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„The role of Tyk2 in IL-22 and IL-27 signalling“

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1 Abstract

Tyrosine kinase 2 (Tyk2) is one of the four members of the Janus kinase (Jak) family, which together with signal transducers and activators of transcription (Stats) compose the Jak/Stat pathway. In mammals, this signalling cascade is utilized by a wide array of cytokines and growth factors. Tyk2 interacts with several distinct receptor chains and is mainly involved in the regulation of immune responses, such as the defence against infections, inflammatory reactions and in carcinogenesis. In addition to its kinase activity, several lines of evidence suggest kinase-independent functions of Tyk2 in murine and human cells.

One focus of this work was to find an experimental system that allows us to investigate the impact of Tyk2 on interleukin (IL)-22 signalling in colonic epithelial cells. IL-22 belongs to the IL-10 cytokine family and acts mainly on epithelial cells and hepatocytes, where it favours antimicrobial defence, protects against tissue damage and facilitates regeneration. From the two murine (CMT93 and MC38) and two human (HT29 and Caco-2) colonic epithelial cell lines available, only HT29 cells showed IL-22 responsiveness. As an alternative to the use of cell lines, several conditions for the cultivation of primary colonic epithelial cells were tested. However, none of the approaches resulted in sufficient numbers of viable and adherent cells to analyse the IL-22 response.

The second focus was to investigate whether the absence of Tyk2 or the presence of a kinase-inactive Tyk2 affects IL-27 responses in distinct cell types. IL-27 is a member of the IL-12 family, acts on specific immune cell types and exerts pro- and anti-inflammatory functions. Using αCD3/αCD28-activated primary T cells derived from wild-type (WT), Tyk2 knockout (Tyk2$^{-/-}$) and mice expressing a kinase-inactive version of Tyk2 (Tyk2^{K923E}), we could show that Tyk2 is not required for the phosphorylation of Stat1 and Stat3 in response to IL-27. Presence of kinase-inactive Tyk2 protein neither enhanced nor reduced IL-27 responsiveness. However, we did not detect upregulation of any of the IL-27 target genes, which have been mainly described in naive CD4^{+} T cells, in our αCD3/αCD28-activated T cells, irrespective of their genotype. Our data furthermore support the notion that non-activated bone marrow-derived macrophages (BMMΦ) are unresponsive to IL-27, an issue that has been reported controversially. In contrast to what has been published using freshly

isolated NK cells, we could show that *in vitro* expanded NK cells do not respond to IL-27.

2 Zusammenfassung

Die Tyrosin-Kinase 2 (Tyk2) ist eine von den vier Janus-Kinasen (Jak), die gemeinsam mit Signaltransduktoren und Transkriptionsaktivatoren (Stats) den Jak/Stat Signalweg bilden. Diese Signalkaskade wird in Säugetieren von zahlreichen Zytokinen und Wachstumsfaktoren verwendet. Tyk2 interagiert mit verschiedenen Rezeptorketten und spielt eine essentielle Rolle in der angeborenen und adaptiven Immunabwehr gegen Pathogene sowie in der Regulation von Entzündungen und in der Tumorgenese. Über die Kinaseaktivität von Tyk2 hinaus, zeigen einige Studien auch Kinase-unabhängige Funktionen von Tyk2 in menschlichen und tierischen Zellen.

Ein Ziel dieser Arbeit war, ein experimentelles System zu etablieren, das eine Untersuchung des Einflusses von Tyk2 auf den Interleukin (IL)-22 Signalweg in Darmepithelzellen ermöglicht. IL-22 gehört zur Familie der IL-10 Zytokine und beeinflusst mit seiner antimikrobiellen und protektiven Wirkung hauptsächlich Epithel- und Leberzellen. Von den getesteten tierischen (CMT93 und MC38) und menschlichen (HT29 und Caco-2) Epithelzelllinien, konnte nur bei HT29 Zellen eine IL-22 Reaktivität nachgewiesen werden. Als Alternative zu Zelllinien, wurden verschiedene Kultivierungsmethoden für primäre Epithelzellen untersucht. Allerdings führte keine der verwendeten Kultivierungsmethoden zu einer ausreichenden Anzahl an adhärenten Zellen, um den IL-22 Signalweg studieren zu können.

Das zweite Ziel dieser Arbeit war es den IL-27 Signalweg in Abwesenheit von Tyk2 oder in der Anwesenheit eines Kinase inaktiven Tyk2 in verschiedenen Zelltypen zu untersuchen. IL-27 wird der IL-12 Zytokin Familie zugeordnet, dessen pro- und anti-inflammatorisches Wirkungsspektrum in verschiedenen Immunzellen zu beobachten ist. Unter Verwendung von αCD3/αCD28-aktivierte T Zellen von Wildtyp und Tyk2-deletierten (Tyk2$^{-/-}$) Mäusen sowie von Mäusen, die ein Kinase-inaktives Tyk2 exprimieren (Tyk2^{K923E}), konnte nachgewiesen werden, dass Tyk2 für die IL-27 vermittelnde Phosphorylierung von Stat1 und Stat3 nicht benötigt wird. Die Anwesenheit von Kinase-inaktivem Tyk2 hatte keine Auswirkung auf die Fähigkeit auf IL-27 zu antworten. Allerdings, konnte keine Regulation von IL-27 Ziel-Genen, die vorher hauptsächlich in CD4^{+} T Zellen beschrieben wurden, festgestellt werden. Im Gegensatz zu bereits publizierten Daten, zeigen die hier vorliegenden Ergebnisse, dass nicht-aktivierte Makrophagen (BMMΦ) keine Reaktivität auf IL-27 aufweisen.

Zusätzlich konnten wir im Gegensatz zu Daten, die mit frisch isolierten NK Zellen publiziert wurden, bei *in vitro* kultivierten NK Zellen keine IL-27 vermittelnde Aktivierung von Stat Proteinen nachweisen.

3 Introduction

3.1 The Jak-Stat signalling pathway

The Jak-Stat (Janus kinase - signal transducer and activator of transcription) pathway has an important role in signalling by many cytokines and some growth factors. Accordingly, this cascade is involved in diverse processes, such as development, metabolism, hematopoiesis and immune responses (Kisseleva et al., 2002; O'Shea et al., 2002). The highly conserved Jak-Stat cascade consists of three main components: a transmembrane receptor, receptor-associated Jak tyrosine kinases and the cytoplasmatic transcription factors, known as Stats. Upon receptor activation by ligand-binding Jaks get activated by trans- and/or auto-phosphorylation. Subsequently, Jaks phosphorylate specific tyrosine residues on receptor chains, which then serve as binding sites for Stat proteins. The Stats get tyrosine phosphorylated, translocate to the nucleus as homo- or heterodimers and regulate target gene transcription (Levy and Darnell, 2002).

The mammalian Jak family has four members: Jak1, Jak2, Jak3 and Tyk2 (tyrosine kinase 2) which all have a protein size ranging from 120 to 140 kDa. The Jaks consist of seven Jak homology (JH) domains. JH1 is the catalytically active kinase (Ki) domain, which gets activated by phosphorylation of critical tyrosines in the inactivation loop thereby releasing the blockade on the catalytic site. JH2 represents the pseudokinase (ψKi) domain and is considered as catalytically inactive (Ghoreschi et al 2009). However, catalytic function has been reported recently for the Jak2 ψKi domain (Ungureanu et al., 2011). JH3 and part of JH4 is a scr-homology 2 (SH2) related domain with no function assigned yet. The amino terminal JH4-JH7 domains build up a four point one, ezrin, radixin, moesin (FERM) domain, which is responsible for association with cytokine receptors. Genetic knockout studies of individual Jaks have shown that each of them has specific roles in various biological processes. Jak1^{-/-} mice suffer from defective neuronal functions and die perinatally. Jak2 gene knockout results in embryonic lethality because of dysfunctions in erythropoiesis. Deletion of Jak3 results in severe combined immunodeficiency (SCID) due to a lack of T and NK cells (Ghoreschi et al., 2009). Tyk2 deficiency and its functions are described in detail below.

There are seven mammalian Stats: Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6. Stats are proteins with a size ranging from 90 to 115 kDa and share seven

functional domains. These include the amino terminal domain (NH_2), the coiled-coil domain, a DNA binding domain (DBD), the linker domain (LK), the SH2 domain and the carboxy-terminal transactivation domain (TAD). The functions of Stat proteins *in vivo* were mainly revealed by studies using gene knockout mice (Kisseleva et al., 2002; O'Shea et al., 2002). Stat1^{-/-} and Stat2^{-/-} mice are highly sensitive to microbial and viral infections due to impaired interferon (IFN) responses. Stat3 deletion results in early embryonic lethality. Due to impaired interleukin (IL)-12 responses, Stat4 deficient mice show increased susceptibility to infections, but are more resistant to Th1 mediated autoimmune diseases. Stat6 deficient mice display defects in Th2 cell development and increased severity of Th1 mediated diseases. They show increased inflammation and lethality during sterile endotoxemia, but reduced lethality upon bacterial infection. Stat5a knockout mice have impaired mammary gland development due to the loss of prolactin responses. Deletion of Stat5b results in sexually dimorphic growth retardation, due to Stat5b functions in male-specific liver gene expression. Stat5a/b double knockout mice die soon after birth, are small and infertile and show dysfunctional mammary gland development.

3.1.1 Tyk2 in cytokine signalling

Tyk2 interacts with IFNα/β receptor (IFNAR1), IL-12Rβ1, IL-10R2, gp130 and IL-13Rα1 receptor chains and is activated in combination with Jak1 and/or Jak2 (Fig.1). Tyk2 was the first member of the Jak family identified and was described as essential for type I IFN signalling in a human fibroblast cell line (Firmbach-Kraft et al., 1990; Velazquez et al., 1992). The type I IFN receptor consists of two chains, the IFNAR1 receptor which is associated with Tyk2 and IFNAR2 which associates with Jak1. This receptor complex activates mainly Stat1:Stat2 heterodimers, which regulate transcription together with IFN regulatory factor (IRF) 9, but can also activate Stat3-6 (Schindler and Plumlee, 2008; van Boxel-Dezaire et al., 2006). Tyk2 deficiency in human cell lines results in unresponsiveness to IFNα and reduced response to IFNβ, which could be explained by the importance of Tyk2 for stable cell surface expression of IFNAR1 (Gauzzi et al., 1997; Ragimbeau et al., 2003). Tyk2 acts as a masking protein for an endocytic motif within the human IFNAR1 cytoplasmic domain and prevents its endocytosis (Kumar et al., 2008). In contrast, murine Tyk2^{-/-} fibroblasts

and macrophages have normal IFNAR1 surface expression and reduced IFNα/β responses (Karaghiosoff et al., 2000; Sheehan et al., 2006; Shimoda et al., 2000).

Tyk2 associates with IL-12Rβ1, which is used by IL-12 and IL-23. IL-12 binding to IL-12Rβ1 and IL-12Rβ2 receptor chains activates Tyk2 and Jak2 and induces the production of IFNγ by T cells and natural killer (NK) cells. Cells derived from Tyk2 deficient mice have strongly reduced IFNγ production and impaired Stat3 and Stat4 activation in response to IL-12 (Langrish et al., 2004; Strobl et al., 2011). IL-23 signals through IL-12Rβ1 and IL-23R *via* Tyk2 and Jak2 and induces Stat3 activation. In the absence of Tyk2 impaired IL-23 induced Stat3 activation in T cell blasts (Shaw et al., 2003) and defective IL-23 mediated IL-17 production in γδ T and Th17 cells was reported (Nakamura et al., 2008; Oyamada et al., 2009).

IL-10 and IL-22 share the Tyk2 associated IL-10R2 receptor chain and mainly activate Stat3 and Stat1 (Ouyang et al., 2011). Reports on the role of Tyk2 in IL-10 signalling are contradictory. Initially it has been shown that Tyk2 is not required for IL-10 mediated Stat3 activation in T cells and macrophages (Karaghiosoff et al., 2000; Shimoda et al., 2000) but defects in IL-10 responses in Tyk2$^{-/-}$ inflammatory macrophages have been proposed in a more recent study (Shaw et al., 2006).

Tyk2 is also activated by the family of ligands utilizing the glycoprotein 130 (gp130) receptor chain, namely IL-6, IL-11, IL-27, leukemia inhibitory factor (LIF) and granulocyte-colony stimulating factor (G-CSF), oncostatin-M, ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1) and cardiotrophin-like cytokine (CLC) (Schindler and Plumlee, 2008). Among these cytokines, it was shown that murine Tyk2 is not required for IL-6 and LIF mediated Stat3 activation (Karaghiosoff et al., 2000). Moreover, Tyk2 can associate with IL-13Rα1 and is required for the induction of IL-13 mediated target gene transcription in human cells (Bhattacharjee et al., 2010; Roy and Cathcart, 1998).
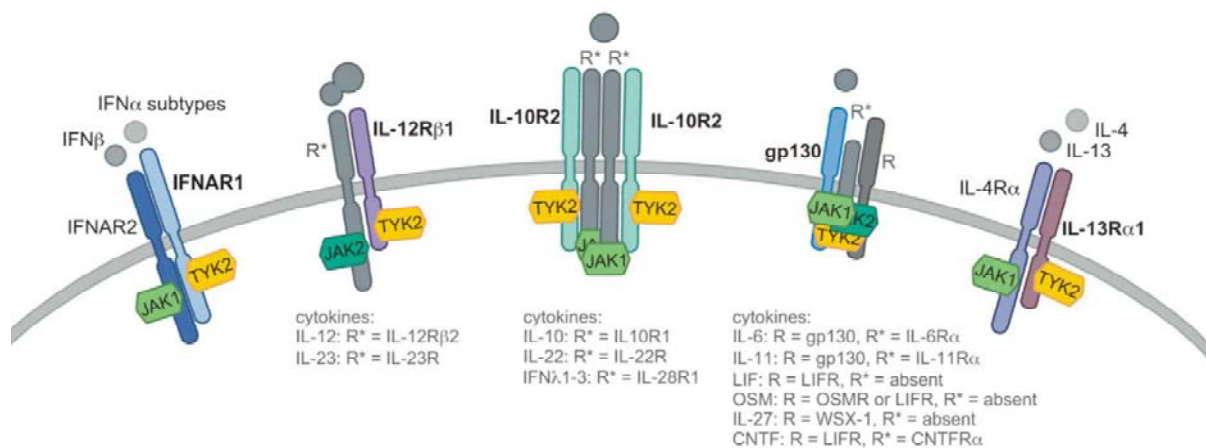
Fig.1: Cytokines which signal through Tyk2 and various receptor (R/R*) chains associated with Jak1/Jak2 and Tyk2 are shown (Strobl et al., 2011). IL-35 was recently characterized to signal trough IL-12Rβ1 and gp130 (Collison et al., 2012). LIF: leukemia inhibitory factor; OSM: oncostatin M; CNTF: ciliary neutrotophic factor.©FB

3.1.2 Tyk2 kinase-independent functions

In addition to the catalytic activity of Tyk2, kinase-independent functions have been described. Tyk2 kinase-activity is dispensable for the surface stabilisation of IFNAR1 and the crosstalk to the phosphoinositol3-kinase pathway in human cells as well as the regulation of mitochondrial functions in murine cells (Li et al., 2013; Potla et al., 2006; Ragimbeau et al., 2003; Rani et al., 1999). However, Tyk2 kinase-activity is required for full IFNα/β signalling in murine cells and mice (Prchal-Murphy et al., 2012).

3.1.3 The role of Tyk2 in immunity

Tyk2 deficient mice are fertile and viable but have many immunological defects. Mice lacking Tyk2 are more sensitive to infectious disease. Tyk2^{-/-} mice have a defective CD8^{+} cytotoxic T lymphocyte (CTL) response after infection with Lymphocytic choriomeningitis virus (LCMV) and *Listeria monocytogenes*. Tyk2^{-/-} mice infected with *L. monocytogenes* showed defects in CD4^{+} and CD8^{+} T cell mediated IFNγ production. Impaired IFNγ production was also observed after *Leishmania major* infection of Tyk2^{-/-} mice and in the mutant Tyk2 mouse strain B10.Q/J upon *Toxoplasma gondii* infection. Another important phenotype in Tyk2 deficient mice is resistance against autoimmune diseases. Tyk2^{-/-} mice and B10.Q/J showed increased resistance to collagen induced arthritis (CIA) and experimental autoimmune encephalomyelitis (EAE), the latter is a mouse model for multiple

sclerosis. Additionally, Tyk2$^{-/-}$ mice are prone to Th2 cell mediated allergic diseases, because of their increased Th2 development. Moreover, Tyk2$^{-/-}$ mice are less susceptible to lipopolysaccaride (LPS) induced endotoxin shock (Strobl et al., 2011).

An involvement of Tyk2 in cancer was first shown in murine lymphoid malignancies. Mice lacking Tyk2 develop Abelson virus-induced B lymphoid leukaemia and TEL-Jak2-induced T cell lymphoid leukaemia with shortened latency and increased incidence. This was attributed to a decreased cytotoxicity of Tyk2$^{-/-}$ NK/NKT cells (Stoiber et al., 2004). Furthermore, Tyk2 triggers effector functions of CTLs in antitumor immunity (Simma et al., 2009). An important role of Tyk2 was also described in immune surveillance of breast cancer using the 4T1 transplant model. Faster tumour growth and more rapid metastasis were observed in Tyk2$^{-/-}$ compared to WT mice (Zhang et al., 2011).

Several germline and somatic Tyk2 mutations have been linked to tumour formation in humans, although the underlying mechanisms are unknown (Strobl et al., 2011). More recently, a cell-intrinsic tumour-promoting function of Tyk2 has been reported in T cell acute lymphoblastic leukemia (T-ALL) and this was associated with a Stat1 dependent upregulation of the anti-apoptotic protein BCL2 (Sanda et al., 2013).

To date two patients with Tyk2 deficiency have been identified (Kilic et al., 2012; Minegishi et al., 2006). Both patients have a frame-shift mutation in the Tyk2 gene, which results in a pre-mature stop codon and the loss of functional Tyk2 protein. They have many similarities, but also some differences in clinical symptoms. In the first patient, hyper-IgE syndrome (HIES), a primary immunodeficiency characterized by skin abscesses, pneumonia and elevated IgE levels, was diagnosed. In addition, this patient suffered from disseminated Bacille Calmette-Guerin (BCG) infection and showed increased susceptibility to viral, bacterial and fungal infections (Minegishi et al., 2006). The second patient also suffered from BCG infection and displayed neurobrucellosis and cutaneous herpes zoster virus infection (Kilic et al., 2012). Furthermore, genome-wide association studies in humans revealed that Tyk2 gene locus polymorphisms are associated with many different inflammatory and autoimmune diseases such as lupus erythematosus, multiple sclerosis, Crohn´s diseases, psoriasis, type I diabetes and endometriosis (Ellinghaus et al., 2012; Lee et al., 2012; Peluso et al., 2013; Strobl et al., 2011).

3.2 IL-22/IL-22 receptor pathway

3.2.1 Structure

IL-22 is a monomeric cytokine and belongs to the IL-10 family. IL-22 consists of six α-helices, which are arranged in an antiparallel conformation. It contains four cysteines which link the glycosylated N-terminus to the loop formation.

IL-22 signals through a receptor complex, which belongs to the cytokine receptor family class 2 (CRF2). The IL-22 heterodimeric receptor complex consist of a type I receptor chain (RI), IL-22R1 and a type II receptor chain (RII), IL-10R2. Their extracellular part contains two tandem fibronectin type III domains and each part includes a ligand-binding site. Each of these domains contains seven β-strands, which are linked by loops. IL-22R1 has a longer intracellular structure, which is important for signalling (Wolk et al., 2010). The IL-10R2 chain is part of the receptor complexes for IL-10, IL-22, IL-26 and the IFN lamda (IFNλ) subtypes (Kotenko, 2002; Prokunina-Olsson et al., 2013). In addition an IL-22 soluble receptor exists, which consists of a single chain and binds with high affinity to IL-22 and is called IL-22 binding protein (IL-22BP). This protein belongs to the CRF2 family, lacks the intracellular and the transmembrane domain and inhibits cellular IL-22 effects (Wolk et al., 2010).

3.2.2 Regulation of IL-22/IL-22R expression

In humans, IL-22 is expressed in activated T cells and at lower levels in NK cells but not in B cells, macrophages, DCs and non-hematopoietic cells (Wolk et al., 2010). Among the T cell subsets IL-22 is preferentially expressed in CD4$^+$ memory cells, Th22, Th1 and Th17 cells (Duhen et al., 2009; Wolk et al., 2002). In contrast, expression of IL-22 in mice could be found in NKT cells, αβ T cells, γδ T cells, Th17 and Th22 cells but not in Th1 cells (Martin et al., 2009; Siegemund et al., 2009). Furthermore, human and murine gut mucosa associated NK cells produce IL-22. It has been shown that intestinal NK cells and T cell subsets express RORγt and could be responsible for constitutive IL-22 expression in the gut (Wolk et al., 2010).

IL-10R2 is ubiquitously expressed on hematopoietic and non-hematopoietic cell lineages (Sonnenberg et al., 2010). In contrast, IL-22R1 is only expressed on a limited number of cell types. Highest expression of IL-22R1 could be found in

pancreas and skin, although, it is also expressed in other organs, such as the small intestine, liver, colon, lung and the trachea. Among distinct cell types, highest IL-22R1 expression was found in keratinocytes. Furthermore, intestinal and respiratory epithelial cells, hepatocytes and pancreatic acinar cells are major targets of IL-22. IL-22 does not act on immune cells and IL-22R1 expression could not be found in bone marrow, blood mononuclear cells, thymus or spleen. Additionally, IL-22R1 is not expressed in isolated resting or activated primary immune cells, including monocytes, B cells, T cells, NK cells, macrophages and immature and mature DCs (Wolk et al., 2010).

The expression of IL-22BP has been detected in the reproductive system (e.g. placenta and breast), lymphatic organs (e.g. thymus, spleen and lymph nodes), the gastrointestinal system (e.g. stomach and colon) and finally in the lung and skin. However, cellular sources remained elusive. Interestingly, reduced IL-22BP production was observed during inflammatory processes associated with increased IL-22 expression. In LPS-treated mice, induced IL-22 expression in various organs results in reduced IL-22BP expression in lymph nodes. In a mouse colitis model high IL-22 expression was associated with decreased IL-22BP expression in the inflamed intestine. Several studies indicate that IL-22BP has inhibitory effects on IL-22 signalling, which is mainly due to a stable complex formation between IL-22BP and IL-22 and overlapping binding-surfaces of IL-22 for IL-22BP and IL-22R1 (Wolk et al., 2010).

3.2.3 Signalling

The interaction of IL-22 with its receptor complex activates primarily the Jak-Stat pathway. IL-22 induces tyrosine phosphorylation of Jak1 and Tyk2, but not Jak2, in a rat hepatoma cell line (H4IIE) (Lejeune et al., 2002). Several studies investigating IL-22 induced signal transduction in cells with endogenous receptor expression demonstrated tyrosine phosphorylation of Stat3 (Boniface et al., 2005; Brand et al., 2006; Nagalakshmi et al., 2004). In tumour cell lines also tyrosine phosphorylation of Stat1 and Stat5 was observed (Wolk et al., 2010). In addition to tyrosine phosphorylation, serine phosphorylation of Stat3 was suggested to be required for full IL-22 mediated gene-induction in H4IIE cells. Furthermore, activation of the p38 kinase, c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase1/2

(ERK1/2) MAPK pathways by IL-22 was reported (Lejeune et al., 2002). In human primary keratinocytes IL-22 induced tyrosine phosphorylation of Stat3, whereas only minor tyrosine and threonine phosphorylation of ERK1/2 and JNK and no activation of p38 kinase were observed (Wolk et al., 2010).

3.2.4 Functions of IL-22 in mice

IL-22 is involved in many biological processes, such as inflammation, cell survival, chemotaxis, host defence and tissue protection. In keratinocytes, IL-22 was first defined to induce antimicrobial proteins (AMPs), which was then also observed in infected bowel and lungs (Wolk et al., 2010). *Citrobacter rodentium* infected IL-22-deficient mice had reduced RegIIIβ and RegIIIγ production in colonic epithelial cells (Zheng et al., 2008). Another study reported that IL-22 produced by a subset of intestinal NK cells is required for immunity against *C. rodentium* (Satoh-Takayama et al., 2008). In pulmonary *Klebsiella pneumoniae* infected mice an IL-22 mediated upregulation of lipocalin 2 and a higher resistance against lung injury was observed. Furthermore, IL-22 neutralization in this infection model led to increased death (Aujla et al., 2008). There is also data suggesting an involvement of IL-22 in immunity against systemic bacterial infections, although this appears to depend on the specific pathogen (Wolk et al., 2010). For example, IL-22 has an important role in the defence against intraperitoneal (i.p) *Salmonella enteritis* infections (Siegemund et al., 2009) but is dispensable for survival upon i.p. infection with *L. monocytogenes* (Zenewicz et al., 2007). Furthermore, IL-22 does not critically impact on the defence against *Mycobacteriam avium* and *Schistostoma mansoni* infections. In contrast, during *T. gondii* infections IL-22 can also promote pathogen induced inflammation in the gastrointestinal tract but not in other organs. The involvement of IL-22 in immunity against fungal infections is reported conflictingly (Sonnenberg et al., 2010; Zenewicz and Flavell, 2011).

IL-22 also exerts protective effects on hepatocytes. IL-22 increases mRNA expression of acute phase proteins, such as serum amyloid A (SAA) and α1-antichymotrypsin and IL-22 injection enhanced hepatic SAA expression. Furthermore, IL-22 induces LPS binding protein and has a protective role in liver injury, a function that could be confirmed in IL-22 deficient mice. IL-22 is also

important for liver regeneration after partial hepatectomy (Wolk et al., 2010; Zenewicz and Flavell, 2011).

Several studies demonstrated a role of IL-22 in the protection against intestinal inflammation. Increased IL-22 expression in the mesenteric lymph nodes and inflamed intestine, correlated with decreased IL-22BP in colitic mice (Wolk et al., 2007). In a Th2 mediated colitis model in mice, which is symptomatically similar to human ulcerative chronic (UC) disease, IL-22 mediated Stat3 activation results in rapid amelioration of local intestinal inflammation (Sugimoto et al., 2008).

Evidence for an involvement of IL-22 in autoimmune diseases came from a study showing less severe collagen-induced arthritis, a mouse model for human rheumatoid arthritis (RA), in the absence of IL-22 (Geboes et al., 2009). In contrast, IL-22 seems not involved in the development of EAE (Kreymborg et al., 2007).

3.2.5 IL-22 in human diseases

IL-22 is associated with the pathogenesis of several human diseases. Increased IL-22 production by T cells in chronic T cell mediated diseases such as psoriasis, IBD and RA suggests that IL-22 plays an important role in pathogenesis of these inflammatory diseases. Regarding psoriasis, it is known that IL-22 and its downstream target IL-20 inhibit keratinocyte cornification, thereby causing the typical epidermal alterations, including epidermal *acanthosis*, *hypogranularity, parakeratosis* and *hyperkeratosis* (Wolk et al., 2010). Regarding IBD it is known that IL-22 expressing CD4^{+} T cells are localized in the inflamed region of the gut, in contrast to active UC, where IL-22 expressing T cells are mainly found in the lamina propria. In addition, the presence of IL-22 in blood is positively correlated with disease activity in Crohn´s disease patients (Andoh et al., 2005; Brand et al., 2006; Wolk et al., 2007). These findings suggest that IL-22 has a potential role in the pathogenesis of UC and Crohn´s diseases. Furthermore studies demonstrated that increased IL-22 expression in synovial tissue and mononuclear cells leads to inflammatory responses in RA patients (Ikeuchi et al., 2005). In addition increased levels of IL-22 positive CD4^{+} cells could be detected in blood of RA patients (Shen et al., 2009). Several studies in humans indicated that overexpression of IL-22 is associated with increased malignancy (Sonnenberg et al., 2010) and it has been reported that IL-22 promotes

tumour cell survival. Neutralizing this cytokine during chemotherapy implicates positive effects in certain cancers (Wolk et al., 2010).

3.3 IL-27/IL-27 receptor pathway

3.3.1 Structure

IL-27 is a heterodimeric cytokine structurally related to IL-12. It consists of two subunits, Epstein-Barr virus induced gene 3 (EBI3) and IL-27p28. EBI3 contains no membrane anchoring motifs and two cytokine binding domains containing highly conserved WSXWS motifs. This structure is characteristic for other known members of the class I cytokine receptor family such as IL-12p40, the soluble receptors IL-6Rα and the ciliary neutrophic factor receptor (CNTFR). The IL-27p28 subunit is a four-helix bundle cytokine and has homology to other helical cytokines such as IL-6, cardiotrophin-like cytokine (CLC), IL-12p35 and IL-23p19 (Hall et al., 2012).

The heterodimeric IL-27 receptor consist of IL-27Rα (WSX-1) and gp130. IL-27Rα has structural homology to gp130 and IL-12Rβ2 (Sprecher et al., 1998). These receptors are characterized by an extracellular cytokine-binding domain which consists of four N-terminal cysteines, three fibronectin-like domains and WSXWS motifs near the C-terminus (Bazan, 1990).

3.3.2 Regulation of IL-27/IL-27R expression

IL-27 is mainly produced by antigen presenting cells upon encounter of inflammatory stimuli. However, various other cell types, such as plasma cells, endothelial cells, microglia, placental trophoblast and NK cells can also produce EBI3 and IL-27p28 (Hall et al., 2012). The subunits EBI3 and IL-27p28 can be secreted independently from each other and are differentially expressed in distinct cell types. This finding suggests that both subunits of IL-27 have biological functions of their own. In human and mouse antigen presenting cells different toll-like receptor (TLR) agonists lead to the induction of EBI3 and IL-27p28 mRNA expression. In response to TLR4, myeloid differentiation factor (MyD) 88-dependent nuclear factor kappa B (NFκB) activation initiates the transcription of IL-27p28. IFNγ can act synergistically through induction of IRF1 and IRF8, which both bind to IRF response elements in the IL-27p28 promoter. Notably, IL-27p28 expression can also be induced by the adaptor protein

MyD88 activated transcription factor AP-1 of the mitogen activated protein kinase (MAPK) pathway. MyD88-independent, TRIF-dependent pathways can also trigger IL-27 production. This occurs by activation of IRF3 for IL-27p28 and possibly IRF7 for EBI3. Furthermore autocrine/paracrine type I IFN signalling and activation of IFN-stimulated gene factor (ISGF) 3 can induce both IL-27p28 and EBI3. Upregulation of IRF1 and IRF7 by IFNs can further increase IL-27 expression (Hall et al., 2012).

Gp130 is ubiquitously expressed, but its level can depend on the cell activation state. IL-27Rα is primarily expressed in lymphoid tissues, with the highest expression in T cells and NK cells. Nevertheless a range of other cells is capable to co-express IL-27Rα and gp130 and to respond to IL-27, including B cells, monocytes, macrophages, dendritic cells (DCs), mast cells, endothelial cells and neurons. Naive CD4$^+$ T cells express high levels of IL-27Rα receptor, but these are decreased after T cell receptor (TCR) stimulation. In contrast, resting NK cells express low levels of IL-27Rα and activation results in an upregulation. In human B cell subsets, B cell receptor (BCR) stimulation reduces the expression of both receptor chains. Moreover, IL-27Rα is also expressed on different human and mouse tumour cell lines, such as HeLa cells and B16 melanoma cells, and was found upregulated in cancer cells from acute myeloid leukemia patients (Hall et al., 2012)

3.3.3 Signalling

Only few studies addressed the molecular signalling pathways activated by IL-27. The binding of IL-27 to its receptor activates the Jak-Stat and the MAPK pathway in lymphocytes. It has been shown that IL-27Rα is associated with Jak1 (see Fig.2A), whereas gp130 is associated with Jak1, Jak2 and Tyk2 (Lutticken et al., 1994; Owaki et al., 2006; Sprecher et al., 1998). Stimulation of CD4$^+$ T cells with IL-27 results in activation of Jak1, Jak2, and Tyk2 and phosphorylation of Stat1, 3, 4 and 5 (Villarino et al., 2004). The best described IL-27 target genes are *T-bet*, *IL-10* and *Gata-3* (Hunter and Kastelein, 2012,). Additionally, several IFN responsive genes, including *Gbp2*, *Irf1* and *Ip-10* can be regulated by IL-27 (Hirahara et al., 2012; Imamichi et al., 2012). IL-27 mediated Stat1 activation promotes the expression of the transcription factor *T-bet* and the IL-12Rβ2 chain in T cells, which are involved in the regulation of Th1 differentiation and responses. IL-27 activated Stat3 promotes IL-10 production through the induction of the transcription factor c-Maf (Hall et al., 2012). Until now

little is known about mechanisms that negatively regulate IL-27 signalling. For gp130 it is known that Stat1 and Stat3 activation lead to increased suppressor of cytokine signalling (*Socs*) 3 expression, which provides a negative feedback to many type I cytokines and inhibits further signalling trough gp130 (Nicholson et al., 2000).
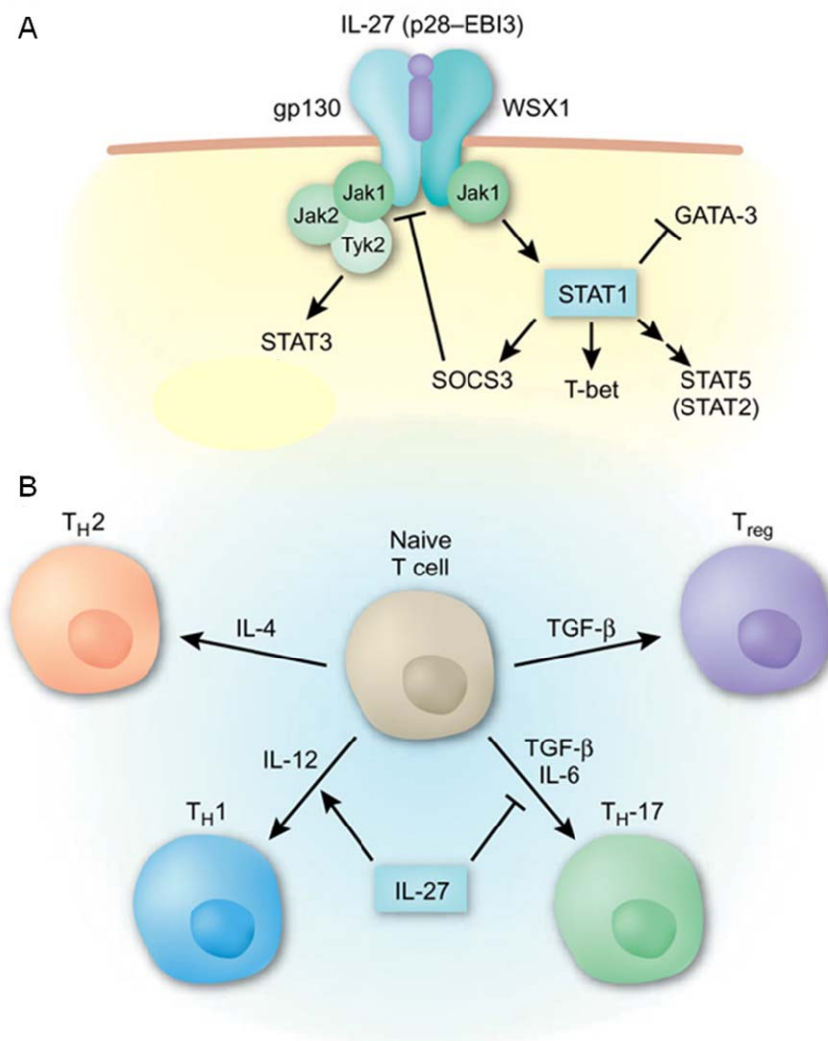

Fig.2: (A) IL-27 subunits (EBI3 and IL-27p28), its receptors (gp130 and WSX1/IL-27Rα) and Jak-Stat signalling. (B) T cell differentiation and involvement of IL-27 and other cytokines. TGF-β: transforming growth factor β, Treg: regulatory T cell (Colgan and Rothman, 2006) ©*2007 Nature publishing group.*

3.3.4 Functions of IL-27 in mice

Early studies suggested that IL-27 has mainly pro-inflammatory properties. However, it is now recognized that IL-27 also exerts anti-inflammatory functions (Fig.2B). The role of IL-27 *in vivo* was characterized by using IL-27p28-, EBI3- and, as the most

specific model, IL-27Rα deficient mice. These studies revealed crucial roles of IL-27 in immunity to infections, during inflammatory diseases and in cancer (Hall et al., 2012). Initially it was reported that IL-27Rα^{-/-} mice show increased susceptibility to *L. monocytogenes* and *L. major* as consequence of defects in Th1 immunity (Chen et al., 2000; Wang et al., 2008). However, this view has been challenged by later studies showing unimpaired Th1 responses and more efficient control of *L. monocytogenes* in the absence of IL-27Rα (Batten et al., 2008; Yang et al., 2008). Increased Th2 responses have been reported in *L. major* infected IL-27Rα^{-/-} mice (Artis et al., 2004), suggesting that increased Th1 responses may be secondary consequences. Nevertheless, *in vivo* studies showed that IL-27 mediates the expression of adhesion molecules, such as ICAM-1/LFA-1, which are important for Th1 differentiation. IL-27 also enhances proliferation, production of IFNγ and IL-12Rβ2 expression in naive CD4^{+} T cells (Hall et al., 2012). In addition to its effects on CD4^{+} T cells, IL-27 promotes CD8^{+} T cell functions. IL-27Rα lacking mice show reduced CD8^{+} T cell responses during acute *T. gondii* and influenza virus infections and during viral hepatitis (Chen et al., 2000).

Another important pro-inflammatory function of IL-27 is the inhibition of Foxp3^{+} T cell populations (Tregs), as demonstrated in a T cell mediated colitis and an oral tolerance model in IL-27Rα^{-/-} mice. In line with this, mice overexpressing IL-27p28 and EBI3 lack peripheral Tregs (Hall et al., 2012).

IL-27 also promotes CD8^{+} T cell effector functions in tumour settings, as illustrated by *in vivo* studies IL-27 expressing cancer cell lines (Hall et al., 2012). In line with this, it was demonstrated that IL-27 mediated tumour regression depends on CD8^{+} T cells but not on NK or CD4^{+} T cells in colon carcinoma and neuroblastoma models (Hisada et al., 2004; Salcedo et al., 2004).

As anti-inflammatory cytokine, IL-27 inhibits Th17 mediated IL-17 production, which leads to inhibition of Th17 development. Furthermore, *in vitro* studies have shown that IL-27 suppresses the expression of RORα and RORγt, two transcription factors that are important for Th17 generation and effector functions. Moreover, IL-27 up-regulates IL-10 in T cells, thereby limiting immune pathology. Reduced IL-10 expression was also correlated with enhanced pathology during chronic toxoplasmic encephalitis and EAE in IL-27Rα^{-/-} mice (Hall et al., 2012).

3.3.5 IL-27 in human diseases

Several studies have shown that a specific single nucleotide polymorphism (SNP) at the IL-27p28 gene locus is associated with autoimmune disease, susceptibility to asthma with increased immunglobuline E (IgE) and eosinophilia. Furthermore, the same polymorphism could be linked with susceptibility to chronic obstructive pulmonary disease (COPD) and inflammatory bowel disease. Genome wide association studies identified two other SNPs to be associated with UC (Hall et al., 2012). These SNPs correlated with decreased mRNA expression of IL-27 in lymphoblastic cell lines and colonic epithelium of UC patients, indicating that IL-27 has a protective role in preventing this disease (Imielinski et al., 2009). A gain of function mutation in Stat1 leads to increased IL-27 mediated downregulation of Th17 responses, which could be relevant for the treatment of Th17 mediated diseases (Liu et al., 2011; van de Veerdonk et al., 2011).

3.4 Background of the project

One of the research interests in our laboratory is to define the role of Tyk2 in dextran sodium sulfate (DSS) induced colitis in mice. Against the expectations it was found that Tyk2 deficient mice develop more severe colitis compared to wild-type (WT) mice. This was correlated with decreased tyrosine phosphorylation of Stat3 in colonic tissues from Tyk2$^{-/-}$ compared to WT mice. The major Stat3 activating cytokines (e.g. IL-6, IL-10 and IL-22) were expressed at similar levels in Tyk2$^{-/-}$ and WT colons. A possible explanation for the observed differences could be reduced signalling in response to any of these cytokines in the absence of Tyk2. Whereas previous reports showed that IL-6 and IL-10 do not rely on Tyk2 for signal transduction, it has not yet been analysed if Tyk2 is required for IL-22 signalling. Furthermore, recent studies suggested that IL-22 is of major importance for epithelial cell wound healing and protection from DSS induced colitis (Pickert et al., 2009; Wolk et al., 2010). Experiments with primary epithelial cells from Tyk2 deficient and WT colons suggested that Tyk2 is needed for full activation of Stat3. However these data need confirmation as IL-22 signalling in primary epithelial cells could only be analysed for a short time (i.e. less than 1 hour) and results showed high variability between experiments.

Another major research topic is to define kinase independent functions of Tyk2. To this end, knockin mice expressing a kinase inactive version of Tyk2 (Tyk2^{K923E}) have been generated. Previous experiments have shown that Tyk2^{K923E} mice are similarly susceptible to virus infection as Tyk2$^{-/-}$ mice, but show intermediate resistance against LPS induced sepsis between Tyk2$^{-/-}$ and WT mice (Prchal-Murphy et al., 2012 and unpublished). Furthermore, Tyk2^{K923E} mice could control growth of transplanted tumour cells (i.e. MC28 adenocarcinoma and EG7 thymoma cells) to a similar extent as WT mice, whereas tumours grew significantly bigger in Tyk2$^{-/-}$ mice (Prchal-Murphy et al., 2012, and manuscript in preparation). Thus Tyk2^{K923E} are phenotypically different from Tyk2$^{-/-}$ mice, clearly supporting the concept that Tyk2 exerts kinase independent functions. Type I IFN and IL-12 signalling was not different between cells derived from Tyk2^{K923E} and Tyk2$^{-/-}$ mice, indicating that other pathways are involved. Current work focuses on signalling by other cytokines that activate Tyk2 and could be relevant for the phenotypic differences. Among these, IL-27 is of major interest, as it is important for the defence against certain infections, is protective during sepsis and is involved in tumour surveillance.

3.5 Aims

My first aim was to find a cell culture system that allows us to test the impact of Tyk2 on IL-22 induced Stat3 activation and target gene induction. In particular, I wanted to (a) test IL-22 responsiveness of human and murine colonic epithelial cell lines and (b) establish conditions for the cultivation of primary colonic epithelial cells for longer time periods. In addition, it should be tested if cells respond to IL-6 and/or IL-10. My second aim was to analyse if absence of Tyk2 or presence of kinase inactive Tyk2 affects IL-27 responses. To this end, I wanted to compare macrophages, T cells and NK cells derived from Tyk2$^{-/-}$, Tyk2^{K923E} and WT mice with respect to IL-27 induced activation of Stat1, Stat3 and Stat4 and the induction of known IL-27 target genes.

4 Material and Methods

4.1 Reagents

Materials	Company	Code/Cat.No
2-Mercaptoethanol	Invitrogen/Gibco, Lofer, Austria	1242924
Amphotericin B	Sigma, St. Louis, Missouri	A9528
Anti-mouse CD28	eBioscience, San Diego, California	37.51
Anti-mouse CD3	eBioscience, San Diego, California	145-25C11
Bio-Rad Protein Dye	BioRad, Hercules, California	5000006
Bovine serum albumin (BSA)	Roth Lactan, Graz, Austria	T844.3
Collagen I	BD Biosciences, Bedford, England	354236
Collagen IV	BD Biosciences, Bedford, England	354428
D (+) Glucose	Sigma, St. Louis, Missouri	RNBB8630
Dexamethasone	Sigma, St. Louis, Missouri	070M8713V
Dithiothreitol (DTT)	Invitrogen/Gibco, Lofer, Austria	D-1532
Dulbecco´s Modified Eagle Medium (DMEM)	PAA Laboratories GmbH, Pasching, Austria	E15-843
ECL Solution	GE Healthcare, Munich, Germany	RPN2106
Epidermal growth factor (EGF)	Sigma, St. Louis, Missouri	E9644
Fetal calf serum (FCS) (heat-inactivated)	Invitrogen/Gibco, Lofer, Austria	10270-106
Gentamycin	Sigma, St. Louis, Missouri	G1397
HAM´s F-12 (HAMF) medium	Sigma, St. Louis, Missouri	P7369
Hepes	Invitrogen/Gibco, Lofer, Austria	916822
IL-10 recombinant mouse	R&D Systems, Minneapolis, Minesota	417-ML
IL-2 recombinant human (rhIL-2)	Cell Signaling, Technology, Danvers, Massachusetts	8907
IL-22 recombinant human	R&D Systems, Minneapolis, Minesota	782-IL

IL-22 recombinant mouse	R&D Systems, Minneapolis, Minesota	582-ML
IL-27 recombinant mouse	R&D Systems, Minneapolis, Minesota	2789-ML
IL-6 recombinant mouse	R&D Systems, Minneapolis, Minesota	406-ML
IL-6Rα recombinant mouse	R&D Systems, Minneapolis, Minesota	1830-SR
Insulin, from bovine pancreas	Sigma, St. Louis, Missouri	120M8702V
Interferon-γ recombinant mouse	Millipore corporation, Billerica, Massachusetts	IF005
Interferon-λ2 recombinant mouse	Peprotech, New Jersey, United States of America	250-33
iScript cDNA synthesis kit	BioRad, Hercules, California	170-8890
Laminin	BD Biosciences, Bedford, England	354232
L-Glutamine	PAA Laboratories GmbH, Pasching, Austria	M11-004
Li-Cor binding buffer (LBB) (10x)	Li-Cor Biosciences, Nebraska, United States of America	829-07910
MACS mCD19 microbeads	Miltenyi Biotech, Bergisch Gladbach, Germany	130052201
MACS mCD49b (DX5) microbeads	Miltenyi Biotech, Bergisch Gladbach, Germany	130052501
Magnetic cell sorting (MACS) buffer	Miltenyi Biotech, Bergisch Gladbach, Germany	130091221
Milk powder	Roth Lactan, Graz, Austria	T145.2
Orange loading dye (10x)	Li-Cor Biosciences, Nebraska, United States of America	829-07910
PageRuler protein ladder	Fermentas, St. Leon-Rot, Germany	26617
Penicillin/Streptomycin (Pen/Strep)	PAA Laboratories GmbH, Pasching, Austria	P11-010
peqGOLD TRIFAST^{TM}	PeqLab, Erlangen, Germany	30-2020
Phenylmethansulfonylfluorid (PMSF)	Sigma, St. Louis, Missouri	78830
Phosphate buffered saline (PBS)	Sigma, St. Louis, Missouri	D8537
Poly (dI:dC)	Li-Cor Biosciences, Nebraska, United States of America	829-07910
Red blood cell lysis buffer	Sigma, St. Louis, Missouri	R7757
RPMI 1640 with L-Glutamine	PAA Laboratories GmbH, Pasching, Austria	E15-840

SigmaFast protease inhibitors	Sigma, St. Louis, Missouri	S8820
Sodium selenite	Sigma, St. Louis, Missouri	109K8736V
Tetramethylethylenediamine (TEMED)	Roth Lactan, Graz, Austria	2367.3
Trypsin-EDTA (1x)	PAA Laboratories GmbH, Pasching, Austria	L11-004
Tween	Sigma, St. Louis, Missouri	P1754

Standard reagents are not listed and were purchased from Sigma *(*St. Louis, Missouri*)* or Merck *(*Darmstadt, Germany*)*.

4.2 Mice

C57BL/6N (WT) mice were purchased from Charles River Laboratories. $Tyk2^{-/-}$ (Karaghiosoff et al., 2000)(B6.129SV-$Tyk2^{tm1Biat}$), $Tyk2^{\Delta CMV}$ ($Tyk2^{fl/fl}$) crossed to Tg(CMV-Cre))* and $Tyk2^{K923E}$ (B6.129P2-$Tyk2^{tm(K923E)3Biat}$) (Prchal-Murphy et al., 2012) mice were on C57BL/6N background. Mice were housed under specific pathogen free conditions according to FELASA guidelines.

*Conditional Tyk2 knockout mice ($Tyk2^{fl/fl}$ (B6.129P2-$Tyk2^{tm1.1Biat}$)) 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). $Tyk2^{fl/fl}$ mice have been crossed to mice expressing the Cre-recombinase under the control of the ubiquitous CMV promoter Tg(CMV-Cre) (Schwenk et al., 1995). After germ line deletion of the floxed allele, the Cre-recombinase was crossed out again and resulting $Tyk2^{\Delta CMV}$ mice were used as control and should phenocopy conventional Tyk2 knockout mice (Karaghiosoff et al., 2000; Shimoda et al., 2000).

4.3 Cell culture

Standard cell culture growth medium

DMEM (High Glucose 4.5g/l)

10% FCS

Pen/Strep (100µg/ml and 100U/ml)

2mM L-Glutamine

For MC38 cells 50µM 2-Mercaptoethanol was added.

4.3.1 Cell lines

The mouse rectum carcinoma cell line CMT93 and the seed bovine aorta derived endothelial cells CW1 were kindly provided by Isabella Rauch (Thomas Decker group, MFPL, Vienna), the murine colon carcinoma cell line MC38 was from Michaela Prchal (Veronika Sexl group, Vetmeduni, Vienna). The human colon adenocarcinoma cell line HT29 and the human epithelial colorectal adenocarcinoma cell line Caco-2 were kindly provided by Brigitte Marian (Meduni, Vienna).
For cell culture a Heraeus multifuge 1S, 75002000 centrifuge was used.

Cultivation of CMT93, MC38, HT29 and Caco-2

Cell lines were routinely cultivated in 10cm petri dishes. For passaging cells were washed with PBS, 1,5ml of trypsin EDTA was added per 10cm plate and dissociation of cells was observed microscopically. Trypsin reaction was stopped by adding 3ml of growth medium per 10cm dish. Cells were resuspended in medium and centrifuged at 1000rpm for 5 minutes. The pellet was resuspended in 5ml of medium and cells were seeded on new plates at the desired density. For experiments cell numbers were determined using a Neubauer counting chamber. For stimulation assays 1×10^{6} cells/well were plated in a 6-well plate.

Cultivation and lysis of CW1 cells as feeder layers

CW1 cells were cultivated on collagen I (1µg/ml) coated 6cm dishes for 3 days at 37°C. To obtain a non-proliferative feeder layer, medium was removed and cells were washed with 5ml of PBS and lysed in 5mM ammonium. Swelling and

destruction of cells were observed under the microscope for several minutes. Cells were washed twice with ice cold PBS and used as feeder layer.

Medium

DMEM (High Glucose 4,5g/l)

20% FCS

100mM Non-Essential Amino Acids

100mM Sodium Pyruvate

25mM Hepes

Primary colonic epithelial cells for short term culture

Medium

RPMI 1640

Pen/Strep (100µg/ml and 100U/ml)

2mM L-Glutamine

Isolation of primary colonic epithelial cells for short term culture

Whole colons from 8-12 week old mice were dissected and put into 10ml 4°C cold PBS. Colon was rinsed and purified with cold PBS by using a 5ml shot and, subsequently, cut into small pieces. These were put into 5ml 20mM EDTA/PBS solution and incubated for 10 minutes by shaking at 37°C. Cells were centrifuged at 1200rpm for 5 minutes and the supernatant was discarded. Pellet was washed 3 times with 5ml PBS and centrifuged at 1200rpm for 5 minutes. Cells were resuspended in 2,5ml medium by up- and down- pipetting. For stimulation assays 0,5ml cells were plated per well in a 6-well plate. Protein isolation of the cells was done as described below.

Primary colonic epithelial cells for long term culture

Medium (I)

DMEM (High Glucose 4,5g/l)

5% FCS

Pen/Strep (100µg/ml and 100U/ml)

Medium (II)

DMEM (High Glucose 4,5g/l)

10% FCS

2mM L-Glutamine

Pen/Strep (100µg/ml and 100U/ml)

Medium (III)

DMEM (High Glucose 4,5g/l) + RPMI 1640 (1:1)

5µg/ml Insulin

50nM Dexamethasone

10ng/ml Selenium

10ng/ml EGF

20mM Hepes

2mM L-Glutamine

10% FCS

10mM D-Glucose

Pen/Strep (100µg/ml and 100U/ml)

50µg/ml Gentamycin

2,5µg/ml Amphotericin B

Cryptomite buffer

1,5mM EDTA

110mM NaCl

2,5mM KCl

1,5mM KH_2PO_4

4,5mM Na_2HPO_4

10mM Glucose

5mM L-Glutamine

Isolation of primary colonic epithelial cells for long term culture

Colons from 8-12 week old mice were taken out and put into petri dishes filled with cold PBS. The crap was removed gently and put on a piece of paper. The colon was gently flushed with a syringe and inserted around the syringe. The colon was transferred to forceps while turning the colon inside-out. It was then transferred to a yellow stick and the end of the stick was blocked with a 10µl tip. The stick was kept in a 50ml Falcon tube with cold PBS until finished with all colons. Sticks containing the colons on it were incubated in 50ml Falcon tubes filled with cryptomite buffer for 50 minutes at 37°C. Sticks were then transferred to a 50ml Falcon containing PBS with 10% FCS. The sticks were mixed 12 times shortly (1 second) (Bio Vortexer, Biospec Products, Bartlesville, United States of America) and once longer (10seconds). Then sticks with containing colons were removed and sedimentation was done for 20 minutes on ice in 50ml PBS with 10% FCS. 35ml of supernatant was removed and fresh PBS with 10% FCS was added to 50ml and sedimentation was done for 20 minutes. 40ml of supernatant was removed and fresh PBS with 10% FCS was added to 50ml and again sedimentation was done for 20 minutes. 47ml of supernatant was removed and the remaining 3ml were divided into 2 centrifuge tubes and centrifuged at 1000rpm for 10 minutes at 4 °C. Supernatant was removed and pellet was resuspended in 1,5ml of medium and plated on coated wells in 6-well plates. Cells were incubated at 37°C and unattached cells were removed every day by gently replacing the medium with fresh one.

Coating of dishes

Collagen I
Collagen I was dissolved in 0,1M HCl to a concentration of 50µg/ml solution. 1ml/well was used to cover a 6-well plate and incubated over night at room temperature. To remove HCl, plates were washed with PBS and then stored at 4°C until use.

Collagen IV
Collagen IV was dissolved in 0,1M HCl to a concentration of 100µg/ml solution. 1ml/well was used to cover a 6-well plate and incubated over night at room temperature. To remove HCl, the plates were washed with PBS and then stored at 4°C until use.

Collagen IV combined with laminin

Collagen IV was diluted as described above. Laminin (conc. 1,62mg/ml) was diluted in serum-free medium to a concentration of 50µg/ml. 0,5ml from each solution/well were mixed and used to coat a 6-well plate as above.

Bone marrow derived macrophages (BMMΦ)

Standard medium L929

DMEM (High Glucose 4,5g/l)

10% FCS

2mM L-Glutamine

1mM Sodium Pyruvate

L929 cell conditioned medium

L929 cells were grown on 15cm cell culture dishes in MEF medium to approximately 70% confluence. Medium was removed and 30ml L929 medium (without FCS) was added. Cells 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

Pen/Strep (100µg/ml and 100U/ml)

2mM L-Glutamine

50µM 2-Mercaptoethanol

15% L929 cell conditioned medium

Isolation and cultivation of BMMΦ

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. Cells were split depending on their density and supplied with 12ml BMMΦ medium per 10cm plate. When plated for experiments (day 6) cell numbers were determined using a Neubauer counting chamber. $1,5 \times 10^6$ cells per well (6-well tissue culture plate) were seeded in 3ml BMMΦ medium and incubated over night at 37°C.

Protein isolation was done as described below.

Isolation of splenocytes

Mice at the age of 8 to 12 week were sacrificed, spleens dissected, transferred to sterile PBS and stored on ice. The spleen was homogenized through a 100µm cell strainer by using a plunger of a syringe. After washing the strainer several times with PBS cell suspensions were centrifuged at 1000rpm for 5 minutes. 1ml red blood cell lysis buffer per spleen was added to the cell pellet and incubated for 2 minutes at room temperature. Cell suspensions were filled up with DMEM to double the volume and centrifuged at 1000rpm for 5 minutes. The pellets were resuspended in 800µl MACS buffer/4 spleens and used for the isolation of NK cells and, subsequently, T cells.

NK cells

NK cell medium
RPMI 1640
10% FCS
50µM 2-Mercaptoethanol
Pen/Strep (100µg/ml and 100U/ml)
5000U/ml recombinant human IL-2 produced in *E.coli*

Isolation and cultivation of NK cells

Splenocytes in 800µl MACS buffer (see above) were incubated with 200µl DX5 microbeads for 20 minutes at 4°C. 4ml MACS buffer was added and cell suspension was centrifuged at 1000rpm for 5 minutes. Cells were resuspended in 1ml MACS buffer and insoluble cell aggregates were resuspended thoroughly. A mini-MACS column was put on the magnet and equilibrated with MACS buffer and cell suspension was applied to the column. The flow-through, which contains mainly B and T cells, was kept for subsequent T cell isolation (see below). The mini-MACS

column was washed with 500µl MACS buffer and removed from the magnet. 1ml MACS buffer was applied to the column and using a plunge and the NK cell fraction was collected in a vial. The NK cell fraction was centrifuged at 800rpm for 5 minutes, resuspended in 900µl NK cell medium and seeded in one well of a 24-well plate. Depending on the cell growth, cells were splitted every second or third day 1:3. NK cells were cultured for 7 days until use for the experiments. Protein and RNA isolation was done as described below.

T cells

T cell medium
RPMI 1640
10% FCS
1x Non-Essential Amino Acids
1x Sodium Pyruvate
50µM 2-Mercaptoethanol
Pen/Strep (100µg/ml and 100U/ml)

Isolation and cultivation of T cells
Splenocytes depleted from DX5^{+} cells were used for T cell isolation by negative selection of CD19^{+} cells. The flow-through (see above), which contains mainly B and T cells, was centrifuged at 1200rpm for 5 minutes. Cells were resuspended in 600µl MACS buffer and 400µl CD19 microbeads and were incubated for 20 minutes at 4°C. 4ml MACS buffer was added and centrifuged at 1000rpm for 5 minutes. Cells were resuspended thoroughly in 1ml MACS buffer. A mini-MACS column was equilibrated with MACS buffer and cell suspension was applied to the column. The flow-through, which contains DX5 and CD19 negative cells (i.e. mainly T cells), was centrifuged at 800rpm for 5 minutes. T cells were resuspended in 3ml T cell medium, αCD3 (0,5µg/µl) and αCD28 (1,5µg/µl) were added and cells were divided into 3 wells in a 6-well plate (1ml per well). T cells were cultured for 3 days. Depending on the cell density, cells were splitted 1:2 at the second day or provided with fresh medium. Protein and RNA isolation was done as described below.

Cell lysis

For RNA isolation, cells were washed with ice cold PBS, lysed in 1ml TRIFAST^{TM} and stored at -80°C until further use. For protein isolation, cells were washed with ice cold PBS and lysed in 100µl 1x Schindler buffer per well (6-well plate). Cell lysates were kept on ice for 30min. To remove cell debris, samples were centrifuged (5000rpm, 2 minutes, 4°C) and supernatant was transferred to new tubes and stored at -80°C. Protein concentrations were determined using Bio-Rad Protein Dye and absorbance was measured at OD_{595nm}.

RIPA buffer

50mM Tris/HCl pH7,4

1% NP-40

0,5% Na-deoxycholate

0,1% SDS

150mM NaCl

2mM EDTA

50mM NaF

1x SigmaFast protease inhibitors

1mM PMSF

1x Schindler lysis buffer

50mM Tris/HCl pH8

150mM NaCl

0,5% NP40 (IGEPAL)

10% Glycerin

2mM DTT

25mM EDTA

0,2mM Na-Vanadate

25mM Na-Fluoride

1x SigmaFast protease inhibitors

1mM PMSF

4.4 SDS-PAGE / Western blot/ electrophoretic mobility shift assay (EMSA)

Antibody solutions

Primary antibodies were diluted 1:1000 in 1% BSA in PY-TBST. To prevent contamination 0,01% Na-Azide was added to the primary antibody solutions. Secondary antibodies were diluted 1:2000 in 1% BSA in PY-TBST without adding Na-Azide.

Separation gel 8%

8ml Acrylamid/Bisacrylamid solution (30%, 37,5:1)

14ml H_2O

7,5ml 1M Tris/HCl pH 8,8

300µl 10% SDS

225µl 10% APS

27µl TEMED (99% p.A.)

Stacking gel

2,5ml Acrylamid/Bisacrylamif solution (30%, 37,5:1)

8,5ml H_2O

2,5ml 0,5M Tris/HCl pH 6,8

150µl 10% SDS

90µl 10% APS

10µl TEMED (99% p.A.)

1x Running buffer

25mM Tris/HCl pH 7,4

192mM Glycine

0,1% SDS

1x Transfer buffer

25mM Tris/HCl pH 7,4

150mM Glycine

20% Methanol

PY-TBST

10mM Tris/HCl pH 7,4

75mM NaCl

1mM EDTA

0,1% Tween

2x Lämmli sample buffer (LSB)

126mM Tris/HCl pH 6,8

4% SDS

20% Glycerine

200mM DTT

0.02% Bromphenol Blue

Strip buffer

2M Glycine/HCl pH 2,5

0.25% SDS

Blocking solution

5% Milk Powder in 1x PY-TBST

SDS-PAGE and Western blot

For SDS-PAGE 10-20µg protein extract were loaded per lane and Page Ruler was used as molecular weight standard. Electrophoresis was run for 1,5-2 hours at 100V. After separation by SDS-PAGE proteins were blotted on nitrocellulose membranes (GE Healthcare Amershan^{TM} Hybond^{TM}-ECL) by semidry blot (1mA/cm^2 of membrane; 2 hours). The membrane was rinsed in PY-TBST and incubated in blocking solution for 1 hour at room temperature. After washing the membrane 3 times in PY-TBST, it was incubated with primary antibody over night at 4°C. The membrane was again washed 3 times and incubated with the secondary antibody for 1 hour at room temperature. After further washing steps (3 times), the membrane was incubated with ECL solution for 1 minute and exposed to a light sensitive film. Membranes were stripped by incubating in strip buffer for 2 hours at room temperature. The membrane was washed with hot water and incubated in blocking solution for 1 hour prior to 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, Massachusetts	9171S
anti-Stat1	rabbit	Cell Signaling, Danvers, Massachusetts	9172S
anti-phospho Stat3 (Tyr785)	rabbit	Cell Signaling, Danvers, Massachusetts	9131S
anti-Stat3	rabbit	Cell Signaling, Danvers, Massachusetts	49045
anti-phospho Stat4	mouse	BD Transduction Laboratories, San Jose,	612738
anti-Stat4	mouse	Cell Signalling, Danvers, Massachusetts	2653
anti-panERK	mouse	BD Biosciences, Franklin Lakes, New Jersey	610124

Electrophoretic mobility shift assay (EMSA)

10µg of whole cell extracts were prepared in 15µl Schindler lysis buffer. 2µl of poly (dI:dC) (0,0625U) were added to each sample and incubated for 5 minutes at room temperature. 8µl of probe mix containing 2,5µl of 10x LiCor binding buffer (LBB) 2,5µl of DTT/Tween (25mM DTT, 2,5%Tween), 2µl H_2O and 1µl of 5´-IRDye 800-labeled double-stranded oligos with a GAS binding site (5´-GTCGACATTTCCCGTAAATC-3', purchased from Metabion, Martinsried, Germany) were added and incubated for 20 minutes at room temperature. 2,5µl of 10x orange loading dye were added and samples were separated on a bandshift gel (6%) and subsequently the gel was scanned with an Odyssey near infrared imager.

10x TBE
890mM Tris
890mM Boric Acid
20mM EDTA

Bandshift gel (6%)

10ml Acrylamid/Bisacrylamid (30%, 37,5:1)

2,5ml 10x TBE

37,2 H_2O

300µl 10%APS

30µl TEMED (99% p.A.)

Run buffer

0,5x TBE

TE buffer

10mM Tris/HCl pH 8

1mM EDTA

4.5 RNA Isolation / cDNA synthesis / quantitative PCR (qPCR)

DEPC-H_2O

0.01% DEPC in sterile water was incubated over night and autoclaved on the next day.

RNA isolation and cDNA synthesis

$1x10^6$ cells were lysed in 1ml peqGOLD TRIFAST^{TM}. Samples were incubated 5 minutes at room temperature. 200µl chloroform was added per sample and after mixing for 15 seconds the samples were incubated at room temperature for 2-3 minutes. Following centrifugation at 12000rpm for 15 minutes 450µl of the clear upper aqueous phase was transferred to a new eppendorf tube and 450µl isopropanol was added. The samples were gently mixed and incubated for 10 minutes at room temperature and then centrifuged at 12000rpm for 10 minutes on 4°C. The supernatant was removed and the pellet was washed once with 75% ethanol. After centrifugation (5 minutes, 7500rpm, 4°C) the supernatant was removed and the RNA pellet was dried at 60°C. RNA was resuspended in 30µl DEPC-H_2O and incubated on ice for 30 minutes. To dissolve properly the samples were pipetted up and down several times. RNA in DEPC-H_2O was stored at -80°C or used directly for cDNA synthesis. The concentration of RNA was determined using 4µl sample in

96µl TE buffer (OD_{260nm}/OD_{280nm}) and the RNA integrity was checked on agarose gels. 1µg total RNA was reverse transcribed using iScript^{TM} cDNA synthesis kit according to the manufacturer´s instructions. For all RNA isolation steps an eppendorf centrifuge 5415R, GE009, F45-24-11 was used.

Quantitative PCR (qPCR)

The following concentrations were used for qPCR analysis:

Stock concentration	Working concentration
$MgCl_2$ (25mM)	4mM
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/20µl
dNTP mix (2mM each)	200 µM
10x Hotfire B buffer	1x
Template (cDNA)	2µl*

* cDNA equivalent to 0,1µg of reverse transcribed RNA

All probes were labelled at their 5´ end with 6-carboxyfluorescin (FAM) and at the 3´ end with Black Hole Quencher (BHQ). For *Lcn2* and Qiagen assays EvaGreen (100nM) was used instead of a probe. Assays were performed with the same conditions and the specificity was proven by melting curve analysis.

The sequences for primers and probes were as follow:

Lcn2 (ID 12012): forward 5´ CCTCCATCCTGGTCAGGGAC 3´

              reverse 5´ TAGTCCGTGGTGGCCACTTG 3´

Ip10 (ID 5249): forward 5´ GTCTGA GTG GGA CTCAAG GGATC 3´

              reverse 5´ CACTGGCCCGTC ATCGATAT 3´

              probe 5´ CATCGATAT 3´

Irf1 (ID 3848): forward 5´ CCGAAGACCTTATGAAGCTCTTTG 3´

              reverse 5´ GCAAGTATCCCTTGCCATCG 3´

              probe 5´ CAGTCTGAGTGGCAGCCGACACACA 3´

RegIIIβ (ID 8856): forward 5´ ATGGCTCCTACTGCTATGCC 3´

reverse 5´ GTGTCCTCCAGGCCTCTTT 3´

probe 5´ TGATGCAGAACTGGCCTGCCA 3´

*RegIIIγ (*ID 8854*)*:forward 5´ CCA GCG CCA AGC ATT CAA TGA GCT 3´

reverse 5´ GAT GTC CTG AGG GCC TCT T 3´

probe 5´ TGG CAG GCC ATA TCT GCA TCA TAC C 3´

Ube2d2 (ID 3377): forward 5´ AGG TCC TGT TGG AGA TGA TAT GTT 3´

reverse 5´ TTG GGA AAT GAA TTG TCA AGA AA 3´

probe 5´ CCA AAT GAC AGC CCC TAT CAG GGT GG 3´

All primers were purchased from Sigma-Aldrich, Missouri, United States of America or Quiagen, Hilden, Germany. *IL-22* (QT00128324), *Isg15* (QT02274335), *Gata3* (QT001708282), *Tbx21* (QT00129822) Qiagen Assays (QuantiTect SYBR Green PCR Kits) were employed. RT-qPCR for *Gbp2* using Eva Green Dye (Biotium Inc., California, United States) was performed as described in (Stockinger et al., 2004).

Samples were analyzed in duplicates using an Eppendorf Real Plex cycler.

Cycling conditions: 15 minutes at 95°C followed by 45 cycles of 20 seconds, 95°C and 1 minute, 60°C.

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

5 Results

5.1 IL-22 responsiveness of colonic epithelial cell lines

To identify cell lines that would enable us to study the role of Tyk2 in IL-22 signalling using knock-down technology, we tested IL-22 responsiveness of the two murine colonic epithelial cell lines available in our lab, namely CMT93, a mouse rectum carcinoma cell line and MC38, a mouse adenocarcinoma cell line. Furthermore, we obtained two human cell lines, namely HT29, a colon carcinoma cell line, and Caco-2, an epithelial colorectal adenocarcinoma cell line.

5.1.1 Murine colonic epithelial cells

CMT93 and MC38 cells were stimulated with 5ng/ml or 10ng/ml IL-22 and Stat3 tyrosine phosphorylation (pY-Stat3) was analyzed by Western blot. As shown in Fig.3 we found constitutive pY-Stat3 in CMT93 cells with a slight increase after IL-22 stimulation. In contrast, pY-Stat3 was not detectable in MC38 cells. Therefore, CMT93 cells were used for further analysis.
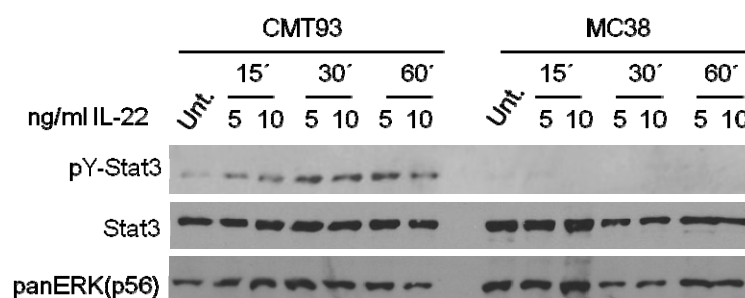
Fig.3: CMT93 and MC38 were treated with IL-22 (5 or 10ng/ml) for 15, 30 and 60 minutes, or left untreated (Unt). 10 µg of total protein extract were separated by 8% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with an antibody against Stat3 tyrosine 705 phosphorylation (pY-Stat3) and reprobed with a Stat3 antibody. Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa, 56kDa and at 85kDa. Depicted is the 56kDa band. Results are from one experiment.

We next treated CMT93 cells with IL-22 over a prolonged time period and analysed phosphorylation of Stat1 and Stat3, since both Stats can be activated by IL-22 (Sonnenberg et al., 2010). As positive control for pY-Stat1, IFNγ treatment was included. In addition, we tested whether CMT93 cells respond to IFNλ, since IFNλ also utilizes Tyk2 and Stat1 and should act on epithelial cells (Sommereyns et al., 2008). As shown in Fig.4A and 4B, neither Stat3 nor Stat1 phosphorylation could be detected after IL-22 stimulation for up to 4 hours. Surprisingly, also IFNλ treatment

did not result in detectable levels of tyrosine phosphorylated Stat1 (pY-Stat1). As expected, IFNγ induced strong pY-Stat1 and weak pY-Stat3.
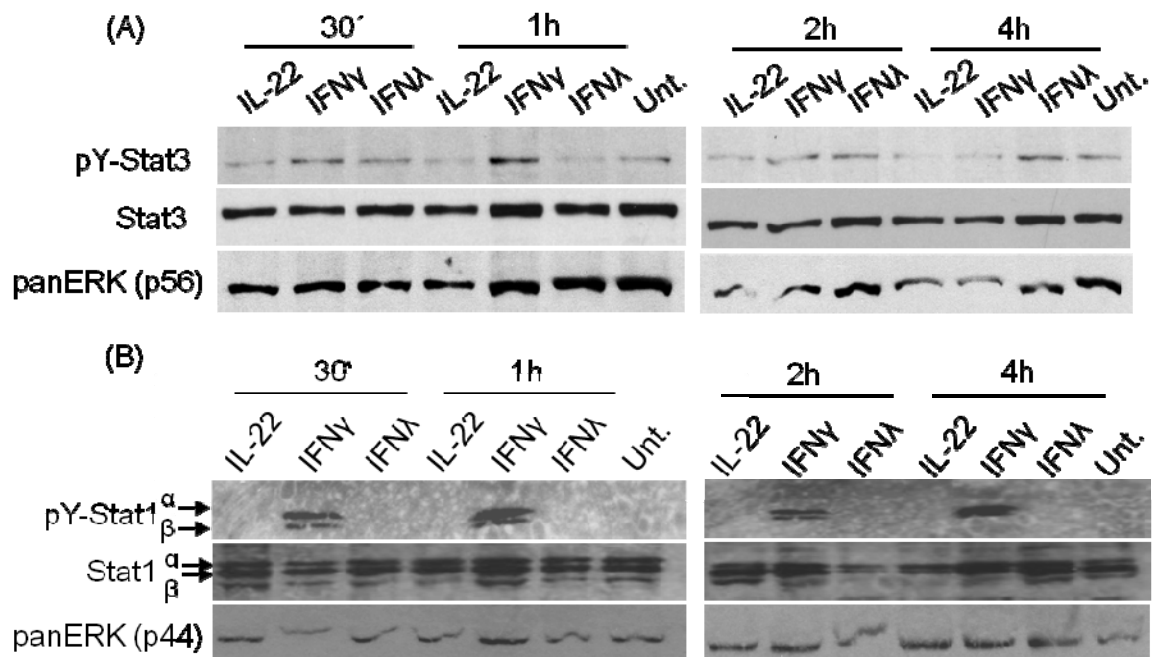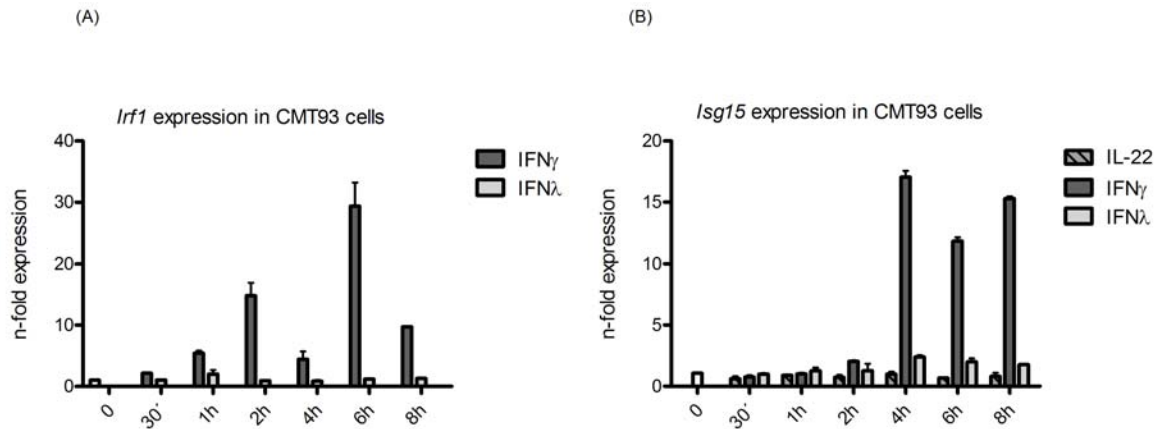

Fig.4: CMT93 cells were stimulated with IL-22 (10ng/ml), IFNγ (100U/ml) or IFNλ (100U/ml) for the times indicated or left untreated (Unt.). (A,B) 10 μg of total protein extract were separated by 8% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with (A) pY-Stat3 or (B) pY705-Stat1 antibody and reprobed with an (A) Stat3 or (B) Stat1 antibody. (A,B) Protein loading was controlled by reprobing with a panERK anibody which detects different ERK proteins at 42kDa, 44kDa 56kDa and 85kDa. Depicted is (A) the 56kDa and (B) the 44kDa band. Results are from one experiment.

To exclude too low Western blot sensitivity for pY-Stat3, we analyzed mRNA expression levels of IL-22 target genes, such as regenerating island derived 3 beta *RegIIIβ*, *RegIIIγ* and lipocalin2 (*Lcn2*) (Wolk et al., 2010). However, we could not detect expression of any of these genes before or after stimulation with IL-22 (data not shown). Consistent with undetectable pY-Stat1, IFNλ treatment did not result in an upregulation of the two classical IFN target genes IFN regulatory factor 1 (*Irf1*) and interferon stimulated gene 15 (*Isg15*) (Fig.5A and Fig.5B). However, IFNγ treatment, which served as positive control, clearly caused an increase in *Irf1* and *Isg15* mRNA levels.

(A) (B)

Irf1 expression in CMT93 cells

Isg15 expression in CMT93 cells

Fig.5: CMT93 cells were treated with IL-22 (10ng/ml), IFNγ (100U/ml) or IFNλ (100U/ml) for the times indicated, or left untreated (0). (A) *Irf1* and (B) *Isg15* mRNA expression was analyzed by RT-qPCR and data were normalized to ubiquitin-conjugating enzyme E2D2 (*Ube2d2*). Expression values from untreated CMT93 cells were set to 1. Mean values ± SD from duplicate measurements are depicted.

Taken together, Western blot and RT-qPCR data indicate that neither CMT93 nor MC38 cells are responsive to IL-22 and IFNλ, while they do respond to IFNγ.

5.1.2 Human colonic epithelial cells

To investigate IL-22 responsiveness of human colonic epithelial cell lines, we have chosen HT29 and Caco-2 cells as they have been previously described to express both IL-22 receptor subunits and to show Stat3 phosphorylation in response to IL-22 (Brand et al., 2006). Following the published conditions, cells were cultivated without FCS for 6 hours and stimulated with 10ng/ml or 100ng/ml human IL-22 (hIL-22) for 15 minutes up to 2 hours. pY-Stat3 and pY-Stat1 levels were analyzed by Western blot. As shown in Fig.6A, Stat3 phosphorylation clearly increased in HT29 cells whereas it was not detectable in Caco-2 cells. Furthermore, strongly induced Stat1 phosphorylation was found in HT29 cells, but not in Caco-2 cells (Fig.6B). Interestingly, phosphorylation of Stat1 and Stat3 was very transient in HT29 cells with a sharp decrease between 15 minutes and 1 hour of IL-22 treatment (Fig.6A and Fig.6B).
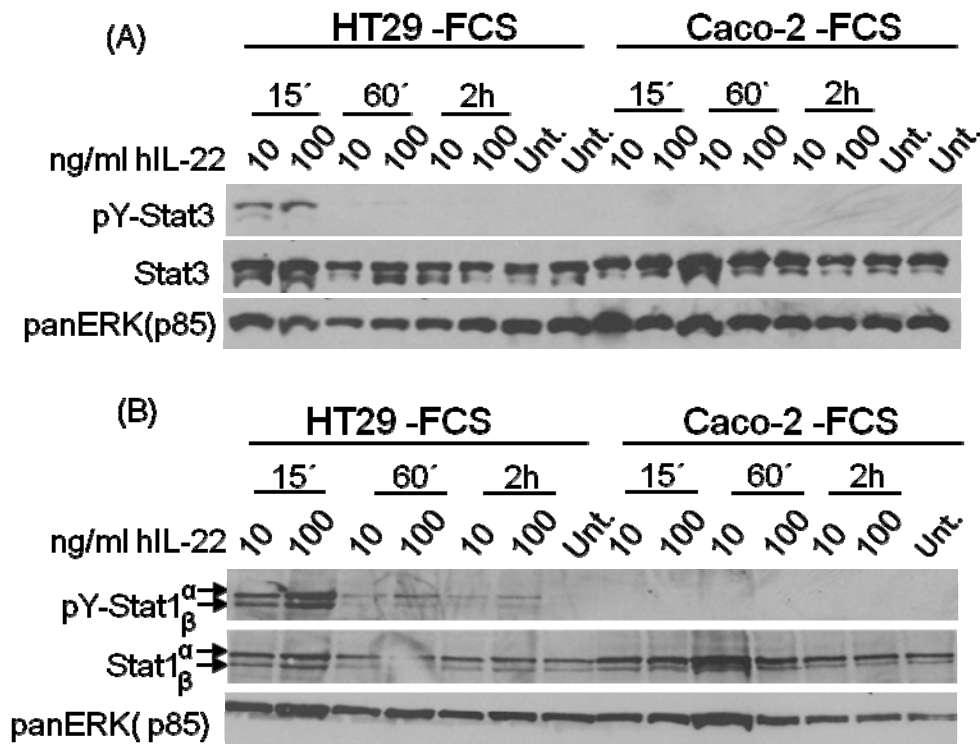
**Fig.6:** HT29 and Caco-2 cells were transferred to medium without FCS (-FCS) for 6h and stimulated with hIL-22 (10 or 100ng/ml) for the times indicated, or left untreated (Unt.). (A,B) 10 µg of total protein extract were separated by 8% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with (A) pY-Stat3 or (B) pY-Stat1 and reprobed with (A) Stat3 or (B) Stat1. (A,B) Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa 56kDa and 85kDa. Depicted is the 85kDa band. Results are from one experiment.

This experiment was repeated by cultivating HT29 cells with FCS containing medium. As shown in Fig.7A, hIL-22 stimulated HT29 cells show high pY-Stat3 after 15 and 30 minutes treatment, pY-Stat3 levels again decreased at later time points. Similar to the results obtained from the experiment with serum free medium (see Fig.6B), pY-Stat1 was increased upon hIL-22 stimulation (Fig.7B). Interestingly, Stat1/3 phosphorylation was slightly more persistent than in the experiment without FCS. For unknown reasons, mainly Stat1α was phosphorylated in this experiment.
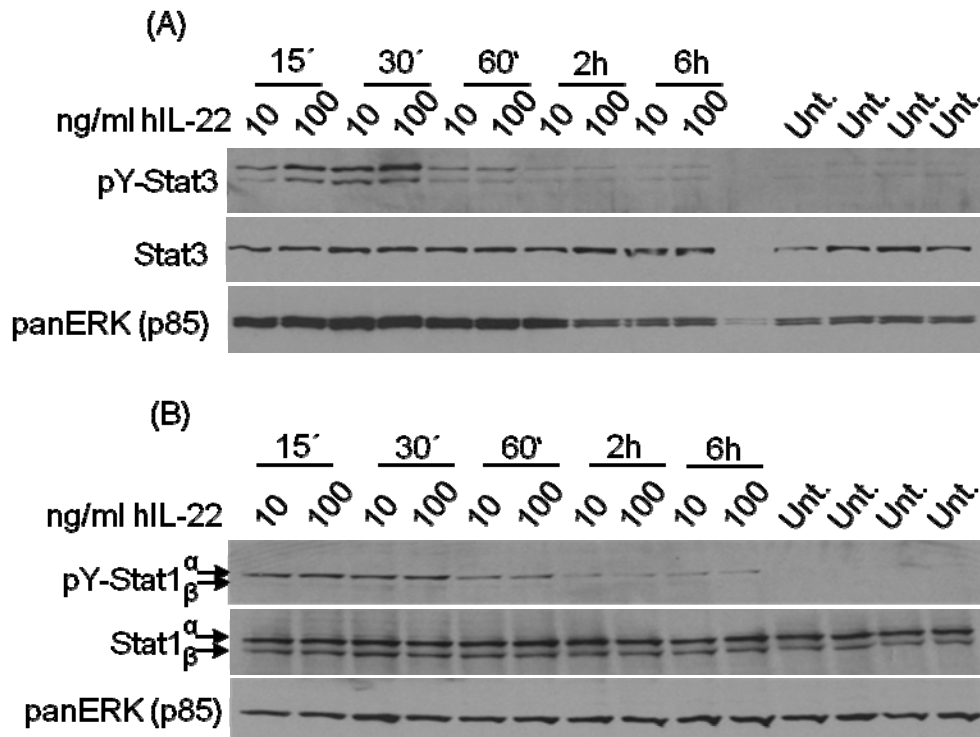
Fig.7: HT29 cells were treated with hIL-22 (10 or 100ng/ml) for the times indicated or left untreated (Unt.). 10µg of total cell extract were separated by 8% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with (A) pY-Stat3 or (B) pY-Stat1 and reprobed with (A) Stat3 or (B) Stat1 antibody. Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa 56kDa and 85kDa. Depicted is the 85kDa band. Results are from one experiment.

In summary, Western blot analyses indicate that HT29 cells respond to hIL-22, whereas Caco-2 cells are unresponsive. Stat3 and Stat1 phosphorylation was evident in IL-22 treated HT29 cells, independent of the presence of FCS during the experiment.

5.1.3 IL-6 responsiveness of CMT93 cells

IL-6 is one of the major Stat3 activating cytokine (Rose-John, 2012) and thus, we additionally investigated IL-6 responsiveness of CMT93 cells. Cells were stimulated with IL-6 and IFNγ for up to 2 hours. As depicted in Fig.8A, IL-6 stimulation slightly increased pY-Stat3 levels in CMT93 cells. However, pY-Stat3 after IL-6 treatment was weak compared to IFNγ induced pY-Stat3. It is known that IL-6 binding to its receptor, IL-6R, results in complex formation and activation of the IL-6R subunit gp130. Many cell types express the gp130 subunit, but lack IL-6R and consequently are unresponsive to IL-6 (Rose-John, 2012). Therefore, we stimulated CMT93 cells with IL-6 and a soluble IL-6R (sIL-6R) and used IFNγ, IFNλ and IL-10 as controls. In

addition, we used two different cell lysis buffers (Schindler buffer vs. RIPA buffer) to test if cell disruption by RIPA buffer results in increased sensitivity for pY-Stat3. Fig.8B shows equal pY-Stat3 levels in cells treated with IL-6 and IL-6/sIL-6R and untreated cells. Different basal pY-Stat3 levels compared to Fig.8A might be due to a new charge of antibody used. No obvious difference between the two lysis buffers could be seen (Fig.8B). Furthermore, pY-Stat3 was not increased after stimulation with IL-22, IFNγ, IFNλ or IL-10.
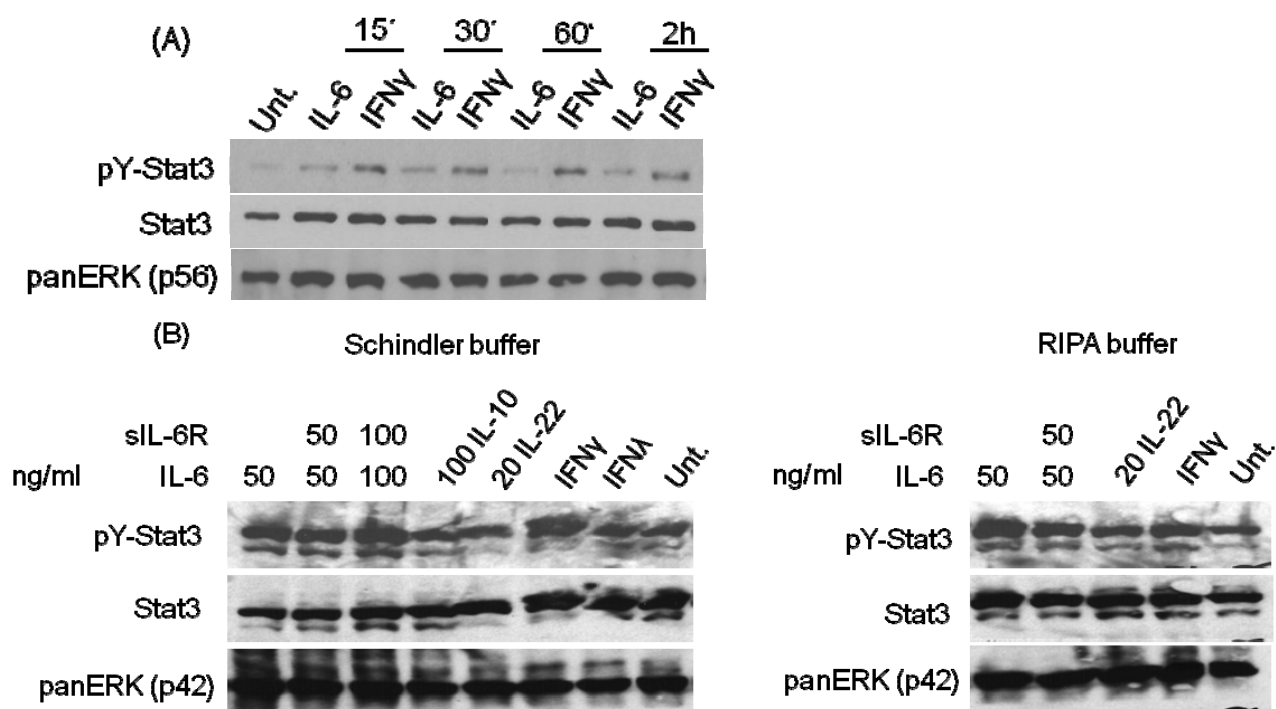
Fig.8: (A) CMT93 cells were stimulated with IL-6 (50ng/ml) or IFNγ (100U/ml) for 15 minutes up to 2 hours, or left untreated (Unt). (B) CMT93 cells were stimulated for 15 minutes with IL-6/sIL-6R at the concentrations indicated, IL-22 (20ngml) and IFNγ (100U/ml), or were left untreated (Unt.). Protein extraction was performed as indicated with either Schindler buffer or RIPA buffer. (A,B) 10µg of total protein extract were separated by 8% SDS-PAGE and subjected to Western blot analysis. Membranes were probed with pY-Stat3 and reprobed with Stat3. Protein loading was controlled by reprobing with panERK, which detects different ERK proteins at 42kDa, 44kDa 56kDa and 85kDa. Depicted are (A) the 56kDa and (B) the 42kDa band. Results are representative for two independent experiments.

In addition to CMT93 cells, we have also analysed primary colonic epithelial cells derived from WT mice before and after stimulation with IL-6 and IL-6/sIL-6R for 15 minutes. However, most likely due to problems with cell purity, viability and cell counting (i.e. too many cell aggregates) we obtained very inconsistent results (data not shown).

5.2 Establishment of a stable cell culture system for primary colonic epithelial cells
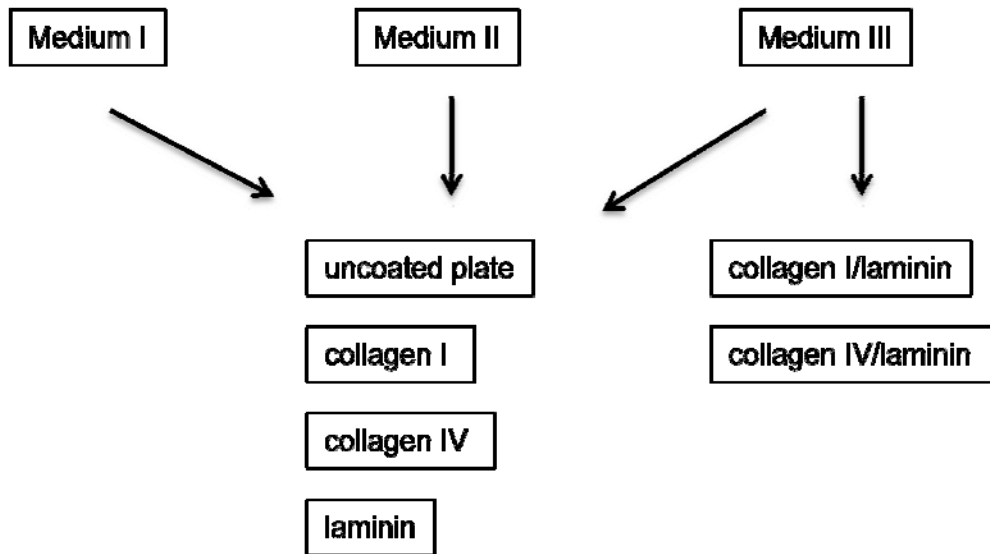
To study signalling in more detail, i.e. kinetics and mRNA induction, we aimed to optimize and prolong the cultivation of primary colonic epithelial cells. As basis for our experiments, two protocols were used to test distinct cultivation methods (Booth et al., 1995; Yamada et al., 2009). These mainly differ in reagents used for coating of tissue culture plates and cell culture media. We tested three different media on four different coating substrates and monitored cell adherence and cell survival microscopically. Primary colonic epithelial cells isolated from 8 week old mice were used. The isolation method was optimized and kept consistent. First, we plated cells in an uncoated 6-well plate in three different media (Table 1) and monitored cells for 3 days. When cultivated with medium I or medium II no adherent cells could be observed and many shrunken cells were visible after a few hours. Although cultivation with medium III did not improve cell adherence, cells appeared more viable and did not shrink.

We next explored cell adherence by using 3 different coating reagents plates (listed in Table 2). Cells were cultivated with medium I, medium II or medium III for 3 days.

Table 1: Media for primary colonic epithelial cells

Medium I	Medium II	Medium III
DMEM 5% FCS Pen/Strep (100µg/ml and 100U/ml)	DMEM 10% FCS L-Glutamine (2mM) Pen/Strep (100µg/ml and 100U/ml)	DMEM/HAMF´12(1:1) Insulin (5µg/ml) Dexamethasone (50nM/ml) Transferrin (5µg/ml) EGF (1µg/ml) Hepes (20mM) L-Glutamine (2mM) 2% FCS D-Glucose (22µg/ml) Pen/Strep (100µg/ml and 100U/ml)

Table 2: Coating plates for primary colonic epithelial cells

Medium I	Medium II	Medium III

```
                          uncoated plate        collagen I/laminin

                          collagen I            collagen IV/laminin

                          collagen IV

                          laminin
```

Cultivation with medium I and medium II on laminin coated plates did not result in any adherent cells. In contrast, plating cells in medium I or medium II on collagen I- or IV-coated plates resulted in a few single adherent cells after one day of cultivation. As depicted in Fig.9A-C, colonic epithelial cells cultivated with medium III on collagen I combined with laminin (collagen I/laminin) coated plates resulted in almost no shrunken cells and adherence of few single cells (see arrows Fig. 9A-C). The best adherence of cells was achieved when we plated cells with medium III on collagen IV/laminin coated plates (Fig.10A-C). In addition, epithelial cells formed "crypte islands" (see arrow Fig.10B), which is a typical structure for epithelial cells, and many cell aggregates (see arrow Fig.10A) were observed. Again cells were only observed visually, as cluster formation did not allow reliable cell counting.

In addition to these coating methods, we also tested whether the use of neonatal mice for the isolation of colonic epithelial cells improves the yield and viability of cells. However, we did not see any differences to cells derived from 8 week old mice when cells were cultivated under the so far best conditions (medium III, collagen IV/laminin coated plates).

To explore further cultivation methods, we tested lysed CW1 cells as feeder layer for colonic epithelial cells. Freshly isolated cells were plated on a monolayer of CW1 cells in medium III and cell adherence and survival was monitored (Fig.11A-C). High numbers of adherent colonic epithelial cells aggregates (arrow Fig.11B), the typically "crypte islands" (arrow Fig.11A) and only few shrunken cells were obtained.

Taken together, colonic epithelial cells cultivated with medium III either in plates coated with collagen IV/laminin or on feeder layer cells gave the best results with respect to cell morphology and adherence. However, this method is still not usable for our purpose since the amount of cells obtained was by far to low.
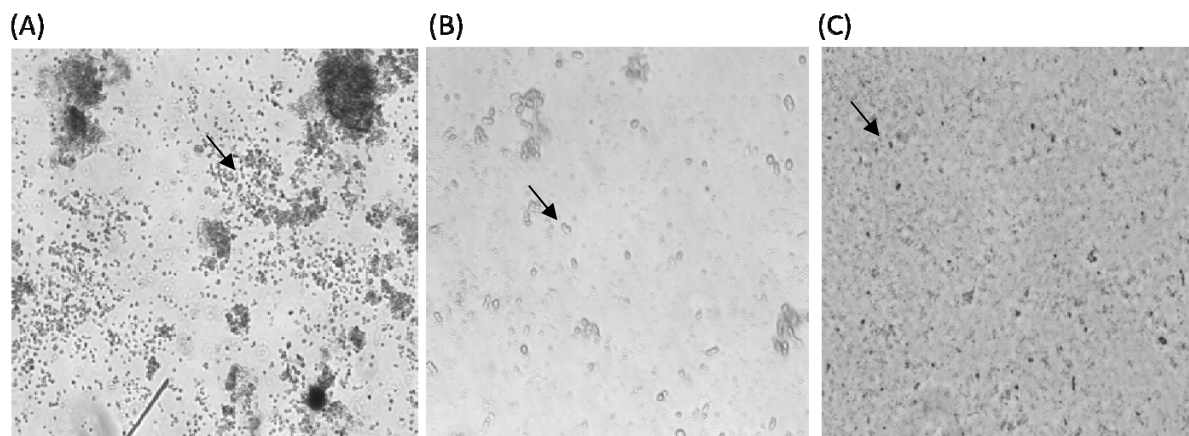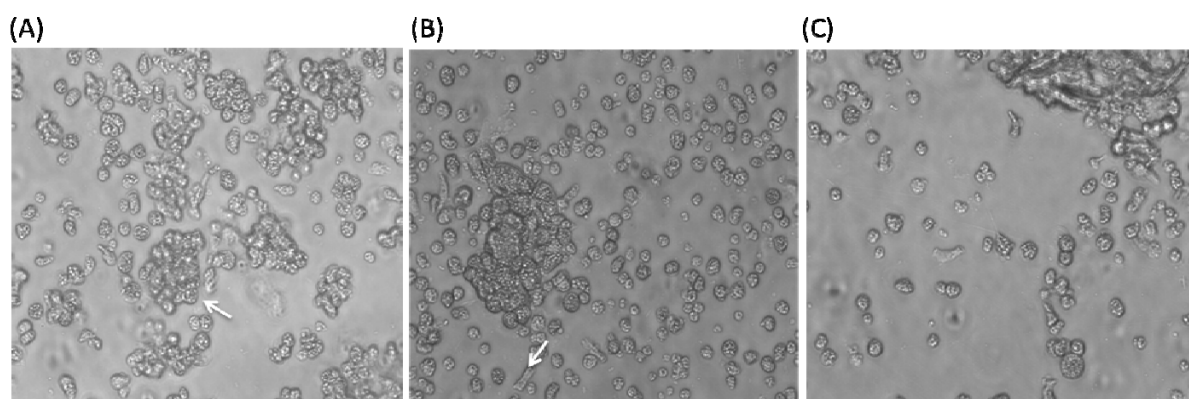

Fig. 9: Colonic epithelial cells cultivated in medium III on collagen I/laminin coated 12-well plates for one day. Three representative pictures were taken at (A,C) 10x or (B) 20x magnification. Arrows, see text.

Fig. 10: Colonic epithelial cells cultivated in medium III on collagen IV/laminin coated 12-well plates for one day. Three representative pictures were taken at 40x magnification. Arrows, see text.
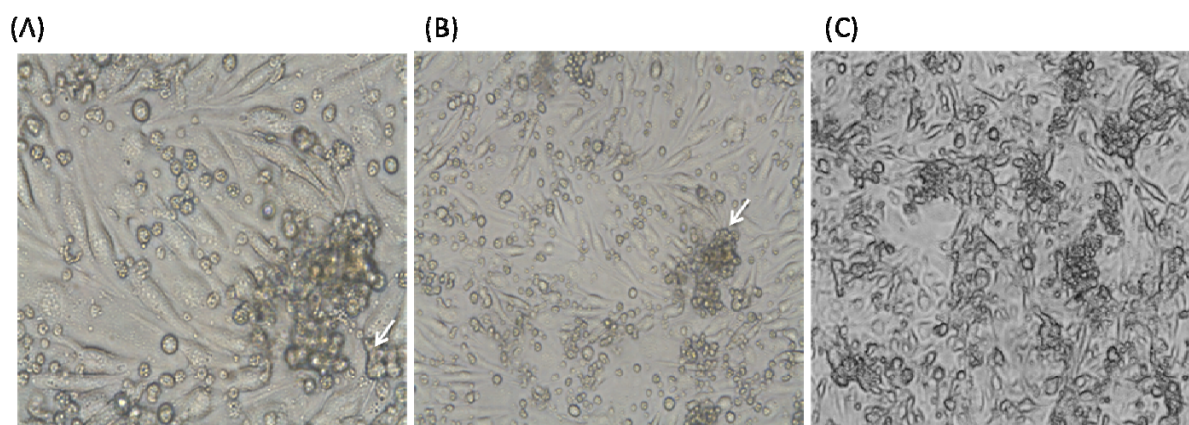
Fig.11: Colonic epithelial cells were cultivated in medium III on CW1 cells for one day. Three representative pictures for each coating method were taken at (A) 40x or (B,C) 20x magnification. Arrows, see text.

5.3 IL-27 responsiveness in distinct immune cells

IL-27 acts on several different cell types (Hall et al., 2012) by activating Stat1 and Stat3-5. Stat1 and Stat3 are required for target gene induction, whereas Stat4 and Stat5 may be activated cell type specifically with unknown biological functions in IL-27 responses. To investigate the role of Tyk2 in IL-27 signalling, we have chosen macrophages and NK cells as these cell types are in the main focus of our research. T cells were included in the analysis, because IL-27 signalling is best described in various T cell subsets. Macrophages, T cells and NK cells derived from WT, Tyk2$^{-/-}$ and Tyk2^{K923E} mice were analysed for IL-27 mediated phosphorylation of Stat proteins and the induction of target genes.

5.3.1 Macrophages

We used bone marrow derived macrophages (BMMΦ) from Tyk2$^{-/-}$, Tyk2^{K923E} and WT mice and investigated IL-27 induced Stat1 and Stat3 activation. IFNγ and IL-10 were used as positive controls for Stat1 and Stat3 activation, respectively. Contrary to published data (Iyer et al., 2010), Western blot analyses of pY-Stat1 (Fig.12A) did not show Stat1 phosphorylation in WT macrophages after IL-27 stimulation. As expected, IFNγ stimulation clearly increased pY-Stat1 levels in WT, Tyk2$^{-/-}$ and Tyk2^{K923E} cells. Compared to WT, Tyk2$^{-/-}$ and Tyk2^{K923E} cells showed slightly lower pY-Stat1 levels. Although less pronounced, this is consistent with previous reports showing reduced Stat1 protein levels and reduced IFNγ induced Stat1 phosphorylation in the absence of Tyk2 or presence of kinase inactive Tyk2 (Karaghiosoff et al., 2000; Prchal-Murphy et al., 2012). Stat3 is the main signal transducer for IL-10, but pY-Stat1 is also induced (Ouyang et al., 2011) and was lower in Tyk2$^{-/-}$ and Tyk2^{K923E} than in WT cells.

As shown in Fig.12B, pY-Stat3 could neither be detected in untreated macrophages nor after IL-27 treatment. However, after IL-10 and IFNγ stimulation pY-Stat3 was readily observable. No gross difference was found between WT, Tyk2$^{-/-}$ and Tyk2^{K923E} cells. The slight reduction in Tyk2^{K923E} compared to WT and Tyk2$^{-/-}$ cells after stimulation with 10ng/ml IL-10 is most likely due to unequal loading.
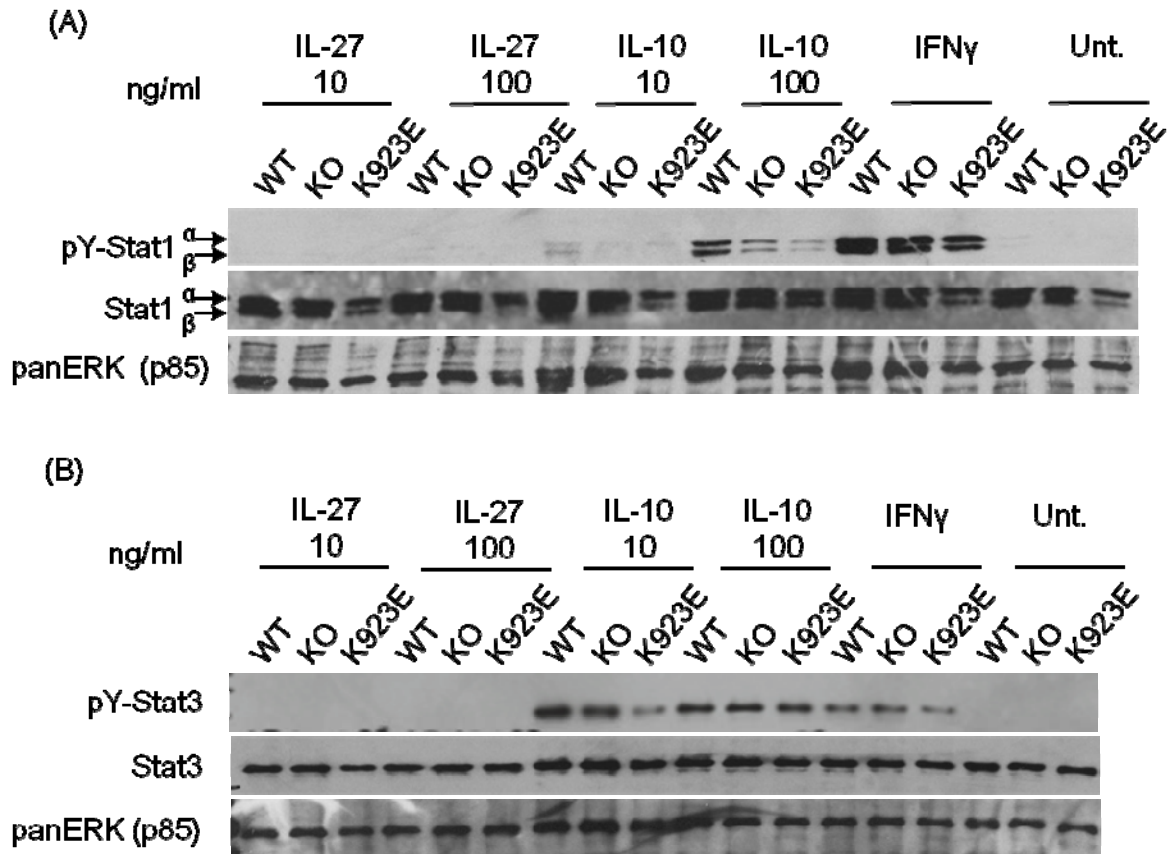
(A)

(B)

Fig.12: BMMΦ from WT, Tyk2$^{-/-}$ and Tyk2^{K923E} were stimulated with IL-27 (10,100ng/ml), IL-10 (10ng/ml) or IFNγ (100U/ml) for 20 minutes, or were left untreated (Unt). Protein extracts were separated with SDS-PAGE (8%, 10µg of total cell extract per lane). Membranes were probed with (A) pY-Stat1 (B) pY-Stat3 and reprobed with (A) Stat1 and (B) Stat3 antibody. (A,B) Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa, 56kDa and 85kDa. Depicted is the 85kDa band. Results are from one experiment.

To also monitor Stat1 and Stat3 DNA binding activity, we used EMSA analysis to detect binding to GAS containing oligonucleotides after IL-27 treatment. As expected, IFNγ stimulation led to strong Stat1 homodimer binding on GAS sites (Fig.13). In line with the Western blot results, we could not detect Stat1 and/or Stat3 DNA binding after IL-27 stimulation. Surprisingly, Stat3 binding could also not be detected after IL-10 treatment. As Stat3:Stat3 and Stat1:Stat3 dimers were well detectable after IL-10 and IFNγ stimulation when radioactive, instead of infrared-dye (IRDye)-labelled oligonucleotides were used, we assume that either the dye disturbs Stat3 binding or that the sensitivity of the EMSA is too low.
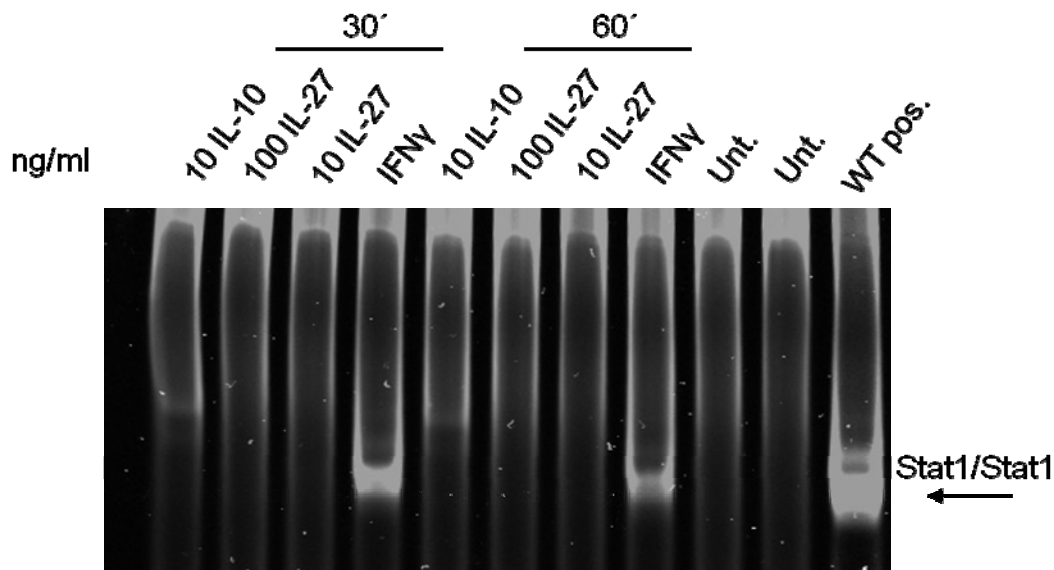
Fig.13: Macrophages from C57BL/6 mice were treated with IL-27 (10 or 100ng/ml), IL-10 (10ng/ml) or IFNγ (100U/ml) for up to 1h or left untreated (Unt). 10µg protein extract were subjected to EMSA, using a 5´-IRDye 800-labeled oligonucleotide containing a GAS consensus site. As positive control macrophages stimulated with IFNγ (100U/ml) for 15 minutes from an independent experiment were used (WT pos.).The picture was created with an Odyssey near-infrared imager. Results are from one experiment.

5.3.2 T cells

T cells were stimulated with IL-27 for up to 2 hours, IFNβ treatment was used as positive control. pY-Stat1, pY-Stat3 and pY-Stat4 levels were analyzed by Western blot. As shown in Fig.14A, IL-27 strongly induced pY-Stat1 in T cells from WT, Tyk2^{K923E} and Tyk2$^{\Delta CMV}$ (i.e. a different mouse model for complete deleted Tyk2, see Material and Methods) mice. pY-Stat1 levels in Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} were lower than in WT T cells. The minor differences in induced pY-Stat1 between Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} were not consistently observed. As expected, stimulation with IFNβ resulted in highly increased pY-Stat1 levels in all 3 genotypes and, consistent with our previous report (Prchal-Murphy et al., 2012) were lower in Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} than in WT cells.

As depicted in Fig.14B, we also observed Stat3 phosphorylation after IL-27 stimulation in WT, Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} T cells. Again, no gross differences between the genotypes were found. Stat4 was not induced by IL-27 in any of the genotypes (Fig.14C), but clearly induced by IFNβ. Consistent with our previous report, IFNβ induced pY-Stat4 levels were clearly higher in WT than in Tyk2$^{\Delta CMV}$ and Tyk2^{K932E} T cells (Prchal-Murphy et al., 2012).
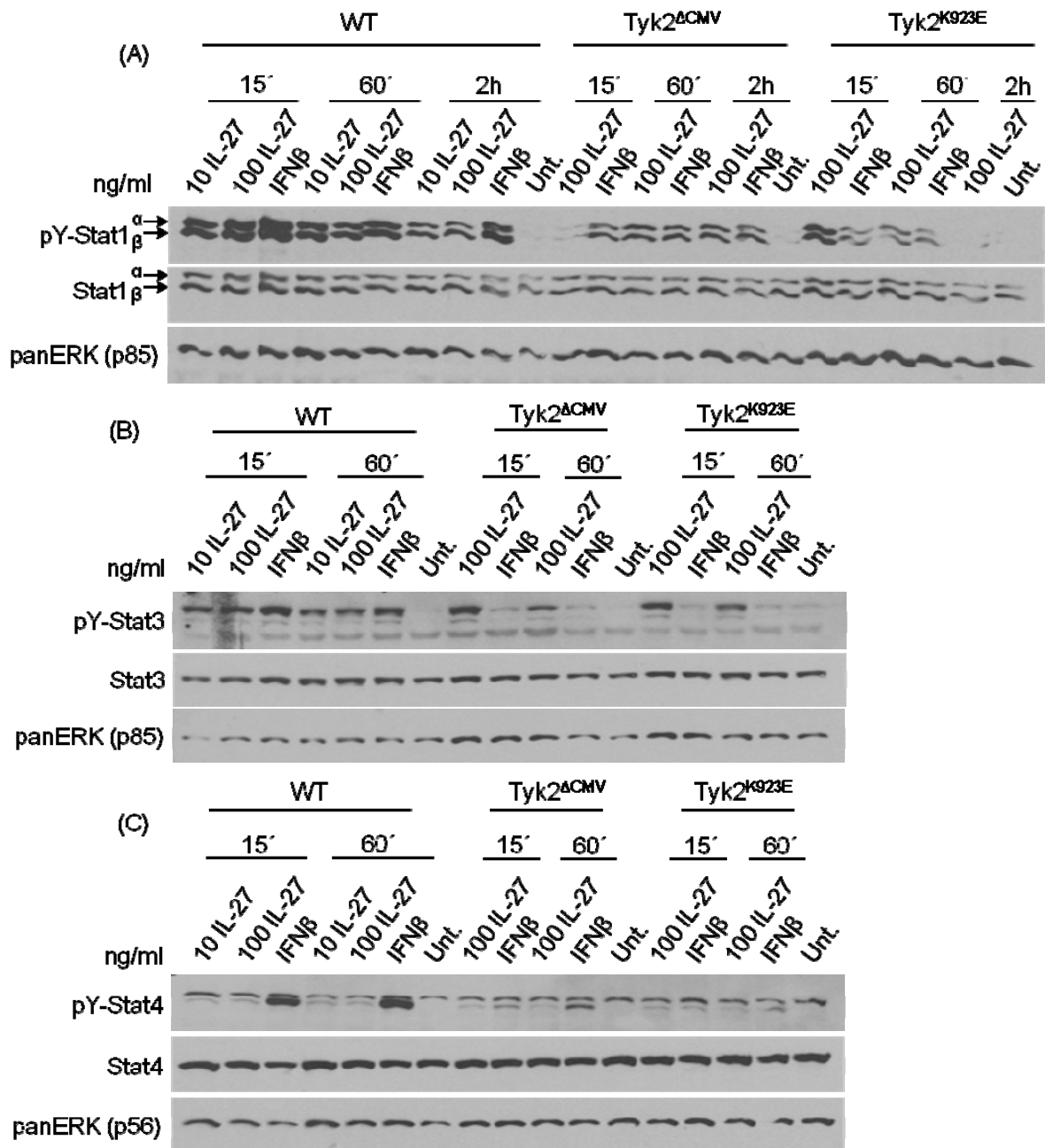
Fig.14: T cells were purified from WT, Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} splenocytes by negative selection for CD19 using MACS and cultured in the presence of αCD3 (0,5µg/µl) and αCD28 (1,5µg/µl) for 3 days. Cells were stimulated with IL-27 (10 or 100ng/ml) or IFNβ (100U/ml) for the times indicated, or left untreated (Unt.). Cells were lysed, separated with SDS-PAGE (8%, 10µg total cell extract per lane) and subjected to Western blot analysis. Membranes were probed with (A) pY-Stat1 (B) pY-Stat3 or (C) pY-Stat4 and reprobed with (A) Stat1 (B) Stat3 or (C) Stat4 antibody. Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa, 56kDa and 85kDa. Depicted is (A, B) the 85kDa or (C) the 56kDa band. Results are representative for three independent experiments.

The same samples as in Fig.14 were analyzed by EMSA, except that only WT cells were used and IFNγ instead of IFNβ treated cells served as controls. As shown in Fig.15, Stat1 dimer binding could neither be detected after IL-27 nor after IFNγ stimulation.
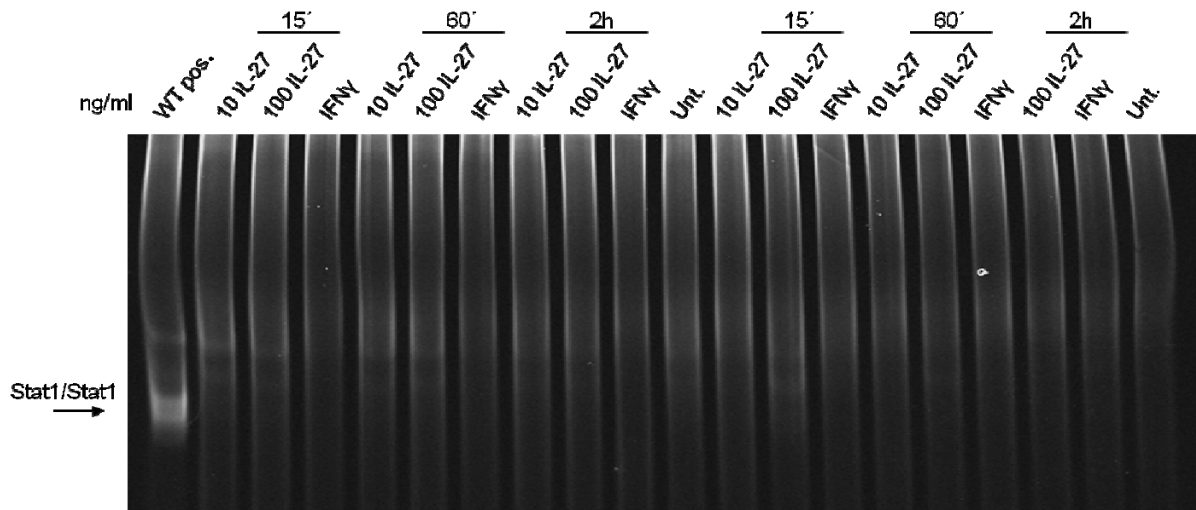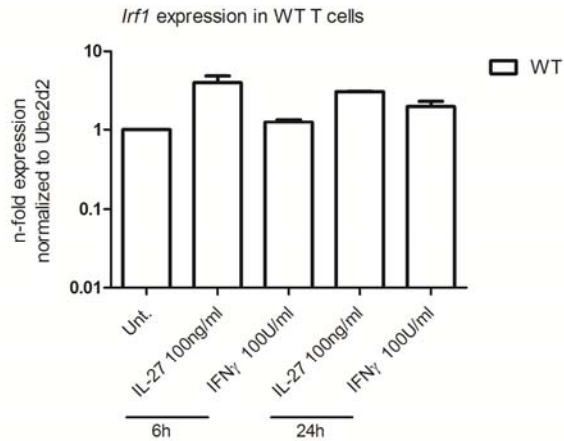
Fig.15: T cells were cultivated as described in the legend to Fig.14. Cells were stimulated with IL-27 (10,100ng/ml) or (IFNγ) (100U/ml) for the times indicated, or left untreated (Unt.). 10µg of total protein extracts were subjected to EMSA, using a 5´-IRDye 800-labelled oligonucleotide containing a GAS consensus sequence. As positive control macrophages were stimulated with IFNγ (100U/ml) for 15 minutes (WT pos.).The picture was created with an Odyssey near-infrared imager. Results are from one experiment.

We next analysed IL-27 mediated gene regulation. Best described is the upregulation of *T-bet* (*Tbx21*) and *Il-10* and the downregulation of *Gata-3* (Hunter and Kastelein, 2012). In addition, several IFN responsive genes, including *Gbp2*, *Irf1* and *Ip-10* (*Cxcl10*), have recently been shown to be regulated by IL-27 in T cells (Hirahara et al., 2012). T cells derived from WT and Tyk2$^{-/-}$ mice were stimulated with IL-27, IFNγ or IFNβ for 6 and 24 hours. *T-bet*, *Gbp2* and *Ip-10* were undetectable by RT-qPCR before and after IL-27, IFNγ or IFNβ treatment in WT and Tyk2$^{-/-}$ T cells (data not shown).

Irf1 expression was slightly increased after IL-27 in WT T cells (Fig.16A), however, this was not observed in an independent experiment (Fig.16B). As expected (Karaghiosoff et al., 2000), we found reduced *Irf1* mRNA expression in unstimulated Tyk2$^{-/-}$ compared to WT cells (Fig.16B). *Irf1* was not upregulated by IL-27 or IFNγ in WT cells and only a slight increase was seen in Tyk2$^{-/-}$ cells after 6 hours IL-27 (100ng/ml) stimulation. Surprisingly, *Irf1* mRNA was not upregulated by IFNβ in WT cells (Fig.16B), despite the strong phosphorylation of Stat1 observed by Western blot analysis (Fig.14A).
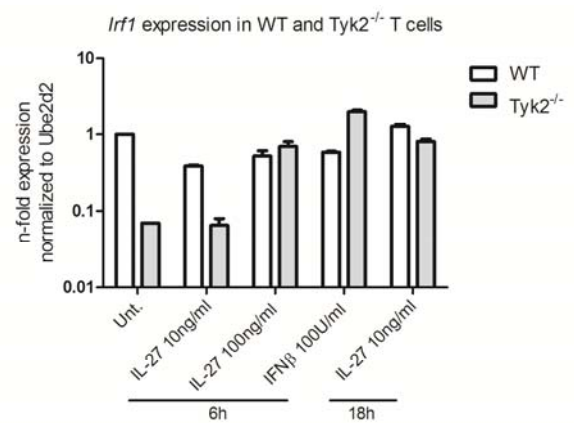
Fig.16: T cells were cultivated as described in the legend to Fig. 14. (A) WT or (B) WT and Tyk2$^{-/-}$ T cells were stimulated with (A) IFNγ (100U/ml) or IL-27 (100ng/ml) and (B) IL-27 (10,100ng/ml) or IFNβ (100U/ml) for the times indicated. (A,B) Total RNA was isolated and *Irf1* mRNA expression determined by RT-qPCR analysis. Values were normalized to *Ube2d2* and expression levels in untreated WT cells were set to 1. Mean values ± SD from duplicate measurements are shown.

5.3.3 NK cells

We stimulated *in vitro* expanded NK cells from WT, Tyk2$^{\Delta CMV}$ and Tyk2^{K923E} mice with IL-27 for up to 2 hours and analyzed Stat1 phosphorylation. As positive control NK cells were stimulated with IFNβ. As shown in Fig.17A and 17B, pY-Stat1 was not induced by IL-27 in NK cells in any of the genotypes. Minor differences in pY-Stat1 levels are most likely due to unequal loading. As expected, IFNβ strongly induced pY-Stat1 in cells from each genotype (Fig.17A and 17B). Again consistent with our previous report (Prchal-Murphy et al., 2012), it was lower in Tyk2$^{-/-}$ and Tyk2^{K923E} compared to WT cells.
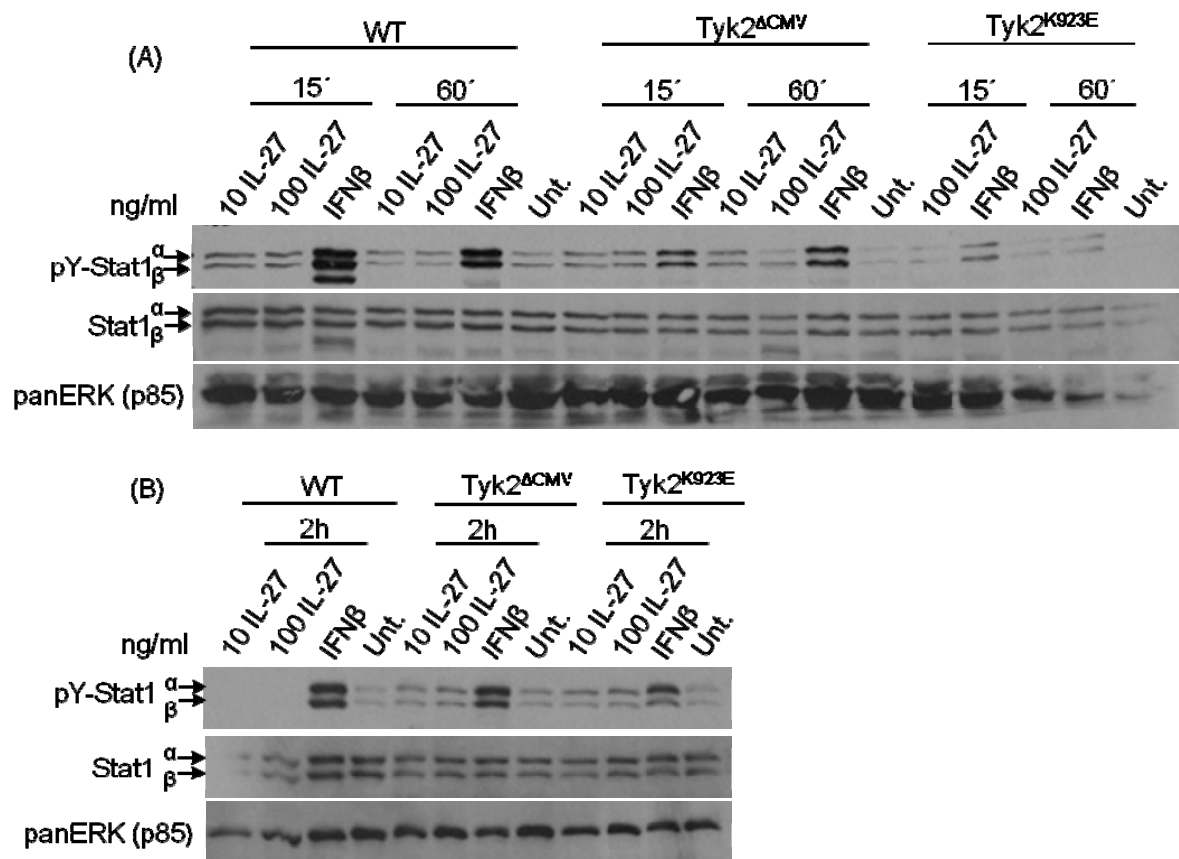
Fig.17: NK/NKT cells were purified from WT, Tyk2[ΔCMV] and Tyk2[K923E] splenocytes by positive selection for DX5 using MACS and cultured in the presence of IL-2 (5000 U/ml) for 7 days. Cells were stimulated with IL-27 (10 or 100ng/ml) or IFNβ (100U/ml) for (A) up to 1 hour or (B) 2 hours or (A,B) left untreated (Unt.). At indicated times cells were lysed, proteins separated with SDS-PAGE (8%, 10µg total cell extract per lane) and subjected to Western blot analysis. Membranes were probed with pY-Stat1 and reprobed with Stat1 antibody. Protein loading was controlled by reprobing with a panERK antibody, which detects different ERK proteins at 42kDa, 44kDa, 56kDa and 85kDa. Depicted is the 85kDa band. Results are representative for two independent experiments.

We next analysed DNA binding of Stat1 homo- and heterodimers by using EMSA. WT NK cells were used for these experiments and WT BMMΦ stimulated with IFNγ for 15 minutes were included as positive control. Consistent with the Western blot analysis, we could not detect IL-27 induced Stat1 DNA binding activity in NK cells (Fig.18). Interestingly, Stat1 DNA binding was also not observed after IFNγ treatment. This is consistent with undetectable pY-Stat1 in IFNγ treated NK cells (not shown) and indicates that *in vitro* expanded NK cells are unresponsive to IFNγ.
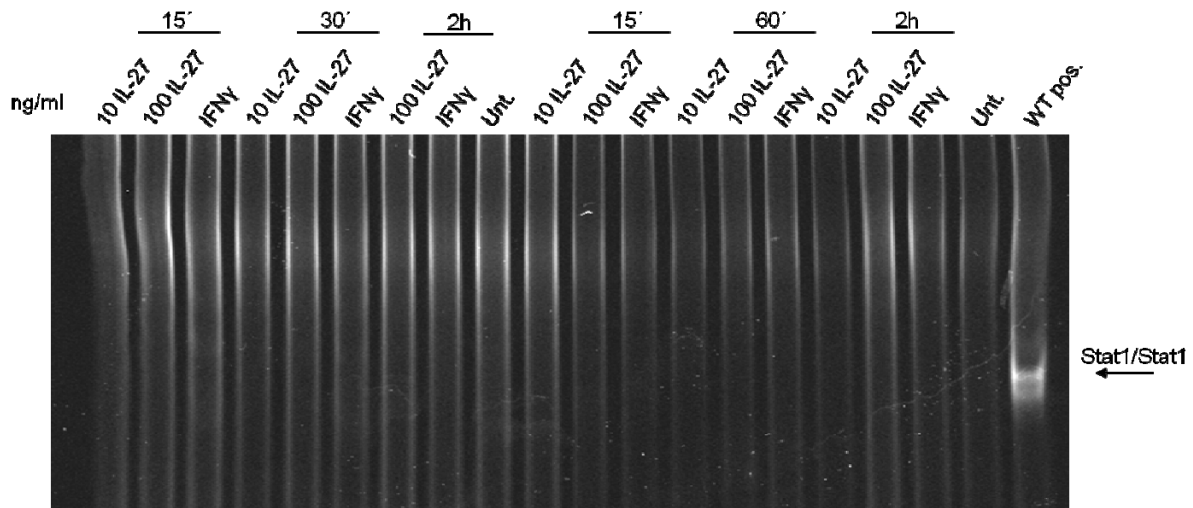
Fig.18 NK/NKT cells were cultivated as described in the legend to Fig.17. Cells were stimulated with IL-27 (10,100ng/ml) or IFNγ (100U/ml) for the times indicated or left untreated (Unt.). 10µg of total protein extract were subjected to EMSA, using a 5′-IRDye 800- labeled oligonucleotide containing a GAS consensus site. As positive control macrophages were stimulated with IFNγ (100U/ml) for 15 minutes (WT pos.). The picture was created with the Odyssey near-infrared imager. Results are from one experiment.

Similar to T cells, *T-bet*, *Gbp2* and *Ip-10* mRNAs were not detectable in NK cells irrespective of the treatment (data not shown). Furthermore, IL-27 treatment did not alter *Irf1* mRNA expression in WT or Tyk2$^{-/-}$ NK cells (Fig.19A and 19B). In contrast to T cells, we did not see any differences in basal *Irf1* mRNA levels between untreated WT and Tyk2$^{-/-}$ NK cells.

Taken together, our results indicate that NK/NKT cells *in vitro* expanded in the presence of IL-2 do not respond to IL-27.
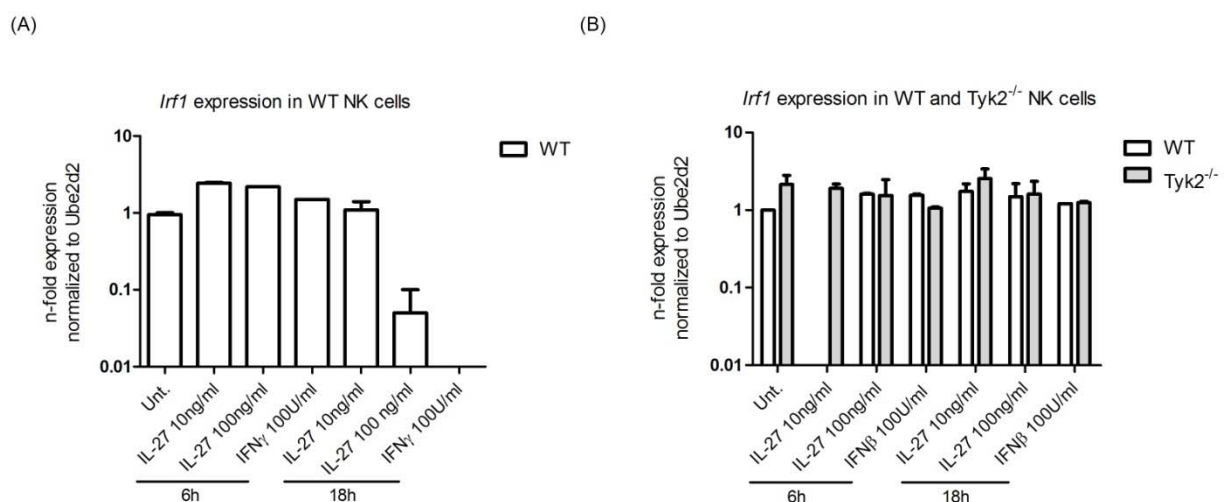
(A) (B)

Fig.19: NK/NKT cells were cultivated as described in the legend to Fig. 17. Cells were stimulated with IL-27 (10,100ng/ml) for the times indicated or left untreated (Unt.). (A) IFNγ (100U/ml) or (B) IFNβ (100U/ml) was used as a control. (A,B) RT-qPCR analysis of *Irf1* mRNA expression in NK cells from (A) WT mice or (B) WT and Tyk2$^{-/-}$ mice. Values were normalized to Ube2d2. Expression levels in untreated WT cells were set to 1. Mean values ± SD from cells derived from 3 mice per genotype are depicted. Results are from one experiment.

6 Discussion

The first aim of the study was to find a cell culture system that allows us to test the impact of Tyk2 on IL-22 signalling. IL-22 responsiveness was analysed in murine and human colonic epithelial cell lines and, as an alternative approach, conditions for the cultivation of primary epithelial cells were tested. The second aim of this work was to investigate whether absence of Tyk2 or presence of a kinase inactive Tyk2 affects IL-27 response in distinct primary cell types.

From the two murine (CMT93 and MC28) and two human (HT29 and Caco-2) epithelial cell lines tested, only HT29 showed IL-22 responsiveness. These findings were quite surprising, as epithelial cells from the digestive system, including the colon, were described to express both IL-22 receptor chains, IL-22R1 and IL-10R2 (Wolk et al., 2004). However, cells may loose receptor chain expression during the immortalisation process. IL-22 responsiveness of HT29 and Caco-2 cells was published previously (Brand et al., 2006), but could not be confirmed for Caco-2 cells in our study. Although the reason for this discrepancy is unclear, it may be due to different subclones used or to the loss of receptors upon extensive consecutive passage. In HT29 cells, IL-22 rapidly induced transient phosphorylation of Stat1 and Stat3 and hence, they can be further used to study the involvement of human Tyk2 in IL-22 induced Stat activation using knock-down technology.

Several different protocols have been described for long-term epithelial cell cultures from the large intestine (colon), using different isolation and cultivation methods, which mainly differ in tissue dissociation techniques (i.e. using different proteolytic enzymes) and culture media (Chopra et al., 2010). In contrast to the cultivation of intestinal fragments as intact organoids (Flint et al., 1994; Macartney et al., 2000; Yamada et al., 2009), we aimed at cultivating EDTA dissociated primary colonic epithelial cells. Cells were grown on coated dishes (collagen I or IV and combinations with laminin), as it was reported previously for the cultivation of small intestinal epithelial cells (Booth et al., 1995). As basis for our experiments we used cultivation methods established by Orest Kuzyk (Diploma thesis 2011, Thomas Decker laboratory, MFPL Vienna). We included more sedimentation steps during isolation, added specific supplements during cultivation and tested different coating reagents. Although we could achieve growth of some adherent cells, serious clumping made quantification of cells impossible. Furthermore, the yield of cells was too low to allow

detailed experiments. We have also cultivated cells on CW1 feeder layer cells in analogy to previous reports describing the cultivation of colon epithelial cells on feeder layer cells or in the presence of fibroblasts (Ren et al., 2011; Sevignani et al., 1998). However, we encountered similar problems as without feeder cells. A future possibility would be to apply a collagenase digestion in addition to EDTA treatment, similar to what has been used previously for epithelial cells from colons of suckling rats. Another possibility would be to isolate intestinal stem cells using specific stem cell markers and subsequently differentiate them into epithelial cells (Chopra et al., 2010).

IL-27 acts on specific cell types and signals mainly trough Stat1 and Stat3, but activation of Stat4 and Stat5 has also been reported. *T-bet, Gata3,* and *IL-10* are well known IL-27 target genes, although transcriptional responses to IL-27 are still ill defined (Hall et al., 2012). IL-27 responses are best described in CD4$^+$ T cells, but other cell types including macrophages and NK cells, have been reported as IL-27 responsive (Hall et al., 2012). Surprisingly, we found that BMMΦ are unresponsive to IL-27. This is in contrast to a previous study demonstrating IL-27 mediated upregulation of IL-10 mRNA and protein in BMMΦ (Iyer et al., 2010) and monocyte-derived macrophages (Imamichi et al., 2012). Consistent with our results, two studies reported low level expression of the IL-27 receptor component IL-27Rα/WSX-1 in BMMΦ (Kalliolias and Ivashkiv, 2008; Ruckerl et al., 2006) and one study showed only weak IL-27 mediated Stat1 and Stat3 activation in non-activated BMMΦ and resident peritoneal macrophages (Kalliolias and Ivashkiv, 2008). IL-27 induced Stat3 activation and synergism with LPS to induced nitric oxide has been shown in thioglycollate elicited peritoneal (i.e. inflammatory) macrophages (Holscher et al., 2005; Shimizu et al., 2012). Furthermore, LPS treatment enhances IL-27Rα/WSX-1 expression in BMMΦ (Holscher et al., 2005; Ruckerl et al., 2006). Alternatively activated macrophages show even higher expression of IL-27Rα/WSX-1 than LPS activated BMMΦ and IL-27 induced Stat3 activation was readily detected (Ruckerl et al., 2006). Taken together, our results support the notion that non-activated BMMΦ do not respond to IL-27 and that IL-27 responsiveness strongly depends on the macrophage activation status.

IL-27 response was also investigated in T cells from Tyk2$^{-/-}$, Tyk2^{K923E} and WT mice. IL-27 mediated Stat1 and Stat3 phosphorylation was evident in all three genotypes, but Stat4 activation could not be detected. No gross differences in Stat1 and Stat3

phosphorylation were found between WT, Tyk2$^{-/-}$ and Tyk2^{K923E} T cells, indicating that Tyk2 is not required for IL-27 signalling and that the presence of kinase inactive Tyk2 does not affect IL-27 responsiveness. Surprisingly, we did not detect upregulation of any of the known IL-27 target genes in T cells, including *T-bet*, *Ip-10*, *Irf1* and *Gbp2*. This cannot be due to the failure to activate Stat4 as these genes are induced by Stat1 and/or Stat3 (Hirahara et al., 2012). One possible reason is that IL-27 target genes may be T cell subtype and/or activation status dependent. We used CD4^{+} and CD8^{+} T cells activated with αCD3/αCD28 for three days, whereas most other studies used naive CD4^{+} T cells or regulatory T cells (Tregs) (Hall et al., 2012). However, T-bet is also induced in naive CD8^{+} T cells (Morishima et al., 2005) and in CD4^{+} T cells activated with αCD3/αCD28 and simultaneously stimulated with IL-27 (Lucas et al., 2003). Although αCD3/αCD28 activation increases IL-27Rα/WSX-1 expression on both CD4^{+} and CD8^{+} T cells (Villarino et al., 2005), IL-27 target genes have to our knowledge not been determined in activated T cells. IL-27 mediated upregulation of IFN inducible genes, such as *Gbp2* and *Ip-10*, was only reported in naive CD4^{+} T cells and human monocyte-derived macrophages (Hirahara et al., 2012; Imamichi et al., 2012). Alternatively, Stat1 and Stat3 activation may simply be too low to induce target genes in our experimental setting. In line with this, we could not detect Stat1 and Stat3 DNA binding activity with EMSA. Further analysis of distinct T cell subtypes with and without *prior* T cell receptor (TCR) activation will be required in order to more precisely define the role of Tyk2 in IL-27 signalling.

In contrast to T cells, we could not observe activation of Stat1 and Stat3 in response to IL-27 in NK cells cultivated in the presence of IL-2 for one week, irrespective of the genotype (WT, Tyk2$^{-/-}$ and Tyk2^{K923E}). Moreover, we could not detect upregulation of *T-bet, Gbp2 and Ip-10*. In the murine system, IL-27 responsiveness has so far only been described in freshly isolated splenic NK cells (Matsui et al., 2009). However, the purity of the NK cell population used was not reported. Hence, the activation of Stats as determined by Western blot and the induction of target genes might be due to contamination with other cell types, in particular with naïve CD4^{+} T cells. Alternatively, only freshly isolated NK cells may be responsive to IL-27 and IL-2 activated *in vitro* expanded NK cells may, for example, loose the expression of either one of the IL-27R chains. In line with this WSX-1 receptor expression is reduced in NK cells from parasite infected mice and IL-2 has been described to negatively regulate the expression of WSX-1 on NK cells (Villarino et al., 2005). Interestingly,

induction of *T-bet* mRNA by IL-27 was shown in human *in vitro* expanded NK cells cultivated in the presence of IL-2 (Pflanz et al., 2002), indicating that there may be species specificity in IL-27 responsiveness.

7 References

Andoh, A., Zhang, Z., Inatomi, O., Fujino, S., Deguchi, Y., Araki, Y., Tsujikawa, T., Kitoh, K., Kim-Mitsuyama, S., Takayanagi, A.*, et al.* (2005). Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. Gastroenterology *129*, 969-984.

Artis, D., Villarino, A., Silverman, M., He, W., Thornton, E.M., Mu, S., Summer, S., Covey, T.M., Huang, E., Yoshida, H.*, et al.* (2004). The IL-27 receptor (WSX-1) is an inhibitor of innate and adaptive elements of type 2 immunity. J Immunol *173*, 5626-5634.

Aujla, S.J., Chan, Y.R., Zheng, M., Fei, M., Askew, D.J., Pociask, D.A., Reinhart, T.A., McAllister, F., Edeal, J., Gaus, K.*, et al.* (2008). IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med *14*, 275-281.

Batten, M., Kljavin, N.M., Li, J., Walter, M.J., de Sauvage, F.J., and Ghilardi, N. (2008). Cutting edge: IL-27 is a potent inducer of IL-10 but not FoxP3 in murine T cells. J Immunol *180*, 2752-2756.

Bazan, J.F. (1990). Structural design and molecular evolution of a cytokine receptor superfamily. Proc Natl Acad Sci U S A *87*, 6934-6938.

Bhattacharjee, A., Mulya, A., Pal, S., Roy, B., Feldman, G.M., and Cathcart, M.K. (2010). Monocyte 15-lipoxygenase gene expression requires ERK1/2 MAPK activity. J Immunol *185*, 5211-5224.

Boniface, K., Bernard, F.X., Garcia, M., Gurney, A.L., Lecron, J.C., and Morel, F. (2005). IL-22 inhibits epidermal differentiation and induces proinflammatory gene expression and migration of human keratinocytes. J Immunol *174*, 3695-3702.

Booth, C., Evans, G.S., and Potten, C.S. (1995). Growth factor regulation of proliferation in primary cultures of small intestinal epithelium. In Vitro Cell Dev Biol Anim *31*, 234-243.

Brand, S., Beigel, F., Olszak, T., Zitzmann, K., Eichhorst, S.T., Otte, J.M., Diepolder, H., Marquardt, A., Jagla, W., Popp, A.*, et al.* (2006). IL-22 is increased in active Crohn's disease and promotes proinflammatory gene expression and intestinal epithelial cell migration. Am J Physiol Gastrointest Liver Physiol *290*, G827-838.

Chen, Q., Ghilardi, N., Wang, H., Baker, T., Xie, M.H., Gurney, A., Grewal, I.S., and de Sauvage, F.J. (2000). Development of Th1-type immune responses requires the type I cytokine receptor TCCR. Nature *407*, 916-920.

Chopra, D.P., Dombkowski, A.A., Stemmer, P.M., and Parker, G.C. (2010). Intestinal epithelial cells in vitro. Stem Cells Dev *19*, 131-142.

Colgan, J., and Rothman, P. (2006). All in the family: IL-27 suppression of T(H)-17 cells. Nat Immunol *7*, 899-901.

Collison, L.W., Delgoffe, G.M., Guy, C.S., Vignali, K.M., Chaturvedi, V., Fairweather, D., Satoskar, A.R., Garcia, K.C., Hunter, C.A., Drake, C.G.*, et al.* (2012). The composition and signaling of the IL-35 receptor are unconventional. Nat Immunol *13*, 290-299.

Duhen, T., Geiger, R., Jarrossay, D., Lanzavecchia, A., and Sallusto, F. (2009). Production of interleukin 22 but not interleukin 17 by a subset of human skin-homing memory T cells. Nat Immunol *10*, 857-863.

Ellinghaus, D., Ellinghaus, E., Nair, R.P., Stuart, P.E., Esko, T., Metspalu, A., Debrus, S., Raelson, J.V., Tejasvi, T., Belouchi, M.*, et al.* (2012). Combined analysis of genome-wide association studies for Crohn disease and psoriasis identifies seven shared susceptibility loci. Am J Hum Genet *90*, 636-647.

Firmbach-Kraft, I., Byers, M., Shows, T., Dalla-Favera, R., and Krolewski, J.J. (1990). tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene *5*, 1329-1336.

Flint, N., Cove, F.L., and Evans, G.S. (1994). Heparin stimulates the proliferation of intestinal epithelial cells in primary culture. J Cell Sci *107 (Pt 2)*, 401-411.

Gauzzi, M.C., Barbieri, G., Richter, M.F., Uze, G., Ling, L., Fellous, M., and Pellegrini, S. (1997). The amino-terminal region of Tyk2 sustains the level of interferon alpha receptor 1, a component of the interferon alpha/beta receptor. Proc Natl Acad Sci U S A *94*, 11839-11844.

Geboes, L., Dumoutier, L., Kelchtermans, H., Schurgers, E., Mitera, T., Renauld, J.C., and Matthys, P. (2009). Proinflammatory role of the Th17 cytokine interleukin-22 in collagen-induced arthritis in C57BL/6 mice. Arthritis Rheum *60*, 390-395.

Ghoreschi, K., Laurence, A., and O'Shea, J.J. (2009). Janus kinases in immune cell signaling. Immunol Rev *228*, 273-287.

Hall, A.O., Silver, J.S., and Hunter, C.A. (2012). The immunobiology of IL-27. Adv Immunol *115*, 1-44.

Hirahara, K., Ghoreschi, K., Yang, X.P., Takahashi, H., Laurence, A., Vahedi, G., Sciume, G., Hall, A.O., Dupont, C.D., Francisco, L.M.*, et al.* (2012). Interleukin-27 priming of T cells controls IL-17 production in trans via induction of the ligand PD-L1. Immunity *36*, 1017-1030.

Hisada, M., Kamiya, S., Fujita, K., Belladonna, M.L., Aoki, T., Koyanagi, Y., Mizuguchi, J., and Yoshimoto, T. (2004). Potent antitumor activity of interleukin-27. Cancer Res *64*, 1152-1156.

Holscher, C., Holscher, A., Ruckerl, D., Yoshimoto, T., Yoshida, H., Mak, T., Saris, C., and Ehlers, S. (2005). The IL-27 receptor chain WSX-1 differentially regulates antibacterial immunity and survival during experimental tuberculosis. J Immunol *174*, 3534-3544.

Hunter, C.A., and Kastelein, R. (2012). Interleukin-27: balancing protective and pathological immunity. Immunity *37*, 960-969.

Ikeuchi, H., Kuroiwa, T., Hiramatsu, N., Kaneko, Y., Hiromura, K., Ueki, K., and Nojima, Y. (2005). Expression of interleukin-22 in rheumatoid arthritis: potential role as a proinflammatory cytokine. Arthritis Rheum *52*, 1037-1046.

Imamichi, T., Yang, J., Huang da, W., Sherman, B., and Lempicki, R.A. (2012). Interleukin-27 induces interferon-inducible genes: analysis of gene expression profiles using Affymetrix microarray and DAVID. Methods Mol Biol *820*, 25-53.

Imielinski, M., Baldassano, R.N., Griffiths, A., Russell, R.K., Annese, V., Dubinsky, M., Kugathasan, S., Bradfield, J.P., Walters, T.D., Sleiman, P*., et al.* (2009). Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet *41*, 1335-1340.

Iyer, S.S., Ghaffari, A.A., and Cheng, G. (2010). Lipopolysaccharide-mediated IL-10 transcriptional regulation requires sequential induction of type I IFNs and IL-27 in macrophages. J Immunol *185*, 6599-6607.

Kalliolias, G.D., and Ivashkiv, L.B. (2008). IL-27 activates human monocytes via STAT1 and suppresses IL-10 production but the inflammatory functions of IL-27 are abrogated by TLRs and p38. J Immunol *180*, 6325-6333.

Karaghiosoff, M., Neubauer, H., Lassnig, C., Kovarik, P., Schindler, H., Pircher, H., McCoy, B., Bogdan, C., Decker, T., Brem, G*., et al.* (2000). Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity *13*, 549-560.

Kilic, S.S., Hacimustafaoglu, M., Boisson-Dupuis, S., Kreins, A.Y., Grant, A.V., Abel, L., and Casanova, J.L. (2012). A patient with tyrosine kinase 2 deficiency without hyper-IgE syndrome. J Pediatr *160*, 1055-1057.

Kisseleva, T., Bhattacharya, S., Braunstein, J., and Schindler, C.W. (2002). Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene *285*, 1-24.

Kotenko, S.V. (2002). The family of IL-10-related cytokines and their receptors: related, but to what extent? Cytokine Growth Factor Rev *13*, 223-240.

Kreymborg, K., Etzensperger, R., Dumoutier, L., Haak, S., Rebollo, A., Buch, T., Heppner, F.L., Renauld, J.C., and Becher, B. (2007). IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol *179*, 8098-8104.

Kumar, K.G., Varghese, B., Banerjee, A., Baker, D.P., Constantinescu, S.N., Pellegrini, S., and Fuchs, S.Y. (2008). Basal ubiquitin-independent internalization of interferon alpha receptor is prevented by Tyk2-mediated masking of a linear endocytic motif. J Biol Chem *283*, 18566-18572.

Langrish, C.L., McKenzie, B.S., Wilson, N.J., de Waal Malefyt, R., Kastelein, R.A., and Cua, D.J. (2004). IL-12 and IL-23: master regulators of innate and adaptive immunity. Immunol Rev *202*, 96-105.

Lee, Y.H., Choi, S.J., Ji, J.D., and Song, G.G. (2012). Associations between PXK and TYK2 polymorphisms and systemic lupus erythematosus: a meta-analysis. Inflamm Res *61*, 949-954.

Lejeune, D., Dumoutier, L., Constantinescu, S., Kruijer, W., Schuringa, J.J., and Renauld, J.C. (2002). Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line. Pathways that are shared with and distinct from IL-10. J Biol Chem *277*, 33676-33682.

Levy, D.E., and Darnell, J.E., Jr. (2002). Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol *3*, 651-662.

Li, Z., Gakovic, M., Ragimbeau, J., Eloranta, M.L., Ronnblom, L., Michel, F., and Pellegrini, S. (2013). Two rare disease-associated Tyk2 variants are catalytically impaired but signaling competent. J Immunol *190*, 2335-2344.

Liu, L., Okada, S., Kong, X.F., Kreins, A.Y., Cypowyj, S., Abhyankar, A., Toubiana, J., Itan, Y., Audry, M., Nitschke, P.*, et al.* (2011). Gain-of-function human STAT1 mutations impair IL-17 immunity and underlie chronic mucocutaneous candidiasis. J Exp Med *208*, 1635-1648.

Lucas, S., Ghilardi, N., Li, J., and de Sauvage, F.J. (2003). IL-27 regulates IL-12 responsiveness of naive CD4+ T cells through Stat1-dependent and -independent mechanisms. Proc Natl Acad Sci U S A *100*, 15047-15052.

Lutticken, C., Wegenka, U.M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A.G., Wilks, A.F., Yasukawa, K., Taga, T., and et al. (1994). Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. Science *263*, 89-92.

Macartney, K.K., Baumgart, D.C., Carding, S.R., Brubaker, J.O., and Offit, P.A. (2000). Primary murine small intestinal epithelial cells, maintained in long-term culture, are susceptible to rotavirus infection. J Virol *74*, 5597-5603.

Martin, B., Hirota, K., Cua, D.J., Stockinger, B., and Veldhoen, M. (2009). Interleukin-17-producing gammadelta T cells selectively expand in response to pathogen products and environmental signals. Immunity *31*, 321-330.

Matsui, M., Kishida, T., Nakano, H., Yoshimoto, K., Shin-Ya, M., Shimada, T., Nakai, S., Imanishi, J., Yoshimoto, T., Hisa, Y., and Mazda, O. (2009). Interleukin-27 activates natural killer cells and suppresses NK-resistant head and neck squamous cell carcinoma through inducing antibody-dependent cellular cytotoxicity. Cancer Res *69*, 2523-2530.

Minegishi, Y., Saito, M., Morio, T., Watanabe, K., Agematsu, K., Tsuchiya, S., Takada, H., Hara, T., Kawamura, N., Ariga, T.*, et al.* (2006). Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity *25*, 745-755.

Nagalakshmi, M.L., Rascle, A., Zurawski, S., Menon, S., and de Waal Malefyt, R. (2004). Interleukin-22 activates STAT3 and induces IL-10 by colon epithelial cells. Int Immunopharmacol *4*, 679-691.

Nakamura, R., Shibata, K., Yamada, H., Shimoda, K., Nakayama, K., and Yoshikai, Y. (2008). 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*, 2071-2075.

Nicholson, S.E., De Souza, D., Fabri, L.J., Corbin, J., Willson, T.A., Zhang, J.G., Silva, A., Asimakis, M., Farley, A., Nash, A.D.*, et al.* (2000). Suppressor of cytokine signaling-3 preferentially binds to the SHP-2-binding site on the shared cytokine receptor subunit gp130. Proc Natl Acad Sci U S A *97*, 6493-6498.

O'Shea, J.J., Gadina, M., and Schreiber, R.D. (2002). Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell *109 Suppl*, S121-131.

Ouyang, W., Rutz, S., Crellin, N.K., Valdez, P.A., and Hymowitz, S.G. (2011). Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol *29*, 71-109.

Owaki, T., Asakawa, M., Fukai, F., Mizuguchi, J., and Yoshimoto, T. (2006). IL-27 induces Th1 differentiation via p38 MAPK/T-bet- and intercellular adhesion molecule-1/LFA-1/ERK1/2-dependent pathways. J Immunol *177*, 7579-7587.

Oyamada, A., Ikebe, H., Itsumi, M., Saiwai, H., Okada, S., Shimoda, K., Iwakura, Y., Nakayama, K.I., Iwamoto, Y., Yoshikai, Y., and Yamada, H. (2009). Tyrosine kinase 2 plays critical roles in the pathogenic CD4 T cell responses for the development of experimental autoimmune encephalomyelitis. J Immunol *183*, 7539-7546.

Peluso, C., Christofolini, D.M., Goldman, C.S., Mafra, F.A., Cavalcanti, V., Barbosa, C.P., and Bianco, B. (2013). TYK2 rs34536443 polymorphism is associated with a decreased susceptibility to endometriosis-related infertility. Hum Immunol *74*, 93-97.

Pflanz, S., Timans, J.C., Cheung, J., Rosales, R., Kanzler, H., Gilbert, J., Hibbert, L., Churakova, T., Travis, M., Vaisberg, E.*, et al.* (2002). IL-27, a heterodimeric cytokine composed of EBI3 and p28 protein, induces proliferation of naive CD4(+) T cells. Immunity *16*, 779-790.

Pickert, G., Neufert, C., Leppkes, M., Zheng, Y., Wittkopf, N., Warntjen, M., Lehr, H.A., Hirth, S., Weigmann, B., Wirtz, S.*, et al.* (2009). STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. J Exp Med *206*, 1465-1472.

Potla, R., Koeck, T., Wegrzyn, J., Cherukuri, S., Shimoda, K., Baker, D.P., Wolfman, J., Planchon, S.M., Esposito, C., Hoit, B.*, et al.* (2006). Tyk2 tyrosine kinase expression is required for the maintenance of mitochondrial respiration in primary pro-B lymphocytes. Mol Cell Biol *26*, 8562-8571.

Prchal-Murphy, M., Semper, C., Lassnig, C., Wallner, B., Gausterer, C., Teppner-Klymiuk, I., Kobolak, J., Muller, S., Kolbe, T., Karaghiosoff, M.*, et al.* (2012). TYK2 kinase activity is required for functional type I interferon responses in vivo. PLoS One *7*, e39141.

Prokunina-Olsson, L., Muchmore, B., Tang, W., Pfeiffer, R.M., Park, H., Dickensheets, H., Hergott, D., Porter-Gill, P., Mumy, A., Kohaar, I., *et al.* (2013). A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet *45*, 164-171.

Ragimbeau, J., Dondi, E., Alcover, A., Eid, P., Uze, G., and Pellegrini, S. (2003). The tyrosine kinase Tyk2 controls IFNAR1 cell surface expression. EMBO J *22*, 537-547.

Rani, M.R., Leaman, D.W., Han, Y., Leung, S., Croze, E., Fish, E.N., Wolfman, A., and Ransohoff, R.M. (1999). Catalytically active TYK2 is essential for interferon-beta-mediated phosphorylation of STAT3 and interferon-alpha receptor-1 (IFNAR-1) but not for activation of phosphoinositol 3-kinase. J Biol Chem *274*, 32507-32511.

Ren, H.J., Cui, J., Wang, Z.Q., and Liu, R.D. (2011). Normal mouse intestinal epithelial cells as a model for the in vitro invasion of Trichinella spiralis infective larvae. PLoS One *6*, e27010.

Rose-John, S. (2012). IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci *8*, 1237-1247.

Roy, B., and Cathcart, M.K. (1998). Induction of 15-lipoxygenase expression by IL-13 requires tyrosine phosphorylation of Jak2 and Tyk2 in human monocytes. J Biol Chem *273*, 32023-32029.

Ruckerl, D., Hessmann, M., Yoshimoto, T., Ehlers, S., and Holscher, C. (2006). Alternatively activated macrophages express the IL-27 receptor alpha chain WSX-1. Immunobiology *211*, 427-436.

Salcedo, R., Stauffer, J.K., Lincoln, E., Back, T.C., Hixon, J.A., Hahn, C., Shafer-Weaver, K., Malyguine, A., Kastelein, R., and Wigginton, J.M. (2004). IL-27 mediates complete regression of orthotopic primary and metastatic murine neuroblastoma tumors: role for CD8+ T cells. J Immunol *173*, 7170-7182.

Sanda, T., Tyner, J.W., Gutierrez, A., Ngo, V.N., Glover, J., Chang, B.H., Yost, A., Ma, W., Fleischman, A.G., Zhou, W., *et al.* (2013). TYK2-STAT1-BCL2 Pathway Dependence in T-cell Acute Lymphoblastic Leukemia. Cancer Discov *3*, 564-577.

Satoh-Takayama, N., Vosshenrich, C.A., Lesjean-Pottier, S., Sawa, S., Lochner, M., Rattis, F., Mention, J.J., Thiam, K., Cerf-Bensussan, N., Mandelboim, O., *et al.* (2008). Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. Immunity *29*, 958-970.

Schindler, C., and Plumlee, C. (2008). Inteferons pen the JAK-STAT pathway. Semin Cell Dev Biol *19*, 311-318.

Schwenk, F., Baron, U., and Rajewsky, K. (1995). A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. Nucleic Acids Res *23*, 5080-5081.

Sevignani, C., Wlodarski, P., Kirillova, J., Mercer, W.E., Danielson, K.G., Iozzo, R.V., and Calabretta, B. (1998). Tumorigenic conversion of p53-deficient colon epithelial cells by an activated Ki-ras gene. J Clin Invest *101*, 1572-1580.

Shaw, M.H., Boyartchuk, V., Wong, S., Karaghiosoff, M., Ragimbeau, J., Pellegrini, S., Muller, M., Dietrich, W.F., and Yap, G.S. (2003). 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*, 11594-11599.

Shaw, M.H., Freeman, G.J., Scott, M.F., Fox, B.A., Bzik, D.J., Belkaid, Y., and Yap, G.S. (2006). Tyk2 negatively regulates adaptive Th1 immunity by mediating IL-10 signaling and promoting IFN-gamma-dependent IL-10 reactivation. J Immunol *176*, 7263-7271.

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., and Schreiber, R.D. (2006). 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*, 804-819.

Shen, H., Goodall, J.C., and Hill Gaston, J.S. (2009). Frequency and phenotype of peripheral blood Th17 cells in ankylosing spondylitis and rheumatoid arthritis. Arthritis Rheum *60*, 1647-1656.

Shimizu, M., Ogura, K., Mizoguchi, I., Chiba, Y., Higuchi, K., Ohtsuka, H., Mizuguchi, J., and Yoshimoto, T. (2012). IL-27 promotes nitric oxide production induced by LPS through STAT1, NF-kappaB and MAPKs. Immunobiology.

Shimoda, K., Kato, K., Aoki, K., Matsuda, T., Miyamoto, A., Shibamori, M., Yamashita, M., Numata, A., Takase, K., Kobayashi, S.*, et al.* (2000). Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. Immunity *13*, 561-571.

Siegemund, S., Schutze, N., Schulz, S., Wolk, K., Nasilowska, K., Straubinger, R.K., Sabat, R., and Alber, G. (2009). Differential IL-23 requirement for IL-22 and IL-17A production during innate immunity against Salmonella enterica serovar Enteritidis. Int Immunol *21*, 555-565.

Simma, O., Zebedin, E., Neugebauer, N., Schellack, C., Pilz, A., Chang-Rodriguez, S., Lingnau, K., Weisz, E., Putz, E.M., Pickl, W.F.*, et al.* (2009). Identification of an indispensable role for tyrosine kinase 2 in CTL-mediated tumor surveillance. Cancer Res *69*, 203-211.

Sommereyns, C., Paul, S., Staeheli, P., and Michiels, T. (2008). IFN-lambda (IFN-lambda) is expressed in a tissue-dependent fashion and primarily acts on epithelial cells in vivo. PLoS Pathog *4*, e1000017.

Sonnenberg, G.F., Fouser, L.A., and Artis, D. (2010). Functional biology of the IL-22-IL-22R pathway in regulating immunity and inflammation at barrier surfaces. Adv Immunol *107*, 1-29.

Sprecher, C.A., Grant, F.J., Baumgartner, J.W., Presnell, S.R., Schrader, S.K., Yamagiwa, T., Whitmore, T.E., O'Hara, P.J., and Foster, D.F. (1998). Cloning and characterization of a novel class I cytokine receptor. Biochem Biophys Res Commun *246*, 82-90.

Stockinger, S., Reutterer, B., Schaljo, B., Schellack, C., Brunner, S., Materna, T., Yamamoto, M., Akira, S., Taniguchi, T., Murray, P.J., *et al.* (2004). IFN regulatory factor 3-dependent induction of type I IFNs by intracellular bacteria is mediated by a TLR- and Nod2-independent mechanism. J Immunol *173*, 7416-7425.

Stoiber, D., Kovacic, B., Schuster, C., Schellack, C., Karaghiosoff, M., Kreibich, R., Weisz, E., Artwohl, M., Kleine, O.C., Muller, M., *et al.* (2004). TYK2 is a key regulator of the surveillance of B lymphoid tumors. J Clin Invest *114*, 1650-1658.

Strobl, B., Stoiber, D., Sexl, V., and Mueller, M. (2011). Tyrosine kinase 2 (TYK2) in cytokine signalling and host immunity. Front Biosci *16*, 3214-3232.

Sugimoto, K., Ogawa, A., Mizoguchi, E., Shimomura, Y., Andoh, A., Bhan, A.K., Blumberg, R.S., Xavier, R.J., and Mizoguchi, A. (2008). IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest *118*, 534-544.

Ungureanu, D., Wu, J., Pekkala, T., Niranjan, Y., Young, C., Jensen, O.N., Xu, C.F., Neubert, T.A., Skoda, R.C., Hubbard, S.R., and Silvennoinen, O. (2011). The pseudokinase domain of JAK2 is a dual-specificity protein kinase that negatively regulates cytokine signaling. Nat Struct Mol Biol *18*, 971-976.

van Boxel-Dezaire, A.H., Rani, M.R., and Stark, G.R. (2006). Complex modulation of cell type-specific signaling in response to type I interferons. Immunity *25*, 361-372.

van de Veerdonk, F.L., Plantinga, T.S., Hoischen, A., Smeekens, S.P., Joosten, L.A., Gilissen, C., Arts, P., Rosentul, D.C., Carmichael, A.J., Smits-van der Graaf, C.A., *et al.* (2011). STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. N Engl J Med *365*, 54-61.

Velazquez, L., Fellous, M., Stark, G.R., and Pellegrini, S. (1992). A protein tyrosine kinase in the interferon alpha/beta signaling pathway. Cell *70*, 313-322.

Villarino, A.V., Huang, E., and Hunter, C.A. (2004). Understanding the pro- and anti-inflammatory properties of IL-27. J Immunol *173*, 715-720.

Villarino, A.V., Larkin, J., 3rd, Saris, C.J., Caton, A.J., Lucas, S., Wong, T., de Sauvage, F.J., and Hunter, C.A. (2005). Positive and negative regulation of the IL-27 receptor during lymphoid cell activation. J Immunol *174*, 7684-7691.

Wang, J., Wang, G., Sun, B., Li, H., Mu, L., Wang, Q., Li, G., Shi, L., Jin, L., and Kostulas, N. (2008). Interleukin-27 suppresses experimental autoimmune encephalomyelitis during bone marrow stromal cell treatment. J Autoimmun *30*, 222-229.

Wolk, K., Kunz, S., Asadullah, K., and Sabat, R. (2002). Cutting edge: immune cells as sources and targets of the IL-10 family members? J Immunol *168*, 5397-5402.

Wolk, K., Kunz, S., Witte, E., Friedrich, M., Asadullah, K., and Sabat, R. (2004). IL-22 increases the innate immunity of tissues. Immunity *21*, 241-254.

Wolk, K., Witte, E., Hoffmann, U., Doecke, W.D., Endesfelder, S., Asadullah, K., Sterry, W., Volk, H.D., Wittig, B.M., and Sabat, R. (2007). IL-22 induces lipopolysaccharide-binding protein in hepatocytes: a potential systemic role of IL-22 in Crohn's disease. J Immunol *178*, 5973-5981.

Wolk, K., Witte, E., Witte, K., Warszawska, K., and Sabat, R. (2010). Biology of interleukin-22. Semin Immunopathol *32*, 17-31.

Yamada, K., Sato, K., Morishita, S., Kaminogawa, S., and Totsuka, M. (2009). Establishment of a primary culture method for mouse intestinal epithelial cells by organ culture of fetal small intestine. Biosci Biotechnol Biochem *73*, 1849-1855.

Yang, J., Yang, M., Htut, T.M., Ouyang, X., Hanidu, A., Li, X., Sellati, R., Jiang, H., Zhang, S., Li, H.*, et al.* (2008). Epstein-Barr virus-induced gene 3 negatively regulates IL-17, IL-22 and RORgamma t. Eur J Immunol *38*, 1204-1214.

Zenewicz, L.A., and Flavell, R.A. (2011). Recent advances in IL-22 biology. Int Immunol *23*, 159-163.

Zenewicz, L.A., Yancopoulos, G.D., Valenzuela, D.M., Murphy, A.J., Karow, M., and Flavell, R.A. (2007). Interleukin-22 but not interleukin-17 provides protection to hepatocytes during acute liver inflammation. Immunity *27*, 647-659.

Zhang, Q., Sturgill, J.L., Kmieciak, M., Szczepanek, K., Derecka, M., Koebel, C., Graham, L.J., Dai, Y., Chen, S., Grant, S.*, et al.* (2011). The role of Tyk2 in regulation of breast cancer growth. J Interferon Cytokine Res *31*, 671-677.

Zheng, Y., Valdez, P.A., Danilenko, D.M., Hu, Y., Sa, S.M., Gong, Q., Abbas, A.R., Modrusan, Z., Ghilardi, N., de Sauvage, F.J., and Ouyang, W. (2008). Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med *14*, 282-289.

Danksagung

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