressed in a variegated pattern resulting in a heterogenous population of NK cells in every individual (55). KIR show great functional homology to Ly49 receptors in mice in that the ligands are classical MHC class I molecules, although they are structurally very different. KIR express two or three extracellular Ig-like domains, designated 2D or 3D. They can also have either long or short cytoplasmic tails. The inhibitory KIR have long cytoplasmic tails containing ITIMs, whereas activating KIR have short cytoplasmic tails (56). Examples of inhibitory KIR are KIR2DL1, KIR2DL2 and KIR3DL1.

2.2.4.2 Activating receptors

An activating receptor can refer to a receptor when triggered leads to release of cytolytic granules or cytokines. Several ligands for NK cells activating receptors are known, but not all. Common for many of these receptors is that they signal through associating with adaptor proteins that carry an ITAM sequence (DAP10, DAP12, Fc γ or the ζ chain). Here follows a brief description of a few activating receptors.

KIR/Ly49

A few members of the KIR and Ly49 families also express activating isoforms. It is not clear why NK cells would express activating receptors

for MHC class I molecules. One hypothesis is that pathogens express proteins to be recognized by inhibitory receptors on NK cells to escape recognition. As a consequence NK cells have evolved activating receptors to recognize these decoy ligands. Members of the activating Ly49 receptors are Ly49D and Ly49H. Ly49H recognizes the viral protein m157 from MCMV and Ly49D can recognize H2-D^d although this may not be its true ligand. Also, two other activating receptors have been found in NOD mice: Ly49P with specificity for H2-D^d and Ly49W that interacts with H2-D^d and H2-D^k. Example of activating KIR are KIR2DS1 recognizing HLA-C, and KIR2DS2.

NKG2D is a type II C-type lectin-like protein expressed on all NK cells in both mouse and human. It is also expressed on all human CD8⁺ T cells and inducible on mouse CD8⁺ T cells and macrophages. It recognizes the stress-inducible proteins MICA, MICB or ULBP in humans and H60, Rae1 and Mult-1 in mice (57-59). Since the ligands for NKG2D are uncommon on normal cells but widely expressed in response to cellular stress in transformed or infected cells, NKG2D may serve as a receptor searching for unhealthy, transformed cells (reviewed in (60)). NKG2D is unique in that it associates primarily with the signaling adaptor molecule DAP10 (61).

NCR, natural cytotoxicity receptors, includes the members NKp30, NKp44 and NKp46 that are expressed on human NK cells. A NKp46 homolog has also been found in mice (62). These receptors have probably evolved recently, since only NKp46 exists in mice. Also, NKp44 is lacking in macaques monkeys (63). The cellular ligands for these receptors have not been discovered, although some viral ligands have been identified (64, 65)

CD94-NKG2C/E are the activating equivalent of CD94-NKG2A. They also bind Qa1 although with a lower affinity than NKG2A.

CD16 is a low affinity Fc γ receptor expressed on a majority of NK cells that recognizes IgG antibody-coated targets resulting in elimination of that target, a process referred to as antibody dependent cell-mediated cytotoxicity (ADCC) (66).

NKRP-1C (NK1.1) has been used as a marker for NK cells in some laboratory mouse strains, such as B6. However it is not expressed on NK cells exclusively. Upon NK1.1 cross-linking, NK cells can degranulate and produce IFN γ . Although the natural ligand has not yet been discovered for NKRP-1C, other NKPR-1 molecules have been found to recognise C-type

lectin-related (Clr) molecules. Some NKRP-1 molecules are also detected on human NK cells.

DNAM-1 (CD226) recognizes nectin (CD112) or polio-virus receptor molecules (CD155). DNAM-1 is not exclusively expressed on NK cells. However, recent studies with DNAM-1 deficient mice have demonstrated its critical role in host defence against tumors (67, 68).

There are also adhesion molecules involved in activation, such as **β 1 and β 2 integrins**. Of course there are several other molecules involved in the activation of NK cells that will not be discussed here in this thesis.

2.2.4.3 Receptors with dual function

2B4 (CD244) is functional in both mice and humans and is expressed on all NK cells and some T cells. It is a member of the SLAM (signaling lymphocyte activation molecule) family of receptors and recognizes CD48, which is a cell surface glycoprotein expressed on hematopoietic cells. Two isoforms generated by alternative RNA splicing exist in mice, 2B4L (long) and 2B4S (short) (69). Initially, 2B4 was considered to be an activating receptor (70), but later studies have shown that this receptor also can inhibit NK cell function (69, 71). More recently, the group of Kumar has described that regulation of the dual function is dependent on surface expression and degree of cross-linking of 2B4 as well as levels of SAP (SLAM-associated protein) expression. High levels of 2B4 expression and cross-linking promote inhibitory function, while the opposite generate activating signals (72).

| Receptor | | | Ligand | Species | |
|--------------------|-------------|--|---|---|-------------------------|
| Inhibitory | | | | | |
| Inhibitory members | Ly49 family | | Various MHC class I molecules (H-2) | Mouse | |
| | | | CD94-NKG2A | Qa1b (mouse) HLA-E (humans) | Mouse, human |
| Inhibitory members | KIR family | | Various MHC class I molecules (HLA) | Human | |
| | | | KLRG1 | Cadherins | Mouse |
| | | | NKRP1-B, -D | Clr-b | Mouse |
| | | | LAIR-1 | Collagen | Mouse, human |
| | | | LILRB1, ILT2 | HLA class I | Human |
| | | | 2B4 | CD48 | Mouse, human |
| Activating | | | | | |
| Activating members | Ly49 family | | MCMV 157 (Ly49H) H2-D ^d (Ly49D) | Mouse | |
| | | | CD226 (DNAM-1) | CD112, CD155 | Mouse, human |
| | | | CD16 | IgG immune complexes | Mouse, human |
| Activating members | KIR family | | HLA class I | Human | |
| | | | CD94-NKG2C/E | Qa1b (mouse) HLA-E (human) | Mouse, human |
| | | | NKG2D | Rae1, MULT-1, H60 (mouse) ULBP, MICA, MICB (human) | Mouse, human |
| | | | NCR (NKp30, 44, 46) | Viral hemagglutinins? | Humans (NKp46 in mouse) |
| | | | 2B4 | CD48 | Mouse, human |
| | | | CD27 | CD70 | Mouse, human |
| | | | NKRP-1C | ? | Some mouse strains (B6) |

Table. I. Examples of activating and inhibitory receptors on NK cells. Additional molecules also contribute to the activation of NK cells, such as β 2 integrins (CD11a-c).

2.2.5 NK cells as regulatory cells

Apart from their role as “killer cells” NK cells also have immunoregulatory properties, especially in their interaction with DC, which will be discussed later. NK cells produce several cytokines, such as IFN γ , TNF, GM-SCF, and chemokines, including MIP-1 α , MIP-1 β and RANTES (12, 22, 73). Perhaps the most prominent cytokine that is released by NK cells is IFN γ . IFN γ is a type II IFN family member that is important for our defense against bacterial and viral pathogens, as demonstrated using mice deficient in IFN γ or its receptor (reviewed in (74)). It has also been shown to be important for tumor immuno-surveillance (75). IFN γ can promote the differentiation of CD4 T-cells into T_H1 cells and up regulate MHC class I

and class II molecules which enables increased activation of T cells. IFN γ also induces a nitric oxide synthase (NOS), an enzyme identified as NOS2 in macrophages and other cells. NOS2 promotes the production of particularly NO that can modify several molecules important for replication of some viruses and therefore inhibit viral spread. NK can also produce TGF β (76, 77) and IL-10 (78, 79) that antagonize proinflammatory cytokines and inhibit DC maturation, resulting in reduced T cell responses (80, 81).

In addition, NK cells can interact with cells of the immune system more directly, such as DC (see later section), T cells, B cells and endothelial cells. In lymph nodes, NK cells can drive the differentiation of CD4⁺ Th1 cells by the production of IFN γ (31, 82). NK cells have also been demonstrated to provide co-stimulation to T cells via 2B4-CD48 interactions (83) and stimulate autologous CD4⁺ T cells (84) in vitro. Furthermore, NK cells can activate B cells to secrete immunoglobulins (85). Although NK cells are able to enhance the activation of cells from the adaptive immunity, studies have also demonstrated a role for NK cells in limiting or terminating the adaptive immune response. In addition to decreasing T cell responses by eliminating DC, NK cells can also kill T cells directly. While resting T cells are insensitive to NK cell mediated lysis, activated T cells can be killed in vitro through induction of NKG2D ligands or NKp46 ligands on their surface (86, 87). In a model of EAE, blocking of CD94/NKG2A inhibitory receptors on NK cells reduced autoimmune disease due to NK cell mediated killing of autoreactive T cells (88). These studies implicate a role for NK cells in the termination of immune responses and thereby preventing the development of immunopathology. NK cells can also kill endothelial cells, a process mediated by fractaline, suggesting a role for NK cells in the pathogenesis of vascular injury (89). In contrast, NK cells have a positive role on angiogenesis during pregnancy where NK cells in the decidua produce pro-angiogenic factors such as VEGF and PLGF (90).

2.2.6 NK cells and infections

NK cells are crucial in the first line of defense against several pathogens (22, 91-93). They can respond directly by recognizing infected cells or via crosstalk with DC that have encountered a pathogen. Upon activation, NK cells can kill infected cells and/or secrete cytokines such as IFN γ or TNF that aid in limiting the infection. NK cells have been found to be involved in the defense against a wide range of pathogens including viruses, bacteria and parasites. Below follows a few examples of NK cells role during some infections.

2.2.6.1 Viral infections

Viruses are the group of pathogens that have been most extensively studied when it comes to NK cells role in protecting hosts against intruders. NK cells have been found to play a role in limiting infection for a number of viruses including herpes virus, influenza virus and papilloma virus by production of IFN γ and killing of infected cells (reviewed in (22)). Furthermore, the role of NK cells during HIV and hepatitis virus infection is being investigated (94, 95). In many viral infections NK cells are activated indirectly via IL-12 and type I interferons produced by other cells such as DC and macrophages. However, more recent studies report engagement of NK cell receptors by viral antigen, as for example MCMV.

Studies using mouse cytomegalovirus (MCMV) gave the first evidence that NK cells are important for host protection against viruses. The MCMV model system has been well studied and has greatly increased our knowledge of how NK cells can respond to viral infection. Early studies showed that NK cells could be activated by MCMV *in vivo* and that NK cells are required for efficient control of MCMV replication in C57BL/6 mice. Ly49H, an activating receptor on NK cells, play a crucial role in resistance to MCMV in C57BL/6 mice (96-98). This receptor binds to the viral glycoprotein m157, which is expressed on the surface on all infected cells.

2.2.6.2 Bacterial infections

NK cells role in bacterial infections is unclear. However, NK cells are the main producers of TNF and IFN γ in the early immune response. These cytokines can lead to production of nitric oxide (NO) in infected cells which can be bacteriostatic or bacterial lethal. Mice and patients defective in IFN γ signaling are more susceptible to bacterial infections (99). This may imply that NK cells play an important role in the immune responses towards intracellular bacteria.

NK cells have some protective effect during *Shigella flexneri* since mice deficient in the *RAG2* gene (*RAG*^{-/-} lacking B and T cells) have lower bacterial titers and increased survival compared to *RAG*^{-/-} γ c^{-/-} that also lack NK cells (100).

Infection by a pathogen can lead to changes in expression of different surface molecules, which affect the activation status of cells. It has been demonstrated that human monocytes infected with *Mycobacterium Tuberculosis* upregulate NKG2D-ligand ULBP1. Co-culturing infected monocytes with human NK cells resulted in upregulation of NKG2D, NKp30 and NKp46 on the NK cells leading to lysis of infected monocytes

(101). However, depletion of NK cells in mice does not lead to a reduction in bacterial load (102), questioning the protective effects of NK cells during *Mycobacterium Tuberculosis* infection.

NK cells have also been studied in terms of *Listeria monocytogenes* (LM) infection. Here, the results are contradictory. Previously NK cells were suggested to play a protective role, but more recent work by Berg *et al.* suggest that bystander CD8⁺ T cells may play a more significant role in the innate immunity against LM (103). In fact, NK cells might even worsen the disease, since it has been reported that type I IFNs reduce host control of fact that mice lacking DAP12 adapter protein associated with many NK cell activating receptors, show enhanced TLR responses and better control of infection (104), further adds to this hypothesis.

2.2.6.3 Parasitic infections

It is clear that NK cells are important in the defense against several protozoan pathogens. Evidence to support this comes from the fact that depletion of NK cells results in more severe parasitaemia following infection of mice with different parasites including species from *Trypanosoma*, *Leishmania* and *Toxoplasma* (105-108). In general, for these pathogens cytokines seem to play a bigger role than cytotoxicity since infected beige mice (that have disrupted lytic function) show the same survival as B6 mice. It has been shown that IFN γ is particularly important for resistance to *L. major*, *T. cruzi* and *T. gondii* to mention a few. Also, for all these parasitic infections, activation of NK cells seems to be an indirect effect by macrophage or dendritic cell-derived cytokines such as IL-12. Since I have been dealing with *Toxoplasma gondii* in my studies, a deeper description of this particular parasite will follow in a later section.

2.3 DENDRITIC CELLS

Dendritic cells (DC) were first visualized in 1868 by Paul Langerhans, who assumed, because of their morphology, that they were nerve cells (109). Modern DC research has its starting point in 1973 when Steinmann and Chon identified a “large stellate cell type” in lymphoid organs, which they named dendritic cells (110). Dendritic cells got their name from the greek word dendron, meaning tree because of their probing, tree-like or dendritic shapes. DC have been called nature’s adjuvants because of their ability to induce the adaptive arm of the immune system. DC have important functions not only in initiating an immune response against infections and tumors, but also in inducing tolerance.

2.3.1 Dendritic cell ontogeny and development

DC can be detected very early in the thymus already at embryonic day 17 (111). On day one post parturition, a substantial number of DC can be detected in the spleen. During ontogeny the numbers and proportion of different subtypes change and by 5 weeks of age the DC have reached adult levels. DC, like other blood cells, derive from hematopoietic stem cells through early progenitor cells. DC were from the beginning thought to have myeloid origin since DC could be produced from BM myeloid precursors in the presence of GM-CSF. In addition, studies demonstrated that transplantation of mouse BM common myeloid progenitors (CMP) into irradiated recipients resulted in reconstitution of the conventional DC (cDC) and plasmacytoid DC (pDC) in the spleen and thymus (DC subtypes will be described in more details in the next section) (112, 113). However, other studies showed that thymic cDC and subpopulations of cDC in spleen and LN express lymphoid markers such as CD8 α , CD4 and CD2, suggesting that some DC have lymphoid origin. It was also shown that that mouse BM common lymphoid progenitors (CLP) can differentiate into DC both in vitro and in vivo (113-115). Today, it is known that both CMP and CLP can give rise to all DC subtypes. It has also been demonstrated that there is restriction at an intermediate stage downstream of these early progenitors (116, 117).

One problem in studying DC in the beginning was difficulties in isolating DC because of lack of specific markers and paucity of DC. In the beginning of the 1990s, there was a major break-through in DC research when it was established that a substantial number of DC could be cultured in vitro from progenitors both in mice and humans. It was first discovered that DC could be cultured from mouse bone marrow and blood in the presence of GM-CSF (118, 119). Cells resembling human dermal DC (120) could then be obtained from human blood monocytes cultured in GM-CSF and IL-4 (121, 122). Addition of TGF β gave rise to Langerhans cells (123). For a long time GM-CSF has played a very central role in the in vitro culturing of DC. However, there is no direct evidence that these cells generated in vitro have an equivalent in vivo during steady-state conditions (124). Thus, injecting mice with GM-CSF does not lead to a clear increase of CD11c⁺ cells (125). These DC seem to increase during inflammation, thus they have been called inflammatory DC. Injecting Flt3L on the other hand, has shown to increase cells with typically DC characteristics markedly (126, 127), indicating that this may be a very important cytokine when it comes to DC development in vivo during steady-state conditions.

2.3.2 Different DC subsets

DC represent a very heterogeneous cell type that can be further divided into several subsets both in mice and humans. The fact that there are also differences in the DC repertoire during steady-state or inflammatory conditions further adds to the complexity. In mice the major subtypes have been segregated according to the markers CD4 and CD8. The integrin CD11b, which is a myeloid marker, 33D1 and CD205 (the multilectin domain molecule DEC-205) are other markers used to describe mouse DC. A common marker for all mature DC is the integrin- α_x chain CD11c. During my studies I have worked with the mouse system so my focus in this thesis is on mouse DC. I will briefly go through some DC subtypes that have been examined during my studies, which is very nicely reviewed by Shortman and Naik (128).

2.3.2.1 Conventional DC

Conventional DC (cDC) can be referred to as cells having dendritic cell form and function. This category of DC includes **lymphoid-tissue-resident DC** and **migratory DC**.

Lymphoid-tissue-resident DC include cDC resident in lymphoid tissues, such as spleen, thymus and lymph nodes. They can be divided into three subtypes consisting of:

- CD205⁺CD11b⁻CD8⁺ (CD8 α^+ DC)
- CD205⁻CD11b⁺CD8⁻ (conventional or CD11b⁺ DC). This subtype can be further divided into CD4⁺ and CD4⁻ subsets.

These lymphoid-tissue-resident DC do not migrate through the lymph, but instead collect and present foreign and self-antigens in this organ. It should be noted that LN and thymus can contain other cDC subtypes that will not be discussed here. The different cDC subsets differ in their cytokine production (129) and presentation of antigens on MHC class I molecules (130, 131) (**Table II**). For example, CD8 α^+ DC, but not CD8 α^- DC are able to cross-prime cytotoxic T cells (132). Activated CD8 α^+ DC also tend to induce a more T_H1-biased cytokine response in CD4⁺ T cells, in contrast to CD8 α^- cDC that seem to induce a T_H2-biased response (133-135). CD8⁻CD4⁺ DC are better producers of chemokines, such as MIP1- α , MIP-1 β and RANTES as compared to the rest of splenic cDC (136). They also differ in their location in the spleen where CD8 α^+ DC are concentrated to the T cell areas and CD8 α^- DC in the marginal zone of mice (137). Equivalents of these splenic cDC can be cultured from bone marrow in vitro with Flt3L (124). They show similar characteristics as splenic cDC in vivo in terms of mRNA expression of TLR and chemokines receptors, production of cytokines/chemokines and expression of CD11b. Although

in vitro Flt3L-derived cDC do not express CD8 α on their surface, they will upregulate its expression when transferred in vivo. Although human DC do not express CD8 α , similar subsets exist in human blood. Human BDCA3⁺ DC are thought to be equivalent to the murine CD8 α ⁺ DC, while human BDCA1⁺ DC are equivalent to the murine CD8 α ⁻ DC (138, 139).

Migratory DC are cells that reside in the periphery, sampling antigens and carrying them to lymph nodes for presentation to T cells. The classical text-book DC, Langerhans cells (LC) and dermal DC belongs to this category. LC express high levels of langerin associated with the Birbeck granules (140) and have a long lifespan in the skin, but turn over quite rapidly once they reach the lymph nodes (141). In humans, they express mRNA for TLR1, 2, 3, 5, 6 and 10 enabling them to respond to viruses and gram-positive bacteria (142)

2.3.2.2 *Plasmacytoid dendritic cells*

Plasmacytoid dendritic cells (pDC) is a relative newly defined subset of DC. When this cell with plasmacytoid appearance and a unique set of surface antigens first was discovered it was named plasmacytoid T cell or plasmacytoid monocyte (reviewed in (143)). In 1997, these cells were renamed to plasmacytoid DC because they had characteristics of precursor DC (144, 145). At the same time, a small cell type and low in number in human blood with a very high capacity to produce type I interferon in response to certain viruses had been described, which was called natural interferon producing cell (NIPC) (146). In 1999, it was shown that pDC, like NIPC, could produce high levels of type I interferons and the two fields of research merged (147, 148). This work was performed primarily with human pDC and the first reports of a murine equivalent came in 2001 (149-151). These cells are a bit mysterious still, since there are many theories about their origin. It is not yet clear if they follow myeloid or lymphoid developmental pathways or simple a pathway of their own. To complicate things further, pDC have been called pre-cDC because upon inflammatory stimuli, they convert into a dendritic form and acquire some antigen-presentation properties of cDC (152). In the text that follows I will refer to mouse steady state pDC characterized by the surface phenotype CD11c^{int}B220^{high}, Ly6C^{high}. pDC can be grown in vitro from bone marrow in the presence of Flt3L. Recently, it has also been demonstrated by Francke *et al.* that M-CSF can drive differentiation of pDC from bone marrow precursors both in vitro and in vivo (153).

pDC are quite rare cells and account for less than 1% of PBMC in blood. They can be found in many tissues like bone marrow, liver, blood, thymus and T-cell areas of lymphoid organs (150, 154-157). They display strong

expression of TLR7 and TLR9, whose ligands are viral and synthetic ssRNA and CpG DNA, respectively (158-160). Upon encounter with certain agents, like viruses and bacteria expressing these molecules, pDC become activated and secrete a number of cytokines and chemokines, perhaps the most prominent one being IFN α . IFN α can inhibit viral replication in infected cells (161) and plays a major role in antiviral defense (162), but can also affect cells of both innate and adaptive immune system. This includes enhancing cytotoxic activity of NK cells and macrophages (163), enhancing survival of T cells (164) and promoting antibody production by B cells (165). In contrast to many other cell types, pDC do not have to be infected themselves to produce type I IFN and they are totally superior in speed and amounts of IFN α produced. The reason for this is probably that pDC constitutively express members of the interferon regulatory factors (IRFs), that play an important role in the regulation of interferon gene transcription (166, 167).

pDC are generally considered to be immune-modulating cells with regards to their production of type I interferons and the antigen presenting capacity of pDC has been a matter of debate. It is clear that pDC upon activation can activate naive T cells, but priming is less efficient than for cDC (168, 169), perhaps due to lower expression of MHC class I and costimulatory molecules on pDC. Furthermore, pDC present mostly endogenous antigens rather than exogenous antigens (170).

2.3.2.3 Inflammatory DC

As mentioned earlier, culturing bone marrow cells in GM-CSF give rise to DC that has not been described in vivo in mice during steady state. However this subtype of cells seems to increase in vivo during inflammation, hence the name. In vitro derived GM-CSF DC have been widely used to study DC biology, including this thesis work, because the simplicity in getting large amounts of cells. These DC mature upon microbial stimulation and upregulate MHC molecules and costimulatory molecules. They are also very capable of antigen presentation and cytokine/chemokine production. A study by Dearman *et al.* demonstrate that murine in vitro derived GM-CSF DC can respond to most TLR ligands, except for ligands for TLR3 and TLR7 (171). Another example of inflammatory DC is the DC population that appears after infection of mice with *Listeria monocytogenes*, called Tip DC because of their production of TNF and iNOS (172)

| | CD8α⁺ CD4⁻ | CD8α⁻ CD4⁺ | CD8α⁻ CD4⁻ | pDC |
|---|---|---|---|------------|
| Phenotype | | | | |
| CD11b | - | + | + | - |
| CD11c | ++ | ++ | ++ | + |
| Ly6C | - | - | - | + |
| B220 | + | + | + | +++ |
| DEC-205 | + | +/- | - | - |
| Function | | | | |
| IL-12 | +++ | - | - | + |
| IFN γ | ++ | - | ++ | - |
| IFN α | + | - | - | +++ |
| Crosspriming of CD8 ⁺ T cells | + | - | - | - |
| MHC class I presentation | +++ | - | - | - |
| MHC class II presentation | + | +++ | ++ | + |

Table II. Phenotype and function of different DC subsets in murine spleen.

2.3.3 Antigen presentation and stimulation of the adaptive immune system

Immature DC act as sentinels in peripheral tissue, sampling antigen from the environment. DC can capture viruses, bacteria, dead/dying cells, protein and immune complexes via processes like phagocytosis, endocytosis and pinocytosis. Upon encounter with pathogenic antigen or tissue damage, DC begin to migrate to draining lymph nodes, a phenomenon first described by Macatonia and colleagues. (173, 174). To facilitate antigen recognition and activation of immunity, DC express an array of receptors called Toll-like receptors (TLR). Ligands for TLR are various pathogen-associated molecules, such as LPS and unmethylated CpG DNA (**Figure 2**). Most TLR signal via the adaptor protein MyD88, although MyD88-independent signalling exists as well. Upon stimulation of TLR by their respective ligands, DC mature by upregulating MHC class I and class II molecules, co-stimulatory molecules and initiate secretion of cytokines/chemokines. This, in turn, enables DC to activate cells of the adaptive immune response (especially T cells), but also innate immune cells, such as NK cells. Different DC subsets express different patterns of

TLR and that in combined with their different localization pattern and cytokine production suggest that DC subtypes have evolved to handle different pathogens.

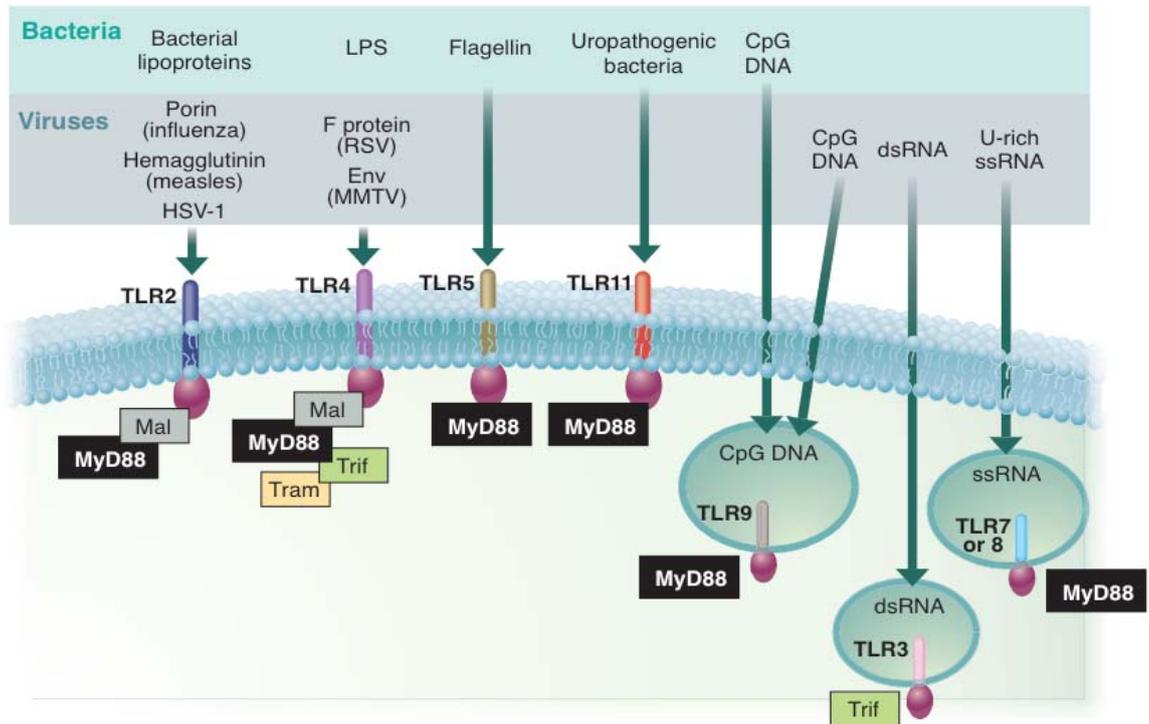


Figure 2. Toll-like receptors and their respective ligands. Modified from (175)

Upon maturation DC lose their ability to take up antigens, but instead up regulate MHC molecules and co-stimulatory molecules required for activation of T cells. Depending on where the antigen is captured, different processes take place. If a dendritic cell captures an exogenous antigen (for example bacteria), the antigen is processed onto MHC class II. However, if a DC is infected with a virus, antigen can be synthesized in the cytosol leading to presentation by MHC class I molecules. The cellular processes leading to antigen presentation by different MHC have been extensively studied and are reviewed in (176-179). In general, endogenous antigens presented on MHC class I are recognized by CD8⁺ T cells, while exogenous antigen from the environment presented on MHC class II is presented to CD4⁺ T cells. However, another term called cross-presentation, is an exception to this rule. This term refers to priming of CD8⁺ T cells as a consequence of presentation of exogenous antigen on MHC class I (180-183).

DC do not only sample foreign antigen, indeed they also present self antigens. Since no PPR is engaged on the DC when sampling self-antigens

in a non-inflammatory environment, no maturation is induced leading to presentation of self-antigen to T cells without co-stimulatory signals. This, in turn, leads to tolerization of potentially self-reactive T cells in the periphery. The concept of tolerance to self-antigens is critical for the prevention of autoimmunity.

2.4 NK-DC INTERACTIONS

Originally, DC were described to capture and present antigens and prime the adaptive immune response. The function of NK cells was to lyse tumors and virally infected cells. Today, it is evident that these two cell types also have an important function in regulating the adaptive immune response by cell-to-cell crosstalk. In the last fifteen years, studies of the interactions between NK cells and DC and their effect on adaptive immune responses have just exploded. Many studies have shown that this crosstalk can result in cellular maturation, activation and also death. In 1985, the first indication came that NK cells might be capable of regulating adaptive T cell responses by eliminating DC that have interacted with antigen (184). Later 1999, Fernandez *et al.* published the first evidence in vivo that DC could trigger NK cell mediated anti-tumor effects (185). Many studies have followed demonstrating that NK cell and DC have reciprocal effects on each other (186, 187).

DC can activate NK cells via both cytokines and cell-to-cell contact. Two DC-produced cytokines important for NK cell activation are IL-12 and type I IFN. IL-12 is important for IFN γ production by NK cells (188-190), while type I interferons have been demonstrated to drive cytotoxicity of NK cells (188, 189). IL-2 has always been used to culture NK cells in vitro and when it was demonstrated that DC can produce this cytokine after certain stimulation, the role of IL-2 in NK-DC crosstalk was investigated. In vitro-derived *E.coli*-activated DC could enhance IFN γ production by NK cells, which was dependent on DC-derived IL-2 (191). IL-2 production by DC is inhibited by IL-4, while IL-4 is needed to enhance IL-12 secretion by DC (192). This may have impact on the results when investigating in vitro-derived DC interactions with NK cells since some research groups include IL-4 when culturing DC, while some do not. IL-15 is also an important factor for activation of NK cell during co-culture with DC (24). In line with this, Koka *et al.* suggested that DC could trans-present IL-15 on their IL-15 receptor, which enhances both killing and IFN γ production by NK cells in vitro (193).

Several *in vivo* studies have shown that *in vitro* derived DC that have been activated by different stimuli, such as TLR ligands, can induce migration of NK cells to draining lymph nodes (31, 194). There, IFN γ from recruited NK cells polarize the adaptive immune response to be T_H1 dominated (31). Lucas and colleagues very nicely demonstrated by inducible ablation of CD11C^{high} cells that NK cell responses to viral and bacterial pathogens *in vivo*, is also dependent on DC. Furthermore, type I interferon-experienced DC could prime NK cells by trans-presenting IL-15 in lymph nodes (20), consistent with what Koka and colleagues described *in vitro*. It has also been shown that inducible ablation of CD11c⁺ DC reduces NK and T-cell responses leading to increased susceptibility to herpes simplex virus type I infection (191).

NK cells can in turn induce maturation and IL-12 production by the DC, which have been reported to depend on cytokines such as TNF and IFN γ , but also cell-to-cell contact, including NKp30 for human cells (186, 187). This could be important for initiation of immune responses during circumstances where DC are not activated directly by infection or tumors. In line with this, it has been demonstrated that recognition of MHC class I tumor cells by NK cells activated DC, which in turn led to induction of CD8⁺ T cell responses (195). One important factor in controlling the outcome of NK-DC interactions, at least *in vitro*, is the NK:DC ratio. It appears as if low NK:DC ratio results in DC maturation and high NK:DC ratio results in inhibition of DC function (187).

In contrast to what is described in the section above, another consequence of NK-DC interactions is lysis of DC by activated NK cells. Initial studies by Chambers *et al.* showed that NK cells could kill immature DC *in vitro* (196). This was also demonstrated *in vitro* for human cells (197-199). In general, immature DC are considered to be targets to NK cells and mature DC, due to upregulation of MHC class I molecules, are protected from NK cell-mediated lysis (200). However, in paper I in this thesis, NKG2A⁻ NK cells seemed to be able to lyse also IFN γ -stimulated DC to some extent. In humans, the NK cell population responsible for lysis of autologous immature DC expresses NKG2A/CD94, but lack KIR (201). NKp30 has been demonstrated to be able to mediated killing of iDC *in vitro* in humans (202), but so far no such receptor has been demonstrated in mice. Recently, another receptor named DNAM-1, recognizing CD112 and CD155, has also been proposed to be involved in NK cell-mediated lysis of DC (203). There are considerable amounts of literature on killing of immature DC *in vitro*, but there is still a bit of debate how common lysis of DC by NK cells is *in vivo*, especially in an autologous setting. Hayakawa *et al.* showed that killing of DC *in vivo* requires TRAIL and that NK cell-mediated elimination of peptide-loaded DC resulted in

reduced anti-tumour responses (41). In relation to transplantation, Ruggieri *et al.* and Velardi *et al.* have demonstrated a role for allogeneic NK cell lysis of host DC for the prevention of graft-versus-host disease in a bone marrow transplantation model (204, 205). More recently, Yu *et al.* demonstrated that NK cell elimination of allo-dendritic cells also led to acceptance of allo-grafted skin in mice (206).

One question that is usually asked is where the crosstalk between these cells actually takes place *in vivo*. Studies have shown that NK cells can be found in close association with DC in the lymph node (207, 208) and also in inflamed skin as shown by Buentke *et al.* studying patients with atopic eczema/dermatitis syndrome (209). In addition, it has been reported that depletion of NK cells affects both the number and activation state of DC in the lymph nodes (208, 210). Since NK cells can be recruited to sites of inflammation it is likely that NK cells and DC can interact also in the periphery upon infection or during anti-tumor responses.

In summary, the outcome of NK–DC interactions depends on several factors, including cytokines, cell surface molecules and cell ratio. It is clear though, that the crosstalk between these cells greatly influence the outcome of the adaptive immune response. It should be noted though, that most studies mentioned above investigating the mechanisms behind NK-DC crosstalk have involved DC derived from bone marrow *in vitro* in GM-SCF +/- IL-4. More studies needs to be performed especially *in vivo* to learn more about the mechanisms behind this interaction enabling us to manipulate this crosstalk to possibly improve therapies targeting these cells in the future.

NK-DC interactions are important in the host defense against many pathogens. In my thesis I have examined NK cell interactions with DC in the course of *Toxoplasma gondii* infection. Below follows a brief presentation of this particular parasite.

2.5 TOXOPLASMA GONDII

Toxoplasma gondii is a crescent-shaped, parasitic protozoan belonging to the phylum Apicomplexa (**Figure 3**). The parasite was first described in 1908 by Nicolle/Manceaux in North Africa (211, 212) and by Splendore in Brazil (213). It can infect a variety of warm-blooded mammals including humans (214) and up to one third of the world's population is estimated to carry a *Toxoplasma* infection (215).. The primary hosts are members of the Felidae family (domestic and wild cats), in which the parasite has its sexual reproduction. The parasite is the causative agent of

Toxoplasmosis, a disease that usually leads to mild flu-like symptoms or no symptoms at all in healthy individuals and the disease is self-limiting. However, in immunocompromised hosts or fetuses that are infected via placenta the disease can cause severe illness and be fatal.

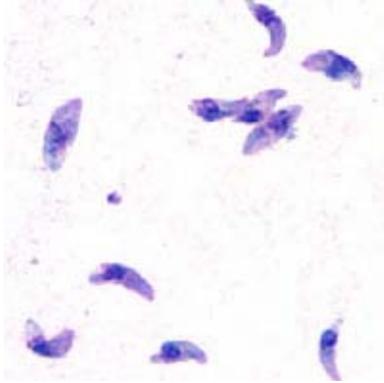


Figure 3. Giemsa staining of *T. gondii* tachyzoites

2.5.1 Life cycle

The life cycle of *Toxoplasma gondii* was described in 1970 when it was discovered that members of the Felidae family are the primary hosts (216, 217). In order to be successful the parasite has to establish long-lasting chronic infections in their immunocompetent intermediate host to increase its chances for transmission to the definitive host, the cat. There are three major routes of transmission: congenitally, via feces or by consumption of poorly cooked infected food (218).

Toxoplasma is an obligate intracellular pathogen and it is able to infect basically any nucleated mammalian or avian cell (219, 220). The life cycle is divided into two phases: the sexual cycle that takes place in feline intestine, and the asexual cycle that occurs in mammals or birds (**Figure 4**). The asexual phase has two distinct stages of growth depending on if the infection is acute or chronic. The tachyzoite stage that is characterized by rapidly growing parasites found during the acute phase of toxoplasmosis. They replicate inside cells until they exit and infect neighbouring cells. Free tachyzoites are usually efficiently cleared by the immune system, but some manage to differentiate into bradyzoites. Bradyzoites are the slowly replicating form of the parasite that forms tissue cysts mainly within muscle tissue and in the central nervous system where the parasite can reside for a lifetime of the host. The development of cysts defines the chronic stage of the asexual cycle. Cysts can be ingested by eating infected tissue and are ruptured when passing the digestive tract. This causes the release of bradyzoites that can infect the epithelium and differentiate back to the tachyzoite stage. The tachyzoites can rapidly divide and disseminate

throughout the body, thereby completing the asexual cycle. Also, reactivation of bradyzoites differentiating into tachyzoite can occur in immunocompromised hosts, which can lead to severe injuries.

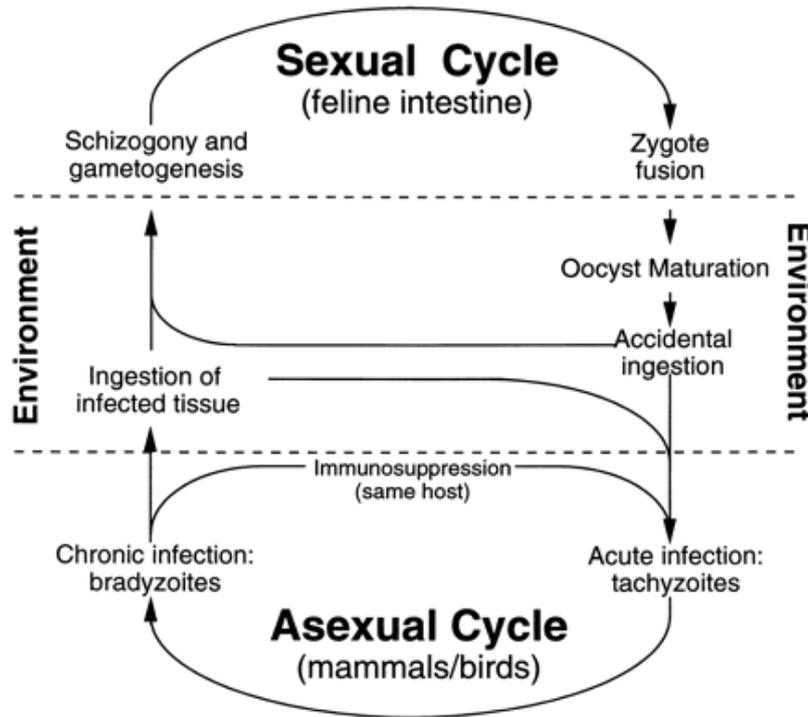


Figure 4. The life cycle of *Toxoplasma gondii*. Adapted from (214)

Tissue cysts can be ingested by cats (by for example eating infected mice) and the bradyzoites released initiate the formation of a number of asexual generations before the sexual cycle begins. During the sexual cycle oocysts are formed, which is secreted in the feces. Oocysts can then be accidentally ingested by the intermediate host and the whole cycle is complete.

2.5.2 Genotypes and pathogenesis

The *T. gondii* population in Europe and North America is dominated by three different clonal lineages, designated strain types I, II and III, with type II infections dominating in humans (221-223). These genotypes display different virulence in the mouse model where type I strains are lethal, whereas type II and III strains are less virulent and can establish chronic infections (224).

As mentioned previously, *Toxoplasma* only causes mild flu-like symptoms or no symptoms at all in healthy individuals. The immune system avoids disease but is not able to clear the infection causing a chronic stage. In contrast, in immunocompromised hosts, i.e. patients with AIDS or patients under immunosuppressive drug treatment, infection can result in life-threatening toxoplasmosis with encephalitis (225). Another risk group of toxoplasmosis are fetuses that can be infected prenatally if the mother is primary infected during pregnancy. This is due to *Toxoplasma*'s ability to transmigrate through the placenta and replicate within different fetal tissues without being recognized by the premature immune system. In the fetus, *T. gondii* usually infects the brain and retina and can cause severe damage to these organs (226).

2.5.3 Immune responses to *Toxoplasma gondii*

T. gondii is one of the most successful intracellular protozoans, which manages to survive and persist in healthy individuals for a lifetime of the host. This is despite of a vigorous immune activation during infection. Here follows a brief description of the immune response to *T. gondii* and the mechanisms by which the parasite can escape immune recognition.

Upon infection *T. gondii* is able to cross biological barriers and rapidly disseminate (227). In this process, the parasite actively invades host cells by a term called gliding motility, which is dependent on actin (219). Within the cell it establishes a non-fusogenic parasitophorous vacuole that remains segregated from host endocytic/lysosomal compartments (228, 229), thereby creating a safe harbour for the parasite. Upon infection, *T. gondii* first encounters the innate immune system, including macrophages, DC and NK cells. Following activation, macrophages and DC produce IL-12, which is an important cytokine for an effective immune response (230, 231). This cytokine induces IFN γ production by NK cells during early infection, and later, by CD4⁺ T cells. IFN γ produced by NK cells and T cells is the crucial cytokine involved in controlling *T. gondii* during the effector phase (232). It activates several anti-parasitic effects in macrophages including inhibition of parasite replication or even destruction induced by oxidative mechanisms (233, 234) or NO production (235). IFN γ and IL-12 also results in a T_H1 response crucial for controlling the acute infection.

Innate immune responses result in the differentiation of activated APC able to stimulate the adaptive response. CD4⁺ and CD8⁺ T cells have been reported to be the main players involved in resistance to *T. gondii* (reviewed in (236)). CD4⁺ T cells are important for the regulation of the

immune response (237, 238), while CD8⁺ T cells play a major role as effector lymphocytes able to kill both tachyzoites or infected cells (239, 240). Because of the intracellular lifestyle of *T. gondii*, antibody responses have been suggested to play a reduced role during acute infection. However, Kang *et al.* reported that mice deficient in B cells survive early infection but die 3-4 week post infection, suggesting an important role for antibodies in resistance to persistent tachyzoite activation (241). Also, antibodies are important tools for diagnosis of toxoplasmosis and protection of fetuses by crossing the placenta.

To date, several mechanisms of immune evasion of *T. gondii* have been described. Below follows some examples of mechanisms of parasite evasion:

- The parasitophorous vacuole. The presence of the parasite inside the parasitophorous vacuole enables it to avoid the humoral immune defense with complement, enzymes and proteolysis. Also, the parasitophorous vacuole does not acidify because it resists phagosome-lysosome fusion.
- Apoptosis. During acute infection in mice, CD4⁺ T cells have been reported to undergo apoptosis leading to immune suppression (242). However, *T.gondii*-infected cells showed reduced apoptosis induced by the parasite (243-246), ensuring survival of the infected cells.
- Immune suppression. Infection of *T. gondii* leads to secretion of both pro-inflammatory and anti-inflammatory cytokines. IL-10 triggered by infection suppresses the host cellular immune response both in mice and humans leading to avoidance of acute inflammation that could lead to death of the host (247). Secondly, IL-10 may also facilitate parasite survival within macrophages by reducing IFN γ -induced toxoplasmaicidal activity (247, 248). Hence, IL-10 production can be beneficial for both parasite and host and favor a stable interaction between these two. Also, *T. gondii* is able to inhibit IL-12 and TNF production resulting in inhibition of T_H1 responses (249-252).

In conclusion, *T. gondii* is a clever parasite that manages to keep a delicate balance between induction and suppression of the immune response in order to guarantee survival of the host and increasing the chances of parasite transmission to its definite host.

3 RESULTS AND DISCUSSION

I will in this section discuss my results with a more personal tone and in the view of other research performed. For further details about methods and figures I refer to the papers included this thesis.

3.1 CRITICAL ROLE OF QA1^b IN THE PROTECTION OF MATURE DENDRITIC CELLS FROM NK CELL-MEDIATED KILLING (PAPER I)

The fact that NK cells can kill immature DC in vitro has been convincingly shown. Still, there is a debate whether this happens in vivo. Mature DC are protected from NK cell-mediated lysis and this has previously been shown to be due to upregulation of classical MHC class I molecules on the surface of the mature DC (198, 199). In this paper we investigated another molecule's role in the protection of mature DC, namely the non-classical molecule Qa1. Qa1 is the ligand for the NK cell inhibitory receptor NKG2A (49) and activating receptors NKG2C/E. Qa1 is expressed on most normal cells and predominantly bind the Qdm (Qa-1 determinant modifier) peptide, sequence AMAPRTLTL, derived from the leader sequence of H-2D/L molecules (50, 253).

3.1.1 Mature DC are protected from NK cell mediated killing both in vitro and in vivo

In line with previous studies (41, 199, 201), we confirmed that DC stimulated with either IFN γ or LPS are less sensitive to NK cell lysis both in vitro and in vivo (Paper I Fig.1). By performing flow cytometry, we found that not only were classical MHC class I molecules up regulated, but also the non-classical MHC class I molecule Qa1^b (Paper I Fig. 2).

3.1.2 NKG2A⁻ NK cells are capable of killing mature DC as well as immature DC

After observing the upregulation of Qa1^b on mature DC, we explored the importance of the Qa1-NKG2A interaction in the protection of mature DC. By sorting IL-2 expanded NK cells into NKG2A⁺ and NKG2A⁻ populations using both FACS sorting and magnetic sorting, we investigated the respective populations ability to lyse immature and mature DC. It has been shown previously by Della Chiesa *et al.* that the human NK cell subset responsible for killing immature autologous GMSCF/IL-4 stimulated monocyte-derived DC express NKG2A but lack KIR (201). In

contrast, I found that both NKG2A⁺ and NKG2A⁻ NK cells were capable of killing immature DC (Paper I Fig. 4). To our surprise, since mature/activated DC are considered to be "protected" from NK cell lysis, we found that NKG2A⁻ NK cells were also quit potent killers of IFN γ -stimulated DC as well, even though these NK cells express other functional inhibitory Ly49 receptors.

3.1.3 Qa1 can protect mature DC from NK cell mediated killing even in the absence of classical MHC class I molecules.

Classical MHC class I molecules in B6 mice is represented by H-2K^b and H-2D^b. To determine which MHC class I molecules are important for protection of stimulated DC from NK cell lysis we purified DC from wt, B6.K^{b-/-}, B6.D^{b-/-} and B6.K^{b-/-}xD^{b-/-} mice. By using a rapid elimination assay, where these DC were stimulated with IFN γ , labeled with ⁵¹Cr and injected i.v. into B6 mice, we investigated their survival in vivo (**Figure 5**). DC generated from wt and B6.K^{b-/-} mice showed greater survival than DC from B6.D^{b-/-} and B6.K^{b-/-}xD^{b-/-} mice, indicating the importance of the Db molecule in protecting DC from elimination in vivo (Paper I Fig. 3A).

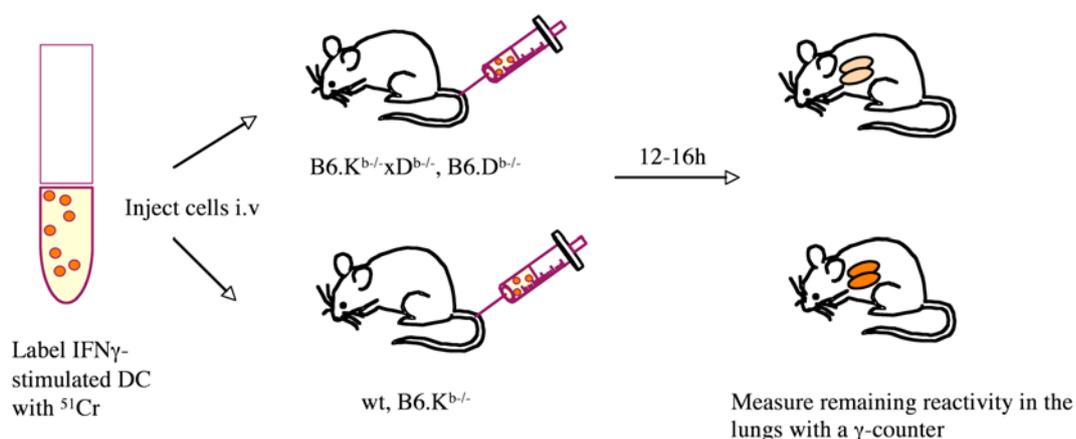


Figure 5. Rapid elimination assay. IFN γ -stimulated DC from wt, B6.K^{b-/-}, B6.D^{b-/-} and B6.K^{b-/-}xD^{b-/-} were labeled with ⁵¹Cr and injected i.v. into mice. 16h later the lungs were removed and remaining radioactivity were mesured in a γ -counter. Lungs from mice injected with DC from wt and B6.K^{b-/-} mice displayed higher radioactivity than from mice injected with DC from B6.D^{b-/-} and B6.K^{b-/-}xD^{b-/-} mice, indicating an increased survival of these DC.

Also, *in vitro*, DC from wt and B6.K^{b-/-} mice showed reduced susceptibility to NK cell lysis after stimulation. In contrast, DC from B6.K^{b-/-}xD^{b-/-} were still sensitive to NK cells after IFN γ -treatment (Paper I Fig. 3B). This led us to the conclusion that expression of the D^b molecule was important, but the question remained if it was actually the D^b molecule itself or the leader peptide crucial for Qa1 signaling through NKG2A?

In order to investigate this question we used TAP^{-/-} mice that are unable to load TAP dependent peptides onto their MHC class I molecules. The result is that these mice basically lack MHC class I molecules on their surface. Furthermore, since Qdm transported by TAP is needed for a stable expression of Qa1 on the cell surface (254), cells from these mice also have reduced surface expression of Qa1, unless Qdm peptide is provided externally. DC from TAP^{-/-} mice did not increase their expression of K^b, D^b or Qa1 upon IFN γ stimulation as observed by FACS analysis. Neither were they less susceptible to NK cell lysis upon maturation (data not shown). By providing IFN γ -stimulated TAP^{-/-} DC with exogenous Qdm, the expression of Qa1 on the cell surface was increased (data not shown). This resulted in reduced killing by NKG2A⁺ NK cells compared to stimulated TAP^{-/-} DC loaded with control peptide (Qdm-C4). However, NKG2A⁻ NK cells killed stimulated TAP^{-/-} DC loaded with Qdm or control peptide equally well (Paper I Fig. 5A). To validate these results *in vivo* we once again used a rapid elimination assay. IFN γ -treated TAP^{-/-} DC were loaded with either Qdm or control peptide, labeled and injected *i.v.* into B6 mice that were untreated or depleted of NK cells. An increased survival was observed for DC loaded with Qdm peptide compared to control peptide (Paper I Fig. 5B). The level of protection was similar to that of B6 mice receiving DC loaded with control peptide that had been depleted of NK cells. Thus, stabilizing the Qa1 expression on mature DC induce protection of mature DC even in the absence of classical class I molecules. These results imply that Qa1-NKG2A interaction is crucial in protecting mature DC from NK cell mediated killing.

3.1.4 Implications of these results

Our results indicate that stabilizing Qa1 expression on the cell surface of DC resulting in signaling through the NK cell inhibitory receptor NKG2A, increase survival of these DC *in vivo*. This might have implications in improving DC-based vaccines. Indeed, it has been shown previously that NK cell-mediated elimination of DC *in vivo* lead to reduced anti-tumor responses and reduced development of anti-peptide-specific cytotoxic T lymphocyte responses (41). Thus loading DC with Qdm peptide on Qa1 in addition to tumor derived peptides on classical MHC class I molecules

prior to inoculation would increase the number of DC available to stimulate T cells and thus enhance any anti-tumor T cell response.

3.2 PLASMACYTOID DC-INDUCED MIGRATION AND ACTIVATION OF NK CELLS IN VIVO (PAPER II)

In **Paper I**, the focus was on NK-cell mediated killing of DC. In **Paper II**, the focus was shifted to another consequence of NK-DC interactions, namely the activation of NK cells induced by activated DC. There is not that much published about NK-DC interactions in vivo and most published work has focused on DC generated in vitro with GM-CSF. In this study, we explored another DC subtype that may actually have more potential to stimulate recruitment and activation of NK cells, namely plasmacytoid DC (pDC). pDC express TLR7 and TLR9 and when stimulated with their respective ligands, ssRNA and CpG, produce the NK cell-activating cytokine IFN α . Also, upon activation, pDC produce more of the NK cell recruiting chemokines CCL3, CCL5 and CXCL10 than conventional DC (136, 255-257). These facts made us curious about the potential of pDC as activators of NK cells in vivo. As many studies regarding DC-induced migration and activation of NK cell in vivo have focused on lymph nodes (31, 194), we found it interesting to also study NK-DC interactions in a peripheral tissue, namely the peritoneum. Recruitment of NK cells to the peritoneum has been studied previously in relation to infection and tumor inoculation (258-260).

3.2.1 pDC induce recruitment to the peritoneal cavity

I began this study by making a comparison between different DC subtypes and their ability to induce migration of NK cells to the peritoneal cavity. **Figure 6** illustrates the method used in this paper to study recruitment. Bone marrow derived DC were cultured in GM-SCF (mDC), conventional and plasmacytoid DC (cDC and pDC) were cultured from bone marrow in Flt3L and then sorted for specific markers. They were all stimulated with TLR ligands, LPS for mDC and cDC and CpG DNA for pDC. All mice given activated DC subtypes exhibited increased numbers of NK cells in the peritoneum when compared to mice given control DC (Paper II Fig. 1). No significant differences were observed in the numbers of NK cells recovered from the peritoneal cavities of mice given PBS or control DC. However, all mice receiving activated pDC seemed to have more NK cells compared with the mice receiving activated GM-CSF-derived DC or activated cDC (Paper II Fig. 1). Splenic pDC were purified and mice receiving activated splenic pDC also had more NK cells in the peritoneal

cavity compared to mice that received control pDC. T cells or NKT cells did not contribute to the increase NK cell numbers since the same results were obtained in B6.RAG1^{-/-} mice (data not shown).

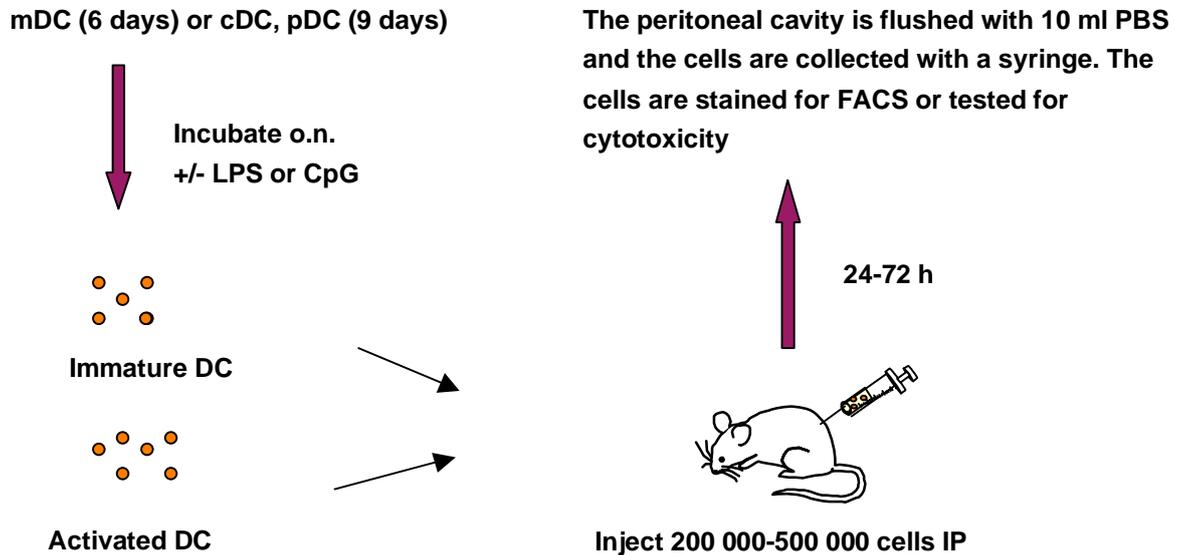


Figure 6. Description of the method used to study recruitment of NK cells to the peritoneum of mice used in paper II and also *T. gondii* infection of NK cells in vivo in paper III.

To demonstrate that the observed increase in numbers of NK cells was recruitment of NK cells to the peritoneum and not just proliferation of resident cells, freshly isolated CFSE labeled NK cells were adoptively transferred i.v. into B6.cyxRAG2^{-/-}, which lack NK cells. One day later, CpG-activated pDC were injected i.p. and the peritoneal exudates were examined after 72 h. Mice receiving CpG-activated pDC had more NK cells in their peritoneal cavities when compared to mice that received control pDC or PBS (Paper II Fig. 2). In addition, if CFSE labeled NK cells were injected i.p., instead of i.v., no division was observed. This demonstrated that the increased number of NK cells in the peritoneum following inoculation with activated pDC was primarily due to recruitment.

Next, I investigated which mechanisms could be involved in this recruitment. CD62L, which is expressed on most murine splenic NK cells, has been shown to be involved in recruitment of NK cells to lymph nodes (31). When mice were pre-treated with anti-CD62L a reduction of recruited NK cells was observed (Paper II Fig. 3). CD62L is known to be

important for the homing to secondary lymph nodes via high endothelial venules. However, the result in Fig. 2 of this paper suggests that NK cells migrate to the peritoneum independent of lymph nodes since B6.cyxRAG2^{-/-} basically lack lymph nodes.

It has been shown that pDC can make chemokines such as CCL3, CCL5 and CXCL10 (136, 255-257). CCL3 and CCL5 are ligands for CCR5, which has previously been shown to be important for recruitment of NK cells during *Toxoplasma gondii* infection (261). CXCL10 is a ligand for CXCR3, which has been associated with NK cell migration to lymph nodes (31). We tested if these receptors were involved in pDC-induced recruitment to the peritoneum by injecting CpG-activated pDC into B6.CCR5^{-/-} and B6.CXCR3^{-/-} mice. Significantly reduced NK cell numbers were observed in B6.CXCR3^{-/-} mice compared to B6 (Paper II Fig. 4). CCR5^{-/-} mice showed reduced numbers as well, but not significantly different from B6 mice. This could possibly be explained by the fact that the CCR5 ligands CCL3 and CCL5 also can bind CCR1 (262-265) and that CCR1 on NK cells may compensate for the lack of CCR5 in the migration of NK cells to the peritoneum. Migration of NK cells to the peritoneal cavity in response to tumor inoculation has been shown to be dependent on TNF (259). However, TNF did not seem to be important in pDC-induced migration since no difference in NK cell numbers was observed in mice treated with anti-TNF antibody compared to isotype control (data not shown).

3.2.2 Activation of NK cells in vivo

One benefit with studying recruitment to a compartment like the peritoneal cavity is that one can easily recover the cells and test their activation ex vivo. So, after discovering that activated pDC can induce recruitment of NK cells to the peritoneum, I examined the activation status of these NK cells. NK cells were sorted from the peritoneal exudates and tested for their ability to kill a classical NK cell target, YAC1, and for production of IFN γ after restimulation ex vivo. Injection of CpG-activated pDC led to enhanced cytotoxicity and IFN γ production by the recovered NK cells (Paper II Fig. 5A and Fig. 6). IFN α has been shown to be critical for the activation of NK cells in vivo (20). In line with that, we showed that IFN α is important for NK cell cytotoxicity, since no killing of YAC1 by NK cells from IFN α -R^{-/-} mice was observed (Paper II Fig. 5B). However, this cytokine had no influence on NK cells recruitment as shown by normal recruitment in IFN α -R^{-/-} mice (data not shown). IL-15 from mDC has previously been reported to be important for priming NK cells in vivo (20). Since, pDC produce little (or no) IL-15 it is possible that activated

pDC stimulate other cells in the peritoneum, such as macrophages or mDC, to produce IL-15, or that IFN α produced by the pDC activate NK cells directly.

CD28-CD80/86 interactions are important for activation of T cells. However, the story is less clear for NK cells. Mouse NK cells express low levels of CD28 and several studies have demonstrated this molecule's involvement in the activation of NK cells both in vitro and in vivo (266-270). Cell lines transfected with CD80/86 molecules can increase the lytic capacity of both mouse and human NK cells compared to non-transfected (271-273). We investigated the role of CD28 in NK cell activation by pDC by injecting CpG-activated pDC into B6.CD28^{-/-} mice and tested the ability of recruited NK cells to lyse YAC1 cells. NK cells recovered from B6.CD28^{-/-} mice displayed decreased cytotoxicity (Paper II Fig. 5C) and also IFN γ production (Paper II Fig. 6) compared to NK cells from wt mice. In addition, injection of pDC from B6 mice into B6.CD80/86^{-/-} mice showed increased NK cells cytotoxicity compared to injection of B6.CD80/86^{-/-} pDC into B6.CD80/86^{-/-} mice. These data suggest that CD28-CD80/86 interaction is at least in part involved in NK cell activation induced by pDC. Whether this is a direct interaction between pDC and NK cells or involves other cell types, such as T cells, is not clear. However, recruitment and increased IFN γ production by NK cells could still be observed in mice lacking T cells, indicating that T cells are not crucial in activating the NK cells in this setting. This does not exclude that T or NKT cells may contribute to NK cell activation when present.

3.2.3 Implications for these results

NK cells are the frontline of the innate defense against infections and tumors. Therefore, the recruitment of NK cells to the site of infection or tumor growth may be important in fighting these battles. Understanding more about how NK cells are recruited to tissues in vivo and the mechanisms underlying their activation can help improving treatments to different diseases. Therapeutic vaccinations with antigen-pulsed myeloid DC against tumors have been studied, but have showed limited efficacy to generate an effective long-lasting immune response to the tumor. Our results suggest that activated pDC, in combination with other therapies, could be more beneficial in anti-tumor vaccines than myeloid DC, since at least in our hands, pDC seemed to be more powerful at recruiting and activating NK cells than myeloid DC. In line with this, Liu and colleagues demonstrated that intra-tumoral injection of activated pDC induced CTL crosspriming against B16 tumor antigens and regression of both treated and non-treated tumors at distant collateral sites. This was dependent on

early recruitment and activation of NK cells at the tumor site induced by activated pDC (274).

3.3 TRANSFER OF *TOXOPLASMA GONDII* FROM INFECTED DC TO NK CELLS (PAPER III)

While working on **Paper II**, we thought it would be very interesting to study an infectious model in the recruitment of NK cells to the peritoneum. *Toxoplasma gondii* (*T. gondii*) is an intriguing and very successful parasite that was available to us and we felt that it would be an interesting model to study.

The role played by NK cells in the immune response against *T. gondii* has been mainly as producers of IFN γ and it has been demonstrated that CCR5-dependent NK cell recruitment to infected liver and spleen is important for survival of mice following a lethal injection of the parasite (261). In this study, I investigated another aspect of NK cell function, namely the killing of infected cells and its consequences.

3.3.1 NK cells gets infected in vivo following inoculation of *T. gondii*-infected DC

In this study, we used the same method to study the peritoneal cavity as in **Paper II**. When GFP⁺ *T. gondii*-infected DC or free parasites were injected i.p. a massive infiltration of cells was observed. Interestingly, among lymphocytes, the NK cells were the population that increased the most in numbers. Even more interestingly, the NK cell population was the lymphocyte population that became highly infected, as calculated by the percentage of GFP⁺ NK1.1⁺ CD3⁻ cells (Paper III Fig. 1A and B). Ex vivo analysis of these cells by microscopy also clearly showed NK cells infected with replicating parasites (Paper III Fig.1C and D). We found it intriguing that NK cells were infected to a greater extent than B cells and T cells and decided to investigate this further. This observation was made both with the type I strain RH-LDM and the type II strain ME49-PTG. For further studies discussed below the virulent type I strain RH-LDM was used.

3.3.2 NK cells effectively kill *T. gondii*-infected DC, which leads to infection of the effector NK cells.

Since our group is interested in NK cell-mediated killing of DC, we asked the question if NK cells could lyse *T. gondii*-infected DC and if so, would that lead to re-infection of the effector NK cells. At present, the “dogma” is that immature DC can be lysed by NK cells while mature DC are spared (**Paper I**). When DC are subject to intracellular infections such as influenza (199) or bacteria (275), a common feature is that they increase MHC class I molecules which, in turn, protects them from NK cell-mediated lysis. However in the case of *T. gondii*-infection, DC exhibited increased sensitivity to NK cell-mediated lysis when compared to untreated DC (Paper III Fig. 2A and C). Stimulating DC with the maturing agents LPS or IFN γ two hours prior to parasite infection did not affect their sensitivity to lysis (data not shown). Experiments using heat-killed *T. gondii*-infected DC or DC treated with *T. gondii* lysate did not show increased lysis indicating that live parasites are required. By adding pyrimethamine to the DC during the infection time with *T. gondii*, the parasite loses its ability to replicate. This, however, did not change *T. gondii*-infected DC’s sensitivity to NK cell-mediated lysis (Paper III Fig. 2B). We stained the infected DC for classical and non-classical MHC class I molecules to see if those were down-regulated compared to untreated DC, but found similar expression patterns. Staining for the NK activating ligand Rae-1 also showed similar results for infected and non-infected DC. The molecules recognised by NK cells on infected DC to induce cytotoxicity were not investigated in this study but it is an interesting question that is looking for an answer.

It has been shown previously (196) and also confirmed in this paper (Paper III Fig. 2A) that NK cell mediated killing of DC in vitro is dependent on perforin. To investigate if lysis of infected DC could facilitate transfer of parasite from DC to NK cells, infected DC were mixed with NK cells from wt mice or perforin (*pfpr*)^{-/-} mice for two hours and then analyzed by flow cytometry. As hypothesized, NK cells from wt mice had a significantly increased infection compared to NK cells from *pfpr*^{-/-} mice (Paper III Fig. 3B).

With these results in mind I went back to see if the same phenomenon with reduced infection of NK cells could be observed in mice lacking killing machinery. Even though NK cells cytotoxicity against DC in vitro is dependent on perforin it has been shown that both perforin and TRAIL is important for lysis of DC in vivo (41). Therefore, *pfpr*^{-/-} mice treated with anti-TRAIL and anti-FASL Ab were used for these experiments. As I observed in vitro, *pfpr*^{-/-} mice treated with anti-TRAIL and anti-FASL Ab inoculated with *T. gondii*-infected DC (Paper III Fig.5A) or free parasites

(Paper III Fig.5B) had significantly fewer infected NK cells compared to wt mice. It has been shown that *T. gondii* can actually sense Ca^{2+} fluxes in the cell membrane induced upon perforin exposure or death receptor ligation and actively egress from the infected cell and reinfect neighbouring cells (276). Thus, even small amounts of perforin that may not be enough to induce apoptosis in DC, may be enough to induce egress of parasite leading to cell death.

Throughout the experiments a comparison has been made to the T cell population. As mentioned previously NK cell was the lymphocyte population that was mostly infected in vivo. Also, in vitro when mixing *T. gondii*-infected DC with a mixture of NK cells and T cells, NK cells were preferentially infected (Paper III Fig. 3A). I do not think that this means that *T. gondii* necessarily has a bias towards infecting NK cells over T cells in general. I propose that during the first 72 h of infection that we are studying here, the cytotoxic cells of the immune system are NK cells. Because of this and the fact that *T. gondii*-infected DC are targets for the NK cells, the close proximity that occur during NK cell mediated killing of the infected DC leads to a greater infection rate in NK cells compared to T cells. That T cells can be infected has been shown by Persson *et al.* who demonstrated that primed T cells trigger parasite egress from infected cells via perforin or death-receptor ligation, which in turn led to infection of effector T cells (276).

3.3.3 NK cells in *T. gondii* infection

As mentioned previously, since *T. gondii* is an intracellular parasite, the infection is mostly controlled by cell-mediated immunity (236). NK cells has been shown to have an important role in the immune response as producers of $\text{IFN}\gamma$ (277-279), induced by IL-12 from infected DC or macrophages (280-282), which drives the immune response towards $\text{T}_{\text{H}}1$. $\text{IFN}\gamma$ from NK cells can also drive cytotoxic CD8^+ T-cell immunity even in the absence of CD4^+ T cells (283). Perforin-mediated cytolysis has been shown previously not to be important for acute infection since preimmunized $\text{pfp}^{-/-}$ mice challenged with a normally lethal acute infection survive. However, perforin-mediated cytolysis seemed to be more involved in the control of the infection during chronic stage (284).

So, where do our results fit into all this? There is no question about NK cells importance as $\text{IFN}\gamma$ producers during acute infection. In contrast though, from our results one can speculate that NK cells might also in fact facilitate *T. gondii* survival, persistence and chronicity. Transfer of *T. gondii* from infected DC to NK cells might provide the parasite with a less

hostile environment where it can proliferate and maybe disseminate to distal parts. Indeed, NK cells do not seem to be targeted by other NK cells (Paper III Fig 2C) and NK cells are not as good at handling intracellular infections as antigen-presenting cells are. It may be hypothesized that *T. gondii* may selectively recruit NK cells (261), and be strong activators of NK cells. This activation could lead to NK cell-mediated lysis of infected cells and production of IFN γ that could eliminate the majority of the parasites. In the process, though, NK cells could become infected, thus creating a niche for the parasite. Therefore, parasites that have secluded themselves within NK cells could reach distant organs directly upon migration of NK cells or indirectly upon lysis of infected NK cells after parasite replication. This may not be an isolated mechanism of *T. gondii*. The closely protozoan relative *Neospora* has been shown to infect bovine NK cells and *Neospora*-infected fibroblast showed increased sensitivity to NK cell lysis (285).

We have started to study infected NK cells in vitro and observed a reduced expression of the activating receptor NKG2D as well as IFN γ production upon IL-12/IL-18 stimulation. Both NKG2D (282) and IFN γ appear to play critical role in host protection from *Toxoplasma* (278). So does the downregulation of these molecules upon infection of NK cells (or T cells for that matter) also enhance the immunoevasion capabilities of *Toxoplasma*?

3.3.4 Future studies

In **Paper III**, we demonstrate that *T. gondii* can be transferred from infected DC to effector NK cells and speculate that this could actually be beneficial for the parasite in terms of inducing chronicity. An interesting question for a NK cell researcher is of course what happens to the NK cell upon infection with *T. gondii* and does it have any consequences in vivo. While NKG2D is downregulated other receptors such as 2B4, NKG2A, NK1.1, ICAM1 and Ly49 receptors were not affected. IFN γ production is reduced upon infection but are other cytokines affected? Thus, future studies should investigate the mechanism by which NK cells are subverted by *Toxoplasma* and how this aids *Toxoplasma* survival in vivo.

4 CONCLUDING REMARKS

Immunology is an extremely complex science and although we have come a long way in unraveling how it all works we still have far to go. To learn how cells of the immune system interact with each other and thereby influence the outcome of the immune response is a critical piece of the puzzle. With this thesis that is dealing with different aspects of the crosstalk between NK cells and DC, I hope that I have contributed with some little piece of that puzzle. Here, I will summarize the main conclusions of my thesis work.

In the first study we investigated the importance of the non-classical MHC class I molecule Qa1 in the protection of mature DC from NK cell mediated killing. Qa1-NKG2A interaction was sufficient to protect mature DC from lysis by NKG2A⁺ NK cells even in the absence of classical MHC class I molecules, both in vitro and in vivo. To further support the observation that Qa1-NKG2A interaction is critical in protecting mature DC, we found that NKG2A⁻ NK cells were capable of killing not only immature DC, but also IFN γ -stimulated DC.

Another aspect of NK-DC crosstalk is activation of NK cells by stimulated DC, which was investigated in the second study. Here, pDC were found to be potent activators of NK cells in vivo in terms of migration, cytotoxicity and IFN γ production. This is one of few studies looking at pDC-induced activation of NK cells in vivo. We found pDC to be more powerful activators of NK cells than DC of myeloid origin, which have previously been mostly studied when it comes to NK cell-DC interactions.

The third study also involved an infectious agent, *T. gondii*. Here, we described that *T. gondii*-infected DC are very susceptible to NK cell mediated killing, which was dependent on infection with live parasites. Effector NK cells were readily infected by the parasite after lysis of infected DC both in vitro and in vivo. We speculate that this could be a way for the parasite to escape immune recognition and induce chronicity of the host.

In the future, more studies need to be performed, especially in vivo, to learn more about the mechanisms behind NK-DC crosstalk. Since most studies have looked at DC derived from bone-marrow or blood in GM-CSF +/- IL-4, it is important to explore if the subtypes of cells existing in vivo play different roles in this cross-talk during certain circumstances, such as infection, tumor outgrowth and autoimmunity.

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6 REFERENCES

1. Thucydides. 1980. The plague in Athens. Thucydides. The history of the Peloponnesian War. Translated by Thomas Hobbes. *North Carolina medical journal* 41:230-232.
2. Jenner, E. 1768. An inquiry into the causes and effects of the variolae vaccinae. a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the Cow-pox. *London: Sampson Low*.
3. Janeway, C. A., Travers P., Walport M. & Shlomchick M. 2001. Immunobiology: the immune system in health and disease. 5th ed, ed. C. Janeway. *New York: Garland*.
4. 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. *Eur J Immunol* 5:112-117.
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. *Eur J Immunol* 5:117-121.
6. Herberman, R. B., M. E. Nunn, and D. H. Lavrin. 1975. Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic acid allogeneic tumors. I. Distribution of reactivity and specificity. *Int J Cancer* 16:216-229.
7. 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.
8. Cooper, M. A., T. A. Fehniger, and M. A. Caligiuri. 2001. The biology of human natural killer-cell subsets. *Trends Immunol* 22:633-640.
9. Hayakawa, Y., and M. J. Smyth. 2006. CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity. *J Immunol* 176:1517-1524.
10. 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. *Nat Immunol* 7:507-516.
11. Sun, J. C., J. N. Beilke, and L. L. Lanier. 2009. Adaptive immune features of natural killer cells. *Nature* 457:557-561.
12. Trinchieri, G. 1989. Biology of natural killer cells. *Advances in immunology* 47:187-376.
13. Fehniger, T. A., and M. A. Caligiuri. 2001. Interleukin 15: biology and relevance to human disease. *Blood* 97:14-32.
14. Vosshenrich, C. A., M. E. Garcia-Ojeda, S. I. Samson-Villeger, V. Pasqualetto, L. Enault, O. Richard-Le Goff, E. Corcuff, D. Guy-Grand, B. Rocha, A. Cumano, L. Rogge, S. Ezine, and J. P. Di Santo. 2006. A thymic pathway of mouse natural killer cell development characterized by expression of GATA-3 and CD127. *Nat Immunol* 7:1217-1224.
15. Zhang, Y., D. L. Wallace, C. M. de Lara, H. Ghattas, B. Asquith, A. Worth, G. E. Griffin, G. P. Taylor, D. F. Tough, P. C. Beverley, and D. C. Macallan. 2007. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. *Immunology* 121:258-265.
16. Jamieson, A. M., P. Isnard, J. R. Dorfman, M. C. Coles, and D. H. Raulet. 2004. Turnover and proliferation of NK cells in steady state and lymphopenic conditions. *J Immunol* 172:864-870.
17. Walzer, T., M. Blery, J. Chaix, N. Fuseri, L. Chasson, S. H. Robbins, S. Jaeger, P. Andre, L. Gauthier, L. Daniel, K. Chemin, Y. Morel, M. Dalod, J. Imbert, M. Pierres, A. Moretta, F. Romagne, and E. Vivier. 2007. Identification, activation, and selective in vivo ablation of mouse NK cells via Nkp46. *Proc Natl Acad Sci U S A* 104:3384-3389.
18. Bryceson, Y. T., M. E. March, H. G. Ljunggren, and E. O. Long. 2006. Synergy among receptors on resting NK cells for the activation of natural cytotoxicity and cytokine secretion. *Blood* 107:159-166.

19. Fehniger, T. A., S. F. Cai, X. Cao, A. J. Bredemeyer, R. M. Presti, A. R. French, and T. J. Ley. 2007. Acquisition of murine NK cell cytotoxicity requires the translation of a pre-existing pool of granzyme B and perforin mRNAs. *Immunity* 26:798-811.
20. 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.
21. Chaix, J., M. S. Tessmer, K. Hoebe, N. Fuseri, B. Ryffel, M. Dalod, L. Alexopoulou, B. Beutler, L. Brossay, E. Vivier, and T. Walzer. 2008. Cutting edge: Priming of NK cells by IL-18. *J Immunol* 181:1627-1631.
22. Biron, C. A., K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather. 1999. Natural killer cells in antiviral defense: function and regulation by innate cytokines. *Annu Rev Immunol* 17:189-220.
23. Cooper, M. A., J. E. Bush, T. A. Fehniger, J. B. VanDeusen, R. E. Waite, Y. Liu, H. L. Aguila, and M. A. Caligiuri. 2002. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood* 100:3633-3638.
24. Ferlazzo, G., M. Pack, D. Thomas, C. Paludan, D. Schmid, T. Strowig, G. Bougras, W. A. Muller, L. Moretta, and C. Munz. 2004. Distinct roles of IL-12 and IL-15 in human natural killer cell activation by dendritic cells from secondary lymphoid organs. *Proc Natl Acad Sci U S A* 101:16606-16611.
25. 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. *Proc Natl Acad Sci U S A* 102:14747-14752.
26. French, A. R., E. B. Holroyd, L. Yang, S. Kim, and W. M. Yokoyama. 2006. IL-18 acts synergistically with IL-15 in stimulating natural killer cell proliferation. *Cytokine* 35:229-234.
27. Liu, B., I. Mori, M. J. Hossain, L. Dong, K. Takeda, and Y. Kimura. 2004. Interleukin-18 improves the early defence system against influenza virus infection by augmenting natural killer cell-mediated cytotoxicity. *The Journal of general virology* 85:423-428.
28. Bellone, G., M. Aste-Amezaga, G. Trinchieri, and U. Rodeck. 1995. Regulation of NK cell functions by TGF-beta 1. *J Immunol* 155:1066-1073.
29. Hunter, C. A., L. Bermudez, H. Beernink, W. Waegell, and J. S. Remington. 1995. Transforming growth factor-beta inhibits interleukin-12-induced production of interferon-gamma by natural killer cells: a role for transforming growth factor-beta in the regulation of T cell-independent resistance to Toxoplasma gondii. *Eur J Immunol* 25:994-1000.
30. Gregoire, C., L. Chasson, C. Luci, E. Tomasello, F. Geissmann, E. Vivier, and T. Walzer. 2007. The trafficking of natural killer cells. *Immunol Rev* 220:169-182.
31. 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. *Nat Immunol* 5:1260-1265.
32. Walzer, T., L. Chiossone, J. Chaix, A. Calver, C. Carozzo, L. Garrigue-Antar, Y. Jacques, M. Baratin, E. Tomasello, and E. Vivier. 2007. Natural killer cell trafficking in vivo requires a dedicated sphingosine 1-phosphate receptor. *Nat Immunol* 8:1337-1344.
33. Vivier, E., E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini. 2008. Functions of natural killer cells. *Nat Immunol* 9:503-510.
34. Smyth, M. J., E. Cretney, J. M. Kelly, J. A. Westwood, S. E. Street, H. Yagita, K. Takeda, S. L. van Dommelen, M. A. Degli-Esposti, and Y. Hayakawa. 2005. Activation of NK cell cytotoxicity. *Mol Immunol* 42:501-510.
35. Smyth, M. J., K. Y. Thia, E. Cretney, J. M. Kelly, M. B. Snook, C. A. Forbes, and A. A. Scalzo. 1999. Perforin is a major contributor to NK cell control of tumor metastasis. *J Immunol* 162:6658-6662.
36. Smyth, M. J., M. D. O'Connor, and J. A. Trapani. 1996. Granzymes: a variety of serine protease specificities encoded by genetically distinct subfamilies. *J Leukoc Biol* 60:555-562.

37. Smyth, M. J., and J. A. Trapani. 1995. Granzymes: exogenous proteinases that induce target cell apoptosis. *Immunol Today* 16:202-206.
38. Kelly, J. M., N. J. Waterhouse, E. Cretney, K. A. Browne, S. Ellis, J. A. Trapani, and M. J. Smyth. 2004. Granzyme M mediates a novel form of perforin-dependent cell death. *J Biol Chem* 279:22236-22242.
39. Degli-Esposti, M. 1999. To die or not to die--the quest of the TRAIL receptors. *J Leukoc Biol* 65:535-542.
40. Takeda, K., Y. Hayakawa, M. J. Smyth, N. Kayagaki, N. Yamaguchi, S. Kakuta, Y. Iwakura, H. Yagita, and K. Okumura. 2001. Involvement of tumor necrosis factor-related apoptosis-inducing ligand in surveillance of tumor metastasis by liver natural killer cells. *Nat Med* 7:94-100.
41. Hayakawa, Y., V. Screpanti, H. Yagita, A. Grandien, H. G. Ljunggren, M. J. Smyth, and B. J. Chambers. 2004. NK cell TRAIL eliminates immature dendritic cells in vivo and limits dendritic cell vaccination efficacy. *J Immunol* 172:123-129.
42. Bradley, M., A. Zeytun, A. Rafi-Janajreh, P. S. Nagarkatti, and M. Nagarkatti. 1998. Role of spontaneous and interleukin-2-induced natural killer cell activity in the cytotoxicity and rejection of Fas+ and Fas- tumor cells. *Blood* 92:4248-4255.
43. Screpanti, V., R. P. Wallin, H. G. Ljunggren, and A. Grandien. 2001. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. *J Immunol* 167:2068-2073.
44. Karre, K., H. G. Ljunggren, G. Piontek, and R. Kiessling. 1986. Selective rejection of H-2-deficient lymphoma variants suggests alternative immune defence strategy. *Nature* 319:675-678.
45. Ljunggren, H. G., and K. Kärre. 1985. Host resistance directed selectively against H-2-deficient lymphoma variants. Analysis of the mechanism. *J Exp Med* 162:1745-1759.
46. Karlhofer, F. M., R. K. Ribaldo, and W. M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly-49+ IL-2-activated natural killer cells. *Nature* 358:66-70.
47. Takei, F., K. L. McQueen, M. Maeda, B. T. Wilhelm, S. Lohwasser, R. H. Lian, and D. L. Mager. 2001. Ly49 and CD94/NKG2: developmentally regulated expression and evolution. *Immunol Rev* 181:90-103.
48. 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.
49. 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). *J Exp Med* 188:1841-1848.
50. Aldrich, C. J., A. DeCloux, A. S. Woods, R. J. Cotter, M. J. Soloski, and J. Forman. 1994. Identification of a Tap-dependent leader peptide recognized by alloreactive T cells specific for a class Ib antigen. *Cell* 79:649-658.
51. 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. *J Exp Med* 192:613-624.
52. Davies, A., S. Kalb, B. Liang, C. J. Aldrich, F. A. Lemonnier, H. Jiang, R. Cotter, and M. J. Soloski. 2003. A peptide from heat shock protein 60 is the dominant peptide bound to Qa-1 in the absence of the MHC class Ia leader sequence peptide Qdm. *J Immunol* 170:5027-5033.
53. Sivakumar, P. V., A. Gunturi, M. Salcedo, J. D. Schatzle, W. C. Lai, Z. Kurepa, L. Pitcher, M. S. Seaman, F. A. Lemonnier, M. Bennett, J. Forman, and V. Kumar. 1999. Cutting edge: expression of functional CD94/NKG2A inhibitory receptors on fetal NK1.1+Ly-49- cells: a possible mechanism of tolerance during NK cell development. *J Immunol* 162:6976-6980.

54. Plougastel, B., T. Jones, and J. Trowsdale. 1996. Genomic structure, chromosome location, and alternative splicing of the human NKG2A gene. *Immunogenetics* 44:286-291.
55. Parham, P. 2005. Immunogenetics of killer cell immunoglobulin-like receptors. *Mol Immunol* 42:459-462.
56. Vilches, C., and P. Parham. 2002. KIR: diverse, rapidly evolving receptors of innate and adaptive immunity. *Annu Rev Immunol* 20:217-251.
57. 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. *Nat Immunol* 1:119-126.
58. 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.
59. 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.
60. Raulet, D. H. 2003. Roles of the NKG2D immunoreceptor and its ligands. *Nat Rev Immunol* 3:781-790.
61. 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* 285:730-732.
62. Moretta, A., C. Bottino, M. Vitale, D. Pende, C. Cantoni, M. C. Mingari, R. Biassoni, and L. Moretta. 2001. Activating receptors and coreceptors involved in human natural killer cell-mediated cytotoxicity. *Annu Rev Immunol* 19:197-223.
63. De Maria, A., R. Biassoni, M. Fogli, M. Rizzi, C. Cantoni, P. Costa, R. Conte, D. Mavilio, B. Ensoli, A. Cafaro, A. Moretta, and L. Moretta. 2001. Identification, molecular cloning and functional characterization of NKp46 and NKp30 natural cytotoxicity receptors in *Macaca fascicularis* NK cells. *Eur J Immunol* 31:3546-3556.
64. 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.
65. 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. *Eur J Immunol* 31:2680-2689.
66. Lanier, L. L. 1998. NK cell receptors. *Annu Rev Immunol* 16:359-393.
67. Gilfillan, S., C. J. Chan, M. Cella, N. M. Haynes, A. S. Rapaport, K. S. Boles, D. M. Andrews, M. J. Smyth, and M. Colonna. 2008. DNAM-1 promotes activation of cytotoxic lymphocytes by nonprofessional antigen-presenting cells and tumors. *J Exp Med* 205:2965-2973.
68. Iguchi-Manaka, A., H. Kai, Y. Yamashita, K. Shibata, S. Tahara-Hanaoka, S. Honda, T. Yasui, H. Kikutani, K. Shibuya, and A. Shibuya. 2008. Accelerated tumor growth in mice deficient in DNAM-1 receptor. *J Exp Med* 205:2959-2964.
69. Schatzle, J. D., S. Sheu, S. E. Stepp, P. A. Mathew, M. Bennett, and V. Kumar. 1999. Characterization of inhibitory and stimulatory forms of the murine natural killer cell receptor 2B4. *Proc Natl Acad Sci U S A* 96:3870-3875.
70. Garni-Wagner, B. A., A. Purohit, P. A. Mathew, M. Bennett, and V. Kumar. 1993. A novel function-associated molecule related to non-MHC-restricted cytotoxicity mediated by activated natural killer cells and T cells. *J Immunol* 151:60-70.
71. Lee, K. M., M. E. McNerney, S. E. Stepp, P. A. Mathew, J. D. Schatzle, M. Bennett, and V. Kumar. 2004. 2B4 acts as a non-major histocompatibility complex binding inhibitory receptor on mouse natural killer cells. *J Exp Med* 199:1245-1254.
72. Chlewicki, L. K., C. A. Velikovsky, V. Balakrishnan, R. A. Mariuzza, and V. Kumar. 2008. Molecular basis of the dual functions of 2B4 (CD244). *J Immunol* 180:8159-8167.

73. Perussia, B. 1991. Lymphokine-activated killer cells, natural killer cells and cytokines. *Curr Opin Immunol* 3:49-55.
74. Schroder, K., P. J. Hertzog, T. Ravasi, and D. A. Hume. 2004. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* 75:163-189.
75. Smyth, M. J., Y. Hayakawa, K. Takeda, and H. Yagita. 2002. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nature reviews* 2:850-861.
76. Gray, J. D., M. Hirokawa, K. Ohtsuka, and D. A. Horwitz. 1998. Generation of an inhibitory circuit involving CD8⁺ T cells, IL-2, and NK cell-derived TGF-beta: contrasting effects of anti-CD2 and anti-CD3. *J Immunol* 160:2248-2254.
77. Horwitz, D. A., J. D. Gray, and K. Ohtsuka. 1999. Role of NK cells and TGF-beta in the regulation of T-cell-dependent antibody production in health and autoimmune disease. *Microbes and infection / Institut Pasteur* 1:1305-1311.
78. Mehrotra, P. T., R. P. Donnelly, S. Wong, H. Kanegane, A. Geremew, H. S. Mostowski, K. Furuke, J. P. Siegel, and E. T. Bloom. 1998. Production of IL-10 by human natural killer cells stimulated with IL-2 and/or IL-12. *J Immunol* 160:2637-2644.
79. Deniz, G., G. Erten, U. C. Kucuksezer, D. Kocacik, C. Karagiannidis, E. Aktas, C. A. Akdis, and M. Akdis. 2008. Regulatory NK cells suppress antigen-specific T cell responses. *J Immunol* 180:850-857.
80. Li, M. O., Y. Y. Wan, S. Sanjabi, A. K. Robertson, and R. A. Flavell. 2006. Transforming growth factor-beta regulation of immune responses. *Annu Rev Immunol* 24:99-146.
81. Moore, K. W., R. de Waal Malefyt, R. L. Coffman, and A. O'Garra. 2001. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 19:683-765.
82. 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. *Eur J Immunol* 36:2394-2400.
83. Assarsson, E., T. Kambayashi, J. D. Schatzle, S. O. Cramer, A. von Bonin, P. E. Jensen, H. G. Ljunggren, and B. J. Chambers. 2004. NK cells stimulate proliferation of T and NK cells through 2B4/CD48 interactions. *J Immunol* 173:174-180.
84. Zingoni, A., T. Sornasse, B. G. Cocks, Y. Tanaka, A. Santoni, and L. L. Lanier. 2004. Cross-talk between activated human NK cells and CD4⁺ T cells via OX40-OX40 ligand interactions. *J Immunol* 173:3716-3724.
85. Snapper, C. M., H. Yamaguchi, M. A. Moorman, R. Sneed, D. Smoot, and J. J. Mond. 1993. Natural killer cells induce activated murine B cells to secrete Ig. *J Immunol* 151:5251-5260.
86. 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.
87. Roy, S., P. F. Barnes, A. Garg, S. Wu, D. Cosman, and R. Vankayalapati. 2008. NK cells lyse T regulatory cells that expand in response to an intracellular pathogen. *J Immunol* 180:1729-1736.
88. Lu, L., K. Ikizawa, D. Hu, M. B. Werneck, K. W. Wucherpfennig, and H. Cantor. 2007. Regulation of activated CD4⁺ T cells by NK cells via the Qa-1-NKG2A inhibitory pathway. *Immunity* 26:593-604.
89. Yoneda, O., T. Imai, S. Goda, H. Inoue, A. Yamauchi, T. Okazaki, H. Imai, O. Yoshie, E. T. Bloom, N. Domae, and H. Umehara. 2000. Fractalkine-mediated endothelial cell injury by NK cells. *J Immunol* 164:4055-4062.
90. Hanna, J., D. Goldman-Wohl, Y. Hamani, I. Avraham, C. Greenfield, S. Natanson-Yaron, D. Prus, L. Cohen-Daniel, T. I. Arnon, I. Manaster, R. Gazit, V. Yutkin, D. Benharroch, A. Porgador, E. Keshet, S. Yagel, and O. Mandelboim. 2006. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nat Med* 12:1065-1074.
91. Bancroft, G. J. 1993. The role of natural killer cells in innate resistance to infection. *Curr Opin Immunol* 5:503-510.
92. Tay, C. H., E. Szomolanyi-Tsuda, and R. M. Welsh. 1998. Control of infections by NK cells. *Curr Top Microbiol Immunol* 230:193-220.

93. Biron, C. A., and L. Brossay. 2001. NK cells and NKT cells in innate defense against viral infections. *Curr Opin Immunol* 13:458-464.
94. De Maria, A., and L. Moretta. 2008. NK cell function in HIV-1 infection. *Current HIV research* 6:433-440.
95. Takehara, T., and N. Hayashi. 2005. Natural killer cells in hepatitis C virus infection: from innate immunity to adaptive immunity. *Clin Gastroenterol Hepatol* 3:S78-81.
96. 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. *Nature genetics* 28:42-45.
97. 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* 296:1323-1326.
98. 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. *Proc Natl Acad Sci U S A* 100:13483-13488.
99. Jouanguy, E., R. Doffinger, S. Dupuis, A. Pallier, F. Altare, and J. L. Casanova. 1999. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 11:346-351.
100. 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.
101. Vankayalapati, R., A. Garg, A. Porgador, D. E. Griffith, P. Klucar, H. Safi, W. M. Girard, D. Cosman, T. Spies, and P. F. Barnes. 2005. Role of NK cell-activating receptors and their ligands in the lysis of mononuclear phagocytes infected with an intracellular bacterium. *J Immunol* 175:4611-4617.
102. Junqueira-Kipnis, A. P., A. Kipnis, A. Jamieson, M. G. Juarrero, A. Diefenbach, D. H. Raulet, J. Turner, and I. M. Orme. 2003. NK cells respond to pulmonary infection with *Mycobacterium tuberculosis*, but play a minimal role in protection. *J Immunol* 171:6039-6045.
103. Berg, R. E., E. Crossley, S. Murray, and J. Forman. 2005. Relative contributions of NK and CD8 T cells to IFN-gamma mediated innate immune protection against *Listeria monocytogenes*. *J Immunol* 175:1751-1757.
104. Hamerman, J. A., N. K. Tchao, C. A. Lowell, and L. L. Lanier. 2005. Enhanced Toll-like receptor responses in the absence of signaling adaptor DAP12. *Nat Immunol* 6:579-586.
105. Rottenberg, M., R. L. Cardoni, R. Andersson, E. L. Segura, and A. Orn. 1988. Role of T helper/inducer cells as well as natural killer cells in resistance to *Trypanosoma cruzi* infection. *Scand J Immunol* 28:573-582.
106. Scharton, T. M., and P. Scott. 1993. Natural killer cells are a source of interferon gamma that drives differentiation of CD4+ T cell subsets and induces early resistance to *Leishmania major* in mice. *J Exp Med* 178:567-577.
107. Laskay, T., M. Rollinghoff, and W. Solbach. 1993. Natural killer cells participate in the early defense against *Leishmania major* infection in mice. *Eur J Immunol* 23:2237-2241.
108. Hunter, C. A., E. Candolfi, C. Subauste, V. Van Cleave, and J. S. Remington. 1995. Studies on the role of interleukin-12 in acute murine toxoplasmosis. *Immunology* 84:16-20.
109. Langerhans, P. 1868. Uber die Nerven det menschlien Haut. *Vircc. Arch. (Pathol. Anat.):*325.
110. Steinman, R. M., and Z. A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 137:1142-1162.
111. Dakic, A., Q. X. Shao, A. D'Amico, M. O'Keeffe, W. F. Chen, K. Shortman, and L. Wu. 2004. Development of the dendritic cell system during mouse ontogeny. *J Immunol* 172:1018-1027.

112. Traver, D., K. Akashi, M. Manz, M. Merad, T. Miyamoto, E. G. Engleman, and I. L. Weissman. 2000. Development of CD8alpha-positive dendritic cells from a common myeloid progenitor. *Science* 290:2152-2154.
113. Wu, L., A. D'Amico, H. Hochrein, M. O'Keeffe, K. Shortman, and K. Lucas. 2001. Development of thymic and splenic dendritic cell populations from different hemopoietic precursors. *Blood* 98:3376-3382.
114. Izon, D., K. Rudd, W. DeMuth, W. S. Pear, C. Clendenin, R. C. Lindsley, and D. Allman. 2001. A common pathway for dendritic cell and early B cell development. *J Immunol* 167:1387-1392.
115. Manz, M. G., D. Traver, T. Miyamoto, I. L. Weissman, and K. Akashi. 2001. Dendritic cell potentials of early lymphoid and myeloid progenitors. *Blood* 97:3333-3341.
116. Diao, J., E. Winter, W. Chen, C. Cantin, and M. S. Cattral. 2004. Characterization of distinct conventional and plasmacytoid dendritic cell-committed precursors in murine bone marrow. *J Immunol* 173:1826-1833.
117. Fogg, D. K., C. Sibon, C. Miled, S. Jung, P. Aucouturier, D. R. Littman, A. Cumano, and F. Geissmann. 2006. A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. *Science* 311:83-87.
118. Inaba, K., M. Inaba, N. Romani, H. Aya, M. Deguchi, S. Ikehara, S. Muramatsu, and R. M. Steinman. 1992. Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J Exp Med* 176:1693-1702.
119. Inaba, K., R. M. Steinman, M. W. Pack, H. Aya, M. Inaba, T. Sudo, S. Wolpe, and G. Schuler. 1992. Identification of proliferating dendritic cell precursors in mouse blood. *J Exp Med* 175:1157-1167.
120. Grassi, F., C. Dezutter-Dambuyant, D. McIlroy, C. Jacquet, K. Yoneda, S. Imamura, L. Boumsell, D. Schmitt, B. Autran, P. Debre, and A. Hosmalin. 1998. Monocyte-derived dendritic cells have a phenotype comparable to that of dermal dendritic cells and display ultrastructural granules distinct from Birbeck granules. *J Leukoc Biol* 64:484-493.
121. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 180:83-93.
122. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *J Exp Med* 179:1109-1118.
123. Strobl, H., E. Riedl, C. Scheinecker, C. Bello-Fernandez, W. F. Pickl, K. Rappersberger, O. Majdic, and W. Knapp. 1996. TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors. *J Immunol* 157:1499-1507.
124. Naik, S. H., A. I. Proietto, N. S. Wilson, A. Dakic, P. Schnorrer, M. Fuchsberger, M. H. Lahoud, M. O'Keeffe, Q. X. Shao, W. F. Chen, J. A. Villadangos, K. Shortman, and L. Wu. 2005. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. *J Immunol* 174:6592-6597.
125. Vremec, D., G. J. Lieschke, A. R. Dunn, L. Robb, D. Metcalf, and K. Shortman. 1997. The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. *Eur J Immunol* 27:40-44.
126. Pulendran, B., J. Banchereau, S. Burkeholder, E. Kraus, E. Guinet, C. Chalouni, D. Caron, C. Maliszewski, J. Davoust, J. Fay, and K. Palucka. 2000. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J Immunol* 165:566-572.
127. Maraskovsky, E., K. Brasel, M. Teepe, E. R. Roux, S. D. Lyman, K. Shortman, and H. J. McKenna. 1996. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J Exp Med* 184:1953-1962.
128. Shortman, K., and S. H. Naik. 2007. Steady-state and inflammatory dendritic-cell development. *Nat Rev Immunol* 7:19-30.

129. Hochrein, H., K. Shortman, D. Vremec, B. Scott, P. Hertzog, and M. O'Keeffe. 2001. Differential production of IL-12, IFN-alpha, and IFN-gamma by mouse dendritic cell subsets. *J Immunol* 166:5448-5455.
130. Pooley, J. L., W. R. Heath, and K. Shortman. 2001. Cutting edge: intravenous soluble antigen is presented to CD4 T cells by CD8- dendritic cells, but cross-presented to CD8 T cells by CD8+ dendritic cells. *J Immunol* 166:5327-5330.
131. Heath, W. R., G. T. Belz, G. M. Behrens, C. M. Smith, S. P. Forehan, I. A. Parish, G. M. Davey, N. S. Wilson, F. R. Carbone, and J. A. Villadangos. 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. *Immunol Rev* 199:9-26.
132. den Haan, J. M., S. M. Lehar, and M. J. Bevan. 2000. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 192:1685-1696.
133. Maldonado-Lopez, R., T. De Smedt, P. Michel, J. Godfroid, B. Pajak, C. Heirman, K. Thielemans, O. Leo, J. Urbain, and M. Moser. 1999. CD8alpha+ and CD8alpha- subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J Exp Med* 189:587-592.
134. Pulendran, B., J. L. Smith, G. Caspary, K. Brasel, D. Pettit, E. Maraskovsky, and C. R. Maliszewski. 1999. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc Natl Acad Sci U S A* 96:1036-1041.
135. Moser, M., and K. M. Murphy. 2000. Dendritic cell regulation of TH1-TH2 development. *Nat Immunol* 1:199-205.
136. Proietto, A. I., M. O'Keeffe, K. Gartlan, M. D. Wright, K. Shortman, L. Wu, and M. H. Lahoud. 2004. Differential production of inflammatory chemokines by murine dendritic cell subsets. *Immunobiology* 209:163-172.
137. Shortman, K., and Y. J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2:151-161.
138. Narbutt, J., A. Lesiak, M. Zak-Prelich, A. Wozniacka, A. Sysa-Jedrzejowska, M. Tybura, T. Robak, and P. Smolewski. 2004. The distribution of peripheral blood dendritic cells assayed by a new panel of anti-BDCA monoclonal antibodies in healthy representatives of the polish population. *Cellular & molecular biology letters* 9:497-509.
139. Robbins, S. H., T. Walzer, D. Dembele, C. Thibault, A. Defays, G. Bessou, H. Xu, E. Vivier, M. Sellars, P. Pierre, F. R. Sharp, S. Chan, P. Kastner, and M. Dalod. 2008. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. *Genome biology* 9:R17.
140. Valladeau, J., O. Ravel, C. Dezutter-Dambuyant, K. Moore, M. Kleijmeer, Y. Liu, V. Duvert-Frances, C. Vincent, D. Schmitt, J. Davoust, C. Caux, S. Lebecque, and S. Saeland. 2000. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12:71-81.
141. Kamath, A. T., S. Henri, F. Battye, D. F. Tough, and K. Shortman. 2002. Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. *Blood* 100:1734-1741.
142. Flacher, V., M. Bouschbacher, E. Verronese, C. Massacrier, V. Sisirak, O. Berthier-Vergnes, B. de Saint-Vis, C. Caux, C. Dezutter-Dambuyant, S. Lebecque, and J. Valladeau. 2006. Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria. *J Immunol* 177:7959-7967.
143. Galibert, L., C. R. Maliszewski, and S. Vandenabeele. 2001. Plasmacytoid monocytes/T cells: a dendritic cell lineage? *Semin Immunol* 13:283-289.
144. Olweus, J., A. BitMansour, R. Warnke, P. A. Thompson, J. Carballido, L. J. Picker, and F. Lund-Johansen. 1997. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc Natl Acad Sci U S A* 94:12551-12556.
145. Grouard, G., M. C. Risoan, L. Filgueira, I. Durand, J. Banchereau, and Y. J. Liu. 1997. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J Exp Med* 185:1101-1111.
146. Fitzgerald-Bocarsly, P. 1993. Human natural interferon-alpha producing cells. *Pharmacology & therapeutics* 60:39-62.

147. Cella, M., D. Jarrossay, F. Facchetti, O. Alebardi, H. Nakajima, A. Lanzavecchia, and M. Colonna. 1999. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* 5:919-923.
148. Siegal, F. P., N. Kadowaki, M. Shodell, P. A. Fitzgerald-Bocarsly, K. Shah, S. Ho, S. Antonenko, and Y. J. Liu. 1999. The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284:1835-1837.
149. Asselin-Paturel, C., A. Boonstra, M. Dalod, I. Durand, N. Yessaad, C. Dezutter-Dambuyant, A. Vicari, A. O'Garra, C. Biron, F. Briere, and G. Trinchieri. 2001. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* 2:1144-1150.
150. Nakano, H., M. Yanagita, and M. D. Gunn. 2001. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J Exp Med* 194:1171-1178.
151. Bjorck, P. 2001. Isolation and characterization of plasmacytoid dendritic cells from Flt3 ligand and granulocyte-macrophage colony-stimulating factor-treated mice. *Blood* 98:3520-3526.
152. O'Keeffe, M., H. Hochrein, D. Vremec, I. Caminschi, J. L. Miller, E. M. Anders, L. Wu, M. H. Lahoud, S. Henri, B. Scott, P. Hertzog, L. Tatarczuch, and K. Shortman. 2002. Mouse plasmacytoid cells: long-lived cells, heterogeneous in surface phenotype and function, that differentiate into CD8(+) dendritic cells only after microbial stimulus. *J Exp Med* 196:1307-1319.
153. Fancke, B., M. Suter, H. Hochrein, and M. O'Keeffe. 2008. M-CSF: a novel plasmacytoid and conventional dendritic cell poietin. *Blood* 111:150-159.
154. O'Keeffe, M., H. Hochrein, D. Vremec, B. Scott, P. Hertzog, L. Tatarczuch, and K. Shortman. 2003. Dendritic cell precursor populations of mouse blood: identification of the murine homologues of human blood plasmacytoid pre-DC2 and CD11c+ DC1 precursors. *Blood* 101:1453-1459.
155. Nikolic, T., G. M. Dingjan, P. J. Leenen, and R. W. Hendriks. 2002. A subfraction of B220(+) cells in murine bone marrow and spleen does not belong to the B cell lineage but has dendritic cell characteristics. *Eur J Immunol* 32:686-692.
156. Okada, T., Z. X. Lian, M. Naiki, A. A. Ansari, S. Ikehara, and M. E. Gershwin. 2003. Murine thymic plasmacytoid dendritic cells. *Eur J Immunol* 33:1012-1019.
157. Lian, Z. X., T. Okada, X. S. He, H. Kita, Y. J. Liu, A. A. Ansari, K. Kikuchi, S. Ikehara, and M. E. Gershwin. 2003. Heterogeneity of dendritic cells in the mouse liver: identification and characterization of four distinct populations. *J Immunol* 170:2323-2330.
158. Ito, T., R. Amakawa, T. Kaisho, H. Hemmi, K. Tajima, K. Uehira, Y. Ozaki, H. Tomizawa, S. Akira, and S. Fukuhara. 2002. Interferon-alpha and interleukin-12 are induced differentially by Toll-like receptor 7 ligands in human blood dendritic cell subsets. *J Exp Med* 195:1507-1512.
159. Krug, A., S. Rothenfusser, V. Hornung, B. Jahrsdorfer, S. Blackwell, Z. K. Ballas, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol* 31:2154-2163.
160. Kadowaki, N., S. Antonenko, and Y. J. Liu. 2001. Distinct CpG DNA and polyinosinic-polycytidylic acid double-stranded RNA, respectively, stimulate CD11c- type 2 dendritic cell precursors and CD11c+ dendritic cells to produce type I IFN. *J Immunol* 166:2291-2295.
161. Isaacs, A., and J. Lindenmann. 1957. Virus interference. I. The interferon. *Proceedings of the Royal Society of London. Series B, Containing papers of a Biological character* 147:258-267.
162. Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science* 264:1918-1921.
163. Ortaldo, J. R., A. Mantovani, D. Hobbs, M. Rubinstein, S. Pestka, and R. B. Herberman. 1983. Effects of several species of human leukocyte interferon on cytotoxic activity of NK cells and monocytes. *Int J Cancer* 31:285-289.

164. Marrack, P., J. Kappler, and T. Mitchell. 1999. Type I interferons keep activated T cells alive. *J Exp Med* 189:521-530.
165. Le Bon, A., G. Schiavoni, G. D'Agostino, I. Gresser, F. Belardelli, and D. F. Tough. 2001. Type I interferons potently enhance humoral immunity and can promote isotype switching by stimulating dendritic cells in vivo. *Immunity* 14:461-470.
166. Taylor, P., T. Tamura, and K. Ozato. 2006. IRF family proteins and type I interferon induction in dendritic cells. *Cell research* 16:134-140.
167. Izaguirre, A., B. J. Barnes, S. Amrute, W. S. Yeow, N. Megjugorac, J. Dai, D. Feng, E. Chung, P. M. Pitha, and P. Fitzgerald-Bocarsly. 2003. Comparative analysis of IRF and IFN- α expression in human plasmacytoid and monocyte-derived dendritic cells. *J Leukoc Biol* 74:1125-1138.
168. Colonna, M., G. Trinchieri, and Y. J. Liu. 2004. Plasmacytoid dendritic cells in immunity. *Nat Immunol* 5:1219-1226.
169. Liu, Y. J. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 23:275-306.
170. Villadangos, J. A., and L. Young. 2008. Antigen-presentation properties of plasmacytoid dendritic cells. *Immunity* 29:352-361.
171. Dearman, R. J., M. Cumberbatch, G. Maxwell, D. A. Basketter, and I. Kimber. 2009. Toll-like receptor ligand activation of murine bone marrow-derived dendritic cells. *Immunology* 126:475-484.
172. Serbina, N. V., T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, and E. G. Pamer. 2003. TNF/ iNOS -producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* 19:59-70.
173. Macatonia, S. E., A. J. Edwards, and S. C. Knight. 1986. Dendritic cells and the initiation of contact sensitivity to fluorescein isothiocyanate. *Immunology* 59:509-514.
174. Macatonia, S. E., S. C. Knight, A. J. Edwards, S. Griffiths, and P. Fryer. 1987. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. Functional and morphological studies. *J Exp Med* 166:1654-1667.
175. O'Neill, L. A. 2004. Immunology. After the toll rush. *Science* 303:1481-1482.
176. Wolf, P. R., and H. L. Ploegh. 1995. How MHC class II molecules acquire peptide cargo: biosynthesis and trafficking through the endocytic pathway. *Annual review of cell and developmental biology* 11:267-306.
177. Heemels, M. T., and H. Ploegh. 1995. Generation, translocation, and presentation of MHC class I-restricted peptides. *Annual review of biochemistry* 64:463-491.
178. Germain, R. N., and D. H. Margulies. 1993. The biochemistry and cell biology of antigen processing and presentation. *Annu Rev Immunol* 11:403-450.
179. Guermonprez, P., J. Valladeau, L. Zitvogel, C. Thery, and S. Amigorena. 2002. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol* 20:621-667.
180. Heath, W. R., and F. R. Carbone. 2001. Cross-presentation, dendritic cells, tolerance and immunity. *Annu Rev Immunol* 19:47-64.
181. Ackerman, A. L., C. Kyritsis, R. Tampe, and P. Cresswell. 2003. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. *Proc Natl Acad Sci U S A* 100:12889-12894.
182. Fonteneau, J. F., M. Larsson, and N. Bhardwaj. 2002. Interactions between dead cells and dendritic cells in the induction of antiviral CTL responses. *Curr Opin Immunol* 14:471-477.
183. Guermonprez, P., L. Saveanu, M. Kleijmeer, J. Davoust, P. Van Endert, and S. Amigorena. 2003. ER-phagosome fusion defines an MHC class I cross-presentation compartment in dendritic cells. *Nature* 425:397-402.
184. Shah, P. D., S. M. Gilbertson, and D. A. Rowley. 1985. Dendritic cells that have interacted with antigen are targets for natural killer cells. *J Exp Med* 162:625-636.
185. 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.
186. 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. *J Exp Med* 195:327-333.
 187. Piccioli, D., S. Sbrana, E. Melandri, and N. M. Valiante. 2002. Contact-dependent Stimulation and Inhibition of Dendritic Cells by Natural Killer Cells. *J Exp Med* 195:335-341.
 188. 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.
 189. Nguyen, K. B., T. P. Salazar-Mather, M. Y. Dalod, J. B. Van Deusen, X. Q. Wei, F. Y. Liew, M. A. Caligiuri, J. E. Durbin, and C. A. Biron. 2002. Coordinated and distinct roles for IFN-alpha beta, IL-12, and IL-15 regulation of NK cell responses to viral infection. *J Immunol* 169:4279-4287.
 190. Borg, C., A. Jalil, D. Laderach, K. Maruyama, H. Wakasugi, S. Charrier, B. Ryffel, A. Cambi, C. Figdor, W. Vainchenker, A. Galy, A. Caignard, and L. Zitvogel. 2004. NK cell activation by dendritic cells (DCs) requires the formation of a synapse leading to IL-12 polarization in DCs. *Blood* 104:3267-3275.
 191. Kassim, S. H., N. K. Rajasagi, X. Zhao, R. Chervenak, and S. R. Jennings. 2006. In vivo ablation of CD11c-positive dendritic cells increases susceptibility to herpes simplex virus type 1 infection and diminishes NK and T-cell responses. *J Virol* 80:3985-3993.
 192. Sauma, D., P. Michea, A. M. Lennon-Dumenil, A. Fierro, J. Morales, M. Roseblatt, and M. R. Bono. 2004. Interleukin-4 selectively inhibits interleukin-2 secretion by lipopolysaccharide-activated dendritic cells. *Scand J Immunol* 59:183-189.
 193. Koka, R., P. Burkett, M. Chien, S. Chai, D. L. Boone, and A. Ma. 2004. Cutting edge: murine dendritic cells require IL-15R alpha to prime NK cells. *J Immunol* 173:3594-3598.
 194. Watt, S. V., D. M. Andrews, K. Takeda, M. J. Smyth, and Y. Hayakawa. 2008. IFN-gamma-dependent recruitment of mature CD27(high) NK cells to lymph nodes primed by dendritic cells. *J Immunol* 181:5323-5330.
 195. Mocikat, R., H. Braumuller, A. Gumy, O. Egeter, H. Ziegler, U. Reusch, A. Bubeck, J. Louis, R. Mailhammer, G. Riethmuller, U. Koszinowski, and M. Rocken. 2003. Natural killer cells activated by MHC class I(low) targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* 19:561-569.
 196. Chambers, B. J., M. Salcedo, and H. G. Ljunggren. 1996. Triggering of natural killer cells by the costimulatory molecule CD80 (B7-1). *Immunity* 5:311-317.
 197. Parajuli, P., Y. Nishioka, N. Nishimura, S. M. Singh, M. Hanibuchi, H. Nokihara, H. Yanagawa, and S. Sone. 1999. Cytolysis of human dendritic cells by autologous lymphokine-activated killer cells: participation of both T cells and NK cells in the killing. *J Leukoc Biol* 65:764-770.
 198. Carbone, E., G. Terrazzano, G. Ruggiero, D. Zanzi, A. Ottaiano, C. Manzo, K. Karre, and S. Zappacosta. 1999. Recognition of autologous dendritic cells by human NK cells. *Eur J Immunol* 29:4022-4029.
 199. Wilson, J. L., L. C. Heffler, J. Charo, A. Scheynius, M. T. Bejarano, and H. G. Ljunggren. 1999. Targeting of human dendritic cells by autologous NK cells. *J Immunol* 163:6365-6370.
 200. Ferlazzo, G., C. Semino, and G. Melioli. 2001. HLA class I molecule expression is up-regulated during maturation of dendritic cells, protecting them from natural killer cell-mediated lysis. *Immunology letters* 76:37-41.
 201. 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. *Eur J Immunol* 33:1657-1666.
 202. 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. *J Exp Med* 195:343-351.
203. Pende, D., R. Castriconi, P. Romagnani, G. M. Spaggiari, S. Marcenaro, A. Dondero, E. Lazzeri, L. Lasagni, S. Martini, P. Rivera, A. Capobianco, L. Moretta, A. Moretta, and C. Bottino. 2006. Expression of the DNAM-1 ligands, Nectin-2 (CD112) and poliovirus receptor (CD155), on dendritic cells: relevance for natural killer-dendritic cell interaction. *Blood* 107:2030-2036.
 204. 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* 295:2097-2100.
 205. Velardi, A., L. Ruggeri, Alessandro, Moretta, and L. Moretta. 2002. NK cells: a lesson from mismatched hematopoietic transplantation. *Trends Immunol* 23:438-444.
 206. Yu, G., X. Xu, M. D. Vu, E. D. Kilpatrick, and X. C. Li. 2006. NK cells promote transplant tolerance by killing donor antigen-presenting cells. *J Exp Med* 203:1851-1858.
 207. Bajenoff, M., B. Breart, A. Y. Huang, H. Qi, J. Cazareth, V. M. Braud, R. N. Germain, and N. Glaichenhaus. 2006. Natural killer cell behavior in lymph nodes revealed by static and real-time imaging. *J Exp Med* 203:619-631.
 208. Laffont, S., C. Seillet, J. Ortaldo, J. D. Coudert, and J. C. Guery. 2008. Natural killer cells recruited into lymph nodes inhibit alloreactive T-cell activation through perforin-mediated killing of donor allogeneic dendritic cells. *Blood* 112:661-671.
 209. Buentke, E., L. C. Heffler, J. L. Wilson, R. P. Wallin, C. Lofman, B. J. Chambers, H. G. Ljunggren, and A. Scheynius. 2002. Natural killer and dendritic cell contact in lesional atopic dermatitis skin--Malassezia-influenced cell interaction. *J Invest Dermatol* 119:850-857.
 210. Winkler-Pickett, R., H. A. Young, J. M. Cherry, J. Diehl, J. Wine, T. Back, W. E. Bere, A. T. Mason, and J. R. Ortaldo. 2008. In vivo regulation of experimental autoimmune encephalomyelitis by NK cells: alteration of primary adaptive responses. *J Immunol* 180:4495-4506.
 211. Nicolle, C. M., L. 1908. Sur une infection á corps de Leishman (ou organismes voisins) du gondi. *C.R. Seances Acad. Sci.* 147:763-766.
 212. Nicolle, C. M., L. 1909. Sur un protozoaire nouveau du gondii. *C.R. Seances Acad. Sci.* 148:369-372.
 213. Splendore, a. 1908. Un nuovo protozoa parassita de' conigli. incontrato nelle lesioni anatomiche d'une malattia che ricorda in molti punti il Kala-azar dell' uomo. Nota preliminare pel. *Rev. Soc. Scient. Sao Paulo* 3:109-112.
 214. Black, M. W., and J. C. Boothroyd. 2000. Lytic cycle of *Toxoplasma gondii*. *Microbiol Mol Biol Rev* 64:607-623.
 215. Montoya, J. G., and O. Liesenfeld. 2004. Toxoplasmosis. *Lancet* 363:1965-1976.
 216. Hutchison, W. M., J. F. Dunachie, J. C. Siim, and K. Work. 1970. Coccidian-like nature of *Toxoplasma gondii*. *British medical journal* 1:142-144.
 217. Frenkel, J. K., J. P. Dubey, and N. L. Miller. 1970. *Toxoplasma gondii* in cats: fecal stages identified as coccidian oocysts. *Science* 167:893-896.
 218. Tenter, A. M., A. R. Heckerth, and L. M. Weiss. 2000. *Toxoplasma gondii*: from animals to humans. *Int J Parasitol* 30:1217-1258.
 219. Dobrowolski, J. M., and L. D. Sibley. 1996. *Toxoplasma* invasion of mammalian cells is powered by the actin cytoskeleton of the parasite. *Cell* 84:933-939.
 220. Joiner, K. A. 1993. Cell entry by *Toxoplasma gondii*: all paths do not lead to success. *Res Immunol* 144:34-38.
 221. Ajzenberg, D., N. Cogne, L. Paris, M. H. Bessieres, P. Thulliez, D. Filisetti, H. Pelloux, P. Marty, and M. L. Darde. 2002. Genotype of 86 *Toxoplasma gondii* isolates associated with human congenital toxoplasmosis, and correlation with clinical findings. *J Infect Dis* 186:684-689.

222. Howe, D. K., S. Honore, F. Derouin, and L. D. Sibley. 1997. Determination of genotypes of *Toxoplasma gondii* strains isolated from patients with toxoplasmosis. *Journal of clinical microbiology* 35:1411-1414.
223. Howe, D. K., and L. D. Sibley. 1995. *Toxoplasma gondii* comprises three clonal lineages: correlation of parasite genotype with human disease. *J Infect Dis* 172:1561-1566.
224. Sibley, L. D., and J. C. Boothroyd. 1992. Virulent strains of *Toxoplasma gondii* comprise a single clonal lineage. *Nature* 359:82-85.
225. Luft, B. J., and J. S. Remington. 1992. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 15:211-222.
226. Ambroise-Thomas, P., and H. Pelloux. 1993. Toxoplasmosis - congenital and in immunocompromised patients: a parallel. *Parasitol Today* 9:61-63.
227. Barragan, A., and L. D. Sibley. 2002. Transepithelial migration of *Toxoplasma gondii* is linked to parasite motility and virulence. *J Exp Med* 195:1625-1633.
228. Joiner, K. A., S. A. Fuhrman, H. M. Miettinen, L. H. Kasper, and I. Mellman. 1990. *Toxoplasma gondii*: fusion competence of parasitophorous vacuoles in Fc receptor-transfected fibroblasts. *Science* 249:641-646.
229. Jones, T. C., and J. G. Hirsch. 1972. The interaction between *Toxoplasma gondii* and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasites. *J Exp Med* 136:1173-1194.
230. Gazzinelli, R. T., M. Wysocka, S. Hayashi, E. Y. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher. 1994. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with *Toxoplasma gondii*. *J Immunol* 153:2533-2543.
231. Reis e Sousa, C., S. Hieny, T. Scharon-Kersten, D. Jankovic, H. Charest, R. N. Germain, and A. Sher. 1997. In vivo microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J Exp Med* 186:1819-1829.
232. Suzuki, Y., M. A. Orellana, R. D. Schreiber, and J. S. Remington. 1988. Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. *Science* 240:516-518.
233. Hughes, H. P. 1988. Oxidative killing of intracellular parasites mediated by macrophages. *Parasitol Today* 4:340-347.
234. Murray, H. W., and Z. A. Cohn. 1979. Macrophage oxygen-dependent antimicrobial activity. I. Susceptibility of *Toxoplasma gondii* to oxygen intermediates. *J Exp Med* 150:938-949.
235. Adams, L. B., J. B. Hibbs, Jr., R. R. Taintor, and J. L. Krahenbuhl. 1990. Microbiostatic effect of murine-activated macrophages for *Toxoplasma gondii*. Role for synthesis of inorganic nitrogen oxides from L-arginine. *J Immunol* 144:2725-2729.
236. Denkers, E. Y., and R. T. Gazzinelli. 1998. Regulation and function of T-cell-mediated immunity during *Toxoplasma gondii* infection. *Clin Microbiol Rev* 11:569-588.
237. Gazzinelli, R. T., F. T. Hakim, S. Hieny, G. M. Shearer, and A. Sher. 1991. Synergistic role of CD4+ and CD8+ T lymphocytes in IFN-gamma production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. *J Immunol* 146:286-292.
238. Gazzinelli, R., Y. Xu, S. Hieny, A. Cheever, and A. Sher. 1992. Simultaneous depletion of CD4+ and CD8+ T lymphocytes is required to reactivate chronic infection with *Toxoplasma gondii*. *J Immunol* 149:175-180.
239. Yano, A., F. Aosai, M. Ohta, H. Hasekura, K. Sugane, and S. Hayashi. 1989. Antigen presentation by *Toxoplasma gondii*-infected cells to CD4+ proliferative T cells and CD8+ cytotoxic cells. *The Journal of parasitology* 75:411-416.
240. Subauste, C. S., A. H. Koniaris, and J. S. Remington. 1991. Murine CD8+ cytotoxic T lymphocytes lyse *Toxoplasma gondii*-infected cells. *J Immunol* 147:3955-3959.
241. Kang, H., J. S. Remington, and Y. Suzuki. 2000. Decreased resistance of B cell-deficient mice to infection with *Toxoplasma gondii* despite unimpaired

- expression of IFN-gamma, TNF-alpha, and inducible nitric oxide synthase. *J Immunol* 164:2629-2634.
242. Khan, I. A., T. Matsuura, and L. H. Kasper. 1996. Activation-mediated CD4+ T cell unresponsiveness during acute *Toxoplasma gondii* infection in mice. *Int Immunol* 8:887-896.
243. Goebel, S., U. Gross, and C. G. Luder. 2001. Inhibition of host cell apoptosis by *Toxoplasma gondii* is accompanied by reduced activation of the caspase cascade and alterations of poly(ADP-ribose) polymerase expression. *J Cell Sci* 114:3495-3505.
244. Keller, P., F. Schaumburg, S. F. Fischer, G. Hacker, U. Gross, and C. G. Luder. 2006. Direct inhibition of cytochrome c-induced caspase activation in vitro by *Toxoplasma gondii* reveals novel mechanisms of interference with host cell apoptosis. *FEMS microbiology letters* 258:312-319.
245. Payne, T. M., R. E. Molestina, and A. P. Sinai. 2003. Inhibition of caspase activation and a requirement for NF-kappaB function in the *Toxoplasma gondii*-mediated blockade of host apoptosis. *J Cell Sci* 116:4345-4358.
246. Nash, P. B., M. B. Purner, R. P. Leon, P. Clarke, R. C. Duke, and T. J. Curiel. 1998. *Toxoplasma gondii*-infected cells are resistant to multiple inducers of apoptosis. *J Immunol* 160:1824-1830.
247. Neyer, L. E., G. Grunig, M. Fort, J. S. Remington, D. Rennick, and C. A. Hunter. 1997. Role of interleukin-10 in regulation of T-cell-dependent and T-cell-independent mechanisms of resistance to *Toxoplasma gondii*. *Infect Immun* 65:1675-1682.
248. Bogdan, C., and C. Nathan. 1993. Modulation of macrophage function by transforming growth factor beta, interleukin-4, and interleukin-10. *Annals of the New York Academy of Sciences* 685:713-739.
249. Aliberti, J., C. Serhan, and A. Sher. 2002. Parasite-induced lipoxin A4 is an endogenous regulator of IL-12 production and immunopathology in *Toxoplasma gondii* infection. *J Exp Med* 196:1253-1262.
250. Butcher, B. A., L. Kim, A. D. Panopoulos, S. S. Watowich, P. J. Murray, and E. Y. Denkers. 2005. IL-10-independent STAT3 activation by *Toxoplasma gondii* mediates suppression of IL-12 and TNF-alpha in host macrophages. *J Immunol* 174:3148-3152.
251. Butcher, B. A., L. Kim, P. F. Johnson, and E. Y. Denkers. 2001. *Toxoplasma gondii* tachyzoites inhibit proinflammatory cytokine induction in infected macrophages by preventing nuclear translocation of the transcription factor NF-kappa B. *J Immunol* 167:2193-2201.
252. Butcher, B. A., and E. Y. Denkers. 2002. Mechanism of entry determines the ability of *Toxoplasma gondii* to inhibit macrophage proinflammatory cytokine production. *Infect Immun* 70:5216-5224.
253. Aldrich, C. J., J. R. Rodgers, and R. R. Rich. 1988. Regulation of Qa-1 expression and determinant modification by an H-2D-linked gene, Qdm. *Immunogenetics* 28:334-344.
254. Kambayashi, T., J. R. Kraft-Leavy, J. G. Dauner, B. A. Sullivan, O. Laur, and P. E. Jensen. 2004. The nonclassical MHC class I molecule Qa-1 forms unstable peptide complexes. *J Immunol* 172:1661-1669.
255. Megjugorac, N. J., H. A. Young, S. B. Amrute, S. L. Olshalsky, and P. Fitzgerald-Bocarsly. 2004. Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. *J Leukoc Biol* 75:504-514.
256. Krug, A., A. Towarowski, S. Britsch, S. Rothenfusser, V. Hornung, R. Bals, T. Giese, H. Engelmann, S. Endres, A. M. Krieg, and G. Hartmann. 2001. Toll-like receptor expression reveals CpG DNA as a unique microbial stimulus for plasmacytoid dendritic cells which synergizes with CD40 ligand to induce high amounts of IL-12. *Eur J Immunol* 31:3026-3037.
257. Krug, A., R. Uppaluri, F. Facchetti, B. G. Dorner, K. C. Sheehan, R. D. Schreiber, M. Cella, and M. Colonna. 2002. IFN-producing cells respond to CXCR3 ligands in the presence of CXCL12 and secrete inflammatory chemokines upon activation. *J Immunol* 169:6079-6083.

258. Prlic, M., J. Gibbs, and S. C. Jameson. 2005. Characteristics of NK cell migration early after vaccinia infection. *J Immunol* 175:2152-2157.
259. Smyth, M. J., J. M. Kelly, A. G. Baxter, H. Korner, and J. D. Sedgwick. 1998. An essential role for tumor necrosis factor in natural killer cell-mediated tumor rejection in the peritoneum. *J Exp Med* 188:1611-1619.
260. Glas, R., L. Franksson, C. Une, M. L. Eloranta, C. Ohlen, A. Orn, and K. Karre. 2000. Recruitment and activation of natural killer (NK) cells in vivo determined by the target cell phenotype. An adaptive component of NK cell-mediated responses. *J Exp Med* 191:129-138.
261. Khan, I. A., S. Y. Thomas, M. M. Moretto, F. S. Lee, S. A. Islam, C. Combe, J. D. Schwartzman, and A. D. Luster. 2006. CCR5 is essential for NK cell trafficking and host survival following *Toxoplasma gondii* infection. *PLoS Pathog* 2:e49.
262. Neote, K., D. DiGregorio, J. Y. Mak, R. Horuk, and T. J. Schall. 1993. Molecular cloning, functional expression, and signaling characteristics of a C-C chemokine receptor. *Cell* 72:415-425.
263. Gao, J. L., D. B. Kuhns, H. L. Tiffany, D. McDermott, X. Li, U. Francke, and P. M. Murphy. 1993. Structure and functional expression of the human macrophage inflammatory protein 1 alpha/RANTES receptor. *J Exp Med* 177:1421-1427.
264. Post, T. W., C. R. Bozic, M. E. Rothenberg, A. D. Luster, N. Gerard, and C. Gerard. 1995. Molecular characterization of two murine eosinophil beta chemokine receptors. *J Immunol* 155:5299-5305.
265. Furuichi, K., J. L. Gao, R. Horuk, T. Wada, S. Kaneko, and P. M. Murphy. 2008. Chemokine receptor CCR1 regulates inflammatory cell infiltration after renal ischemia-reperfusion injury. *J Immunol* 181:8670-8676.
266. Maier, S., C. Tertilt, N. Chambron, K. Gerauer, N. Huser, C. D. Heidecke, and K. Pfeffer. 2001. Inhibition of natural killer cells results in acceptance of cardiac allografts in CD28^{-/-} mice. *Nat Med* 7:557-562.
267. Nandi, D., J. A. Gross, and J. P. Allison. 1994. CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. *J Immunol* 152:3361-3369.
268. Geldhof, A. B., G. Raes, M. Bakkus, S. Devos, K. Thielemans, and P. De Baetselier. 1995. Expression of B7-1 by highly metastatic mouse T lymphomas induces optimal natural killer cell-mediated cytotoxicity. *Cancer Res* 55:2730-2733.
269. Geldhof, A. B., M. Moser, L. Lespagnard, K. Thielemans, and P. De Baetselier. 1998. Interleukin-12-activated natural killer cells recognize B7 costimulatory molecules on tumor cells and autologous dendritic cells. *Blood* 91:196-206.
270. Hunter, C. A., L. Ellis-Neyer, K. E. Gabriel, M. K. Kennedy, K. H. Grabstein, P. S. Linsley, and J. S. Remington. 1997. The role of the CD28/B7 interaction in the regulation of NK cell responses during infection with *Toxoplasma gondii*. *J Immunol* 158:2285-2293.
271. Wilson, J. L., J. Charo, A. Martin-Fontecha, P. Dellabona, G. Casorati, B. J. Chambers, R. Kiessling, M. T. Bejarano, and H. G. Ljunggren. 1999. NK cell triggering by the human costimulatory molecules CD80 and CD86. *J Immunol* 163:4207-4212.
272. Azuma, M., M. Cayabyab, D. Buck, J. H. Phillips, and L. L. Lanier. 1992. Involvement of CD28 in MHC-unrestricted cytotoxicity mediated by a human natural killer leukemia cell line. *J Immunol* 149:1115-1123.
273. Galea-Lauri, J., D. Darling, S. U. Gan, L. Krivochchapov, M. Kuiper, J. Gaken, B. Souberbielle, and F. Farzaneh. 1999. Expression of a variant of CD28 on a subpopulation of human NK cells: implications for B7-mediated stimulation of NK cells. *J Immunol* 163:62-70.
274. Liu, C., Y. Lou, G. Lizee, H. Qin, S. Liu, B. Rabinovich, G. J. Kim, Y. H. Wang, Y. Ye, A. G. Sikora, W. W. Overwijk, Y. J. Liu, G. Wang, and P. Hwu. 2008. Plasmacytoid dendritic cells induce NK cell-dependent, tumor antigen-specific T cell cross-priming and tumor regression in mice. *J Clin Invest* 118:1165-1175.

275. Ferlazzo, G., B. Morandi, A. D'Agostino, R. Meazza, G. Melioli, A. Moretta, and L. Moretta. 2003. The interaction between NK cells and dendritic cells in bacterial infections results in rapid induction of NK cell activation and in the lysis of uninfected dendritic cells. *Eur J Immunol* 33:306-313.
276. Persson, E. K., A. M. Agnarson, H. Lambert, N. Hitziger, H. Yagita, B. J. Chambers, A. Barragan, and A. Grandien. 2007. Death receptor ligation or exposure to perforin trigger rapid egress of the intracellular parasite *Toxoplasma gondii*. *J Immunol* 179:8357-8365.
277. Johnson, L. L., F. P. VanderVegt, and E. A. Havell. 1993. Gamma interferon-dependent temporary resistance to acute *Toxoplasma gondii* infection independent of CD4+ or CD8+ lymphocytes. *Infect Immun* 61:5174-5180.
278. Hunter, C. A., C. S. Subauste, V. H. Van Cleave, and J. S. Remington. 1994. Production of gamma interferon by natural killer cells from *Toxoplasma gondii*-infected SCID mice: regulation by interleukin-10, interleukin-12, and tumor necrosis factor alpha. *Infect Immun* 62:2818-2824.
279. Denkers, E. Y., R. T. Gazzinelli, D. Martin, and A. Sher. 1993. Emergence of NK1.1+ cells as effectors of IFN-gamma dependent immunity to *Toxoplasma gondii* in MHC class I-deficient mice. *J Exp Med* 178:1465-1472.
280. Sher, A., I. P. Oswald, S. Hieny, and R. T. Gazzinelli. 1993. *Toxoplasma gondii* induces a T-independent IFN-gamma response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-alpha. *J Immunol* 150:3982-3989.
281. 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.
282. Guan, H., M. Moretto, D. J. Bzik, J. Gigley, and I. A. Khan. 2007. NK cells enhance dendritic cell response against parasite antigens via NKG2D pathway. *J Immunol* 179:590-596.
283. Combe, C. L., T. J. Curiel, M. M. Moretto, and I. A. Khan. 2005. NK cells help to induce CD8(+)-T-cell immunity against *Toxoplasma gondii* in the absence of CD4(+) T cells. *Infect Immun* 73:4913-4921.
284. Denkers, E. Y., G. Yap, T. Scharton-Kersten, H. Charest, B. A. Butcher, P. Caspar, S. Heiny, and A. Sher. 1997. Perforin-mediated cytolysis plays a limited role in host resistance to *Toxoplasma gondii*. *J Immunol* 159:1903-1908.
285. Boysen, P., S. Klevar, I. Olsen, and A. K. Storset. 2006. The protozoan *Neospora caninum* directly triggers bovine NK cells to produce gamma interferon and to kill infected fibroblasts. *Infect Immun* 74:953-960.