universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

„Cell type-specific functions of TYK2 in the antiviral defense against MCMV“

Verfasser

David Popp

angestrebter akademischer Grad

Magister der Naturwissenschaften (Mag.rer.nat.)

Wien, Februar 2012

Studienkennzahl lt. Studienblatt:	A 441
Studienrichtung lt. Studienblatt:	Diplomstudium Genetik-Mikrobiologie
Betreuer:	Univ. Prof. Dr. Pavel Kovarik

1. Abstract

Tyrosine kinase 2 (TYK2) is one of the four mammalian receptor-associated Janus kinases (JAKs), which together with signal transducers and activators of transcription (STATs) constitute the JAK/STAT pathway, a crucial signaling route utilized by many cytokines and growth factors. TYK2 is critically involved in the innate and adaptive immune defense against viruses and other pathogens. Cytomegaloviruses, members of the Herpesviridae family, had great evolutionary success in evading their host's immune systems. Human Cytomegalovirus (HCMV) is prevalent in most populations worldwide, causes the majority of infection-related congenital birth defects and is responsible for considerable morbidity and mortality in immune suppressed patients. TYK2 has been shown to be a key factor in the defense against murine Cytomegalovirus (MCMV). The general aim of the project is to investigate cell type-specific functions of TYK2 in MCMV defense with a special emphasis on dendritic cells (DC) and macrophages. To this end, conditional TYK2 knockout mice ($TYK2^{fl/fl}$) have been generated in the laboratory and crossed with mice expressing cre recombinase transgene under the control of cell type-specific (i.e. CD11c, LysM) promoters. The topic of this diploma thesis was to characterize $TYK2^{\Delta CD11c}$ mice with respect to TYK2 deletion efficiencies in DCs and to analyze the effect on anti-MCMV defense. We could show highly efficient deletion of TYK2 in bone marrow-derived DCs (BMDCs) and in freshly isolated splenic DCs. Similar to what has been shown in macrophages, we found a strongly increased MCMV replication in DCs derived from $TYK2^{\Delta CD11c}$ and $TYK2^{\Delta/\Delta}$ (i.e. ubiquitous deletion of TYK2) mice *in vitro*. This was accompanied by reduced activation of STAT1 and STAT2 and thus most likely reflects impaired autocrine/paracrine type I interferon (IFN) signaling. Upon MCMV infections *in vivo*, we found increased viral load in livers of $TYK2^{\Delta CD11c}$ compared to $TYK2^{fl/fl}$ and $TYK2^{\Delta LysM}$ mice at 3 days post infection. Surprisingly, this was only observed upon low-dose infection and virus was almost entirely cleared from livers at day seven post infection, irrespective of the genotype and infectious dose. In contrast, increased virus load was found in salivary glands from $TYK2^{\Delta CD11c}$ and $TYK2^{\Delta LysM}$ mice at seven days post infection upon high-dose infection, whereas virus was not detectable in $TYK2^{\Delta LysM}$ and $TYK2^{\Delta/\Delta}$ upon low-dose infection. In summary, our data show that TYK2 is required in DCs to control MCMV replication *in vitro* and indicate an organ-specific and infectious dose-dependent contribution of TYK2 in DCs and macrophages in MCMV defense *in vivo*. This study gives insight in which cellular context TYK2 is needed to control MCMV infections and may be important to reveal mechanisms of host immunity to MCMV infections in greater detail in the future.

2. Zusammenfassung

Die Tyrosin-Kinase 2 (TYK2) ist eine der vier rezeptorassoziierten Janus Kinasen (JAKs) der Säugetiere. Gemeinsam mit Signal Transduktoren und Aktivatoren der Transkription (STATs) konstituiert TYK2 den JAK/STAT Signalweg, eine bedeutende, von vielen Zytokinen und Wachstumsfaktoren genutzte, Signalroute. TYK2 ist entscheidend bei der angeborenen und adaptiven Immunabwehr gegen Viren und andere Pathogene beteiligt. Cytomegaloviren, Mitglieder der Herpesviridae Familie, hatten großen evolutionären Erfolg im Umgehen der Immunsyteme ihrer Wirte. Der humane Cytomegalovirus (HCMV) ist prävalent in den meisten Populationen weltweit, verursacht einen Großteil der infektionsbedingten angeborenen Geburtsdefekte und ist verantwortlich für vermehrte Krankheitsanfälligkeit und Sterblichkeit in immunsuppremierten Patienten. TYK2 ist ein bekannter Schlüsselfaktor in der Abwehr gegen den murinen Cytomegalovirus (MCMV). Das Ziel dieses Projekts ist die Untersuchung zelltypspezifischer Funktionen von TYK2 in der MCMV Abwehr mit speziellem Augenmerk auf Dendritischen Zellen (DCs) und Makrophagen. Zu diesem Zweck wurden im Labor konditionelle TYK2 Knockout-Mäuse generiert und mit Mäusen gekreuzt, welche das Cre Rekombinase Transgen unter der Kontrolle von zelltypspezifischen (z.B. CD11c, LysM) Promotoren exprimieren. Gegenstand dieser Diplomarbeit war $TYK2^{\Delta CD11c}$ Mäuse hinsichtlich der Deletionseffizienz von TYK2 in DCs zu charakterisieren und den Effekt auf die MCMV Abwehr zu analysieren. Wir konnten zeigen, dass TYK2 in Knochenmark-DCs (BMDCs) und frisch isolierten Milz-DCs sehr gut deletiert wird. Ähnlich zu der bereits bekannten Situation in Makrophagen, konnten wir auch in DCs von $TYK2^{\Delta CD11c}$ und $TYK2^{\Delta/\Delta}$ (entspricht ubiquitärer Deletion von TYK2) Mäusen *in vitro* stark erhöhte MCMV Replikation feststellen. Gemeinsam mit der ebenfalls beobachteten, reduzierten Aktivierung von STAT1 und STAT2 reflektiert dies höchstwahrscheinlich eine Beeinträchtigung der autokrinen/parakrinen Typ I Interferon (IFN) Signalwirkung. MCMV Infektion *in vivo* führte drei Tage nach der Infektion zu einer erhöhten Virenlast in Lebern von $TYK2^{\Delta CD11c}$ Mäusen verglichen mit denen von $TYK2^{fl/fl}$ und $TYK2^{\Delta LysM}$ Mäusen. Überraschenderweise war dies nur nach der Infektion mit niedrigen Infektionsdosen zu beobachten. Sieben Tage nach der Infektion waren die Lebern nahezu zur Gänze von Viren befreit, unabhängig vom Genotyp und der Infektionsdosis. Im Gegensatz dazu wurde eine erhöhte Virenlast in Speicheldrüsen von $TYK2^{\Delta CD11c}$ und $TYK2^{\Delta LysM}$ Mäusen sieben Tage nach der Infektion mit hoher Infektionsdosis gefunden, wohingegen keine Viren in $TYK2^{\Delta LysM}$ und $TYK2^{\Delta/\Delta}$ Mäusen nach Infektion mit niedriger Dosis detektierbar waren. Zusammenfassend zeigen unsere Daten, dass TYK2 in DCs benötigt wird um die MCMV Replikation *in vitro* zu kontrollieren. Zudem weisen die Ergebnisse auf eine organ- und infektionsdosisabhängige Beteiligung von TYK2 in DCs und Makrophagen an der MCMV Abwehr *in vivo* hin. Alles in allem, trägt

diese Studie zum besseren Verständnis der zellulären Zusammenhänge bei, in denen TYK2 benötigt wird, um MCMV Infektionen zu kontrollieren und ist somit ein entscheidender Beitrag zur Aufklärung der Abwehrmechanismen gegen MCMV Infektionen.

3. Introduction

3.1. The JAK/STAT Pathway

The Janus kinase (JAK)/signal transducer and activator of transcription(STAT) pathway is an essential signal transduction pathway for cytokines and various growth factors[1]. It basically consists of three elements: a transmembrane receptor complex which binds extracellular peptides, JAK proteins which are associated at the cytoplasmic tails of the receptor chains, and STAT proteins which are able to migrate to the nucleus and induce target gene expression. When ligands bind to the receptor complex a conformational change on the cytoplasmic side of the receptor is induced. Due to this conformational change associated JAK proteins come in close proximity and get activated by trans- and/or autophosphorylation. JAKs then phosphorylate tyrosine residues of the receptor tails and therefore create docking sites for STAT proteins. Recruited STAT proteins get phosphorylated by JAKs and undergo a conformational change which allows them to dissociate from the receptor. Activated STATs then migrate as homo- and/or heterodimers to the nucleus where they bind promoter regions of target genes to activate transcription[1, 2].

Four JAKs (JAK1, JAK2, JAK3 and TYK2) and seven STATs (STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6) have been identified in mammals[3]. Although they were initially discovered in human cell lines[4], the importance of members of the JAK/STAT pathway *in vivo* was mainly revealed by studies using knockout mice. JAK1$^{-/-}$ mice for instance develop neurological lesions causing a postnatal lethal failure in suckling[5]. JAK2 knockouts die on embryonic day 12.5, due to defects in definitive erythropoiesis[6, 7]. Mice lacking JAK3 are viable but display severe defects in lymphoid development leading to a severe combined immunodeficiency (SCID-) like phenotype. STAT1 and STAT2 knockouts show a dramatically increased susceptibility to infectious diseases due to impaired innate immune defense mechanisms. STAT3 is known to regulate cell growth and STAT3$^{-/-}$ mice show an early embryonic lethal phenotype. Due to impaired interleukin (IL-) 12 signaling STAT4$^{-/-}$ mice have defects in Th1 cell development, while STAT6$^{-/-}$ mice show severely impaired Th2 cell differentiation. STAT5a and STAT5b share 96% sequence homology and are to some extend functionally redundant. Impaired mammary gland development and lymphohematopoietic defects were reported in STAT5a-deficient mice, whereas STAT5b-deficient males are significantly smaller than wild type males, express higher levels of female-specific liver genes and show a loss in expression of male-specific liver genes. Double knockout mice show a combined phenotype with severe defects in development and expansion of myeloid and lymphoid cell lineages[8].

3.2. TYK2

3.2.1. Structure

Tyrosine kinase 2 (TYK2) was the first described member of the JAK family of non-receptor tyrosine kinases[9, 10]. It is considered to be ubiquitously expressed although unpublished data from our laboratory suggest differences in expression levels between cell types and organs. In humans the TYK2 gene is located on chromosome 19 and in mice on chromosome 9. The TYK2 protein is a multi-domain protein, consists of about 1200 amino acids and shares seven homology regions with all other JAKs (JH1-JH7)[11, 12]. At the C-terminal end the JH1 domain functions as active kinase with the catalytic activity located at the so called activation loop around two conserved tyrosine residues. The pseudokinase domain (JH2) takes over eminent regulatory functions. Further toward the N-terminal end, the src-homology 2 (SH2) domain is located (JH3/4), which until now has no known function. The largest domain is a four-point-one, ezrin, radixin, moesin (FERM) homology domain spanning JH4, JH5, JH6 and JH7. This domain mediates the interaction and specificity of JAKs with their appropriate receptor[12].

3.2.2. TYK2 Functions in Signaling

The function of TYK2 was first analyzed by studies with a TYK2-deficient human fibrosarcoma cell line[13]. Today the B10.Q/J (B10.D1-H2^{q}/SgJ.) mouse strain, carrying a natural mutation in TYK2, and three TYK2 knockout mice, which were generated by different groups using different targeting strategies, are available to study TYK2 functions[14-17].

TYK2 is associated with different receptor complexes and gets activated by several cytokines (Fig. 1).

It is localized on the cytoplasmic side of IFN-α/β receptor 1 (IFNAR1) in the type I interferon (IFN) receptor complex consisting of IFNAR1/TYK2 and IFNAR2/JAK1. This complex mainly induces STAT1 and STAT2 activation but, dependent on the cell type, all other STATs can get phosphorylated as well[18]. In human cell lines lacking TYK2 type I IFN signaling is dramatically impaired due to a significant reduction of IFNAR1 surface expression[19]. In contrast, murine TYK2$^{-/-}$ macrophages and fibroblasts show normal IFNAR1 levels and just partially impaired IFN-α and IFN-β signaling[15, 17].

Associated with IL-10 receptor 2 (IL-10R2) TYK2 also takes part in IL-10 family signaling. The role of TYK2 in most of these pathways has not yet been analyzed but at least for IL-10 signaling a redundant function of TYK2 can be suggested as it has been shown that the IL-10 response remains unchanged in splenocytes lacking TYK2[15, 16]. Contrarily, in TYK2-deficient macrophages, reduced IL-10 dependent STAT3 activation and decreased suppression of IFN-induced nitrite production was observed in a different study[20].

Two members of the IL-12 family, IL-12 and IL-23, signal through the TYK2-associated IL-12Rβ1 in combination with JAK2-associated IL-12Rβ2 or IL-23R. Absence of TYK2 in this complex significantly impairs IL-12 signaling[15, 21, 22]. Nevertheless, costimmulatory effects of IL-12 on T-cell proliferation remain unchanged[16]. Reduced IL-23 mediated STAT3 activation has been reported in T-cells derived from a mutant TYK2 defective mouse strain[14].

Additionally, TYK2 can be activated by cytokines like IL-6, IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM) and ciliary neurotrophic factor (CNTF) which utilize gp130 consisting receptor complexes. Signal transduction by this complex is mainly dependent on JAK1. TYK2 was shown to have a redundant role in IL-6 and LIF signaling[15]. TYK2 can also associate with IL-13Rα1 and was shown to be needed for the induction of IL-13 target genes in human cells[23].

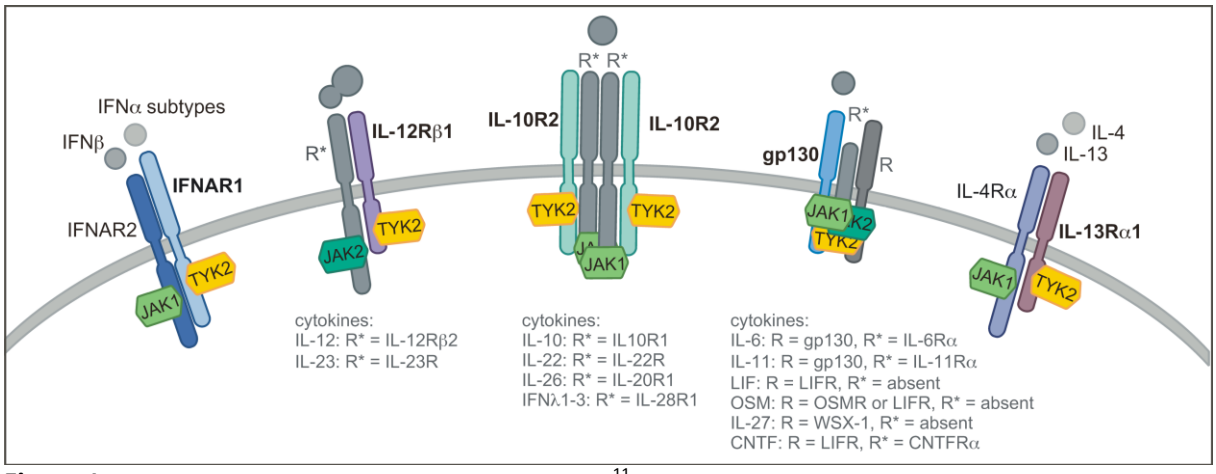

Figure 1: Receptor complexes where TYK2 is associated[11]

3.2.3. Role of TYK2 in Infectious Diseases

Most importantly, it has been shown that the absence of TYK2 leads to a significantly higher susceptibility to infectious diseases. During Lymphocytic Choriomeningitis Virus (LCMV) infection, for example, TYK2$^{-/-}$ mice fail to instigate CD8^{+} cytotoxic T-cells (CTL), which are important effector cells of the adaptive immune system[15]. TYK2$^{-/-}$ mice show higher virus titers in spleens, lungs, livers and salivary glands and increased lethality during murine Cytomegalovirus (MCMV) infection[24]. Furthermore, after intravenous infection of TYK2-deficient mice with Vaccina Virus (VV), increased virus titers were detected in the spleens[15]. Facing protozoan pathogens a higher susceptibility against *Leishmania major* and *Toxoplasma gondii* due to impaired IFN-γ production in natural killer (NK-) cells and T-cells was reported[25, 26]. TYK2$^{-/-}$ mice infected with the bacterium *Listeria monocytogenes* show increased mortality compared to wild type mice. This might be attributed to impaired dendritic cells (DC) priming of CD8^{+} T-cells and defective T-cell dependent IFN-γ production[27, 28]. In *Escherichia*

coli infections, TYK2$^{-/-}$ mice showed higher bacterial load and expressed less IL-17 which correlated with reduced neutrophile granulocyte numbers in the peritoneal cavity[29].

In summary, the most prominent defects in the immune defense against infectious diseases in TYK2$^{-/-}$ mice is the impaired IL-12 mediated IFN-γ production plus, to a less well-defined extend, impaired IL-23-mediated IL-17 expression and reduced IFN-α/β responses (Fig. 2). However, TYK2 is activated by a number of other cytokines (as outlined above) which are crucial for immune responses or have still unknown functions. Future analysis will be required in order to fully understand the many functions of TYK2, in particular with respect to cell type and pathogen specificities.

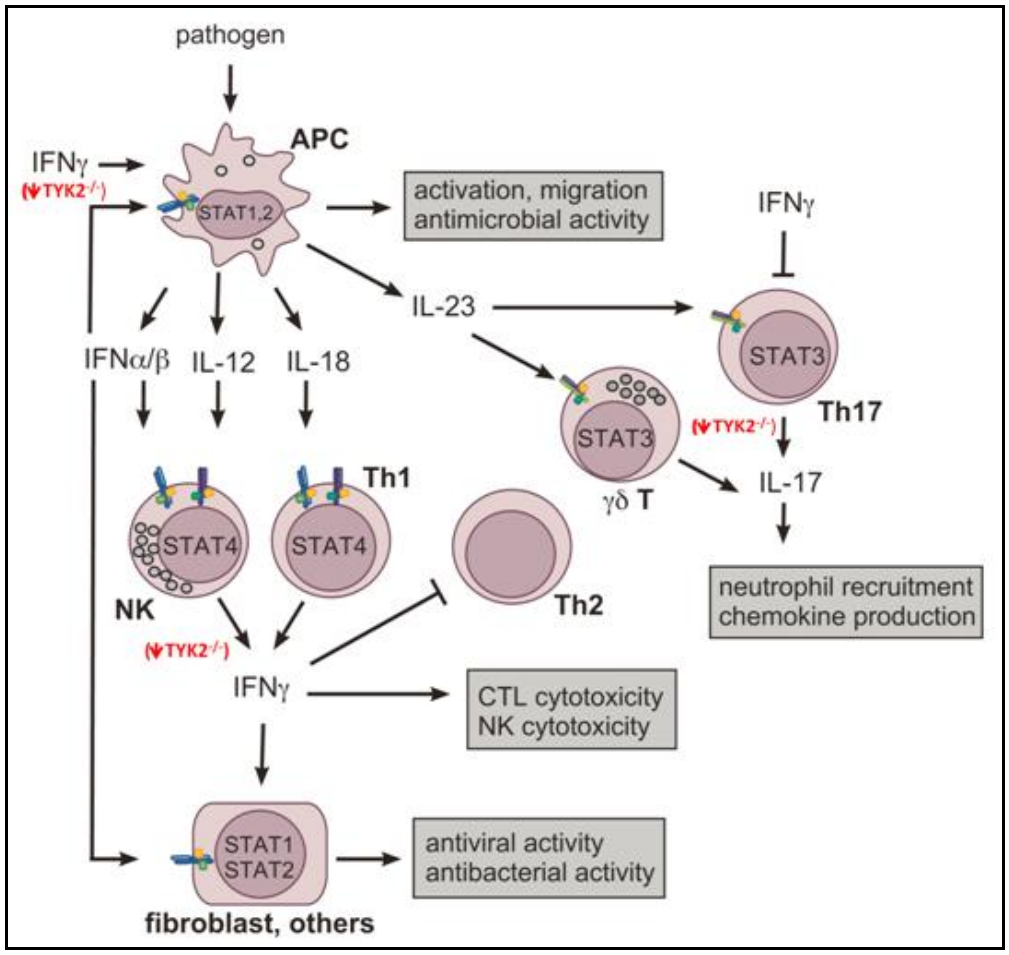

Figure 2: TYK2 in infections. Pathogen encounter induces IFN-α/β, IL-12 and IL-18 secretion of antigen presenting cells (APCs). Upon IFN-α/β (type I IFN) and IL-12 stimulation NK-cells and T-cells produce and release IFN-γ (type II IFN) in a STAT4- and TYK2-dependent manner. Additionally IFN-γ can also be induced by type I IFN in synergy with IL-18. IFN-γ propels cytotoxicity of NK-cells and CTLs, inhibits Th2 and Th17 differentiation and, together with IFN-α/β induces, antiviral and antibacterial actions in most cell types. Type I and II IFNs activate macrophages and DCs and, in a positive feedback loop, induce production of more IL-12 by these cells. TYK2 is also needed for the production of IL-17 by γδ T-cells and most likely also by Th17 cells which can be co-induced by IL-23 and also require STAT3[11].

3.2.4. TYK2 in Other Diseases

In inflammatory and autoimmune diseases a lack of TYK2 has been shown to mitigate disease phenotypes. TYK2-deficient mice survive high-dose LPS-induced endotoxin shock and are resistant to collagen induced arthritis[22, 30, 31]. TYK2 is also involved in development of experimental autoimmune encephalomyelitis (EAE), contributes to intestinal ischemia/reperfusion injury and has been reported to worsen collagen-induced arthritis (CIA)[22, 32-34]. An exception to that is found in contact hypersensitivity (CHS), where the disease phenotype is aggravated in $TYK2^{-/-}$ mice[35]. Although impaired IFN-α/β, IL-12 and IL-23 signaling in the absence of TYK2 likely contributes to the observed phenotypes, the precise roles of TYK2 in specific inflammatory and autoimmune diseases are still largely unknown.

Furthermore, TYK2 has a role in cancer development and progression. $TYK2^{-/-}$ mice have increased susceptibility to lymphoid tumors as a result of impaired tumor immune surveillance by NK/NKT- and T-cells[36, 37]. Recently, enhanced tumor growth and metastasis of 4T1 breast cancer cells in $TYK2^{-/-}$ mice was observed[38]. Tumor cell intrinsic effects of TYK2 seem to be less important as TYK2 deficiency did not affect Abelson murine leukaemia virus-induced transformation[36]. However, overexpression of TYK2 has been reported in human breast cancer cell lines as well as in prostate cancer and squamous cervical carcinomas[39-41].

Moreover, single nucleotide polymorphisms (SNP) in the human TYK2 gene are associated with an increased risk of multiple sclerosis, Crohn´s disease, systemic lupus erythematosus, psoriasis and type I diabetes and one SNP has been shown to potentially promote tumor formation[11].

Until now there is only one patient with a homozygous TYK2 defieciency known. This patient suffers from atopic dermatitis, shows an autosomal recessive hyper IgE-like syndrome and struggles from reoccurring infectious diseases[42].

3.3. Innate Immunity to Viruses

3.3.1. Innate Immune System

The innate immune system is among the first lines of defense when pathogens like parasites, bacteria, fungi or viruses invade a host. Its main functions are the recognition and the early battle against entering pathogens in addition to the coordination and stimulation of the later acting adaptive immune system. To achieve those tasks the innate immune system relies on different sets of specialized immune cells, most importantly macrophages, DCs, neutrophils and NK cells[43].

3.3.2. Virus Recognition

The detection of invading virus particles mainly depends on the recognition of pathogen-associated molecular patterns (PAMPs). PAMPs are special motifs common to pathogens which can be identified as foreign by pattern recognition receptors (PRRs). PRRs detecting viral surface proteins and viral nucleic acids can be roughly divided into two different receptor systems: membrane associated receptors called Toll-like receptors (TLRs) and cytoplasmic receptors.

Viral genomes and surface glycoproteins are mainly, but not exclusively, recognized by six of the 13 known mammalian TLRs. TLR9 detects unmethylated CpG motives of viral DNA, TLR3 recognizes foreign dsRNA, TLR7 and TLR8 are specialized in perceiving ssRNA whereas TLR2 and TLR4 sense viral surface glycoproteins[44].

Cytoplasmic receptors also function as sensors in the antiviral immune response. Prominent subgroups are retinoid acid-inducible gene (RIG)-I-like receptors (RLRs) and nucleotide oligomerization domain containing (NOD)-like receptors (NLRs). But recently, a lot of novel antiviral cytoplasmic receptors were found in diverse protein families[45].

RIG-I and melanoma differentiation-associated gene 5 (MDA-5), members of the subclass of RIG-I-like receptors (RLRs), are cytoplasmic RNA helicases and recognize viral RNA[46]. The molecular signature for virus detection *via* RIG-I was shown to be uncapped 5´-triphosphate RNA, a motif also produced by preceding receptor activity of polymerase III in response to a synthetic mimic of an AT-rich nucleotide sequence known as poly(dA-dT)[47-49]. MDA-5 senses web-like RNA structures produced during viral life cycle[50].

NOD2 and NACHT-, LRR-, and PYD domains-containing protein 3 (NALP3) belong to the NLRs subgroup of cytoplasmic PRRs. NOD2 can drive antiviral host responses by detecting and binding single stranded RNA[51]. In response to Adenovirus and Influenza Virus infection NALP3 has been shown to stimulate host inflammatory response[52, 53]. The same function is carried out by absent in melanoma 2 (AIM2) in response to VV and cytosolic DNA[54, 55]. Another cytoplasmic DNA sensor, called DNA-dependent activator of interferons (DAI), has been shown to be crucial in antiviral response to synthetic DNA, Herpes Simplex Virus 1 (HSV-1) and human Cytomegalovirus (HCMV)[56, 57].

3.3.3. Early Antiviral Host Responses

In early innate immune response the production and secretion of type I IFNs and proinflammatory cytokines are crucial events to fight viral infections. Most PRRs that recognize viruses trigger signal transduction cascades leading to the activation of IFN regulatory factor 3 (IRF3) and nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB). IRF3 and NF-κB are essential transcription factors to drive production and secretion of IFN-β, whereas NF-κB also propels the production of

proinflammatory cytokines like IL-6, IL-1β and tumour necrosis factor alpha (TNF-α). Type II IFN (IFN-γ) is produced by NK- and Th-cells and together with type I IFNs upregulate antiviral effector molecules that can inhibit different stages of viral life cycle and elicit an antiviral state which allows the cells to react more effectively against (re)-entering pathogens. In addition, IFNs can shape innate and adaptive immune responses. For example, they promote the activity of NK-cells, DCs, macrophages and CTLs[58, 59].

3.4. Cytomegalovirus (CMV)

3.4.1 Introduction

Cytomegaloviruses belong to the family of Herpesviridae and form the subfamily of Betaherpesviridae alongside with the genera Muromegalovirus, Roseolovirus and Proboscivirus[60]. These enveloped dsDNA viruses range among the mammalian viruses with the largest coding capacity with genome sizes of about 230 kilo base pairs and more than 200 open reading frames[61].
HCMV is found in all geographic locations and socioeconomic groups. According to recent data 90% of all adults are seropositive. Immune suppressed individuals can develop severe diseases like pneumonia or encephalitis when infected with CMV. It has also been shown that CMV triggers the majority of infection-related congenital birth defects. Furthermore, there is evidence that HCMV is involved in vascular diseases and has a role in chronic inflammatory disorders and other human malignancies[62, 63]. CMV can infect several different cell types and tissues but is also known for its high degree of species specificity. Therefore, no animal model exists for examining HCMV pathogenesis, but similar viruses are found in almost all mammalian species[64]. Due to the fact, that MCMV infection in mice shares many characteristics with HCMV infection in humans, the mouse model is well established for studying questions that cannot be addressed in clinical research[61].

3.4.2. Experimental MCMV Infection and Early Host Responses

In nature the primary route of MCMV entering the host is through the epithelium of the gastrointestinal and upper respiratory tract, although sexual transmission or a transmission by bite are also possible[65]. In experimental infections MCMV can be administered intraperitoneally, subcutaneously, cerebrally or intravenously. MCMV from two different sources is used in research: tissue culture-derived virus (TC-MCMV) and salivary gland-derived virus (SG-MCMV). Disease manifestation differs depending on the type of virus administered. SG-MCMV is much more virulent

in vivo than TC-MCMV when used at equal titers, whereby TC-MCMV is usually asymptomatic in immunocompetent mice[65].

Inbred mouse strains differ considerably in their susceptibility to MCMV infection. A major determinant of host resistance could be identified as the NK-cell activating receptor Ly49H. Ly49H is expressed in the more resistant C57BL/6 mouse strains but is absent in highly susceptible BALB/c mice[66]. The following paragraph discusses studies done by using mice on C57BL/6 background.

Figure 3 shows the most important host responses in early MCMV defense. Upon intraperitoneal infection of C57BL/6 mice, MCMV spreads hematologically and enters various organs. High levels of virus replication in spleens and livers with loss of liver function and immunosuppression are the primary features of infection[65]. In the spleen $LT\beta R^+$ marginal zone stromal cells become infected first, which then produce and secrete the first wave of type I IFNs about eight hours after infection. These stromal cells require $LT\alpha_1\beta_2$ expressing B-cells to maintain their differentiation state. Most of the systemic type I IFNs from this time point until 44-48h post infection are produced in the spleen. At the same time type I IFNs and IL-12 are also produced in the liver, but underlying mechanisms and responsible cell types are not yet identified. Initial IFN-α/β production in the liver is required to recruit $CCR2^+$ macrophages/monocytes, which then also contribute to type I IFN and IL-12 production in the liver. In addition those $CCR2^+$ cells together with resident macrophages are considered to be important for NK-cell recruitment to the liver. IFN-α/β directly inhibits virus replication and together with IL-12 activates immune effector cells, most importantly NK-cells. The first round of replication of MCMV lasts 30-36 hours. In spleens, approximatly 36 hours post infection plasmacytoid DCs (pDCs) have migrated from the red pulp to the marginal zone and from 36-48 hours post infection produce most of splenic and systemic IFN-α/β in a TLR9-dependent manner. At that time point myeloid DCs (mDCs) also produce small amounts of IFN-α/β in livers and spleens but in a TLR9-independent manner. Interestingly, 44-48h post infection the major contribution to type I IFN production in the spleen is switched from pDCs to mDCs. In livers similar effects were described, indicating importance of DC- or monocyte-derived IFN-α/β production after 44-48h, but the exact cell type responsible is not yet identified. Secretion of IL-12/IL-18 in addition to IFN-α/β by pDCs and mDCs leads to stimulation of IFN-γ production by NK/NKT- and T-cells[67]. IFN-γ in synergy with TNF-α is capable of inducing an antiviral state early during MCMV infection and supports T-cell mediated killing of infected cells by upregulation of major histocompatibility complex class I (MHC I) at later stages[68]. Whereas Ly49H expressing NK-cells are the main effector cells in controlling MCMV infection for the first two days of infection, $CD8^+$ T-cells adopt this regulating function later in infection[67].

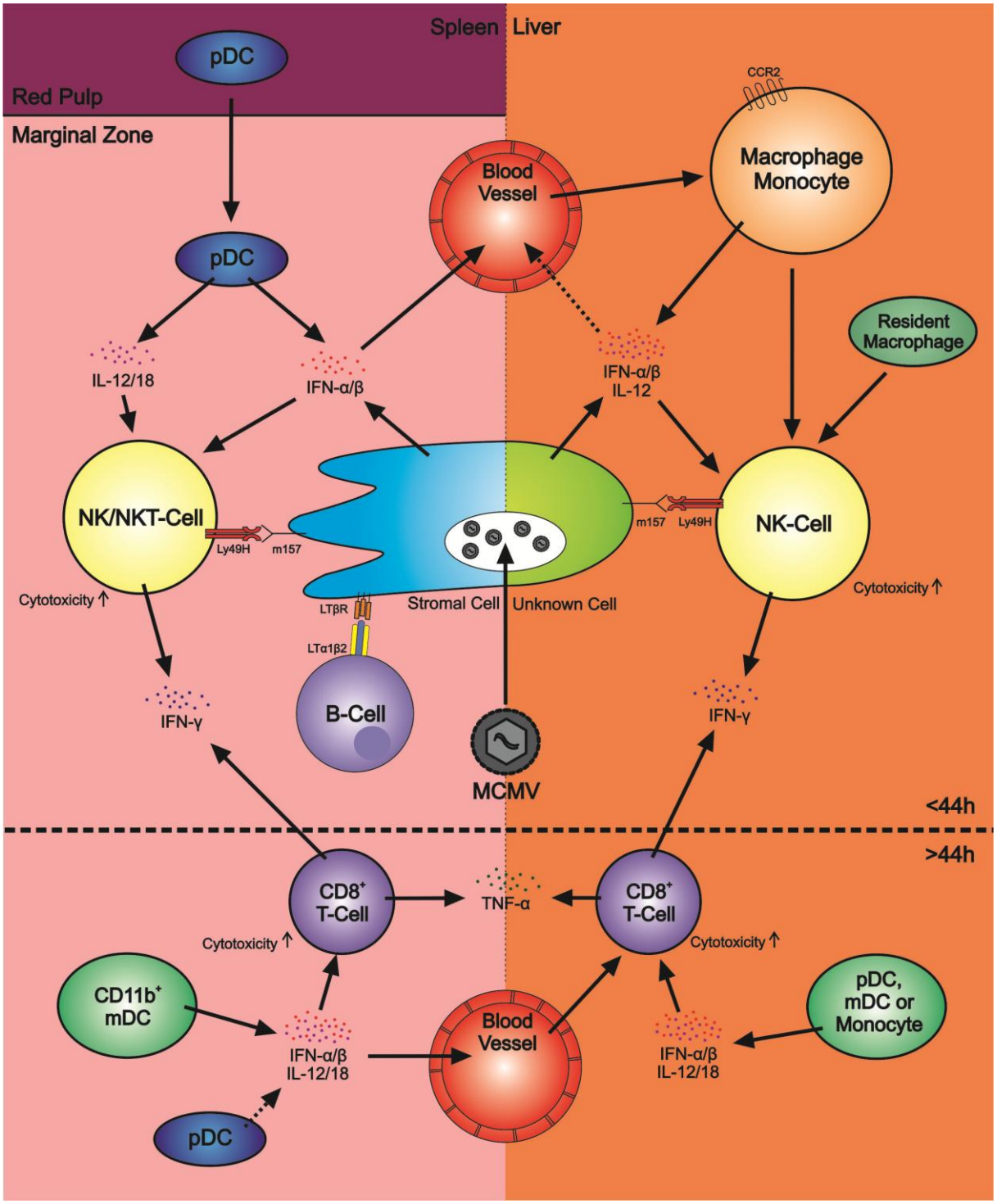

Figure 3: Early innate and adaptive MCMV defenses in the spleen (left, pink) and liver (right, orange). Events until 44 hours (<44h) post infection are shown above the dotted line, whereas subsequent events are shown below (>44h).

3.4.3. Signaling Cascades in MCMV Infection

As shown in Figure 4, MCMV is detected by TLR3 and/or TLR9 and there is evidence that also TLR7 functions redundantly with TLR9 in MCMV infection[67, 69]. These receptors are all located in the endosomes of innate immune cells where many viruses arrive through the endocytic pathway or

uptake in apoptotic bodies of previously infected cells[70]. TLR9 seems to be the most important receptor in MCMV response[67]. Like most other TLRs, TLR9 signaling depends on myeloid differentiation primary response gene 88 (MYD88). After binding of unmethylated CpG DNA in endosomes, the cytoplasmic domain of the receptor activates MYD88. MYD88 than recruits and phosphorylates IL-1 receptor associated kinase (IRAK) which interacts with TNF receptor associated factor 6 (TRAF6). *Via* the IKK complex IRAK is able to activate NF-κB. NF-κB drives IFN-β and proinflammatory cytokine production whereas IRF7, an IFN-inducible factor, which is constitutively expressed in pDCs and also gets activated through IRAK[71], is needed for the upregulation of most IFN-α subtypes[72].

In addition, TLR3 is able to recognize MCMV. Upon ligand binding, TLR3 gets phosphorylated on its cytoplasmic tail and recruits TIR domain-containing adaptor inducing IFN-β (TRIF)[73, 74]. *Via* receptor interacting protein (RIP) TRIF then activates the IKK complex, which further on leads to the phosphorylation and degradation of an NF-κB inhibitory protein. Released NF-κB translocates into the nucleus and among others binds the IFN-β promoter region to activate transcription. TRIF can additionally signal through the TANK-binding kinase 1 (TBK1) complex. IRF3 molecules become phosphorylated dimerize and like NF-κB translocate to the nucleus to stimulate IFN-β production[75]. Secreted type I IFNs function in an autocrine and paracrine manner and activate the JAK/STAT pathway, which was already schematically described in chapter “3.1. The JAK/STAT Pathway”. In response to IFN-a/β mainly STAT1/2 heterodimers are activated and associate with IRF9. STAT1/STAT2/IRF9 complexes then activate IFN stimulated genes (ISGs) by binding so-called IFN stimulated response elements (ISREs). STAT1 homodimers on the other hand bind preferentially to IFN-γ activated site (GAS) elements[76, 77].

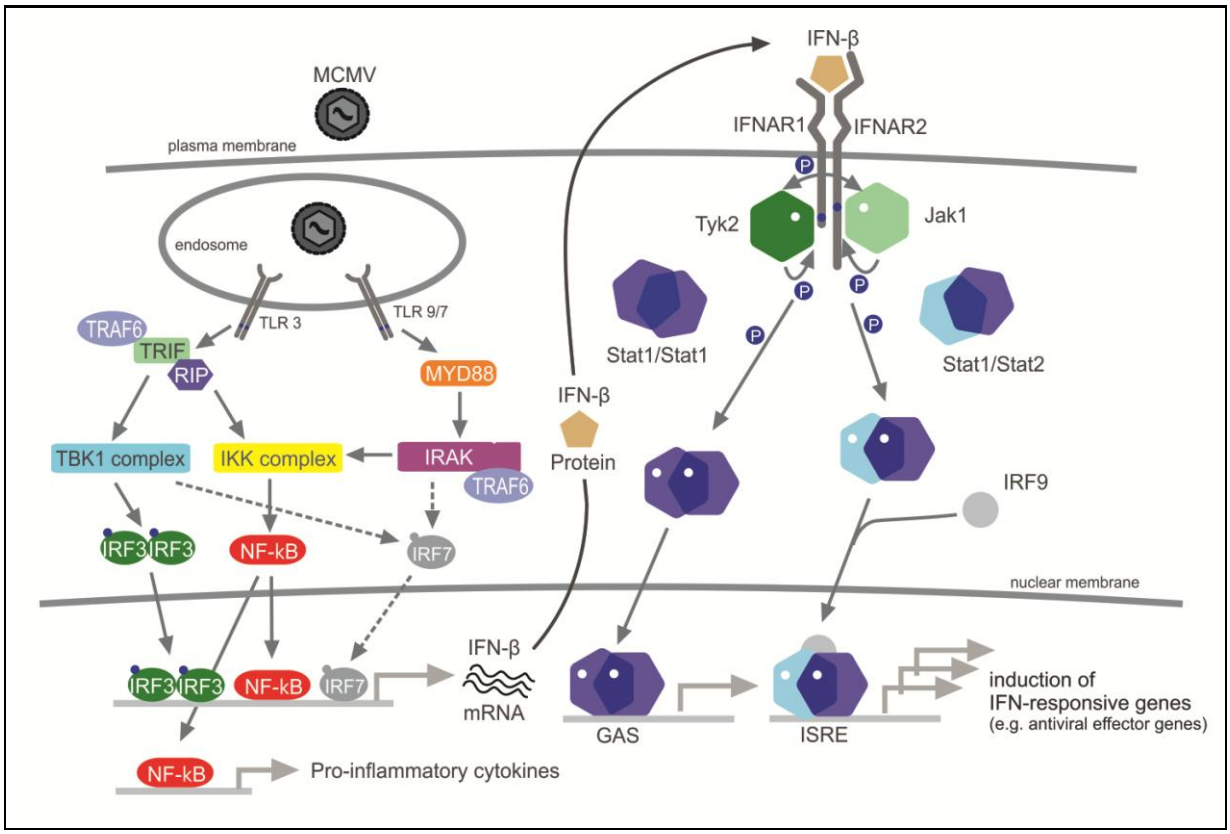

Figure 4: Signaling cascades activated in response to MCMV infection

3.4.4. Innate Immune System Evasion Strategies of MCMV

From the evolutionary perspective, viruses are in constant battle with their host´s immune systems. In order to survive and replicate in a host, viruses have developed a variety of evasion strategies to overcome different immune responses. These strategies can be roughly divided in three major mechanisms[78]:

The first effective strategy to evade the immune response is to avoid detection by immune cells. This can be achieved for example by changing immunodominant epitopes on the surface of the virus particle. MCMV also uses this strategy since m157, a surface glycoprotein of MCMV, which is detected by Ly49H receptors of NK-cells, mutates during infection and therefore lowers the affinity to these receptors[79, 80] . The second successful strategy to evade the immune system is to directly interfere with immune responses to infected cells. The MCMV proteins m06 and m152 are able to inhibit major MHC I expression on the cell surface of infected cells and therefore prevent antigen presentation to $CD8^+$ CTLs[81]. The third immune evasion strategy of viruses targets effector functions of immune cells like IFN-α/β signaling. This mechanism can be found in MCMV, as the protein m27 is able to mediate STAT2 degradation and therefore blocks IFN type I and partially IFN type II signaling[82, 83].

Due to these and many other immune evasion strategies, the immune system is not capable to clear MCMV infections entirely. Therefore, like for all Herpesviridae, MCMV infection leads to a lifelong latent infection. During the latent phase MCMV genomes but no infectious particles can be detected. Reactivation of the virus and resulting reoccurrence of the disease is frequently seen when the hosts immune system is affected, for instance by immunosuppression, or as a result of an additional infection[84].

3.5. Conditional Knockouts

3.5.1. The cre/loxP System

The cre/loxP system is a genetic tool to target and remove pieces of DNA in a living organism. Key enzyme is the cre recombinase (cre) which is able to recombine two loxP sites in proximity. Adapted to every model organism, it has huge fields of application. In mice for instance, the cre/loxP system is used to create conditional knockouts when a complete knockout of a specific gene is not viable or to express/delete a gene tissue or cell-type specifically. In the latter case the cre gene is controlled by an inducible or tissue/cell-type specific promoter. When the promoter is active, cre protein is produced and cuts out the floxed (i.e. region flanked by loxP sites) target sequence of the genome[85, 86].

3.5.2. Conditional TYK2 Knockout Mice

By using the cre/loxP system, conditional TYK2 knockout mice ($TYK2^{fl/fl}$) have been created in our laboratory [Vielnascher et al., unpublished]. Two loxP sites were inserted in the mouse genome, flanking exon 3 of the TYK2 gene, which contains the start codon for translation (ATG)[16]. In order to study cell type-specific functions of TYK2, $TYK2^{fl/fl}$ mice have been crossed to mouse lines expressing cre under the control of the cell type-specific promoters of the CD11c[87], LysM[88], Lck[89] and CMV[90] genes (Fig. 5 for CD11c). CD11c is highly expressed on DCs, and to a lower extent, also on macrophages, monocytes and neutrophils. By using the LysM promoter to control cre expression TYK2 is deleted in cells of the myeloid cell lineage (macrophages, granulocytes, neutrophils), whereas Lck/cre targets T-lymphocytes. The CMV promoter is active in all cell types and this conditional knockout system is used as a control, mimicking the situation in conventional TYK2 knockout mice.

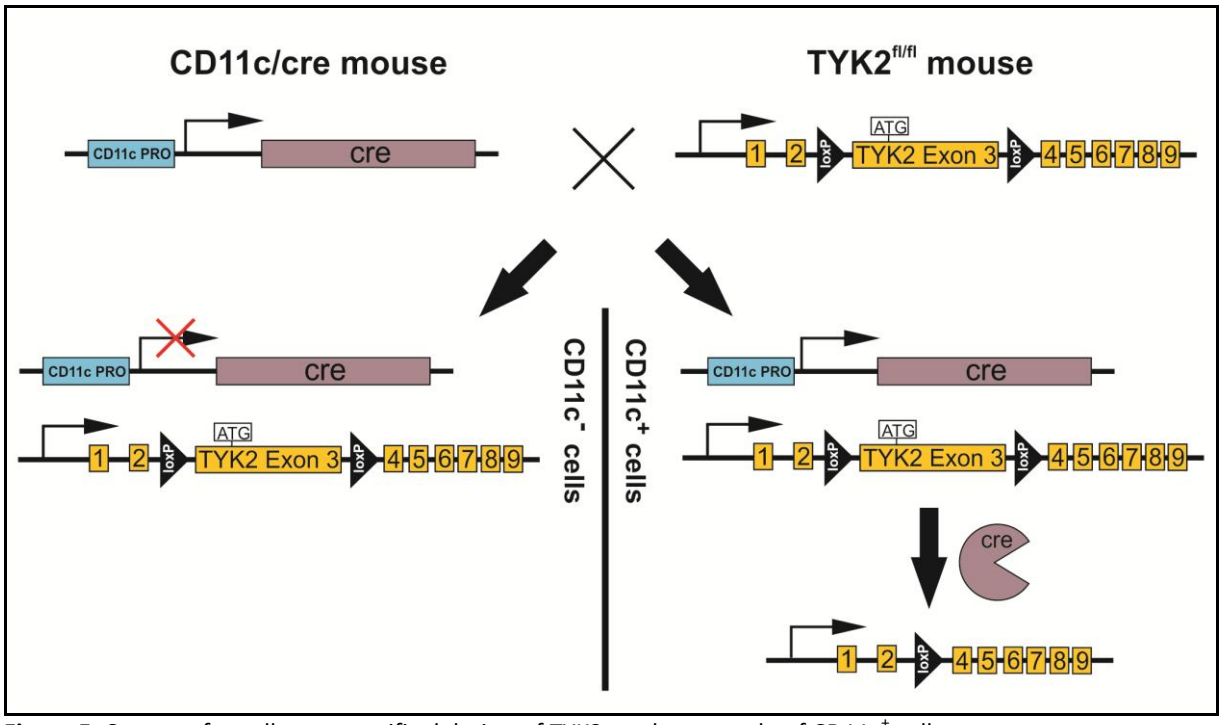

Figure 5: Strategy for cell type-specific deletion of TYK2 on the example of CD11c^{+} cells

3.6. Dendritic Cells in Immunity

3.6.1. General Features

DCs are bone marrow-derived lymphocytes and can be found in surface epithelia like the skin, all lymphoid tissues, mucosal epithelium and organ parenchyma. Morphologically most noticeable are the membranous projections or dendrites which give those cells their name. DCs act as sentinels, which capture pathogens on the periphery, process them, migrate to lymph nodes and present antigenic peptides of the degraded pathogens to lymphocytes. Resting DCs in peripheral organs are called immature DCs which get activated by uptake of pathogens. The following maturation steps involve morphological changes, redistribution of MHC molecules from intracellular endocytic compartments to the cell surface, down-regulation of antigen internalization, an increase in the surface expression of co-stimulatory molecules, secretion of chemokines, cytokines and proteases, and surface expression of adhesion molecules and chemokine receptors[91, 92]. DCs are able to interact with B-cells causing the modulation of B-cell proliferation and differentiation program to become plasma cell[93, 94]. Furthermore, to regulate the humoral response, it has been reported that DCs deliver signals for isotype class switching of antibody producing plasma B-cells[95]. DCs are able to activate naïve NK-cells and also play a major role in early cytokine production in response to infections[96, 97]. Another property of DCs is the cross-presentation of antigen. Thereby exogenous antigens can be presented attached to MHC class I to directly activate CD8+ CTLs[98]. But DCs also

participate in establishing tolerance to self antigen by deleting self reacting lymphoid cells in central lymphoid organs in order to prevent autoimmune responses[99, 100]. Taken together DCs have multiple important functions in immune response and act as key players in linking innate and adaptive immunity. Therefore, DCs are an exciting field of research and are gaining more and more interest by immunological researchers.

3.6.2. Subsets of Dendritic Cells

Different subsets of DCs have been described and can be characterized based on tissue distribution, cell surface markers and general functions. Lymphoid tissue DCs are most commonly divided in myeloid (mDCs) (also called conventional DCs (cDCs)) and pDCs. mDCs can be further subclassified in migratory and lymphoid tissue-resident mDCs[101]. In contrast to mDCs, pDCs look more like plasma cells morphologically and are usually just seen upon pathogen encounter. Both cell types have different surface markers and distinct functions in immunity. pDCs express B220 which was originally described as a pro-B cell marker and the classical DC cell marker CD11c at low levels whereas mDCs show high expression levels of CD11c and no B220 protein on the cell surface[102, 103]. The different subsets of DCs also play individual roles when it comes to their function in innate immune response. pDCs constantly express IRF7 and therefore are the first to react against viral infections by producing IFN-α *via* the TLR7/9 pathway. Because of that feature pDCs were formerly also referred as IFN producing cells (IPCs)[104, 105]. mDCs on the other hand are more specialized in antigen presentation and TLR3 mediated IFN-β response[67, 101, 106].

3.7. Questions and Objectives

Our lab showed previously that TYK2 deficiency in mice leads to higher susceptibility to MCMV infection[24]. Data gained from *in vitro* cultures revealed a surprisingly strong impact of autocrine/paracrine IFN signaling in macrophages upon MCMV challenge. This effect was expected to be even more striking in DCs, as they, contrarily to macrophages, were described as important IFN producers in early MCMV response[67]. Therefore, we wanted to investigate the responses of DCs in MCMV infection analogous to the studies done in macrophages. In addition, benefiting from the availability of conditional knockout mice with TYK2 deletion either in macrophages ($TYK2^{\Delta LysM}$) or DCs ($TYK2^{\Delta CD11c}$), we wanted to analyze the *in vivo* consequences of TYK2 deficiency in MCMV infection. This work is part of a combined effort with Michael Rammerstofer to analyze cell type-specific

functions of TYK2 in macrophages and DCs. Specific questions to be addressed in this diploma thesis are:

1) How efficient is the TYK2 deletion in DCs derived from TYK2$^{\Delta CD11c}$ mice?

For further investigation of TYK2-specific functions in DCs we at first wanted to determine the TYK2 deletion efficiency in TYK2$^{\Delta CD11c}$ DCs. A cell culture to grow bone marrow-derived DCs (BMDCs) *in vitro* had to be established in our laboratory to compare TYK2 expression of TYK2$^{fl/fl}$, TYK2$^{\Delta CD11c}$ and TYK2$^{\Delta/\Delta}$ DCs on mRNA and protein level. In addition, splenic DCs should be isolated to quantify the TYK2 mRNA expression of CD11c^{+} cells *in vivo*.

2) Are TYK2$^{\Delta CD11c}$ BMDCs more permissive to MCMV infection and is the autocrine/paracrine STAT1/2 activation affected in TYK2$^{\Delta CD11c}$ BMDCs?

Previous data showed that MCMV replicates to higher titers in TYK2-deficient bone marrow-derived macrophages[24]. Using a similar approach, the importance of TYK2 in BMDCs in controlling MCMV infections had to be analyzed. As shown in macrophages, resistance against MCMV depends on the presence of functional IFN-α/β signaling and the presence of STAT1. Therefore the phosphorylation status of STAT1 and STAT2 in BMDCs from the respective mice infected with MCMV had to be analyzed.

3) What are the consequences of TYK2 deletion in DCs or macrophages *in vivo* during MCMV infection?

Previous data showed higher MCMV titers in organs of infected TYK2$^{-/-}$ as compared to wild type mice[24]. Infection of TYK2$^{\Delta CD11c}$ and TYK2$^{\Delta LysM}$ mice with MCMV would give insight to the contribution of DCs and macrophages to the observed phenotype. To this end, viral load in livers, spleens, salivary glands and lungs of infected animals had to be determined. TYK2$^{\Delta Lck}$ mice were used as a control, since T-cell contribution in early immune response should be negligible. This part of the study was performed together with Michael Rammerstorfer, who did the *in vivo* infections of mice and determined virus titers of spleens, salivary glands and lungs. Focus of the presented diploma thesis was on DCs and MCMV titers in livers.

4. Materials and Methods

4.1. Reagents

Materials	Company	Code/Cat.No.
Bio-Rad Protein Dye	BioRad, Hercules, CA	5000006
Bovine Serum Albumin (BSA)	Roth Lactan, Graz, Austria	T844.3
Dulbecco´s Modified Eagle Medium (DMEM)	PAA Laboratories GmbH, Pasching, Austria	E15-843
ECL Solution	GE Healthcare, Munich, Germany	RPN2106
Fetal Calf Serum (FCS)	Invitrogen/Gibco, Lofer, Austria	10270-106
Hank´s Balanced Salt Solution (HBSS)	Invitrogen, Lofer, Austria	14175129
iScript cDNA synthesis kit	BioRad, Hercules, CA	170-8890
L-Glutamine 200mM	PAA Laboratories GmbH, Pasching, Austria	M11-004
Low Melting Agarose f. PFA	Biozym, Oldendorf, Germany	840100
Magnetic Cell Sorting (MACS) Buffer	Miltenyi Biotech, Bergisch Gladbach, Germany	130091221
MACS CD11c MicroBeads (N418)	Miltenyi Biotech, Bergisch Gladbach, Germany	130052001
PageRuler Protein Ladder	Fermentas, St. Leon-Rot, Germany	26617
Phosphate Buffered Saline (PBS)	Sigma, St. Louis, MO	D8537
Pen/Strep (100x)	PAA Laboratories GmbH, Pasching, Austria	P11-010
peqGOLD TRIFAST	peqLab, Erlangen, Germany	30-2020
ProteinA Sepharose CL-4B	GE Healthcare, Munich, Germany	17-0780-01
Rat Serum	Roth Lactan, Graz, Austria	S7648
Red Blood Cell Lysis Buffer	Sigma, St. Louis, MO	R7757
SigmaFast Protease Inhibitors	Sigma, St. Louis, MO	S8820
Trypsin-EDTA (1:250)	PAA Laboratories GmbH, Pasching, Austria	L11-004

Standard reagents are not listed and were ordered from *Sigma (St. Louis, MO)* or *Merck (Darmstadt, GER)*.

4.2. Mice

All mice were on C57BL/6 background. Conditional TYK2 mice have been generated in our lab (see Figure 5 or 6). CD11c/cre (B6.Cg-Tg(CD11c-Cre)) mice were described previously[87] and crossed with $TYK2^{fl/fl}$ (B6.129P2-$TYK2^{fl1BIAT}$) mice to generate $TYK2^{\Delta CD11c}$ mice. Similarly LysM/cre (B6.129P2-Lyz2tm1(cre)Ifo/j)[88], Lck/cre (B6.129P2-Tg(LckCre)[89] and CMV/cre (B6.c-Tg(CMV-Cre)Cgn/j)[90] mice were crossed with $TYK2^{fl/fl}$ mice to generate $TYK2^{\Delta LysM}$, $TYK2^{\Delta Lck}$ and $TYK2^{\Delta CMV}$ mice. In case of $TYK2^{\Delta CMV}$ mice, the cre recombinase transgene was crossed out again. All mice were housed under specific pathogen-free conditions according to FELASA guidelines. Animal experiments were discussed and approved by the institutional ethics committee and are conform to Austrian laws (BM.W_F^a ref. 68.205/0204-C/GT/2007 and ref. 68.205/0233-II/10b/2009).

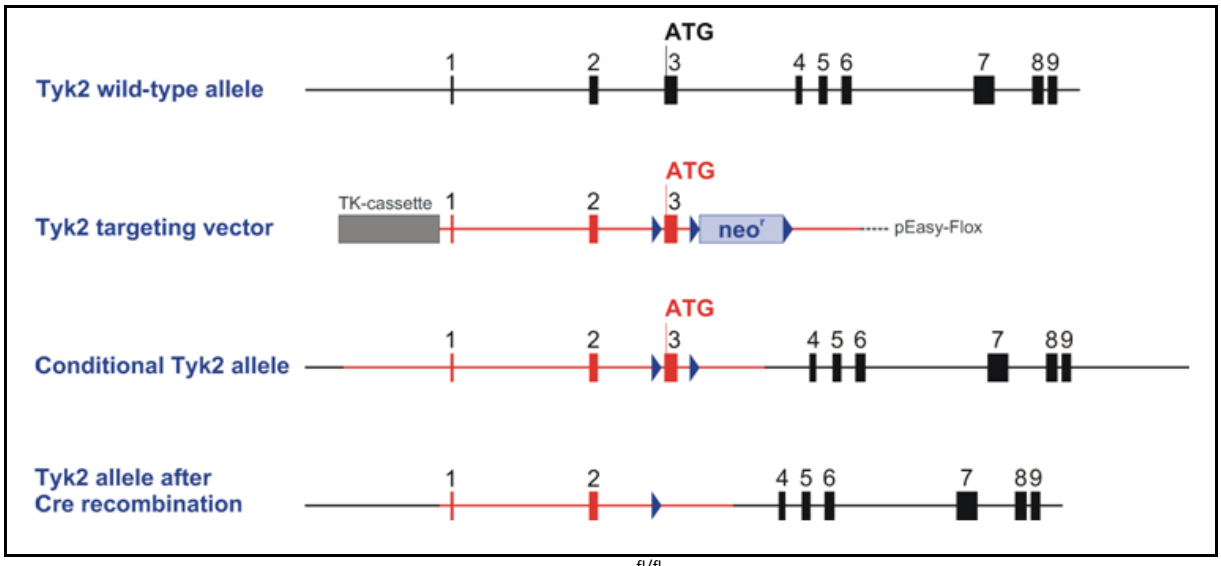

Figure 6: Generation of conditional TYK2 knockout (TYK2$^{fl/fl}$) mice. Genomic organisation of the TYK2 locus is shown from exon 1 to 9 (indicated as black boxes). The targeting vector consists of two homologous arms (indicated in red) and the floxed sequence (exon 3 with flanking loxP sites). A floxed neomycin resistance cassette (neo^r, blue box) is located 3´of exon 3 and a thymidine kinase (TK) cassette (grey box) 5´ of the construct. The conditional TYK2 allele shows the genomic organisation after homologous recombination and deletion of the neomycin resistance cassette. After cre recombination the allele is deficient for exon 3 and hence lacks the transcriptional start site. LoxP sites are represented as blue triangles.

4.3. Cell Culture

If not stated otherwise, "Heraeus Multifuge 1S" centrifuge was used for all cell culture methods.

4.3.1. Murine Embryonal Fibroblasts (MEFs)

MEF Medium

DMEM (high glucose 4.5g/l)

10% FCS (heat-inactivated)

Pen/Strep (100µg/ml and 100U/ml)

50µM 2-mercaptoethanol

2mM L-Glutamine

PBS + Pen/Strep (50µg/ml and 50U/ml)

Isolation and Passaging of MEFs for Experiments

Embryos on day 13.5 of gestation were dissected from the uterus. The embryos were collected in PBS + Pen/Strep containing petri dishes. Livers and heads of the embryos were removed, the embryos were minced with scissors and forceps and pipetted through Pasteur pipettes in MEF medium. The suspension was passed through a 100µm cell strainer and centrifuged for 5min at 1000rpm, cells

were washed twice with PBS, resuspended in MEF medium and seeded into two 10cm cell culture plates/embryo. Next day the cells were washed with PBS twice. On day 3-4, when cells reached near confluency, cells were either frozen or expanded for experiments.

For passaging, MEFs were washed with PBS once, 1.5 ml of Trypsin-EDTA (1:250) was added per 10cm plate and dissociation of cells was observed *via* microscope. Trypsin reaction was stopped by adding 3ml MEF medium per 10cm plate. Cells were resuspended in medium and centrifuged at 1000rpm for 5min. The pellet was resuspended in 5ml MEF medium and cells were seeded on new plates at the desired dilution. For experiments the cell number was determined using a Neubauer counting chamber. For plaque forming assays $2x10^4$ cells were plated per well of a 24 well plate.

4.3.2. Bone Marrow-Derived Macrophages (BMMΦs)

L929 Cell Conditioned Medium

L929 cells were described previously [107]. The cells were grown on 15cm cell culture dishes in MEF medium to approximately 70% confluency. Medium was removed and 30ml DMEM (+Pen/Strep, +L-Glutamine) were added. The plates were incubated for 10 days at 37°C before the supernatant was collected and sterile filtrated. Aliquots of L929 cell conditioned medium were stored at -20°C.

BMMΦ Medium

DMEM (high glucose 4.5g/l)
10% FCS (heat-inactivated)
Pen/Strep (100µg/ml and 100U/ml)
2mM L-Glutamine
50µM 2-mercaptoethanol
15% L929 conditioned medium (CM)

1x Schindler Buffer

50mM Tris/HCl pH8
150mM NaCl
0.5% NP40 (IPEGAL)
10% Glycerol
2mM DTT
0.1mM EDTA
0.2mM Na-Vanadate
25mM Na-fluoride
1x SigmaFast protease inhibitors
1mM PMSF

Isolation, Passaging and Harvesting of BMMΦs

Mice at the age of 8 to 12 weeks were sacrificed by cervical dislocation and tibia and femur were prepared. Under sterile conditions the bones were cut on both ends and bone marrow was flushed with 5ml medium into petri dishes. Cells were resuspended and plated on four 10cm petri dishes per mouse adding BMMΦ medium to a total of 12ml per plate. After 3 days medium was changed. To split BMMΦs (usually on day five after isolation) the cells were washed with 5ml PBS. Then cells were scraped in 5ml BMMΦ medium using a silicon scraper and were resuspended thoroughly. The cells

were split depending on their density and supplied with 12ml BMMΦ medium per 10cm plate. When plated for experiments (day 6) the cell count was determined using a Neubauer counting chamber. $1.5x10^6$ cells per well (6-well tissue culture plate) were seeded in 3ml BMMΦ medium and incubated over night at 37°C.

To harvest whole cell extracts, cells were washed twice with ice cold PBS, 100µl 1x Schindler buffer per well (6-well plate) were added and cells were scraped off using silicon scrapers. Cell lysates were kept on ice for 30min. To remove cell debris, samples were centrifuged (5000rpm [Eppendorf Centrifuge 5415r], 2min, 4°C), supernatant was transferred to new tube and stored at -80°C.

4.3.3. Bone Marrow-Derived Dendritic Cells (BMDCs)

X63 Conditioned Medium

Cells of the mouse myeloma cell line X63 (kind gift of Volker Thiel, Basel), producing GM-CSF, were grown in cell culture flasks in DMEM supplemented with 10% FCS, Pen/Strep (100µg/ml and 100u/ml) and 2mM L-Glutamine. The cells were split 1:3 (usually every 2-3 days) by rinsing from the bottom of the flasks and transferring to a new flask before adding medium. For the production of X63 conditioned medium cells were incubated at 37°C for 10 days when they were very dense. The medium was taken off and sterile filtrated. Aliquots of X63 conditioned medium (6-8ml) were stored at -20°C to avoid freeze/thaw cycles.

BMDC Medium

DMEM (high glucose 4.5g/l)
10% FCS (heat-inactivated)
Pen/Strep (100µg/ml and 100U/ml)
2mM L-Glutamine
10% X63 conditioned medium (added right before use)

Isolation, Passaging and Harvesting of BMDCs

Mice at the age of 8 to 10 weeks were sacrificed. Femur and tibia of mice were isolated and bone marrow was flushed with 5ml MEF medium into non-cell culture plates. The cells were suspended by pipetting up and down several times and centrifuged at 1000rpm for 5min. The pellet was resuspended in 1ml red blood cell lysis buffer and incubated for 1min under constant shaking. The lysis was stopped by adding 3ml MEF medium. The suspension was centrifuged at 1000rpm for 5min and the pellet was resuspended in BMDC medium. 10^7 cells were plated in 5ml BMDC medium per 10cm non-cell culture plate. On day 3 after isolation 5ml BMDC medium containing 10% X63 conditioned medium was added per plate.

For experiments, BMDCs were transferred onto cell culture plates on day six after isolation. The cells were scraped off using silicon scrapers and collected by centrifugation (1000rpm for 5min). The pellet was resuspended in 5ml BMDC medium and the cells were counted. For Western blot and RNA isolation 10^7 BMDCs were seeded on 10cm cell culture plates in 10ml BMDC medium. For infection experiments with MCMV $5x10^5$ cells in 500µl BMDC medium were plated per well at 24 well plates.
On day 8 after isolation BMDCs were scraped off the bottom of the 10cm plates and transferred to tubes. The cells were collected by centrifugation at 1000rpm for 5min. For RNA isolation pellets were resuspended in 1ml TriFast and stored at -80°C. For protein isolation the cells were resuspended in 1ml 1x Schindler buffer and incubated on ice for 30min. Cell debris was removed by centrifugation at 5000rpm [Eppendorf Centrifuge 5415r] for 2min at 4°C. Samples were stored at -80°C.

Infection of BMDCs with MCMV

BMDCs were infected in 24 well plates with TC-MCMV at an MOI of 5. For infection 250µl of the 500µl medium in every well were taken off and 250µl medium containing MCMV were added. The plates were centrifuged at 800rpm for 30min and then incubated for one hour at 37°C. The infection medium was taken off and the wells were washed with PBS. 500µl fresh BMDC medium was added and the plates were incubated at 37°C for the times needed.
To harvest MCMV infected BMDCs for the virus replication experiment the plates were sealed with parafilm and stored at -80°C. After thawing, lysed cells were harvested pipetting up and down and the samples were directly used for plaque forming assays. To harvest protein lysates of MCMV infected BMDCs the cells were scraped off the bottom of the well and the suspension was transferred to eppendorf tubes. The cells were collected by spinning them down at 5000rpm for 5min, lysed in 50µl 1x Schindler buffer and incubated on ice for 30min. Cell debris was removed by centrifugation at 5000rpm [Eppendorf Centrifuge 5415r] for 2min at 4°C, before storing at -80°C.

4.4. Murine Cytomegalovirus (MCMV)

Sucrose/Virus Standard Buffer (VSB)

50mM Tris/HCl, pH7.8

12mM KCl

5mM Na_2EDTA

15% w/v sucrose

Reagents were mixed and sterile filtrated through a 0.2µm filter. VSB can be stored up to 8 months at 4°C.

Agar for Plaque Forming Assays (PFA)

4% low melting agarose was suspended in PBS, autoclaved and stored at RT. Before use agarose was melted in a microwave and incubated for 10min at 37°C to cool down. Three parts of MEF medium at 37°C were mixed with one part of 4% agarose and used immediately to overlay cells.

TC-MCMV Growth and Purification

TC-MCMV was propagated in C57BL/6 MEFs. MEFs were grown in complete MEF medium in 15cm cell culture plates to 70-80% confluency and infected with TC-MCMV (MOI=0.01). Plates were rocked every 30min for 2 hours to ensure equal infection. 20ml complete MEF medium was added and plates were incubated at 37°C. On day 4-5 post infection (complete cytopathic effect visible) MEFs and supernatants were collected and frozen at -80°C in 50ml Falcon tubes. By using dry ice at least 3 freeze/thaw cycles were performed to ensure complete lysis of cells. Virus suspension was centrifuged at 6000rpm [Beckman Avanti J-25, Rotor JA25/50] for 20min at 4°C. The supernatant was kept on ice while the pellet was dounced 20 times (Wheaton tight fitting tissue grinder) on ice and centrifuged at 12000rpm [Beckman Avanti J-25, Rotor JA25/50] for 10min at 4°C. The pellet was discarded. Both supernatants were pooled and centrifuged at 14000rpm [Beckman Avanti J-25, Rotor JA25/50] for 3.5 hours at 4°C in JA-25 tubes to pellet TC-MCMV particles. The supernatant was removed and the pellets were resuspended in medium and incubated on ice over night. Next day the TC-MCMV suspension was dounced 20 times on ice and carefully pipeted on a 25ml VSB cushion in ultra centrifuge SW-28 tubes. After centrifugation with 25000rpm [Sorvall Ultra Pro 80] for two hours at 4°C, supernatants were discarded, the pellets were overlaid with VSB and incubated on ice over night. Next day the virus pellet was resuspended, dounced 80-100 times on ice and passed through a 0.45µm sterile filter to get rid of multicapsids and virus aggregates. Virus aliquots were stored at -80°C.

Homogenization of Livers of MCMV-Infected Mice

Mice infected with TC-MCMV were sacrificed by cervical dislocation and livers were dissected. Single cell suspensions were prepared by mashing livers through 100µm cell strainers. To release virus particles from liver cells they were dounced in 2ml PBS using a tissue grinder (Wheaton tight fitting). Every liver was dounced 20 times and aliquots were stored at -80°C.

Plaque Forming Assay (PFA)

MEFs were grown to 50-60% confluency in 24 well cell culture plates (Falcon). After the medium was taken off cells were infected with 500µl virus inoculum. Plates were centrifuged with 800rpm [Heraeus Multifuge 1S] for 30min and incubated at 37°C for two hours. To ensure proper MCMV

distribution plates were rocked every 20 minutes while incubating. After incubation the inoculum was removed and cells were overlaid with agar-medium. Plates were kept at room temperature until agar was hardened and then incubated at 37°C for 4-5 days before counting plaques.

4.5. Immunoprecipitation(IP) / SDS-PAGE / Western Blot

ProteinA-Sepharose Beads

Freeze-dried ProteinA-Sepharose was washed four times with sterile water and then twice with 20% ethanol. ProteinA-Sepharose beads were stored in 20% ethanol at 4°C.

Antibody Solutions

Primary antibodies were diluted 1:1000 in 1% BSA in PY-TBST. To prevent contamination 0.01% Na-Azide was added to primary antibody solution. Secondary antibodies were diluted 1:2000-1:4000 in 1% BSA in PY-TBST without adding Na-Azide.

1x Transfer Buffer
25mM Tris/HCl
150mM Glycine
20% Methanol

1x Running Buffer
192mM Glycine
25mM Tris/HCl pH 7.4
0.1% SDS

2x Lämmli Sample Buffer (LSB)
126mM Tris/HCl pH 6.8
4% SDS
20% Glycerine
200mM DTT
0.02% Bromphenol Blue

PY-TBST
10mM Tris/HCl pH7.4
75mM NaCl
1mM EDTA pH8
0.1% Tween20

Stacking Gel
5% Acrylamid/Bisacrylamid
126mM Tris/HCl pH 6.8
0.1% SDS
0.06% APS
0.07% TEMED

Separation Gel
6,5% Acrylamid/Bisacrylamid
375mM Tris/HCl pH 8.8
0.1% SDS
0.075% APS
0.09% TEMED

Strip Buffer
2M Glycine/HCl pH 2.5
0.25% SDS

Blocking Solution
5% milk powder in 1x PY-TBST

.

Immunoprecipitation

Protein concentration of whole cell extracts was determined using Bio-Rad Protein Dye according to manufacturer's instructions. 1mg protein in 1ml 1x Schindler buffer was mixed with either 4µg TYK2 antibody or 2µg JAK1 antibody and incubated rotating over night at 4°C. Next day 50µl protein-sepharose slurry (washed 3 times in 1x Schindler buffer and resuspended to ~50% v/v slurry prior to use) was added to each sample. After rotating at 4°C for two hours the samples were pelleted (13000rpm [Eppendorf Centrifuge 5415r], 20sec, 4°C) and washed with 1x Schindler buffer 3 times. Then the pellets were resuspended in 50µl 2x LSB and stored at -20°C. Prior to loading on SDS-PAGE gels, the samples were heated at 96°C for 10min and centrifuged for 2min at 12000rpm [Eppendorf Centrifuge 5415r] and 4°C. 20µl-25µl of the supernatants were loaded.

SDS-PAGE and Western blot

For SDS-PAGE 10-20µg protein extract were loaded per lane and Page Ruler (Fermentas) was used as molecular weight standard. Electrophoresis was run for 1.5-2 hours on 100V in 1x running buffer. After proteins were separated by SDS-PAGE the proteins were blotted on nitrocellulose membranes by semidry blot (1mA/cm^2 of membrane; 2h). The membrane was rinsed in PY-TBST and incubated with blocking solution for 1h. Then the membrane was washed 3 times in PY-TBST and incubated with primary antibody over night at 4°C. The membrane was washed 3 times and incubated with the secondary antibody for 1h. After another washing step (3 times) the membrane was incubated with ECL solution for 1min and exposed to a light sensitive film. Membranes were stripped by incubating in strip buffer for 2 hours. Then the membrane was washed with hot water before it was reblocked in blocking solution and used for reprobing.

Antibodies	Source	Company	Code/Cat.No.
anti-mouse IgG	*sheep*	GE Healthcare, Munich, Germany	NA9310
anti-rabbit IgG	*donkey*	GE Healthcare, Munich, Germany	NA9340
anti-phospho-STAT1 (Tyr701)	*rabbit*	Cell Signaling, Danvers, MA	9167
anti-STAT1	*rabbit*	Cell Signaling, Danvers, MA	9172
anti-phospho-STAT2 (Tyr689)	*rabbit*	Millipore, Billerica, MA	07-224
anti-STAT2	*rabbit*	Santa Cruz Biotechnology, Santa Cruz, CA	sc-839
anti-phospho-STAT5 (Tyr694)	*mouse*	Cell Signaling, Danvers, MA	9356
anti-STAT5	*rabbit*	Santa Cruz Biotechnology, Santa Cruz, CA	sc-835
anti-JAK1	*rabbit*	Santa Cruz Biotechnology, Santa Cruz, CA	sc-277
anti-TYK2	*rabbit*	piCHEM, Graz, Austria, custom made	--------
anti-panERK	*mouse*	BD Biosciences	610124

4.6. RNA Isolation / cDNA Synthesis / Quantitative PCR (qPCR)

DEPC-H_2O

0.01% DEPC in sterile water were incubated over night and autoclaved next day.

RNA Isolation and cDNA Synthesis

"Eppendorf Centrifuge 5415r" centrifuge was used for RNA isolation. Cells were lysed in 1ml peqGOLD Trifast. Samples were stored at -80°C or were used directly for RNA isolation after 5min incubation at room temperature. 0.2vol chloroform was added per sample and after shaking for 15sec the samples were incubated at room temperature for 2-3min. Following centrifugation at 12000rpm for 15min the clear upper aqueous phase was transferred to a new eppendorf tube and 1vol isopropanol was added. The samples were incubated for 10min at room temperature and then centrifuged at 12000rpm for 10min on 4°C. The supernatant was removed and the pellet was washed once with 75% ethanol. After pelleting (5min, 7500rpm, 4°C) the supernatant was removed and the RNA pellet was dried at 60°C. The pellet was resuspended in DEPC-H_2O and incubated on ice for 30min. To dissolve properly the samples were heated to 56°C for 30min and pipeted up and down several times. RNA in DEPC-H_2O was stored at -80°C or used directly for cDNA synthesis. The concentration of isolated RNA was determined using 4µl sample in 96µl TE buffer (OD_{260}/OD_{280}) and the RNA integrity was checked on agarose gels. Then total RNA was reverse transcribed using iScript cDNA synthesis kit.

Quantitative PCR (qPCR)

The following concentrations were used for qPCR analysis:

$MgCl_2$ (25mM)	4 mM
Primer fw (10 pmol/µl)	300 nM
Primer rev (10 pmol/µl)	300 nM
Probe (10 pmol/µl)	100 nM
HotfirePol (5 U/µl)	1 U/rxn
dNTP mix (2mM each)	200 µM
10x Hotfire B buffer	1x
Template (cDNA)	2µl*

* cDNA equivalent to 1µg of reverse transcribed RNA

The primers for the TYK2 qPCR reaction were designed to bind in the deleted region of exon 3, excluding signals from truncated TKY2 mRNA present in $TYK2^{\Delta CD11c}$ BMDC. Ube2d2 was used as endogenous reference gene.

Primer and Probes	Sequence
Ube2d2 fw:	5´-AGGTCCTGTTGGAGATGATATGTT-3´
Ube2d2 rev:	5´-TTGGGAAATGAATTGTCAAGAAA-3´
Ube2d2 TaqMan Probe:	5´-CCAAATGACAGCCCCTATCAGGGTGG-3´
$TYK2^{fl/fl}$ fw:	5´-GGGTCACCTTCAGCCAGACA-3´
$TYK2^{fl/fl}$ rev:	5´-GACCTTAGCCTGTGCATTGTAGAGT-3´
$TYK2^{fl/fl}$ TaqMan Probe:	5´-CACATCGCACACAAAGTCGGCATCA-3´

Samples were analyzed in duplicates using Eppendorf Real Plex cycler.
Cycling conditions: 15 min at 95°C then 45 cycles of 20 sec, 95°C and 1 min, 60°C

Fluorescence data were collected during the extension phase (step 3). Target gene expression was calculated by normalizing cycle threshold (C_t) values to endogenous control (Ube2d2) and were shown relative to unstimulated wild type samples.

4.7. Magnetic Cell Sorting (MACS)

Isolated spleens were passed through 100µm cell strainers under sterile conditions. Splenocytes were homogenized in 5ml MEF medium and centrifuged for 5min at 1000rpm [Heraeus Multifuge 1S]. The pellet was resuspended in 1ml red blood cell lysis buffer and incubated under constant shaking for 2min. The lysis was stopped by adding 3ml MEF medium. After centrifugation at 1000rpm [Heraeus Multifuge 1S] for 5min the pellet was resuspened in MEF medium first and after a second centrifugation step in 900µl MACS buffer. 100µl MACS beads (CD11c) were added. The suspension was incubated for 20min at 4°C and shaken occasionally to ensure proper binding of MACS beads. Then 4ml MACS buffer were added, the sample was centrifuged as above and the supernatant was discarded. The pellet was resuspended in 1ml MACS buffer and pipeted on an MACS column. The column was rinsed twice with 500µl MACS buffer. After removing columns from the magnetic field, positively selected cells were eluted with 1ml MACS buffer and either used directly for FACS analysis or RNA isolation.

4.8. FACS Analysis

4% Paraformaldehyde

100ml HBSS were heated to 60-70°C in beaker using heat stirrer. 4g paraformaldehyde were added and after 3-4 hours of stirring (covered with aluminum foil) the pH was adjusted with NaOH to 7.4. The solution was sterile filtrated and stored for maximal 4 weeks at 4°C (protected from light).

Staining Buffer

HBSS

25mM Hepes

5mM $MgCl_2$

0.1% BSA

Blocking Solution

5% aggregated rat serum

Mouse BD FC-Block (1µg/10^6 cells)

+up to 10µl with staining buffer

Antibodies	Chromophore	Company	Code/Cat.No.
anti-mouse CD11c	*PE/Cy7*	BioLegend, Uithoorn, Netherlands	117316
anti-mouse CD11b	*A488*	BioLegend, Uithoorn, Netherlands	101217
anti-mouse F4/80	*PE*	BioLegend, Uithoorn, Netherlands	123110
anti-mouse B220	*Pacific Blue*	BD Biosciences, San Diego, CA	558108
anti-mouse NK1.1	*A488*	BioLegend, Uithoorn, Netherlands	108718
anti-mouse DX5	*PE*	BioLegend, Uithoorn, Netherlands	108907
anti-mouse Gr1	*APC*	BioLegend, Uithoorn, Netherlands	108411
7-AAD viability stain	*PE*	BioLegend, Uithoorn, Netherlands	420403
Mouse BD FC-Block	--	BD Biosciences, San Diego, CA	553142

Surface Staining for FACS Analysis

10µl blocking solution were added to 5x10^5 cells in 50µl staining buffer in 1.5ml tubes, vortexed and incubated for 10min on ice. Then 10µl of staining solution (250ng antibody/5x10^5 cells) was added to each sample. After vortexing the samples were incubated 20min at 4°C, protected from light. Two washing steps were performed by centrifuging at 5000rpm [Eppendorf Centrifuge 5415r] for 4min at 4°C and resuspending the pellet in 200µl staining buffer. Stained cells were either used directly for FACS analysis or stored over night at 4°C in 1% paraformaldehye prior to analysis. The samples were analyzed using FACS Canto 2 and BD´s FACS-Diva Software 6.

5. Results

5.1. *In vitro* Differentiation and Characterization of Bone Marrow-Derived DCs (BMDCs)

To study functions of TYK2 in DCs and also to quantify the deletion efficiency of the CD11c promoter-driven cre recombination, we established and characterized BMDC cultures. For *in vitro* differentiation of mouse bone marrow cells to mDCs, granulocyte macrophage colony-stimulating factor (GM-CSF) had to be provided in the culture medium. GM-CSF was produced using cells from a mouse myeloma cell line (X63), which secretes GM-CSF under starving conditions. To determine the quality of X63 cell-derived GM-CSF, tyrosine 694 (Tyr694) phosphorylation of STAT5 in BMMΦs after stimulation with different concentrations of either X63 cell-derived or recombinant GM-CSF (rGM-CSF) was determined by Western blot analysis (Fig. 7).

STAT5-Tyr694 phosphorylation was not detectable in unstimulated BMMΦs, whereas BMMΦs showed similar STAT5 phosphorylation levels when stimulated with medium containing 5%, 10% or 15% X63-conditioned medium. Comparable levels could also be observed when BMMΦ were treated with rGM-CSF at different concentrations.

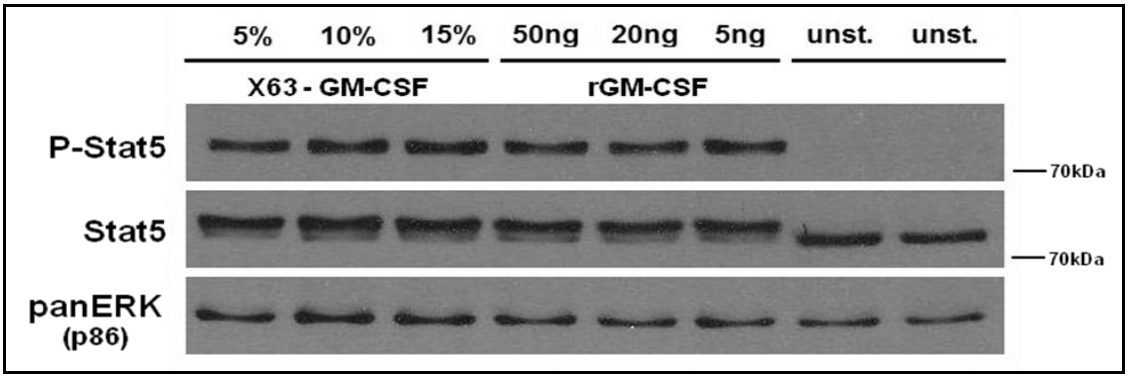

Figure 7: GM-CSF induced STAT5 phosphorylation in BMMΦs. Wild type BMMΦs were treated for ten minutes with various concentrations of X63-conditioned medium, rGM-CSF or left untreated. Twenty microgram of total cell extract per lane were separated by 7% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with an anti-phosphoTyr694-STAT5 (P-Stat5), and reprobed with anti-STAT5 antibody (Stat5). Protein loading was controlled by reprobing with anti-panERK. unst.: unstimulated

Since both 5% and 10% X63-conditioned medium prominently induced Stat5 phosphorylation, we next tested the capability to drive *in vitro* differentiation of BMDCs. BMDCs are usually analyzed between day 6 and day 8 of *in vitro* differentiation. Therefore, we decided to compare $TYK2^{fl/fl}$ BMDC cultures grown 6 days in the presence of either 5% or 10% of X63-conditioned medium and BMDCs cultivated for 8 days using 10% X63-conditioned medium. The purity of the BMDC cultures was

analyzed by characterization of the surface expression of CD11c, a prominent DC-cell marker, and was determined by FACS analysis (Fig. 8).

We set a gate according to a viability staining to exclude dead cells from analysis (Fig. 8a and b). On day 6 of *in vitro* differentiation BMDCs cultures grown in 5% X63-conditioned medium contained already more than 88% $CD11c^+$ cells (Fig. 8c). The amount of CD11c-expressing cells could not be increased by the use of a higher concentration of X63 cell-derived GM-CSF (Fig. 8d) and on average no significant difference regarding CD11c expression was observed between these two conditions (Fig. 8f). But, as shown in Fig. 8e and 8f, the numbers of $CD11c^+$ cells further increased with time since more than 95% of all living cells were expressing CD11c on day eight.

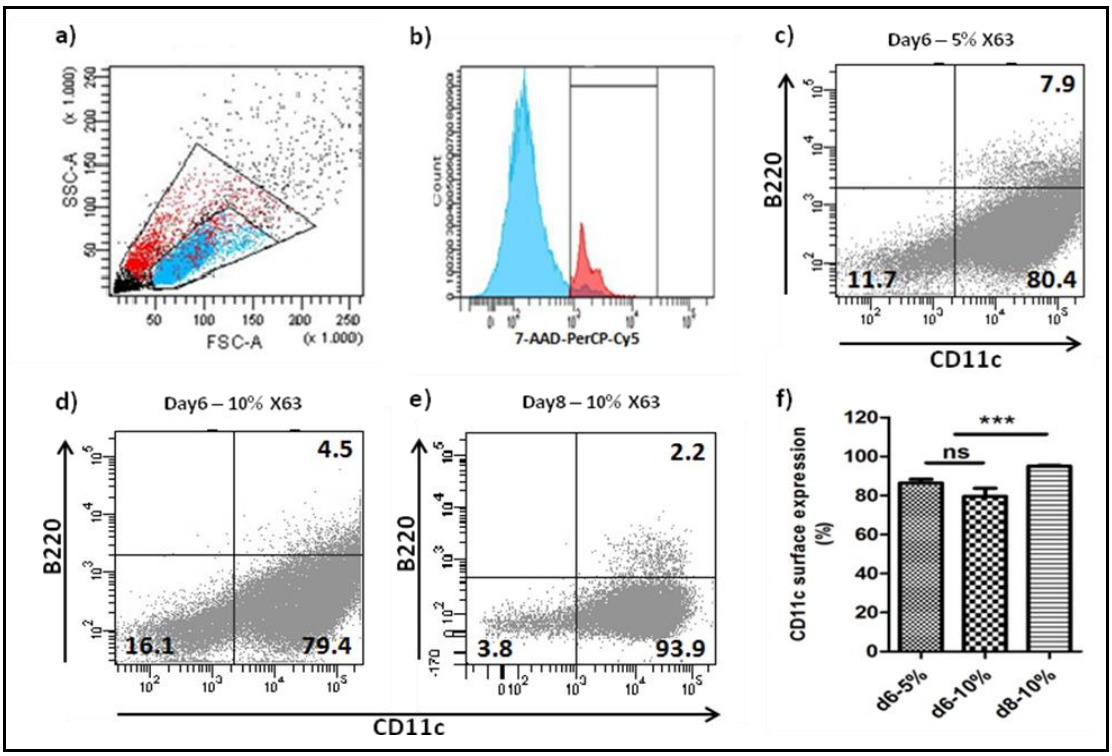

Figure 8: FACS analysis of BMDC cultures **a)** Forward and side scatter analysis of BMDC culture cells. The living gate was set according to the viability staining shown in b). **b)** Dead cells (red) incorporate 7-AAD due to decreased membrane integrity. Living cells are shown in blue. **c)-e)** CD11c expression in BMDCs after 6 and 8 days in culture. Numbers in quadrants represent the percentage of living cells in this quadrant. **c)** $TYK2^{fl/fl}$ BMDCs after six days using 5% X63- or **d)** 10% X63-conditioned medium. **e)** $TYK2^{fl/fl}$ BMDCs after eight days using 10% X63-conditioned medium. **f)** Mean values ± SD of CD11c-expressing cells of 2 mice in 1 experiment for BMDCs at day 6 in 5% X63-conditioned medium (d6-5%) and 10% X63-conditioned medium (d6-10%) and 10 mice in 3 experiments for BMDCs at day 8 in 10% X63-conditioned medium (d8-10%) are shown. ***: $p<0.0002$

Next, we characterized BMDC cultures in more detail by analyzing surface expression of CD11c, CD11b, B220, F4/80, NK1.1, Gr-1 and DX5 (Fig. 9). BMDCs were analyzed on day 8 of *in vitro* differentiation in 10% X63-conditioned medium, representing the conditions chosen for future experiments.

Beside high levels of CD11c, approximately 95% of BMDCs also expressed CD11b but not B220 (Fig. 9a and b), thus representing conventional DCs ($CD11c^+CD11b^+B220^-$). As expected, hardly any cells expressed the NK cell-specific markers DX5 and NK1.1 or F4/80, a marker specific for macrophages (Fig. 9c and data not shown). Less than 20% of the cells were Gr-1^+, a marker characteristic for pDCs and other cell types of the myeloid lineage (Fig. 9d). In summary we could see that BMDCs after 8 days of differentiation were a homogenous cell population with characteristic mDC marker expression (Fig. 9e).

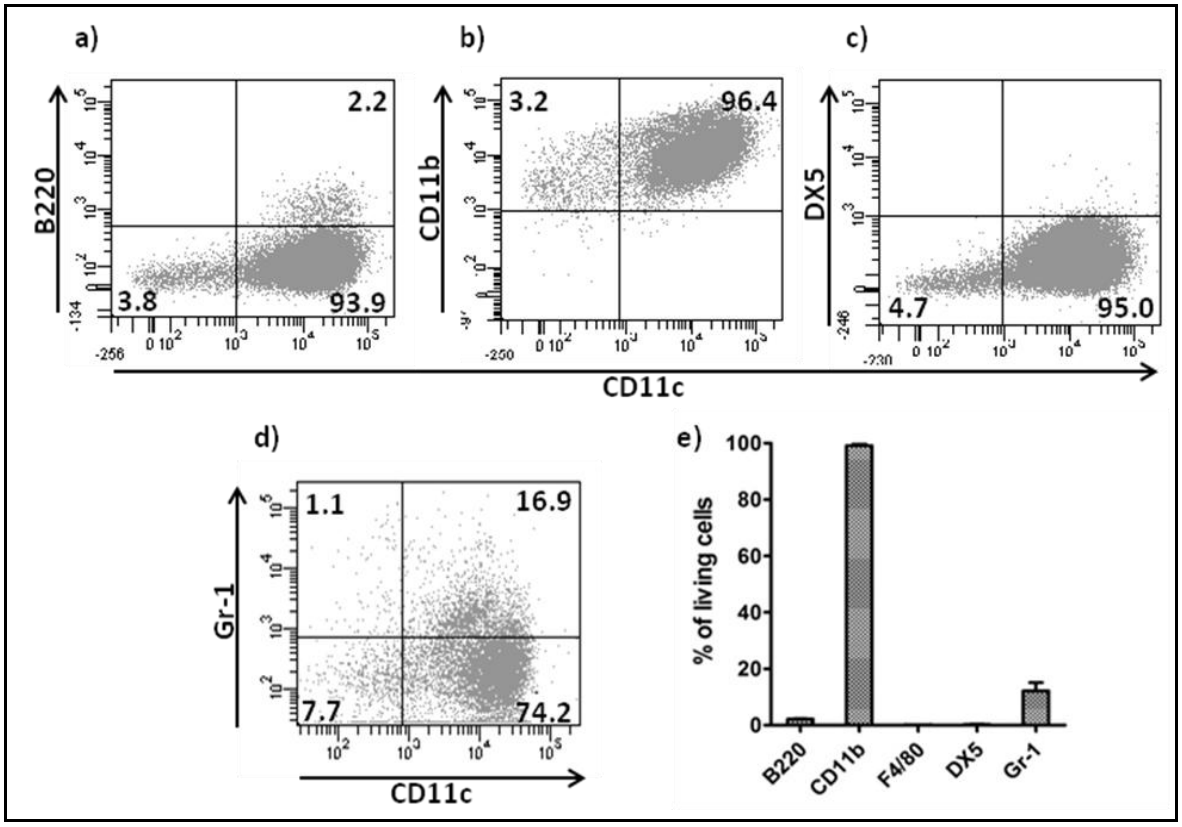

Figure 9: Characterization of unstimulated $TYK2^{fl/fl}$ BMDCs on day 8 of *in vitro* differentiation. **X-axis:** expression levels of CD11c increase from left to right. **Y-axis:** expression of surface marker indicated increases from bottom to top. Numbers in quadrants represent the percentage of cells in this quadrant. **a-d)** Figures are representative for cells derived from 3 mice in 2 independent experiments. **e)** Mean values ± SD (3 mice in 2 independent experiments) of $TYK2^{fl/fl}$ BMDCs positive for the indicated cell surface markers are shown.

5.2. TYK2 mRNA and Protein Levels are Significantly Reduced in $TYK2^{\Delta CD11c}$ BMDCs

Cre activity in CD11c-expressing cells, like BMDCs, should lead to deletion of the floxed TYK2 gene in these cells. To determine the deletion efficiency, we compared TYK2 mRNA levels of $TYK2^{\Delta CD11c}$ to $TYK2^{fl/fl}$ and $TYK2^{\Delta/\Delta}$ BMDCs *via* RT-quantitative PCR (qPCR)(Fig. 10a) and TYK2 protein levels of the same genotypes using IP-Western blot analysis (Fig. 10b and c).

As shown in Fig. 10a, we could observe an effective reduction of TYK2 mRNA in TYK2$^{\Delta CD11c}$ BMDCs compared to TYK2$^{fl/fl}$ mRNA levels. TYK2 mRNA was reduced by 73% on average, even though remaining CD11c^{-} cells in the cell culture still made up approximately 5% of all living cells. In TYK2$^{\Delta/\Delta}$ BMDCs TYK2 mRNA was not detectable (Fig. 10a).

On protein level TYK2 protein was readily detectable in TYK2$^{fl/fl}$ BMDCs and, as expected, was absent in TYK2$^{\Delta/\Delta}$ cells (Fig. 10b). TYK2 protein deletion in TYK2$^{\Delta CD11c}$ BMDCs was comparable to data gained from mRNA analysis (Fig. 10c). Although it has to be considered, that quantification of Western blot pictures is afflicted with increased error rates compared to qPCR analysis, the mean TYK2 reduction was calculated with 88% from TYK2$^{fl/fl}$ BMDCs to TYK2$^{\Delta CD11c}$ BMDCs (Fig. 10c). JAK1 protein levels were analyzed to control IP procedure and they were clearly not influenced by deletion of TYK2 which is consistent with previous data (Fig. 10b)[15, 16].

Taken together, we could show that TYK2 is reduced drastically in TYK2$^{\Delta CD11c}$ BMDCs on mRNA and protein level when compared to TYK2$^{fl/fl}$ BMDCs.

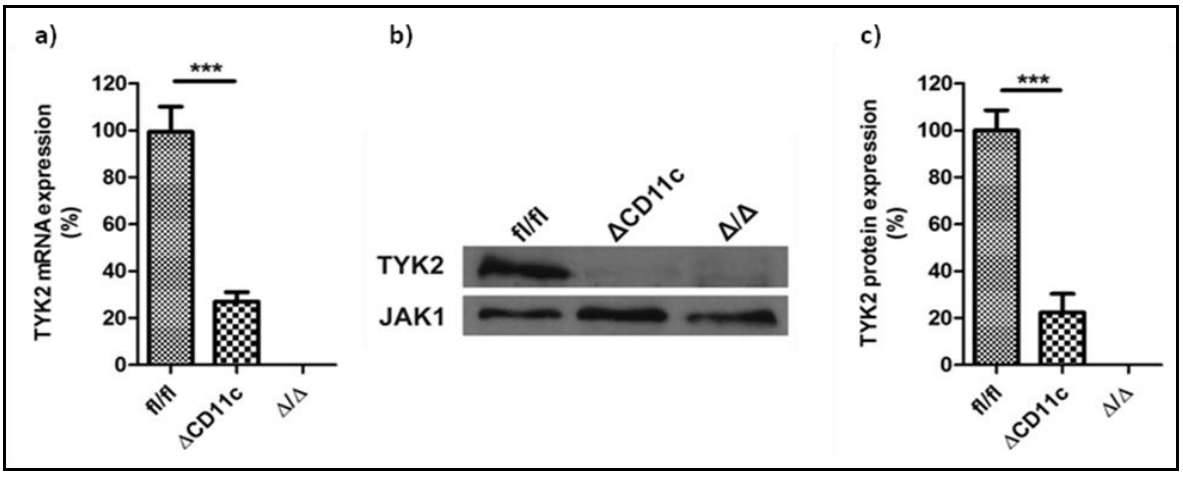

Figure 10: a) TYK2 mRNA levels in BMDCs. TYK2$^{fl/fl}$ (fl/fl), TYK2$^{\Delta CD11c}$ (ΔCD11c) and TYK2$^{\Delta/\Delta}$ (Δ/Δ) BMDCs were analyzed after 8 days of differentiation in 10% X63-conditioned medium. Mean values ± SD of 3 TYK2$^{\Delta/\Delta}$, 5 TYK2$^{fl/fl}$ and 6 TYK2$^{\Delta CD11c}$ mice in 3 independent experiments were normalized to 1 TYK2$^{fl/fl}$ mouse (100%). ***: p<0.0001 **b)** TYK2 and JAK1-IP-Western blot analysis of BMDCs after 8 days of differentiation. Protein lysates of 2 mice per genotype were pooled. Results are representative for 3 individual experiments. Same samples were used for JAK1 IP. **c)** TYK2 protein levels in BMDCs were quantified with ImageJ 1.45. Mean values ± SD of cells derived from 5 mice per genotype in 3 independent experiments were normalized to 1 TYK2$^{fl/fl}$ mouse (100%). ***: p<0.0002

5.3. TYK2 mRNA Levels Are Significantly Reduced in CD11c^{+} TYK2$^{\Delta CD11c}$ Splenocytes

The deletion efficiencies observed in BMDC culture might not be reflecting an actual *in vivo* situation, as TYK2 deletion might vary in different DC subsets. Next we determined the efficiency of cre

deletion of TYK2 in CD11c-expressing cells *in vivo*. Spleens of $TYK2^{fl/fl}$, $TYK2^{\Delta CD11c}$ and $TYK2^{\Delta/\Delta}$ mice were isolated and single cell suspensions were positively selected for CD11c expression using Magnetic Cell Sorting (MACS). TYK2 mRNA levels of $CD11c^+$ cells were subsequently analyzed *via* RT-qPCR. We found an 87% reduction of TYK2 mRNA levels of $CD11c^+$ $TYK2^{\Delta CD11c}$ compared to $CD11c^+$ $TYK2^{fl/fl}$ splenocytes on average (Fig. 11).

In order to examine the effectiveness of the cell enrichment method used, the purity of $CD11c^+$ splenocytes was analyzed. The proportion of CD11c-expressing cells after positive selection *via* MACS was determined by FACS analysis. In addition, we conducted one experiment where we performed a second round of MACS to quantify the increase of purity from single to double MACSed cell populations (Fig. 12). On average 87.9% of single MACSed and 99.2% of double MACSed splenocytes were expressing CD11c on the cell surface. Applying a second round of cell sorting clearly increased the purity of $CD11c^+$ splenocytes (Fig. 12c). Since we used single MACSed $TYK2^{\Delta CD11c}$ splenocytes to determine TYK2 mRNA levels, a reduction of 87.1% of TYK2 mRNA in splenocytes with a purity of 87.9%, indicated very effective TYK2 deletion in $CD11c^+$ cells. To further characterize $CD11c^+$ splenocytes we analyzed surface expression of prominent marker proteins of immune cells similar to BMDC characterization (Fig. 13).

Beside expressing CD11c, FACS analysis of single MACSed splenocytes showed that approximately 18% of all living cells were expressing B220 (Fig. 12a and 13a). $CD11c^+B220^+$ cells likely constitute pDCs, whereas $CD11c^-B220^+$ represent splenic B-cells. Contrarily to *in vitro* differentiated mDCs, splenic $CD11c^+$ cells were $CD11b^+$ or $CD11b^-$ (Fig. 13b). Although pDCs also do not express CD11b, the negative population for this marker might in addition be constituted by splenic mDCs, which can be, depending on the subtype, either CD11b positive or negative[108]. In agreement with previous studies, freshly isolated splenic DCs were $F4/80^+$ (Fig. 13c)[109]. DX5 was not expressed, with the exception of a small population of $CD11c^+DX5^{low}$ cells (Fig. 13d). $CD11c^+DX5^{low}$ cells were probably NK/NKT-progenitor cells, because the second NK-cell specific marker NK1.1 was not yet expressed on those cells (data not shown) [110]. Gr-1 could be found on 31% of $CD11c^+$-enriched splenocytes (Fig. 13e). In addition to granulocytes and some myeloid progenitor cells, low amounts of Gr-1 can also be expressed on DCs[102, 111]. Overall we could detect about 80% mDCs ($CD11c^+CD11b^{\pm}F4/80^+$), 10% pDCs ($CD11c^{low}B220^+CD11b^-$) and 10% of other cells which probably consist of B-cells, T-cells, NK/NKT-cells and macrophages. Importantly, marker distribution was not dependent on the genotype of mice analyzed (Fig. 13f).

In summary, we could observe a very effective TYK2 deletion as TYK2 mRNA was reduced by 87% from $TYK2^{fl/fl}$ to $TYK2^{\Delta CD11c}$ $CD11c^+$ splenocytes in cell fractions with a purity of 88%. Although the majority (80%) of single MACSed $CD11c^+$ splenocytes showed characteristic mDC marker expression,

it has to be kept in mind, that a second MACSing step should be performed if a higher purity of $CD11c^+$ splenocytes is required.

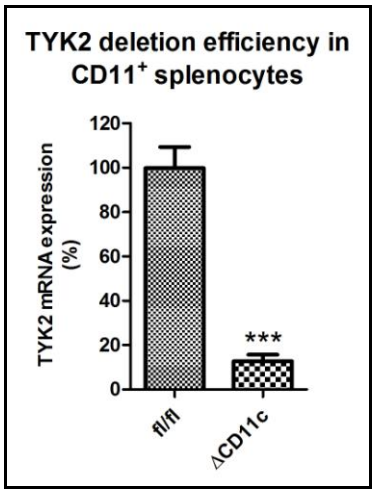

Figure 11: TYK2 mRNA levels of $CD11c^+$ fractions of $TYK2^{\Delta CD11c}$ and $TYK2^{fl/fl}$ splenocytes are shown. Mean values ± SD of cells derived from 6 mice per genotype in 3 independent experiments were normalized to 1 $TYK2^{fl/fl}$ mouse ***: p<0.0001; fl/fl: $TYK2^{fl/fl}$; ΔCD11c: $TYK2^{\Delta CD11c}$

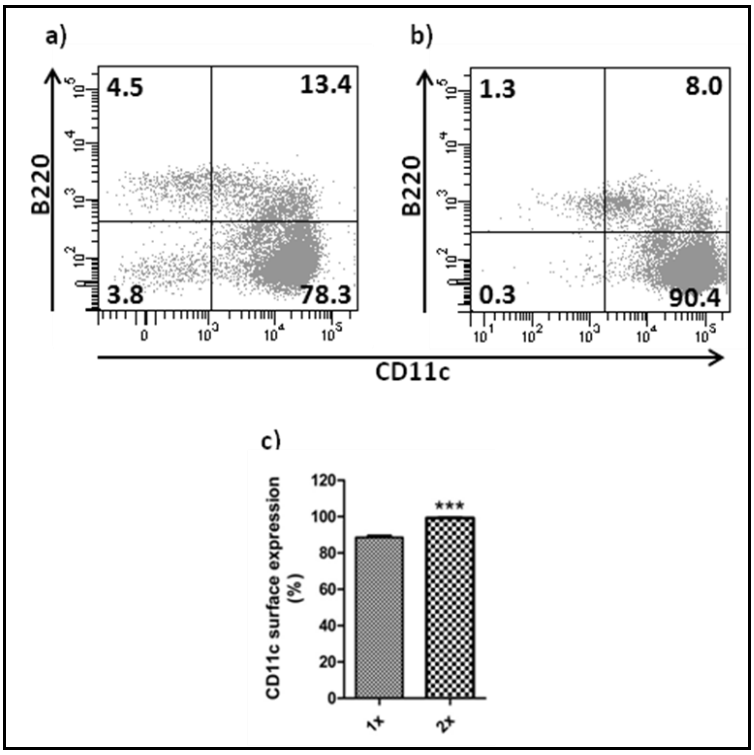

Figure 12: CD11c expression in $CD11c^+$ fractions of MACSed splenocytes. CD11c expression increases from left to right. **a)** Single MACSed splenocytes. Data shown are representative for 12 mice in 3 independent experiments. **b)** Double MACSed splenocytes representative for 2 mice in 1 experiment. **c)** Mean values ± SD of CD11c-expressing cells of single (1x) and double (2x) MACSed splenocytes are shown ***: p<0.0001

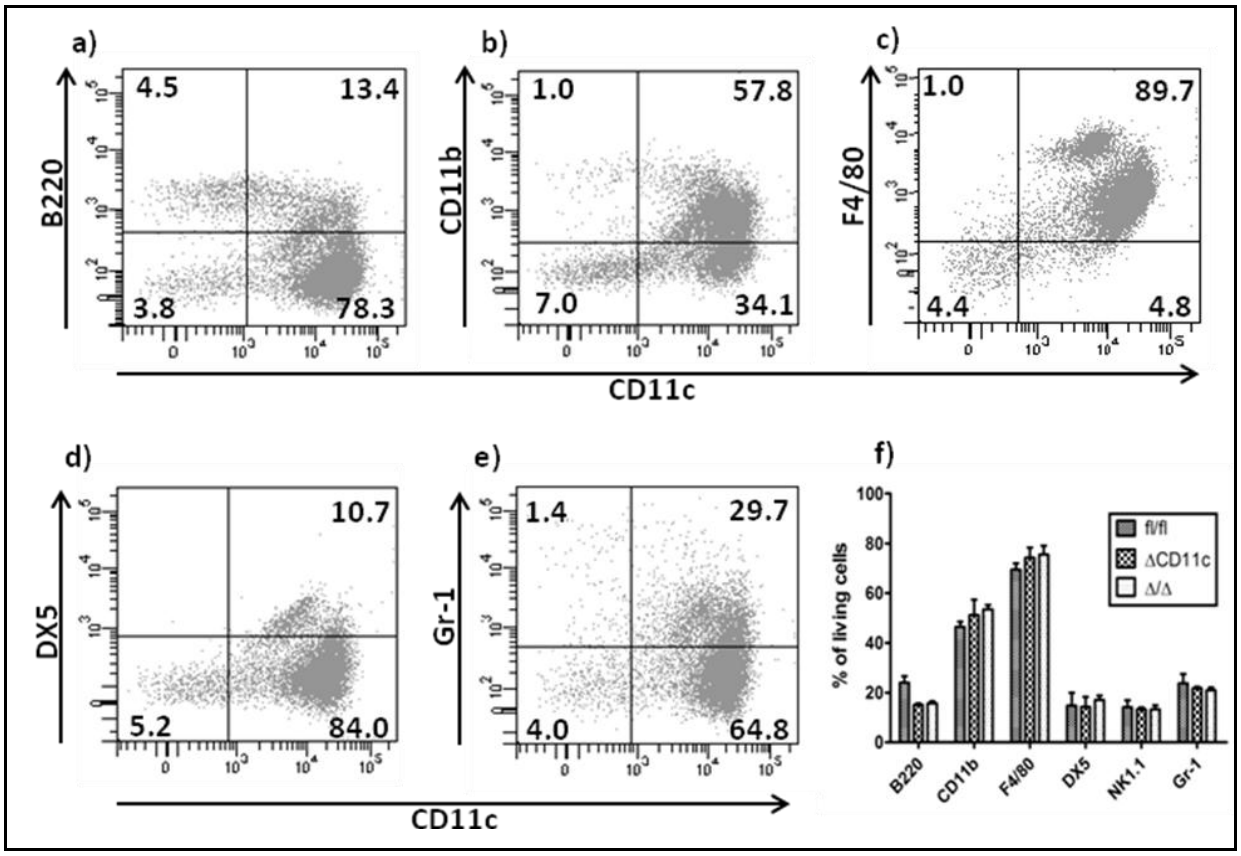

Figure 13: Characterization of single MACSed CD11c^{+} splenocytes. X-axis: expression levels of CD11c increase from left to right. Y-axis: expression of surface marker indicated increases from bottom to top. Figures are representative for 12 mice in 2 independent experiments. **f)** Mean values ± SD of splenocytes positive for the indicated cell surface markers of 3 mice in 2 independent experiments for TYK2$^{fl/fl}$ (fl/fl) and TYK2$^{\Delta CD11c}$ (ΔCD11c) and 2 mice in 2 experiments for TYK2$^{\Delta/\Delta}$ (Δ/Δ) are shown.

5.4. MCMV Replicates to Higher Titers in TYK2$^{\Delta CD11c}$ BMDCs *in vitro* Compared to TYK2$^{fl/fl}$ BMDCs

It has been shown previously in our lab that macrophages, in contrast to what is known from fibroblasts, are very resistant against MCMV infection. This resistance is mediated by autocrine/paracrine IFN-α/β signaling and depends on the presence of TYK2[24]. In order to test if this is also true for DCs, we analyzed MCMV replication in TYK2$^{\Delta CD11c}$ BMDCs and compared it to TYK2$^{fl/fl}$ and TYK2$^{\Delta/\Delta}$ BMDCs. Viral titers were determined 0, 1, 3 and 5 days post infection by using plaque forming assays (Fig. 14). Right after infection MCMV titers of all three genotypes were similar, indicating equal binding and entry of MCMV particles into the cells. MCMV replicated poorly in TYK2$^{fl/fl}$ BMDCs compared to TYK2$^{\Delta/\Delta}$ BMDCs. Three and five days post infection the MCMV titers were 100-fold higher in TYK2$^{\Delta/\Delta}$ BMDCs than in TYK2$^{fl/fl}$ cells. MCMV titers in TYK2$^{\Delta CD11c}$ BMDC were increased 10-fold when compared to TYK2$^{fl/fl}$ BMDCs. The difference to TYK2$^{\Delta/\Delta}$ BMDCs might come

from a contribution of other ($CD11c^-$) cells in the culture or from ineffective TYK2 deletion in DCs (TYK2 deletion efficiency in BMDCs approx. 75% s. Fig. 10, purity of population approx. 95% s. Fig. 8).
In summary, we could see that the MCMV resistance of BMDC was clearly dependent on TYK2 and that small amounts of remaining TYK2 in $TYK2^{\Delta CD11c}$ are sufficient to have an impact on MCMV resistance.

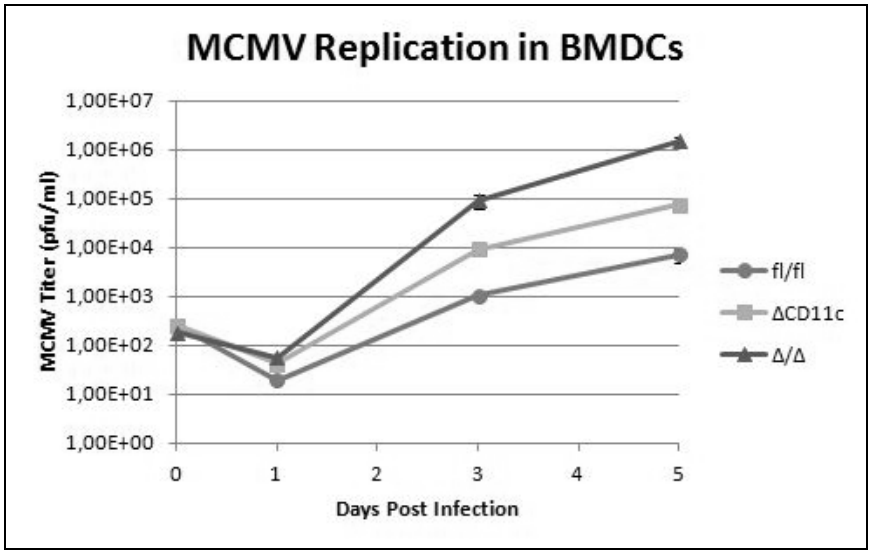

Figure 14: TC-MCMV replication in BMDCs. BMDCs of the indicated genotypes were infected with TC-MCMV (MOI=5). TC-MCMV titers were determined 0, 1, 3 and 5 days post infection by plaque forming assays. 1 representative out of 3 independent experiments is shown. fl/fl: $TYK2^{fl/fl}$; ΔCD11c: $TYK2^{\Delta CD11c}$; Δ/Δ: $TYK2^{\Delta/\Delta}$

5.5. TYK2-Dependent STAT1/2 Activation in $TYK2^{\Delta CD11c}$ BMDCs After MCMV Infection

Infection with MCMV most likely results in an IFN type I induced activation of STAT1 and STAT2 in BMDCs, as DCs produce a lot of IFN-α/β in response to MCMV infection and are responsive to type I IFN treatment[77, 112, 113]. Due to the lack of TYK2 in $TYK2^{\Delta CD11c}$ BMDCs, MCMV-induced IFN signaling could be affected. Therefore, STAT1 and STAT2 phosphorylation was determined after 0, 2 and 5 hours post infection with TC-MCMV using Western blot analysis (Fig. 15).
In agreement with previous studies on BMMΦs[24], STAT1 and STAT2 protein levels were decreased in $TYK2^{\Delta/\Delta}$ BMDCs. In $TYK2^{fl/fl}$ BMDCs Tyr701 phosphorylation of STAT1 was detected right after infection with MCMV (30min), was slightly increased after 2 hours and started to decline 5 hours post infection. In contrast, STAT1 phosphorylation in $TYK2^{\Delta/\Delta}$ BMDCs was hardly detectable immediately after infection, whereas similar to $TYK2^{fl/fl}$ BMDCs the phosphorylation signal was increased after 2 and back to basal levels 5 hours post infection. $TYK2^{\Delta CD11c}$ BMDCs resembled the

situation observed in TYK2$^{\Delta/\Delta}$ BMDCs, except that the induction of STAT1 phosphorylation was slightly higher. The kinetics of STAT2 Tyr689 phosphorylation in TYK2$^{fl/fl}$, TYK2$^{\Delta/\Delta}$ and TYK2$^{\Delta CD11c}$ BMDCs were similar to STAT1 phosphorylation. Although STAT2 phosphorylation was still induced significantly in TYK2$^{\Delta/\Delta}$ and TYK2$^{\Delta CD11c}$ BMDCs it did not reach TYK2$^{fl/fl}$ levels.

Type I IFN response upon MCMV infection induces upregulation of STAT1 and STAT2 proteins. Decreased activation of STAT1/2 genes due to impaired TYK2 functions in DCs would lead to decreased levels of STAT1 and STAT2 later in MCMV infection. Additionally, MCMV was reported to directly degrade STAT2 in fibroblasts[83]. To analyze STAT1 and STAT2 protein levels in BMDCs at later time points of MCMV infection, we performed Western blot analysis with whole cell extracts 0, 1, 3 and 5 days post infection (Fig. 16). As seen previously, STAT1 and STAT2 levels of TYK2$^{\Delta CD11c}$ and TYK2$^{\Delta/\Delta}$ BMDCs were reduced right after MCMV infection (30min) compared to TYK2$^{fl/fl}$ cells. In TYK2$^{fl/fl}$ BMDCs STAT1 levels were increased on day 1 and 3 and slightly declined on day 5 post infection. STAT1 protein levels in TYK2$^{\Delta/\Delta}$ BMDCs were also increased on day 1 and 3 post infection, but were significantly lower than protein levels in TYK2$^{fl/fl}$ cells. Surprisingly, although STAT1 levels in TYK2$^{\Delta CD11c}$ were decreased to levels observed in TYK2$^{\Delta/\Delta}$ cells immediately after infection, within 24 hours they reached TYK2$^{fl/fl}$ levels and remained high until day 5 post infection. STAT2 levels were similar to STAT1, except for day 5, where STAT2 levels of all three genotypes were decreased to equal amounts. Remarkably, STAT2 protein was detectable in all genotypes 5 days post infection, although MCMV titers were increased 100-fold in TYK2$^{\Delta/\Delta}$ BMDCs compared to TYK2$^{fl/fl}$ cells. Thus, similar to what was reported in BMMΦs, MCMV infection does not lead to a detectable STAT2 degradation in BMDCs[24].

Taken together, early after MCMV infection we could observe decreased STAT1 and STAT2 phosphorylation in TYK2$^{\Delta CD11c}$ and TYK2$^{\Delta/\Delta}$ BMDCs compared to TYK2$^{fl/fl}$ cells, which might be partially caused by lower basal levels of these proteins. In contrast to TYK2$^{\Delta/\Delta}$ BMDCs, STAT1 and STAT2 protein expression reached wild type levels in TYK2$^{\Delta CD11c}$ cells on day 1 post infection. STAT1/2 phosphorylation of TYK2$^{\Delta CD11c}$ BMDCs was intermediate between TYK2$^{fl/fl}$ and TYK2$^{\Delta/\Delta}$. Intermediate STAT1/2 phosphorylation of TYK2$^{\Delta CD11c}$ BMDCs is consistent with intermediate MCMV replication in those cells in the previous experiments.

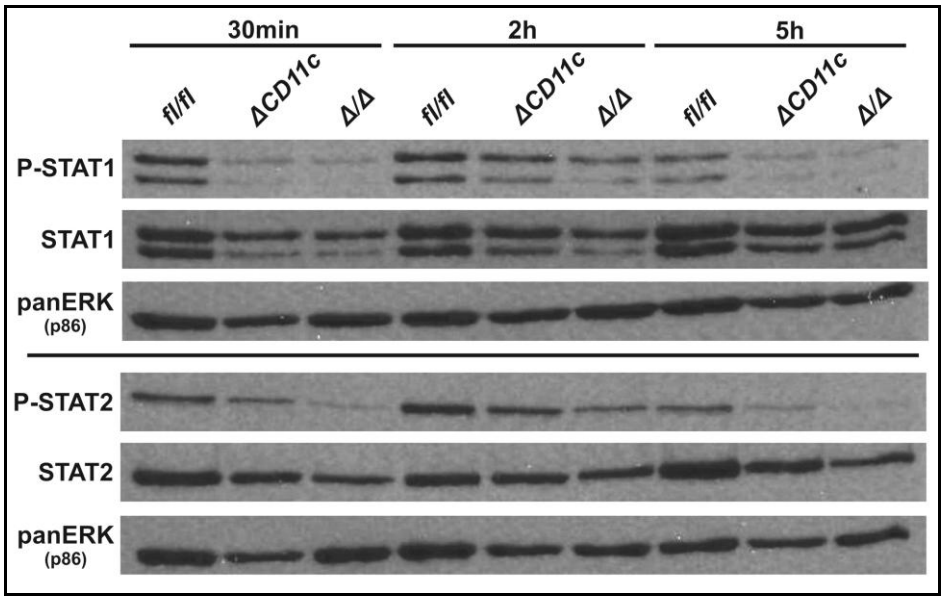

Figure 15: Tyrosine phosphorylation of STAT1 and STAT2 after TC-MCMV infection; BMDCs were infected with MCMV (MOI=5). At indicated times cells were lysed, separated with SDS-PAGE (6.5%, 20µg total cell extract per lane) and subjected to Western blot analysis. Membranes were probed with the indicated antibodies specific for phosphorylated Tyr701 of STAT1 (P-STAT1), phosphorylated Tyr689 of STAT2 (P-STAT2), STAT1 (STAT1) or STAT2 protein (STAT2). Protein loading was controlled by re-probing with anti-ERK antibody (panERK). The figure is representative for 2 individual experiments. fl/fl: $TYK2^{fl/fl}$; ΔCD11c: $TYK2^{\Delta CD11c}$; Δ/Δ: $TYK2^{\Delta/\Delta}$

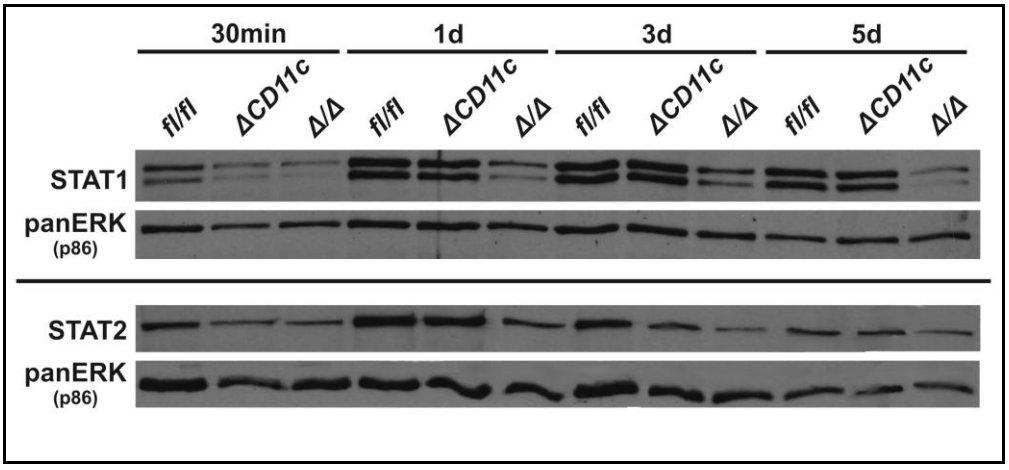

Figure 16: STAT1 and STAT2 expression in BMDCs of indicated genotypes after TC-MCMV infection (MOI=5); At indicated times cells were lysed, separated with SDS-PAGE (6.5%, 20µg total cell extract per lane) and subjected to Western blot analysis. Membranes were probed with the indicated antibodies specific for STAT1 protein (STAT1) or STAT2 protein (STAT2). Protein loading was controlled by re-probing with anti-ERK antibody (panERK). The figure is representative for cells derived from 2 mice per genotype in 1 experiment. fl/fl: $TYK2^{fl/fl}$; ΔCD11c: $TYK2^{\Delta CD11c}$; Δ/Δ: $TYK2^{\Delta/\Delta}$

5.6. Contribution of TYK2 in DCs, Macrophages/Neutrophils and T-Cells to MCMV Defense

It has been reported by our group that TYK2 knockout mice showed impaired clearance of MCMV from organs[24]. In order to test our hypothesis that TYK2 in macrophages and DCs contributes to early innate immune responses, we investigated the consequences of cell type-specific TYK2 deletion during MCMV infection. $TYK2^{fl/fl}$, $TYK2^{\Delta CD11c}$, $TYK2^{\Delta LysM}$ and $TYK2^{\Delta/\Delta}$ mice were infected with a sublethal dose of MCMV and virus replication/clearance was determined in livers. In addition to the deletion of TYK2 in macrophages/neutrophils ($TYK2^{\Delta LysM}$) or DCs ($TYK2^{\Delta CD11c}$), we included T-cell specific TYK2 knockout mice ($TYK2^{\Delta Lck}$) in order to exclude contributions of TYK2 in T-cells to the early anti-MCMV response. Viral titers were determined in livers at three and seven days post infection. As shown in Fig. 17a, viral titers were increased around five-fold in the absence of TYK2 at three days post infection. Increased viral titers were also found in livers from $TYK2^{\Delta CD11c}$ mice, whereas the virus load appeared unchanged in livers from $TYK2^{\Delta LysM}$ and $TYK2^{\Delta Lck}$ mice. Again, by using a higher infection dose ($2.5x10^5$ pfu/mouse), elevated MCMV titers could be found in livers of $TYK2^{\Delta/\Delta}$ mice, but no significant differences between $TYK2^{fl/fl}$, $TYK2^{\Delta CD11c}$, $TYK2^{\Delta LysM}$ and $TYK2^{\Delta Lck}$ mice were observed three days post infection (Fig. 17c). We also observed an increase of experimental variation from lower to higher infection doses used for experiments (Fig. 17a and c). In contrast to what has been shown previously[24], MCMV was almost entirely cleared from livers on day seven post infection irrespective of the genotype and the infection dose (Fig. 17b and d). Other reports demonstrated that MCMV titers remain high in livers up to seven days post infection [114] and the reason for the discrepancy is unclear.

In summary, these data indicate an impact of TYK2 in DCs on the control of MCMV replication and/or clearance in livers early after infection and no contribution of TYK2 in macrophages/neutrophils and T-cells. However, the differences were small and data need to be confirmed (higher sample size). In parallel, virus titrations of spleen, lung and salivary gland homogenates of these mice (done by Michael Rammerstorfer in the laboratory), indicate a requirement of TYK2 in macrophages/neutrophils and DCs for the control of MCMV in salivary glands seven days post infection (Fig. 18a and b). Virus titers in spleen and lungs were too low for conclusive analysis (data not shown).

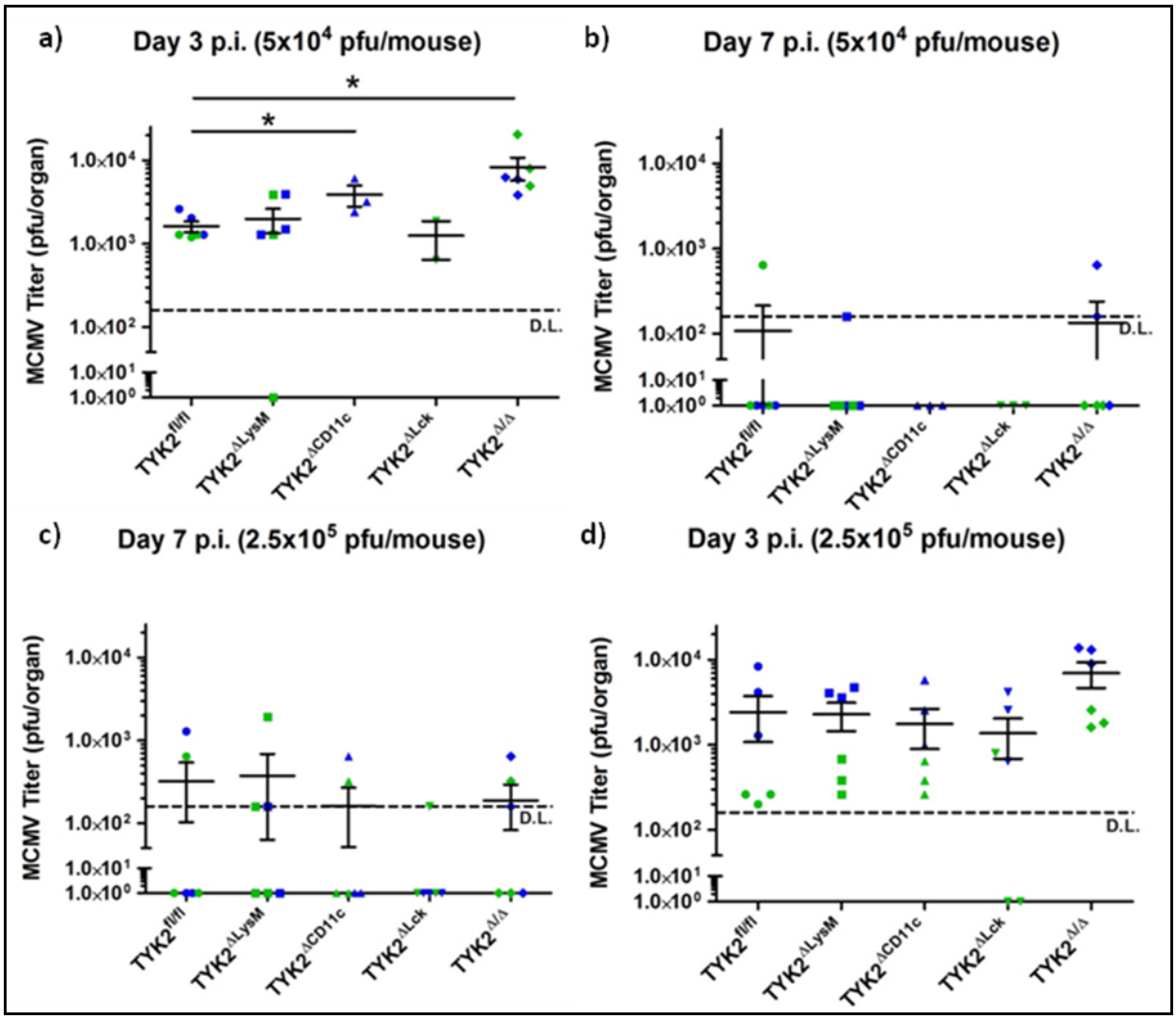

Figure 17: Virus titer in livers of TC-MCMV infected mice. MCMV titers were determined by plaque forming assays 3 **(a/c)** and 7 **(b/d)** days post infection. 1 dot represents 1 mouse and green/blue dots represent the 2 individual experiments. **a)** and **b)** Mice were infected with $5x10^4$ pfu/mouse. Mean values ± SD of 6 mice in 2 experiments are shown, except for ΔLck and ΔCD11c (3 mice in 1 experiment). *: $p<0.025$ **c)** and **d)** Mice were infected with $2.5x10^5$ pfu/mouse. Mean values ± SD of 3 mice in 1 experiment are shown. *: $p<0.04$; D.L.: detection limit; p.i.: post infection; pfu: plaque forming units

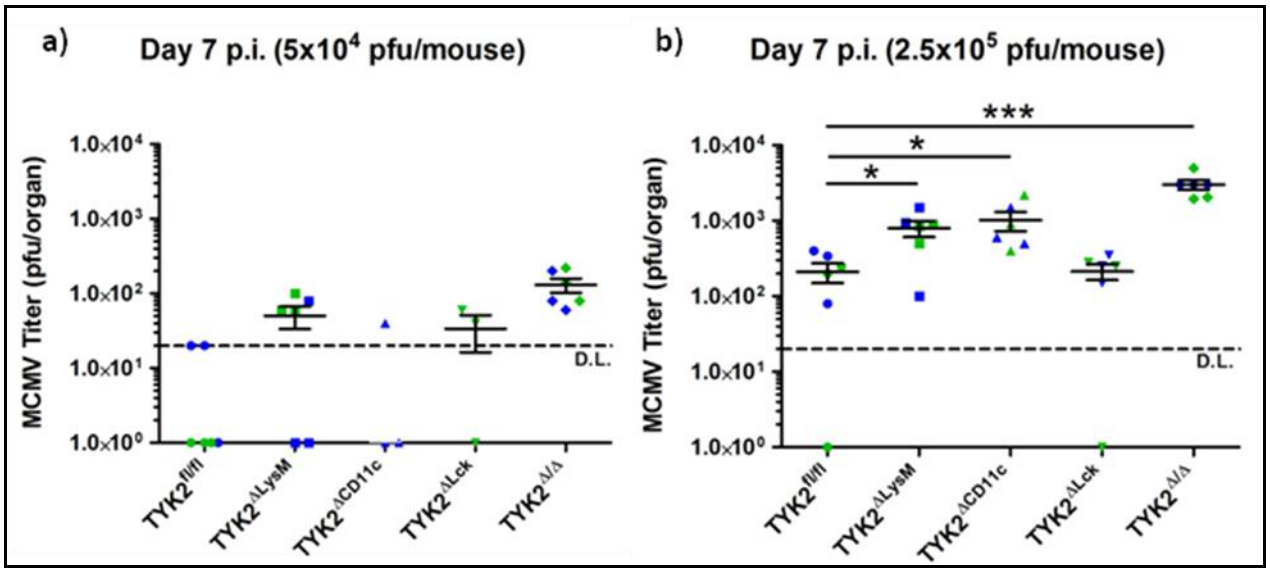

Figure 18: Virus titer in salivary glands of MCMV infected mice. MCMV titers were determined by plaque forming assays 7 days post infection. 1 dot represents1 mouse and green/blue dots represent 2 individual experiments. **a)** Mice were infected with $5x10^4$ pfu/mouse. Mean values ± SD of 6 mice in 2 experiments are shown, except for ΔLck and ΔCD11c (3 mice in 1 experiment). **b)** Mice were infected with $2.5x10^5$ pfu/mouse. Mean values ± SD of 6 mice in 2 experiment are shown. *: $p<0.021$; ***: $p<0.0006$; Data from Michael Rammerstorfer; D.L.: detection limit; p.i.: post infection; pfu: plaque forming units

6. Discussion

The aims of this work about "cell type-specific functions of TYK2 in the antiviral defense against MCMV" were to characterize TYK2$^{\Delta CD11c}$ mice with respect to TYK2 deletion efficiencies in DCs and to analyze the effect on anti-MCMV defense. We established a BMDC culture protocol and showed that more than 95% of our BMDCs were expressing CD11c on day 8 of *in vitro* differentiation. TYK2 mRNA in TYK2$^{\Delta CD11c}$ cells was reduced by 75% when compared to TYK2$^{fl/fl}$ BMDCs and a similar reduction could be detected by Western blot analysis for TYK2 protein. In CD11c^{+} splenocytes we observed a reduction of 87% of TYK2 mRNA from TYK2$^{fl/fl}$ to TYK2$^{\Delta CD11c}$ cells in populations where 88% of the living cells were expressing CD11c. These results are comparable to what was published previously. In the initial characterization of the CD11c/cre mice, Caton et al. could see recombination of the floxed allele from about 51% in CD11c^{low} to about 96% in CD11c^{high} splenocytes[87]. Using almost the same analyzing methods that we used in this work, but targeting STAT3, one group reported deletion efficiencies of 70% and 90% in BMDCs and splenic DCs respectively[115].

When we investigated the replication of MCMV in BMDCs, we could find a clear impact of CD11c-cre-mediated TYK2 deletion. MCMV titers in TYK2$^{\Delta CD11c}$ BMDCs was around 10-fold higher than in TYK2$^{fl/fl}$ controls. However, MCMV replication was lower than in BMDCs derived from TYK2$^{\Delta/\Delta}$ mice. This difference may be explained by the incomplete deletion of TYK2, as BMDCs still containing TYK2 might additionally have a survival/proliferative advantage during MCMV infection. Further insights might be gained by analyzing cell purity and deletion efficiency of TYK2 after 5 days of infection with MCMV. Notably, BMDCs appeared generally more sensitive to MCMV infection than what has been shown for macrophages[24], however, a direct comparison was not performed.

Tyrosine phosphorylation of STAT1 and STAT2 was reduced in TYK2$^{\Delta CD11c}$ and TYK2$^{\Delta/\Delta}$ BMDCs compared to TYK2$^{fl/fl}$ BMDCs until 5 hours post infection with MCMV. This may in part be caused by the reduced basal protein levels of STAT1 and STAT2 in cells of these genotypes. This is similar to what was seen in macrophages[24]. Interestingly, slightly higher STAT1/2 phosphorylation in TYK2$^{\Delta CD11c}$ BMDCs seems to be sufficient to induce STAT1/2 expression to levels observed in TYK2$^{fl/fl}$ cells at 1 day post infection. Since STAT1/2 upregulation upon MCMV infection seems to be TYK2-dependent and the majority of TYK2$^{\Delta CD11c}$ BMDCs should not express TYK2, we expected to see a more pronounced difference between TYK2$^{\Delta CD11c}$ BMDCs and TYK2$^{fl/fl}$ BMDCs. A possible explanation might again be that BMDCs still expressing TYK2 are accumulated during MCMV infection due to a protective or proliferative advantage. Other cells, not expressing CD11c, might also contribute to this observation. Nevertheless, both MCMV replication and activation of STAT1 and STAT2 were clearly affected by the DC-specific TYK2 deletion in BMDC cultures *in vitro*.

In vivo we could show that TYK2 in DCs has an impact on MCMV defense in livers as MCMV titers were elevated 3 days post infection in $TYK2^{\Delta CD11c}$ mice compared to $TYK2^{fl/fl}$ mice. In salivary glands, a similar effect could be found after 7 days of infection in $TYK2^{\Delta CD11c}$ and $TYK2^{\Delta LysM}$ mice. These data indicate an organ-specific TYK2 dependence in the defense against MCMV of at least macrophages/neutrophils and DCs, but this requires confirmation. Nevertheless, these findings refine the current picture of early host defense mechanisms during MCMV infections. In livers, macrophage-specific TYK2 deficiency did not elevate MCMV levels. Although macrophages definitely have a role in MCMV defense in the liver, their major function is the recruitment of NK-cells. Whether or not this is affected in $TYK2^{\Delta LysM}$ mice remains to be investigated. TYK2 deletion in DCs clearly has an impact on controlling MCMV infection in livers. Reasons therefore might be that elevated MCMV replication in infected resident DCs lacking TYK2 is able to overcome NK-cell dependent anti-MCMV response. In addition, killing of DCs by MCMV might lead to decreased IFN-α/β production and therefore reduce NK-cell responses. Furthermore, TYK2 deficiency in DCs could lead to impaired effector cell recruitment to the liver. The same might also apply for salivary glands 7 days post infection. Further insights in cell type-specific TYK2 functions in the ultimate control of MCMV *in vivo* will come from survival experiments which are currently run.

In addition to these findings we observed almost entire MCMV clearance from livers in all tested genotypes 7 days post infection. In similar experiments, MCMV titers in livers were steadily increasing from day 7 to day 28 post infection[24]. Furthermore and again in contrast to previous data, MCMV titers in our experiments were comparably low and often close to the detection limit. Reasons are unclear, but might include the different hygiene status/housing conditions of the mice or differences in handling and determination of virus titers.

In summary, using conditional TYK2 knockout mice, we could demonstrate a requirement of TYK2 in DCs to counteract MCMV infection *in vitro* and *in vivo*. Additional experiments will be required to substantiate these findings and to reveal the underlying mechanisms.

Frequently Used Abbreviations

APC	antigen presenting cell
BMDC	bone marrow-derived dendritic cell
CTL	cytotoxic T-lymphocyte
DC	dendritic cell
HCMV	human cytomegalovirus
IFN	interferon
IFNAR	interferon-α/β receptor
IL	interleukin
IP	intraperitoneal
IRF	interferon regulatory factor
ISG	interferon stimulated gene
JAK	Janus kinase
MCMV	murine cytomegalovirus
mDC	myeloid dendritic cell
MHC	major histocompatibility complex
NK-cell	natural killer cell
PAMP	pathogen-associated molecular pattern
pDC	plasmacytoid dendritic cell
PRR	pattern-recognition receptor
SG	salivary gland
SNP	single nucleotide polymorphism
STAT	signal transducer and activator of transcription
TC	tissue culture
TLR	Toll-like receptor
TYK2	tyrosine kinase 2

7. References

1. Kisseleva, T., Bhattacharya, S., Braunstein, J. & Schindler, C.W. Signaling through the JAK/STAT pathway, recent advances and future challenges. *Gene* **285**, 1-24 (2002).
2. Darnell, J.E., Jr., Kerr, I.M. & Stark, G.R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**, 1415-21 (1994).
3. O'Shea, J.J., Gadina, M. & Schreiber, R.D. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. *Cell* **109 Suppl**, S121-31 (2002).
4. Schindler, C. & Darnell, J.E., Jr. Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem* **64**, 621-51 (1995).
5. Rodig, S.J., Meraz, M.A., White, J.M., Lampe, P.A., Riley, J.K., Arthur, C.D., King, K.L., Sheehan, K.C., Yin, L., Pennica, D., Johnson, E.M., Jr. & Schreiber, R.D. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. *Cell* **93**, 373-83 (1998).
6. Neubauer, H., Cumano, A., Muller, M., Wu, H., Huffstadt, U. & Pfeffer, K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. *Cell* **93**, 397-409 (1998).
7. Parganas, E., Wang, D., Stravopodis, D., Topham, D.J., Marine, J.C., Teglund, S., Vanin, E.F., Bodner, S., Colamonici, O.R., van Deursen, J.M., Grosveld, G. & Ihle, J.N. Jak2 is essential for signaling through a variety of cytokine receptors. *Cell* **93**, 385-95 (1998).
8. Levy, D.E. & Darnell, J.E., Jr. Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol* **3**, 651-62 (2002).
9. Firmbach-Kraft, I., Byers, M., Shows, T., Dalla-Favera, R. & Krolewski, J.J. tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. *Oncogene* **5**, 1329-36 (1990).
10. Krolewski, J.J., Lee, R., Eddy, R., Shows, T.B. & Dalla-Favera, R. Identification and chromosomal mapping of new human tyrosine kinase genes. *Oncogene* **5**, 277-82 (1990).
11. Strobl, B., Stoiber, D., Sexl, V. & Mueller, M. Tyrosine kinase 2 (TYK2) in cytokine signalling and host immunity. *Front Biosci* **17**, 3214-32 (2011).
12. Ghoreschi, K., Laurence, A. & O'Shea, J.J. Janus kinases in immune cell signaling. *Immunol Rev* **228**, 273-87 (2009).
13. Velazquez, L., Fellous, M., Stark, G.R. & Pellegrini, S. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. *Cell* **70**, 313-22 (1992).
14. Shaw, M.H., Boyartchuk, V., Wong, S., Karaghiosoff, M., Ragimbeau, J., Pellegrini, S., Muller, M., Dietrich, W.F. & Yap, G.S. A natural mutation in the Tyk2 pseudokinase domain underlies altered susceptibility of B10.Q/J mice to infection and autoimmunity. *Proc Natl Acad Sci U S A* **100**, 11594-9 (2003).
15. Karaghiosoff, M., Neubauer, H., Lassnig, C., Kovarik, P., Schindler, H., Pircher, H., McCoy, B., Bogdan, C., Decker, T., Brem, G., Pfeffer, K. & Muller, M. Partial impairment of cytokine responses in Tyk2-deficient mice. *Immunity* **13**, 549-60 (2000).
16. Shimoda, K., Kato, K., Aoki, K., Matsuda, T., Miyamoto, A., Shibamori, M., Yamashita, M., Numata, A., Takase, K., Kobayashi, S., Shibata, S., Asano, Y., Gondo, H., Sekiguchi, K., Nakayama, K., Nakayama, T., Okamura, T., Okamura, S. & Niho, Y. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. *Immunity* **13**, 561-71 (2000).
17. Sheehan, K.C., Lai, K.S., Dunn, G.P., Bruce, A.T., Diamond, M.S., Heutel, J.D., Dungo-Arthur, C., Carrero, J.A., White, J.M., Hertzog, P.J. & Schreiber, R.D. Blocking monoclonal antibodies specific for mouse IFN-alpha/beta receptor subunit 1 (IFNAR-1) from mice immunized by in vivo hydrodynamic transfection. *J Interferon Cytokine Res* **26**, 804-19 (2006).
18. van Boxel-Dezaire, A.H., Rani, M.R. & Stark, G.R. Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* **25**, 361-72 (2006).

19. Ragimbeau, J., Dondi, E., Alcover, A., Eid, P., Uze, G. & Pellegrini, S. The tyrosine kinase Tyk2 controls IFNAR1 cell surface expression. *EMBO J* **22**, 537-47 (2003).
20. Shaw, M.H., Freeman, G.J., Scott, M.F., Fox, B.A., Bzik, D.J., Belkaid, Y. & Yap, G.S. Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN-gamma-dependent IL-10 reactivation. *J Immunol* **176**, 7263-71 (2006).
21. Shimoda, K., Tsutsui, H., Aoki, K., Kato, K., Matsuda, T., Numata, A., Takase, K., Yamamoto, T., Nukina, H., Hoshino, T., Asano, Y., Gondo, H., Okamura, T., Okamura, S., Nakayama, K., Nakanishi, K., Niho, Y. & Harada, M. Partial impairment of interleukin-12 (IL-12) and IL-18 signaling in Tyk2-deficient mice. *Blood* **99**, 2094-9 (2002).
22. Ortmann, R., Smeltz, R., Yap, G., Sher, A. & Shevach, E.M. A heritable defect in IL-12 signaling in B10.Q/J mice. I. In vitro analysis. *J Immunol* **166**, 5712-9 (2001).
23. Roy, B. & Cathcart, M.K. Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. *J Biol Chem* **273**, 32023-9 (1998).
24. Strobl, B., Bubic, I., Bruns, U., Steinborn, R., Lajko, R., Kolbe, T., Karaghiosoff, M., Kalinke, U., Jonjic, S. & Muller, M. Novel functions of tyrosine kinase 2 in the antiviral defense against murine cytomegalovirus. *J Immunol* **175**, 4000-8 (2005).
25. Schleicher, U., Mattner, J., Blos, M., Schindler, H., Rollinghoff, M., Karaghiosoff, M., Muller, M., Werner-Felmayer, G. & Bogdan, C. Control of Leishmania major in the absence of Tyk2 kinase. *Eur J Immunol* **34**, 519-29 (2004).
26. Yap, G.S., Ortmann, R., Shevach, E. & Sher, A. A heritable defect in IL-12 signaling in B10.Q/J mice. II. Effect on acute resistance to Toxoplasma gondii and rescue by IL-18 treatment. *J Immunol* **166**, 5720-5 (2001).
27. Li, W., Yamada, H., Yajima, T., Nakagawa, R., Shimoda, K., Nakayama, K. & Yoshikai, Y. Tyk2 signaling in host environment plays an important role in contraction of antigen-specific CD8+ T cells following a microbial infection. *J Immunol* **178**, 4482-8 (2007).
28. Aizu, K., Li, W., Yajima, T., Arai, T., Shimoda, K., Nimura, Y. & Yoshikai, Y. An important role of Tyk2 in APC function of dendritic cells for priming CD8+ T cells producing IFN-gamma. *Eur J Immunol* **36**, 3060-70 (2006).
29. Nakamura, R., Shibata, K., Yamada, H., Shimoda, K., Nakayama, K. & Yoshikai, Y. Tyk2-signaling plays an important role in host defense against Escherichia coli through IL-23-induced IL-17 production by gammadelta T cells. *J Immunol* **181**, 2071-5 (2008).
30. Karaghiosoff, M., Steinborn, R., Kovarik, P., Kriegshauser, G., Baccarini, M., Donabauer, B., Reichart, U., Kolbe, T., Bogdan, C., Leanderson, T., Levy, D., Decker, T. & Muller, M. Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. *Nat Immunol* **4**, 471-7 (2003).
31. Kamezaki, K., Shimoda, K., Numata, A., Matsuda, T., Nakayama, K. & Harada, M. The role of Tyk2, Stat1 and Stat4 in LPS-induced endotoxin signals. *Int Immunol* **16**, 1173-9 (2004).
32. Oyamada, A., Ikebe, H., Itsumi, M., Saiwai, H., Okada, S., Shimoda, K., Iwakura, Y., Nakayama, K.I., Iwamoto, Y., Yoshikai, Y. & Yamada, H. Tyrosine kinase 2 plays critical roles in the pathogenic CD4 T cell responses for the development of experimental autoimmune encephalomyelitis. *J Immunol* **183**, 7539-46 (2009).
33. Spach, K.M., Noubade, R., McElvany, B., Hickey, W.F., Blankenhorn, E.P. & Teuscher, C. A single nucleotide polymorphism in Tyk2 controls susceptibility to experimental allergic encephalomyelitis. *J Immunol* **182**, 7776-83 (2009).
34. Costantino, G., Egerbacher, M., Kolbe, T., Karaghiosoff, M., Strobl, B., Vogl, C., Helmreich, M. & Muller, M. Tyk2 and signal transducer and activator of transcription 1 contribute to intestinal I/R injury. *Shock* **29**, 238-44 (2008).
35. Hosogi, M., Tonogaito, H., Aioi, A., Hamada, K., Shimoda, K., Muromoto, R., Matsuda, T. & Miyachi, Y. Hapten-induced contact hypersensitivity is enhanced in Tyk2-deficient mice. *J Dermatol Sci* **36**, 51-6 (2004).
36. Stoiber, D., Kovacic, B., Schuster, C., Schellack, C., Karaghiosoff, M., Kreibich, R., Weisz, E., Artwohl, M., Kleine, O.C., Muller, M., Baumgartner-Parzer, S., Ghysdael, J., Freissmuth, M. &

Sexl, V. TYK2 is a key regulator of the surveillance of B lymphoid tumors. *J Clin Invest* **114**, 1650-8 (2004).
37. Simma, O., Zebedin, E., Neugebauer, N., Schellack, C., Pilz, A., Chang-Rodriguez, S., Lingnau, K., Weisz, E., Putz, E.M., Pickl, W.F., Felzmann, T., Muller, M., Decker, T., Sexl, V. & Stoiber, D. Identification of an indispensable role for tyrosine kinase 2 in CTL-mediated tumor surveillance. *Cancer Res* **69**, 203-11 (2009).
38. Zhang, Q., Sturgill, J.L., Kmieciak, M., Szczepanek, K., Derecka, M., Koebel, C., Graham, L.J., Dai, Y., Chen, S., Grant, S., Cichy, J., Shimoda, K., Gamero, A., Manjili, M., Bear, H., Conrad, D. & Larner, A.C. The role of tyk2 in regulation of breast cancer growth. *J Interferon Cytokine Res* **31**, 671-7 (2011).
39. Zhu, X., Lv, J., Yu, L., Wu, J., Zou, S. & Jiang, S. Proteomic identification of differentially-expressed proteins in squamous cervical cancer. *Gynecol Oncol* **112**, 248-56 (2009).
40. Song, X.C., Fu, G., Yang, X., Jiang, Z., Wang, Y. & Zhou, G.W. Protein expression profiling of breast cancer cells by dissociable antibody microarray (DAMA) staining. *Mol Cell Proteomics* **7**, 163-9 (2008).
41. Ide, H., Nakagawa, T., Terado, Y., Kamiyama, Y., Muto, S. & Horie, S. Tyk2 expression and its signaling enhances the invasiveness of prostate cancer cells. *Biochem Biophys Res Commun* **369**, 292-6 (2008).
42. Minegishi, Y., Saito, M., Morio, T., Watanabe, K., Agematsu, K., Tsuchiya, S., Takada, H., Hara, T., Kawamura, N., Ariga, T., Kaneko, H., Kondo, N., Tsuge, I., Yachie, A., Sakiyama, Y., Iwata, T., Bessho, F., Ohishi, T., Joh, K., Imai, K., Kogawa, K., Shinohara, M., Fujieda, M., Wakiguchi, H., Pasic, S., Abinun, M., Ochs, H.D., Renner, E.D., Jansson, A., Belohradsky, B.H., Metin, A., Shimizu, N., Mizutani, S., Miyawaki, T., Nonoyama, S. & Karasuyama, H. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. *Immunity* **25**, 745-55 (2006).
43. Abul K. Abbas, A.H.L., Shiv Pillai. in Cellular and Molecular Immunology (2009).
44. Akira, S., Uematsu, S. & Takeuchi, O. Pathogen recognition and innate immunity. *Cell* **124**, 783-801 (2006).
45. Brennan, K. & Bowie, A.G. Activation of host pattern recognition receptors by viruses. *Curr Opin Microbiol* **13**, 503-7 (2010).
46. Yoneyama, M. & Fujita, T. RNA recognition and signal transduction by RIG-I-like receptors. *Immunol Rev* **227**, 54-65 (2009).
47. Hornung, V., Ellegast, J., Kim, S., Brzozka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.K., Schlee, M., Endres, S. & Hartmann, G. 5'-Triphosphate RNA is the ligand for RIG-I. *Science* **314**, 994-7 (2006).
48. Ablasser, A., Bauernfeind, F., Hartmann, G., Latz, E., Fitzgerald, K.A. & Hornung, V. RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. *Nat Immunol* **10**, 1065-72 (2009).
49. Chiu, Y.H., Macmillan, J.B. & Chen, Z.J. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. *Cell* **138**, 576-91 (2009).
50. Pichlmair, A., Schulz, O., Tan, C.P., Rehwinkel, J., Kato, H., Takeuchi, O., Akira, S., Way, M., Schiavo, G. & Reis e Sousa, C. Activation of MDA5 requires higher-order RNA structures generated during virus infection. *J Virol* **83**, 10761-9 (2009).
51. Sabbah, A., Chang, T.H., Harnack, R., Frohlich, V., Tominaga, K., Dube, P.H., Xiang, Y. & Bose, S. Activation of innate immune antiviral responses by Nod2. *Nat Immunol* **10**, 1073-80 (2009).
52. Muruve, D.A., Petrilli, V., Zaiss, A.K., White, L.R., Clark, S.A., Ross, P.J., Parks, R.J. & Tschopp, J. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* **452**, 103-7 (2008).
53. Ichinohe, T., Lee, H.K., Ogura, Y., Flavell, R. & Iwasaki, A. Inflammasome recognition of influenza virus is essential for adaptive immune responses. *J Exp Med* **206**, 79-87 (2009).

54. Hornung, V., Ablasser, A., Charrel-Dennis, M., Bauernfeind, F., Horvath, G., Caffrey, D.R., Latz, E. & Fitzgerald, K.A. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. *Nature* **458**, 514-8 (2009).
55. Burckstummer, T., Baumann, C., Bluml, S., Dixit, E., Durnberger, G., Jahn, H., Planyavsky, M., Bilban, M., Colinge, J., Bennett, K.L. & Superti-Furga, G. An orthogonal proteomic-genomic screen identifies AIM2 as a cytoplasmic DNA sensor for the inflammasome. *Nat Immunol* **10**, 266-72 (2009).
56. DeFilippis, V.R., Alvarado, D., Sali, T., Rothenburg, S. & Fruh, K. Human cytomegalovirus induces the interferon response via the DNA sensor ZBP1. *J Virol* **84**, 585-98 (2010).
57. Takaoka, A., Wang, Z., Choi, M.K., Yanai, H., Negishi, H., Ban, T., Lu, Y., Miyagishi, M., Kodama, T., Honda, K., Ohba, Y. & Taniguchi, T. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature* **448**, 501-5 (2007).
58. Biron, C.A. Role of early cytokines, including alpha and beta interferons (IFN-alpha/beta), in innate and adaptive immune responses to viral infections. *Semin Immunol* **10**, 383-90 (1998).
59. Schroder, K., Hertzog, P.J., Ravasi, T. & Hume, D.A. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol* **75**, 163-89 (2004).
60. Davison, A.J. Herpesvirus systematics. *Vet Microbiol* **143**, 52-69 (2010).
61. Brune, W., Hengel, H. & Koszinowski, U.H. A mouse model for cytomegalovirus infection. *Curr Protoc Immunol* **Chapter 19**, Unit 19 7 (2001).
62. Soderberg-Naucler, C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? *J Intern Med* **259**, 219-46 (2006).
63. Michaelis, M., Doerr, H.W. & Cinatl, J. The story of human cytomegalovirus and cancer: increasing evidence and open questions. *Neoplasia* **11**, 1-9 (2009).
64. McGeoch, D.J., Dolan, A. & Ralph, A.C. Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *J Virol* **74**, 10401-6 (2000).
65. Krmpotic, A., Bubic, I., Polic, B., Lucin, P. & Jonjic, S. Pathogenesis of murine cytomegalovirus infection. *Microbes Infect* **5**, 1263-77 (2003).
66. Fodil-Cornu, N., Lee, S.H., Belanger, S., Makrigiannis, A.P., Biron, C.A., Buller, R.M. & Vidal, S.M. Ly49h-deficient C57BL/6 mice: a new mouse cytomegalovirus-susceptible model remains resistant to unrelated pathogens controlled by the NK gene complex. *J Immunol* **181**, 6394-405 (2008).
67. Loewendorf, A. & Benedict, C.A. Modulation of host innate and adaptive immune defenses by cytomegalovirus: timing is everything. *J Intern Med* **267**, 483-501 (2010).
68. Hengel, H., Esslinger, C., Pool, J., Goulmy, E. & Koszinowski, U.H. Cytokines restore MHC class I complex formation and control antigen presentation in human cytomegalovirus-infected cells. *J Gen Virol* **76 (Pt 12)**, 2987-97 (1995).
69. Bowie, A.G. & Haga, I.R. The role of Toll-like receptors in the host response to viruses. *Mol Immunol* **42**, 859-67 (2005).
70. Gauzzi, M.C., Del Corno, M. & Gessani, S. Dissecting TLR3 signalling in dendritic cells. *Immunobiology* **215**, 713-23 (2010).
71. Zhang, L. & Pagano, J.S. Structure and function of IRF-7. *J Interferon Cytokine Res* **22**, 95-101 (2002).
72. Takeshita, F., Gursel, I., Ishii, K.J., Suzuki, K., Gursel, M. & Klinman, D.M. Signal transduction pathways mediated by the interaction of CpG DNA with Toll-like receptor 9. *Semin Immunol* **16**, 17-22 (2004).
73. Tabeta, K., Georgel, P., Janssen, E., Du, X., Hoebe, K., Crozat, K., Mudd, S., Shamel, L., Sovath, S., Goode, J., Alexopoulou, L., Flavell, R.A. & Beutler, B. Toll-like receptors 9 and 3 as essential components of innate immune defense against mouse cytomegalovirus infection. *Proc Natl Acad Sci U S A* **101**, 3516-21 (2004).
74. Sarkar, S.N., Smith, H.L., Rowe, T.M. & Sen, G.C. Double-stranded RNA signaling by Toll-like receptor 3 requires specific tyrosine residues in its cytoplasmic domain. *J Biol Chem* **278**, 4393-6 (2003).

75. Matsumoto, M., Oshiumi, H. & Seya, T. Antiviral responses induced by the TLR3 pathway. *Rev Med Virol* (2011).
76. Decker, T., Kovarik, P. & Meinke, A. GAS elements: a few nucleotides with a major impact on cytokine-induced gene expression. *J Interferon Cytokine Res* **17**, 121-34 (1997).
77. Perry, A.K., Chen, G., Zheng, D., Tang, H. & Cheng, G. The host type I interferon response to viral and bacterial infections. *Cell Res* **15**, 407-22 (2005).
78. Vossen, M.T., Westerhout, E.M., Soderberg-Naucler, C. & Wiertz, E.J. Viral immune evasion: a masterpiece of evolution. *Immunogenetics* **54**, 527-42 (2002).
79. Scalzo, A.A., Corbett, A.J., Rawlinson, W.D., Scott, G.M. & Degli-Esposti, M.A. The interplay between host and viral factors in shaping the outcome of cytomegalovirus infection. *Immunol Cell Biol* **85**, 46-54 (2007).
80. French, A.R., Pingel, J.T., Kim, S., Yang, L. & Yokoyama, W.M. Rapid emergence of escape mutants following infection with murine cytomegalovirus in immunodeficient mice. *Clin Immunol* **115**, 61-9 (2005).
81. Krmpotic, A., Busch, D.H., Bubic, I., Gebhardt, F., Hengel, H., Hasan, M., Scalzo, A.A., Koszinowski, U.H. & Jonjic, S. MCMV glycoprotein gp40 confers virus resistance to CD8+ T cells and NK cells in vivo. *Nat Immunol* **3**, 529-35 (2002).
82. Marshall, E.E. & Geballe, A.P. Multifaceted evasion of the interferon response by cytomegalovirus. *J Interferon Cytokine Res* **29**, 609-19 (2009).
83. Zimmermann, A., Trilling, M., Wagner, M., Wilborn, M., Bubic, I., Jonjic, S., Koszinowski, U. & Hengel, H. A cytomegaloviral protein reveals a dual role for STAT2 in IFN-{gamma} signaling and antiviral responses. *J Exp Med* **201**, 1543-53 (2005).
84. Britt, W.J. & Alford, C.A. Cytomegalovirus. *Fields Virology* **3**, 2493-2523 (1996).
85. Friedel, R.H., Wurst, W., Wefers, B. & Kuhn, R. Generating conditional knockout mice. *Methods Mol Biol* **693**, 205-31 (2011).
86. Metzger, D. & Chambon, P. Site- and time-specific gene targeting in the mouse. *Methods* **24**, 71-80 (2001).
87. Caton, M.L., Smith-Raska, M.R. & Reizis, B. Notch-RBP-J signaling controls the homeostasis of CD8- dendritic cells in the spleen. *J Exp Med* **204**, 1653-64 (2007).
88. Clausen, B.E., Burkhardt, C., Reith, W., Renkawitz, R. & Forster, I. Conditional gene targeting in macrophages and granulocytes using LysMcre mice. *Transgenic Res* **8**, 265-77 (1999).
89. Orban, P.C., Chui, D. & Marth, J.D. Tissue- and site-specific DNA recombination in transgenic mice. *Proc Natl Acad Sci U S A* **89**, 6861-5 (1992).
90. Schwenk, F., Baron, U. & Rajewsky, K. A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. *Nucleic Acids Res* **23**, 5080-1 (1995).
91. Banchereau, J. & Steinman, R.M. Dendritic cells and the control of immunity. *Nature* **392**, 245-52 (1998).
92. Thery, C. & Amigorena, S. The cell biology of antigen presentation in dendritic cells. *Curr Opin Immunol* **13**, 45-51 (2001).
93. Dubois, B., Vanbervliet, B., Fayette, J., Massacrier, C., Van Kooten, C., Briere, F., Banchereau, J. & Caux, C. Dendritic cells enhance growth and differentiation of CD40-activated B lymphocytes. *J Exp Med* **185**, 941-51 (1997).
94. Wykes, M., Pombo, A., Jenkins, C. & MacPherson, G.G. Dendritic cells interact directly with naive B lymphocytes to transfer antigen and initiate class switching in a primary T-dependent response. *J Immunol* **161**, 1313-9 (1998).
95. Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P. & Cerutti, A. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. *Nat Immunol* **3**, 822-9 (2002).
96. Rinaldo, C.R., Jr. & Piazza, P. Virus infection of dendritic cells: portal for host invasion and host defense. *Trends Microbiol* **12**, 337-45 (2004).

97. Adema, G.J. Dendritic cells from bench to bedside and back. *Immunol Lett* **122**, 128-30 (2009).
98. Albert, M.L., Sauter, B. & Bhardwaj, N. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. *Nature* **392**, 86-9 (1998).
99. Dhodapkar, M.V. & Steinman, R.M. Antigen-bearing immature dendritic cells induce peptide-specific CD8(+) regulatory T cells in vivo in humans. *Blood* **100**, 174-7 (2002).
100. Steinman, R.M., Hawiger, D. & Nussenzweig, M.C. Tolerogenic dendritic cells. *Annu Rev Immunol* **21**, 685-711 (2003).
101. Kushwah, R. & Hu, J. Complexity of dendritic cell subsets and their function in the host immune system. *Immunology* **133**, 409-19 (2011).
102. Nakano, H., Yanagita, M. & Gunn, M.D. CD11c(+)B220(+)Gr-1(+) cells in mouse lymph nodes and spleen display characteristics of plasmacytoid dendritic cells. *J Exp Med* **194**, 1171-8 (2001).
103. Webb, T.J., Sumpter, T.L., Thiele, A.T., Swanson, K.A. & Wilkes, D.S. The phenotype and function of lung dendritic cells. *Crit Rev Immunol* **25**, 465-91 (2005).
104. Ito, T., Wang, Y.H. & Liu, Y.J. Plasmacytoid dendritic cell precursors/type I interferon-producing cells sense viral infection by Toll-like receptor (TLR) 7 and TLR9. *Springer Semin Immunopathol* **26**, 221-9 (2005).
105. Asselin-Paturel, C., Boonstra, A., Dalod, M., Durand, I., Yessaad, N., Dezutter-Dambuyant, C., Vicari, A., O'Garra, A., Biron, C., Briere, F. & Trinchieri, G. Mouse type I IFN-producing cells are immature APCs with plasmacytoid morphology. *Nat Immunol* **2**, 1144-50 (2001).
106. Barchet, W., Cella, M. & Colonna, M. Plasmacytoid dendritic cells--virus experts of innate immunity. *Semin Immunol* **17**, 253-61 (2005).
107. Baccarini, M., Bistoni, F. & Lohmann-Matthes, M.L. In vitro natural cell-mediated cytotoxicity against Candida albicans: macrophage precursors as effector cells. *J Immunol* **134**, 2658-65 (1985).
108. Vremec, D. & Shortman, K. Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. *J Immunol* **159**, 565-73 (1997).
109. Leenen, P.J., Radosevic, K., Voerman, J.S., Salomon, B., van Rooijen, N., Klatzmann, D. & van Ewijk, W. Heterogeneity of mouse spleen dendritic cells: in vivo phagocytic activity, expression of macrophage markers, and subpopulation turnover. *J Immunol* **160**, 2166-73 (1998).
110. Pellicci, D.G., Hammond, K.J., Uldrich, A.P., Baxter, A.G., Smyth, M.J. & Godfrey, D.I. A natural killer T (NKT) cell developmental pathway iInvolving a thymus-dependent NK1.1(-)CD4(+) CD1d-dependent precursor stage. *J Exp Med* **195**, 835-44 (2002).
111. Bronte, V., Apolloni, E., Cabrelle, A., Ronca, R., Serafini, P., Zamboni, P., Restifo, N.P. & Zanovello, P. Identification of a CD11b(+)/Gr-1(+)/CD31(+) myeloid progenitor capable of activating or suppressing CD8(+) T cells. *Blood* **96**, 3838-46 (2000).
112. Longman, R.S., Braun, D., Pellegrini, S., Rice, C.M., Darnell, R.B. & Albert, M.L. Dendritic-cell maturation alters intracellular signaling networks, enabling differential effects of IFN-alpha/beta on antigen cross-presentation. *Blood* **109**, 1113-22 (2007).
113. Dalod, M., Salazar-Mather, T.P., Malmgaard, L., Lewis, C., Asselin-Paturel, C., Briere, F., Trinchieri, G. & Biron, C.A. Interferon alpha/beta and interleukin 12 responses to viral infections: pathways regulating dendritic cell cytokine expression in vivo. *J Exp Med* **195**, 517-28 (2002).
114. Bukowski, J.F., Woda, B.A. & Welsh, R.M. Pathogenesis of murine cytomegalovirus infection in natural killer cell-depleted mice. *J Virol* **52**, 119-28 (1984).
115. Melillo, J.A., Song, L., Bhagat, G., Blazquez, A.B., Plumlee, C.R., Lee, C., Berin, C., Reizis, B. & Schindler, C. Dendritic cell (DC)-specific targeting reveals Stat3 as a negative regulator of DC function. *J Immunol* **184**, 2638-45 (2010).

8. Danksagung

Zuerst möchte ich Univ. Prof. Dr. Mathias Müller für die Ermöglichung der Diplomarbeit, sowie die Bereitstellung des Diplomarbeitsthemas danken.

Danken möchte ich auch Univ. Prof. Dr. Pavel Kovarik für die Begutachtung und die Betreuung der Diplomarbeit von Seiten der Universität Wien.

Der größte Dank gilt meinen Betreuern Dr. Birgit Strobl und Dr. Rita Stiefvater für die ausgezeichnete Betreuung und Unterstützung. Die beeindruckende fachliche Kompetenz, sowie die exzellente technische Versiertheit meiner Betreuer waren ausschlaggebend für die Motivation das ganze letzte Jahr hindurch und haben maßgeblichen Anteil am Erfolg der Diplomarbeit. Besonders möchte ich mich für die überaus freundliche Arbeitsatmosphäre und gewissenhafte Korrektur der Arbeit bedanken.

Weiters möchte ich mich sehr herzlich bei Mag. Michael Rammerstofer für die vielfältige Unterstützung im Labor und die fortwährende Hilfsbereitschaft bedanken.

Großen Dank schulde ich auch Dr. Nicole Leitner, Mag. Christian Semper, Dipl.Ing. Michaela Prchal, Mag. Eva Hainzl, sowie allen anderen Laborkollegen für die Hilfe und Motivation und das freundschaftliche Arbeitsklima.

Zuletzt möchte ich mich noch bei meiner Familie und meinen Freunden bedanken, die mich während des ganzen Studiums unterstützt und immer an mich geglaubt haben.

9. Curriculum Vitae

David Popp
Rafings 26
A-3841 Windigsteig
+43 660 4951701
david_popp@gmx.at

Personal Data

Date of Birth	02.08.1986
Place of Birth	Vienna
Nationality	Austria

Academic

since 11/2011	Diploma Thesis in the laboratory of Univ. Prof. Dr. Mathias Müller at the University of Veterinary Medicine Vienna Title: "Cell type-specific functions of TYK2 in the antiviral defense against MCMV"
01/2010-04/2010	Study of Microbiology at the University of California Riverside
since 10/2007	Study of Genetics-Microbiology at the University of Vienna
10/2005-10/2007	Study of Biology at the University of Vienna
2004-2005	Military Service
06/2004	General qualification for university entrance (=Matura) in Waidhofen/Thaya
1996-2004	Secondary School Waidhofen/Thaya

Published Abstracts

1. Rita Stiefvater, Raimund M. Vielnascher, Elisabeth Hofmann, Ursula Reichart, Thomas Kolbe, Wilhelm Gerner, David Popp, Claus Vogl, Marina Karaghiosoff, Mathias Müller, Birgit Strobl *Conditional knockout of tyrosine kinase 2 (Tyk2): dissecting cell type-specific functions in innate IL-17 production*
   Cytokine, Volume 56, Issue 1, October 2011, Page 90

Meetings

1. 3rd international PhD workshop 2011: "Brothers in Arms"-From Basic Research to Clinical Application, Vienna, Austria
2. 5th international PhD workshop 2012: "Bridging the Gap"-Cell Communication in Health and Disease, Vienna, Austria

Work Experience

10/2011-1/2012	Teaching Assistant for "Molecular Cell Biology II" at the University of Veterinary Medicine, Vienna
3/2011-6/2011	Teaching Assistant for "Practical Course in Immunology" (Part Steinborn) at the University of Vienna
8/2002-2007	Internships at Moeller GmbH (now: Eaton), Schrems, Austria