Interleukin 15 and 17 in *Staphylococcus aureus* arthritis

Louise Henningsson

2011

Department of Rheumatology and Inflammation Research,
The Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

The cover was deigned by David Bliman.

ISBN 978-91-633-9569-7
http://hdl.handle.net/2077/26268

Printed by Geson Hylte Tryck, Göteborg, Sweden 2011.

ABSTRACT

Staphylococcus aureus–induced arthritis leads to severe joint destruction and high mortality despite antibiotic treatment. Thus, there is a need to identify new treatment targets in addition to antibiotic therapy. Interleukin (IL)-15 has been implicated both in osteoclastogenesis and in bacterial clearance – two important issues in *S. aureus*–induced joint destruction. Interleukin-17A has been discovered as an important mediator of aseptic arthritis both in mice and men, while its function in *S. aureus*–induced arthritis is largely unknown. The aim of this thesis was to investigate the importance of IL-15 and IL-17A and in addition, the interaction between IL-17A and interleukin-23 in *S. aureus*–induced arthritis. Wildtype, IL-15 knockout and IL-17A knockout mice were inoculated (systemically or locally) with a defined number of toxic shock syndrome toxin-1 (TSST-1) producing *S. aureus.* At sacrifice, tissues were collected and further analysed. We found that mice genetically lacking IL-15 or treated with anti-IL-15 antibodies developed less severe and destructive arthritis compared with control mice. In neither situation the bacterial clearance was negatively influenced. Furthermore, the IL-15 knockout mice had fewer osteoclasts in the joints compared with wildtype mice. We suggest that due to IL-15 absence, the mice developed milder arthritis probably because of less bone and cartilage destruction. We observed that IL-17A was of minor importance in systemic *S. aureus* arthritis but played a major role in local *S. aureus* arthritis. In the systemic model of arthritis we found elevated levels of IL-17F in the IL-17A knockout mice, suggesting that IL-17F compensates for the absence of IL-17A and that IL-17F in a normal wildtype mice is inhibited by IL-17A. Furthermore we found that IL-17A regulates the production of IL-23, a cytokine that is known to regulate the production of IL-17A, in a negative feedback manner, which means that IL-17A may have regulatory properties. Thus, we have found that IL-15, but not IL-17A, could represent a promising treatment target along with antibiotics in *S. aureus*–induced arthritis, and that IL-17A negatively regulates its upstream inducer, IL-23.

Key words: IL-15, IL-17A, IL-17F, IL-23, arthritis, mice, osteoclasts, *S. aureus.*

LIST OF PUBLICATIONS

The thesis is based on the following papers, which will be referred to in the text by their Roman numerals (I-III).

I. **Louise Henningsson**, Pernilla Jirholt, Yalda Rahpeymai Bogestål, Tove Eneljung, Martin Adiels, Catharina Lindholm, Iain McInnes, Silvia Bulfone-Paus, Ulf H. Lerner and Inger Gjertsson.
Interleukin-15 mediates joint destruction in *Staphylococcus aureus* arthritis.
Accepted in *The Journal of Infectious Diseases*, October 2011.

II. **Louise Henningsson**, Pernilla Jirholt, Catharina Lindholm, Tove Eneljung, Elin Silverpil, Yoichiro Iwakura, Anders Lindén and Inger Gjertsson.
Interleukin-17A during local and systemic *Staphylococcus aureus*–induced arthritis in mice.
Infection and Immunity 2010 Sep; 78(9):3783-90.

III. Elin Silverpil, Adam K.A. Wright, Marit Hanson, Pernilla Jirholt, **Louise Henningsson**, Margareta E. Smith, Stephen B. Gordon, Yoichiro Iwakura, Inger Gjertsson, Pernilla Glader and Anders Lindén.
Negative feedback on Interleukin-23 by Interleukin-17A during airway inflammation.
Submitted to *PLoS ONE*, October 2011.

TABLE OF CONTENTS

DISCUSSION

ABBREVIATIONS

APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
C3, C3b, C5a	Complement factor 3, 3b, 5a
CD	Cluster of differentiation
CFU	Colony forming units
CLP	Cecal ligation and puncture
CP5, 8	Capsule 5, 8
CXCL	CXC chemokine ligand
DC	Dendritic cell
EAE	Experimental autoimmune encephalomyelitis
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FOXP3	Forkhead box P3
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
HSC	Haematopoietic stem cell
ICAM	Intercellular adhesion molecule
IFN	Interferon
IL-	Interleukin
IL-(17, 15, 23)R	Interleukin receptor
LPS	Lipopolysaccharide
M-CSF	Macrophage-colony stimulating factor
MCP	Monocyte chemotactic protein
MHC II	Major histocompatibility complex class II
MIP	Macrophage inflammatory protein
MMP	Matrix metalloproteinases
MØ	Macrophage
MPO	Myeloperoxidase
MRSA	Methicillin resistant *Staphylococcus aureus*
NAP	Neutrophil attracting protein
NF-κB	Nuclear factor kappa B
NK	Natural killer
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
RA	Rheumatoid arthritis
Rac1	Ras-related C3 botulinum toxin substrate 1
RANK(L)	Receptor activator of nuclear factor kappa-B (ligand)
ROR	Retinoic acid-related orphan receptor
TCR	T cell receptor
TGF	Transforming growth factor
Th-	T helper
TLR	Toll like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cells
TSST-1	Toxic shock syndrome toxin-1
TUNEL	Terminal deoxynucleotidyl transferase dUTP Nick and Labelling
VCAM	Vascular cell adhesion molecule

INTRODUCTION

The immune system

Our body is constantly exposed to foreign substances, including hazardous pathogens like bacteria, virus and parasites. The first line of defence is the skin and mucosa, which produce e.g. bactericidal peptides and mechanically prevents the pathogen to enter the body. Should this defence fail, we are still protected against pathogen invasion thanks to our immune system. The immune system has evolved during hundreds and thousands of years and is extremely sophisticated as it recognises foreign substances but does not react to self-structures. When viruses, bacteria, fungi etc. enter the body the innate immune response is immediately activated by conserved pathogen structures. This part of the immune system reacts similarly every time it encounters a group of pathogens e.g. Gram-positive bacteria and has no acquired memory. Second, the adapted immune response is activated. This part of the immune system recognises a specific protein from the invaders and has the capacity to remember a previous infection. The adaptive immune system provides us e.g. with specific antibodies which enhances the clearance of pathogens and thanks to its memory, the response is more effective and faster upon a second encounter with the same antigen and therefore we are often completely protected against a second challenge of certain pathogens.

Staphylococcus aureus *arthritis & sepsis*

Staphylococcus aureus [1] is an omnipotent Gram-positive coccus that can be found in the nasal mucosa and on the skin of healthy individuals. Under certain conditions such as hospitalisation and old age it can invade the host and cause severe infections. During the last decades new risk factors have appeared e.g. immunosuppressive therapy, joint prosthesis, AIDS, intravenous drug abuse and importantly, drug resistance. Today's treatment is entirely dependent on antibiotics, however many bacterial strains have developed antibiotic resistance, including methicillin resistant *S. aureus* (MRSA) and these strains are very hard to combat with conventional antibiotics.

S. aureus causes a variety of infections in humans. It can cause a) minor superficial lesions such as skin abscesses or wound infections, b) more systemic and life-threatening infections such as pneumonia, mastitis, meningitis, septic arthritis, endocarditis, sepsis and septicemia, c) toxinoses such as toxic shock syndrome and food poisoning. The bacterium is the main cause of hospital

acquired infection i.e. surgical wounds and infections associated with foreign devices in the body such as catheters and prosthesis. *S. aureus* is also the most common pathogen that causes septic arthritis.

If *S. aureus* enters the joint a highly erosive and rapidly progressive arthritis may evolve. Usually the staphylococci are spread to different compartments of the host (including the joints) via the blood stream, and even though the bacteria can be found in most tissues, they persist mainly in kidneys and joints. *S. aureus* arthritis is characterised by a swollen, red and painful joint. The yearly incidence of septic arthritis in general is 0.002-0.01% in the general population and 0.03-0.07% in patients with rheumatoid arthritis (RA) and patients with joint prostheses [2]. Despite antimicrobial therapy 25-50% of the patients develop permanent joint damage and the mortality rate is high (5-20%) mainly due to the profound inflammatory response evoked by the bacteria [1,2]. In a patient with RA the meantime from bacterial colonisation to treatment is approximately 7 days and during this period the bacterial load has increased considerably and thereby also the risk of joint destruction and sepsis [1]. Although substantial efforts have been made to understand the immunological mechanisms that lead to *S. aureus*–induced joint destruction, it remains difficult to treat the infection (by maintaining the host's ability to clear bacteria) whilst simultaneously limiting the joint destruction (by suppressing the immunological response).

Sepsis is defined as bacteria spread from a local infection into the blood stream and the following hyperthermia/hypothermia, tachypnea and tachycardia. In severe sepsis, septic shock is the worst possible manifestation of the infection, a state with multiple organ dysfunction and death. The reason for death in sepsis is currently not clear. It is speculated whether it is due to immunosuppression or immunostimulation, and most data point in the direction of an over-stimulated immune response. High cytokine levels of proinflammatory tumor necrosis factor (TNF)-α are correlated with septic death [3]. A combination of antibiotics and unspecific inhibition of the immune system with glucocorticosteroids during *S. aureus*–induced arthritis has reduced the prevalence, the severity of arthritis and mortality rate in both mice and humans [4,5]. Combination treatment with antibiotics and TNF-α inhibitors improves survival and reduces the severity of arthritis in mice, while the combination treatment in human sepsis point towards a survival enhancement however the data are not clear cut [6,7].

As *S. aureus*–induced arthritis and sepsis is often caused by haematogenously spread bacteria we use our well-established mouse model based on intravenous inoculation of arthritogenic *S. aureus* strain, LS-1 [8] to study the host's immune response to the infection.

Features of Staphylococcus aureus

In order for the bacterium to protect itself from the immune system it expresses virulence factors, which on the one hand help the bacterium to evade the immune system but on the other hand evokes an immune response towards the bacterium. The bacterium consists of a slime layer, a capsule, a cell wall, a cell membrane and it also produces a variety of surface proteins, enzymes and toxins (Figure 1).

The *slime layer* produced by the bacterium is a viscous substance that consists of polysaccharides, glycoproteins and glycolipids and promotes bacterial adhesion to both endogenous surfaces i.e. cartilage and foreign devices such as catheters and prosthesis [9]. The slime protects the bacterium from environmental damages e.g. it interferes with the antimicrobial effect of certain antibiotics [10,11].

The bacterial *capsule* is a well-organised structure composed of polysaccharides. Many different subtypes of capsules exist where the subtypes CP5 and CP8 are most common in diseases [12]. Encapsulated *S. aureus* strains are more resistant to phagocytosis, as opsonisation by antibodies and complement is hindered, although in the presence of capsular antibodies phagocytosis is enhanced [13,14]. It has been shown that encapsulated strains for CP5 are more virulent than non-encapsulated strains in *S. aureus*–induced arthritis, leading to more arthritis and higher mortality probably due to a reduced ability to phagocytose bacteria [15].

The *cell wall* of *S. aureus* consists of peptidoglycans (mainly N-acetyl glucosamine and N-acetyl muramic acid), teichoic acid and lipoteichoic acid. Peptidoglycans and lipoteichoid acid bind toll-like receptor 2 (TLR2) which leads to activation of nuclear factor kappa B (NF-κB) [16] and the subsequent production of proinflammatory cytokines such as TNF-α, interleukin (IL)-6 and IL-1 [17].

The bacterium expresses a number of virulent *proteins*, which either are anchored to the bacterial cell wall or produced as soluble factors: First, *Protein A* exists both as a cell wall bound protein and as a soluble protein. It binds the Fc part of the IgG molecule, which leads to disruption of

opsonisation and phagocytosis which aggravates the severity of *S. aureus*–induced arthritis [18]. Second, there are a family of proteins called microbial surface components recognising adhesive matrix molecules (MSCRAMM) including A) F*ibronectin-binding protein A & B* bind fibronectin which are found mainly in the extracellular matrix and covers foreign materials such as catheters in the body. B) *Collagen-binding protein* is of importance in binding the bacterium to cartilage in the joints and it has been shown that the collagen-binding protein is an important virulent factor in *S. aureus* induced arthritis [19,20]. C) *Clumping factor A & B* bind fibrinogen [21] and it has been show that it is an important virulence factor both in septic arthritis and endocarditis [22].

In addition to surface proteins, the bacterium secretes molecules including enzymes and toxins. Extracellular *enzymes* such as proteases, lipase, hyaluronidase, β-lactamase, catalase, nuclease, coagulase and staphylokinase facilitate tissue invasion. The toxins produced include hemolysins (α, β, δ, γ toxins), leukocidin and superantigens. The hemolysins and leukocidins are all cytolytic toxins forming a transmembrane channel in the cell membrane resulting in cell lysis and e.g. α and γ toxins aggravates the severity of *S. aureus*–induced arthritis [23].

S. aureus produces *superantigens*, which include enterotoxins A, B, C1-3, D, E and G, and toxic shock syndrome toxin-1 (TSST-1). The superantigens cause fever, hypotension and other acute toxic-shock-like symptoms by release of proinflammatory cytokines, such as interferon (IFN)-γ and TNF- α [1]. Superantigens are capable of activating 5-20% of T cells simultaneously compared to a normal protein antigen, which activates about 0.01% of the T cell population. The T cell is not stimulated in its normal way by binding an antigen presenting cell (APC) in the presens of a cognate antigen on major histocompatibility complex class II (MHC II), instead the superantigen cross-links the Vβ region in the αβ T cell receptor (TCR) to the outside of the MHC II molecule (Figure 2) [24,25]. Thus, the activation of T cells is not antigen specific and any T cell that share the same Vβ family structure in their T cell receptor may get activated.

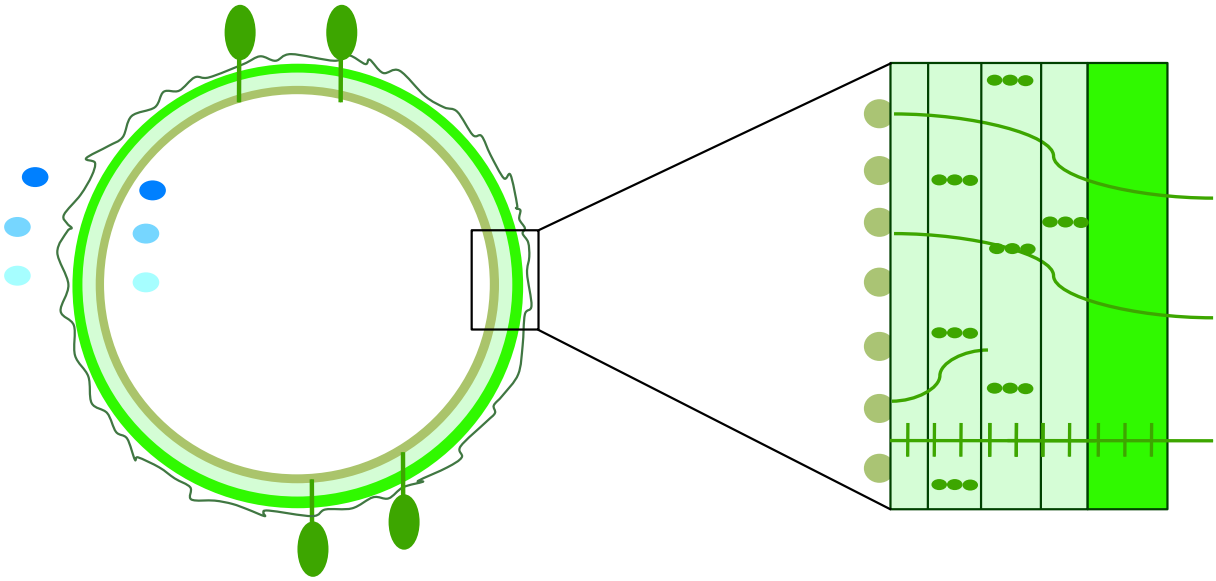


Figure 1. *Schematic view of the structure of* S. aureus.

From the right: Slime layer, capsule, cell wall, cytoplasmatic membrane. Surface proteins. Enzymes and toxins. Peptidoglycans. Teichonic acid, lipoteichonic acid. Adapted from Lowy [26].

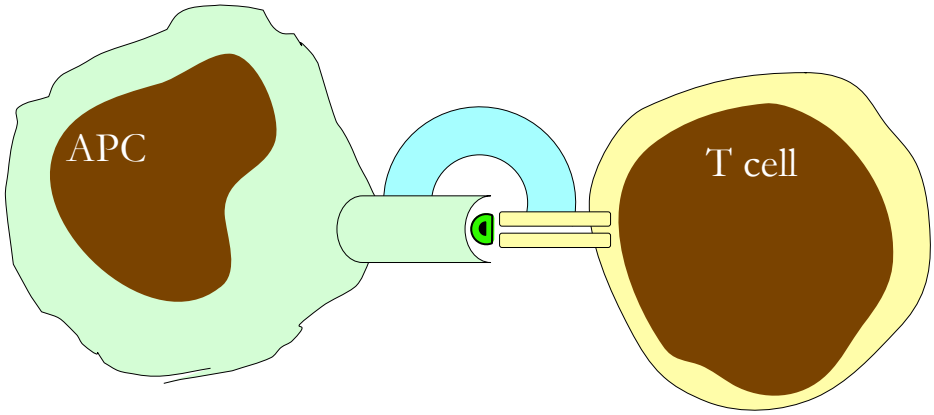


Figure 2. *Superantigen activation of T cells.*

Superantigens (turquoise) bind to the outside of the MHC II molecule of the APC and to the Vβ region of the TCR on the T cell.

The initial trigger in chronic inflammatory diseases of the respiratory tract is not known, but the conditions are characterised by lymphocyte, neutrophil and monocyte infiltration. These cells produce cytokines and chemokines, which recruit and activate more immune cells – creating a vicious circle. Epithelial cells and macrophages release transforming growth factor (TGF)-β which induce proliferation of fibroblasts, and subsequent fibrosis of the small airways. Neutrophils release enzymes including proteases e.g. elastase which causes degradation of elastin in the connective tissue, which leads to emphysema, and mucus hypersecretion by goblet cells [27]. Although the cellular source of IL-17A in the airways is unknown it plays an important role in host defence towards Gram-negative bacteria in the airway but also in chronic inflammatory diseases [28-30]. Lipopolysaccharide (LPS) (Figure 3) is a component of the Gram-negative bacterial outer cell membrane and an endotoxin, which binds to TLR4, activates NF-κB and mount a profound inflammatory response by inducing the production of a substantial amount of proinflammatory cytokines [31].

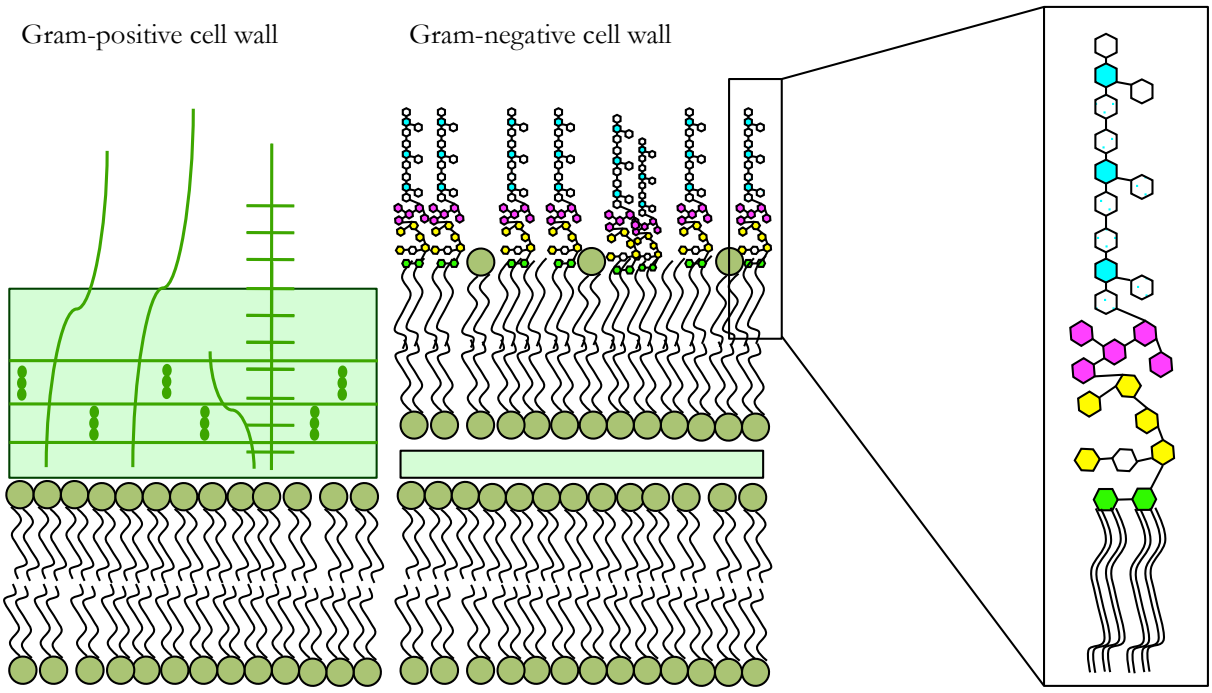


Figure 3. *Gram-positive and Gram-negative bacterial cell wall and LPS.*

Comparison of Gram-positive and Gram-negative bacterial cell wall and a magnification of LPS from Gram-negative bacteria. Gram-positive bacteria from bottom: Inner membrane and peptidoglycans. Gram-negative bacterial from bottom: Inner membrane, peptidoglycans, outer membrane and LPS.

The immune response serves to protect the host against harmful pathogens, but as mentioned above in some situations the pathogen can trigger an overwhelming reaction which can be devastating for the host e.g. in *S. aureus* arthritis and sepsis. The balance between a sufficient response to eliminate the bacteria and limiting the response in order to not cause destruction of the joints and sepsis can be difficult to regulate. An ineffective immune response allows the pathogen to grow in the host and to continue produce potentially harmful products, while an exaggerated response can cause substantial tissue damage and large amounts of cytokines are released, such as TNF-α and IL-6, which can lead to septic shock and in the worst-case scenario – death [32].

The innate defence against S. aureus *infection*

Complement plays an important role in the opsonisation and phagocytosis of encapsulated *S. aureus* [13,33] and complement activation is vital for mouse survival after inoculation of subtype 5 capsule *S. aureus*. C3 depleted mice show a significant reduction in survival [34] as do mice depleted in complement receptor 1 and in C5a [35,36]**.** In the presence of clumping factor A, *S. aureus* is protected against phagocytosis due to enhanced binding of factor 1 and hence enhanced cleavage of C3b [37,38].

Neutrophils are important in host defence against all kind of pathogens and are always the first cells to be recruited to the infected tissue. Already within 24 h after *S. aureus* inoculation, large numbers of neutrophils have entered the joint and they are prominent in the synovial membrane [39]. Neutrophils are protective and extremely important in *S. aureus* arthritis, as depletion increase mortality, frequency of arthritis and bacterial load compared with mice intact in their neutrophil count [40]. The findings are probably due to a decrease in phagocytosis by neutrophils which leads to a higher activation of macrophages and hence an increased production of IL-6 and TNF-α. A high production of these cytokines are correlated to enhanced sepsis related death [32].

Monocytes/macrophages have dual roles in *S. aureus* infection. The cells are crucial for survival as the bacterial load and the severity of sepsis is significantly increased while the development and severity of arthritis are decreased after monocyte depletion [41]. Macrophages are known to produce substantial amounts of e.g. TNF-α which have profound effects on both neutrophil activation [42] and cell migration; TNF-α contributes to the upregulation of intercellular

adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin on endothelial cells and induces the production of chemokines such as IL-8 and monocyte chemotactic protein-1 (MCP-1) [43-45]. Thus, the possibility for neutrophils and lymphocytes to enter the infected joint is impaired in the absence of macrophages, which results in less severe arthritis but also in an increased bacterial load and decreased survival [41]. In addition, the phagocytic capacity of the macrophages *per se* is essential for bacterial clearance [41]. The direct activation of neutrophils by TNF-α promotes a rapid increase in cell surface molecules and hence adherence to endothelium, enhances the ability of neutrophils to phagocytose, degranulate and release hydrogen peroxide [42,46,47].

Natural killer (NK) cells are capable of killing cells that do not express MHC I. They are activated early after bacterial infections via IL-12 released from macrophages and produce substantial amounts of IFN-γ. One previous study has shown that NK cells protect against *S. aureus*–induced arthritis [48].

The adapted defence against S. aureus *infection*

It is well known that T cells contribute to the development of *S. aureus* arthritis and they appear in the synovial membrane of an arthritic joint around 48 h after *S. aureus* inoculation. These cells are predominantly of the $CD4^+$ phenotype and depletion ameliorates the severity of arthritis [49] which also is true for blockade of the αβTCR [50]. Superantigens such as TSST-1 lead to a vigorous activation and clonal expansion of Vβ11 TCR T cells [24]. Staphylococcal strains which produce superantigens are more virulent than their isogenic counterparts [51] and depletion of $V\beta11^+$ T cells reduces mortality and abolishes the development of arthritis [24]. Although a prominent polyclonal B cell activation occurs after *S. aureus* inoculation [39,50] and antibodies play an important role in the phagocytosis of encapsulated *S. aureus* [14] the depletion of B cells does not influence the course of *S. aureus*–induces arthritis or sepsis [52].

Cytokines in S. aureus *infection*

During *S. aureus* infection a substantial amount of proinflammatory cytokines are produced, such as IL-1, IL-6, IL-12, IFN-γ and TNF-α [53,54]. Also, we have shown that IL-15 is increased late during *S. aureus*–induced infection and it has also been shown that IL-17A is upregulated from human peripheral blood mononuclear cells after TSST-1–producing *S. aureus* stimulation *in vitro* [55].

Interleukin-6

IL-6 is one of the main proinflammatory cytokines and is produced both by immune cells e.g. T cells and macrophages, and by non-immune cells including fibroblasts, osteoblasts and endothelial cells. It induces expression of adhesion molecules on endothelial cells and enhances chemokine production, thereby increasing cell migration into tissue [56]. T helper (Th) 17 cell differentiation is promoted by IL-6 which suppresses TGF-β induced regulatory T cell differentiation [57]. Also, IL-6 induces the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) which stimulates osteoclastogenesis and subsequent degradation of bone [58]. Via these mechanisms IL-6 upregulation contributes to destruction of the joints in aseptic arthritis [56]. The role of IL-6 in *S. aureus* infection is not clear. Although IL-6 contributes to increased morbidity and mortality by promoting an overwhelming immune response [39,59], it is most likely also needed for bacterial elimination by recruitment of neutrophils and most importantly macrophages to site if infection. Serum levels of IL-6 are elevated within hours after *S. aureus* inoculation and levels are high throughout the infection [39,50].

Interleukin-12

IL-12 is produced by macrophages and polarises the T cell towards a Th1 phenotype. It promotes secretion of IFN-γ by Th1 cells and NK cells. IL-12 is an important cytokine during *S. aureus* infection, and contributes to an increased survival during sepsis due to an increased bacterial clearance, probably via increased levels of IFN-γ and subsequent macrophage activation [60,61].

Interferon-γ

IFN-γ is the main cytokine produced by Th1 cells and NK cells. IFN-γ activates macrophages and neutrophils but also has a role in the recruitment of these cells to the site of infection. It induces the production of MCP-1 and hence enhances the recruitment of mononuclear cells but seems to inhibit the production of IL-8 and the recruitment of polymorphonuclear cells to site of infection [62-64]. However, a combination of IL-17A and IFN-γ seems to synergistically enhance the secretion of chemokines IL-8 and MCP-1 [65]. The role of IFN-γ in *S. aureus* infection is dual. Administration of IFN-γ shows a protective role of this cytokine during *S. aureus* sepsis, as it seems to increase phagocytosis and thereby bacterial clearance and survival. On the other hand, IFN-γ seems to contribute to the severity of arthritis. This is probably due to the increased activation of macrophages to phagocytose and the enhanced production of e.g. TNF-α and IL-6 from macrophages which contributes to the severity of arthritis [61].

Tumor necrosis factor-α

TNF-α is mainly produced by macrophages but also by T cells, NK cells and endothelial cells. It is a potent proinflammatory cytokine as it induces chemokine secretion, it upregulates adhesion molecules on endothelial cells and on leukocytes, activates neutrophils and macrophages to phagocyte efficiently and promotes bone resorption by inducing RANKL expression in osteoblast. TNF-α is known to be one of the major causative factors involved in degradation of cartilage and subchondral bone in aseptic arthritis, and anti-TNF treatment has revolutionised the life of many patients suffering from e.g. RA [66]. In *S. aureus* infection TNF-α levels in serum are increased throughout infection [39]. Although a treatment combination of TNF-α and antibiotics in *S. aureus*–induced arthritis in mice has been shown to ameliorate both septic arthritis and septic death [6], lack of TNF-α leads to an amelioration of *S. aureus*–induced arthritis whilst *S. aureus*–induced mortality is aggravated [67]. TNF-α upregulates the expression of adhesion molecules by endothelial cells [44] which results in inflammatory cells invading the tissue. This is probably one of the reasons for decreased frequency of synovitis in the TNF-α deficient mice, which displayed a higher mortality rate due to reduced phagocytic activity of macrophages and decreased bacterial clearance.

Cytokines of particular interest in the thesis

Interleukin-15

IL-15 is a proinflammatory pleiotropic cytokine, which share many structural properties with IL-2 and is produced by a wide range of cells including epithelial, endothelial cells, fibroblasts, muscle cells, macrophages, dendritic cells and mast cells [68-70]. Its receptor consists of a β and a γ subunit, which are both included in the IL-2 receptor, and a unique IL-15 receptor α (Rα) subunit. There are two different isoforms of the cytokine due to alternative splicing; one form is retained intracellularly while the other binds the IL-15Rα [71,72]. The cytokine/receptor complex (IL-15/IL-15Rα) may stay bound to the cell surface, where it can be transpresented to cells expressing the β and γ subunits of the receptor or the complex may be secreted. The soluble IL-15/IL-15Rα complex binds with 10-100 fold higher affinity to the IL-15R β and γ subunits compared with IL-15 alone [73]. IL-15 is a potent prosurvival factor, enhancing the expression of the antiapoptotic factors Bcl-xL and Bcl-2, and inhibiting the expression of proapoptotic factor Bax [74-76]. It promotes maturation and survival of NK and dendritic cells (DC) and is a

selective growth factor for $CD8^+$, but not $CD4^+$ T cells [77-79]. In addition, IL-15 transpresentation by DCs is required for NK and $CD8^+$ cell priming [80,81]. IL-15 is important in the protection against viral infections e.g. IL-15 knockout mice have enhanced susceptibility to vaccinia virus infections, as well as in the protection against bacterial infections [74,79,82-84]. It is a potent stimulator of neutrophils and macrophages, as it enhances phagocytosis and proinflammatory cytokine/chemokine production in these cells [85]. In human monocytes IL-15 also has a role in cell recruitment to the site of infection via upregulation of IL-8 and MCP-1 [86]. A new role of intracellular IL-15 in mast cells was described in a cecal ligation and puncture (CLP) sepsis model, as IL-15 knockout mice displayed an increased bacterial clearance due to enhanced recruitment of neutrophils [70]. The suggested mechanism was suppression of an IL-15 dependent inhibition of neutrophil attracting protein-2 (NAP-2) (also called CXCL7). In addition, IL-15 plays an important role in osteoclast differentiation in inducing RANKL expression from not only stromal cells/osteoblasts but also from NK cells and synovial fibroblasts (Figure 4) [87-90].

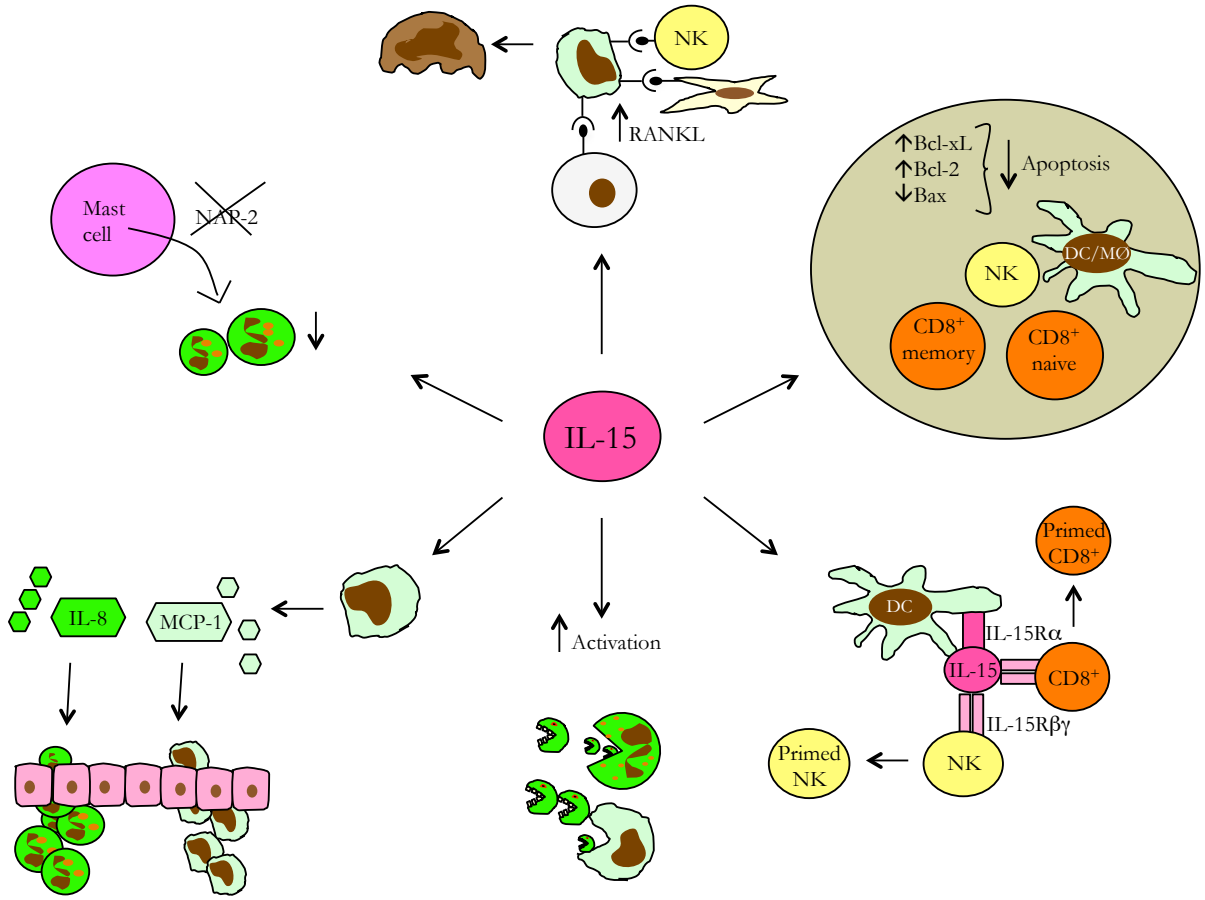


Figure 4. *Functions of IL-15.*

Bacteria, fibroblast, neutrophil, phagocytic neutrophil, macrophage, phagocytic macrophage, osteoclast, stromal cell/osteoblast.

Interleukin-17A

Within the IL-17 superfamily there are six known cytokine members, IL-17A-IL-17F [91], where IL-17A and IL-17F share the highest homology and IL-17E (IL-25) is most different from IL-17A [92]. Within the IL-17 receptor family, five receptor subunits have been identified, IL-17RA-IL-17RE (Figure 5). T cell differentiation towards either Th1, Th2, Th17 or regulatory T (Treg) cells is dependent on cytokine environment, which in turn is dependent on the pathogen invading the host. Naïve T cells exposed to TGF-β will differentiate towards Treg cells, due to activation of the transcription factor forkhead box P3 (FOXP3) [93]. If IL-6 also is present this will reduce the expression of FOXP3 and instead induce the expression of the transcription factor retinoic acid-related orphan receptor (ROR) γt and RORα and the T cell will differentiate towards a Th17 cell producing mainly IL-17A and F [57,94-97]. IL-17A was first described in 1993 but it was not until 2005, when Harrington *et al.* [98] described the Th17 subset, that the relevance of this cytokine was widely recognised among immunologists [91,99,100]. IL-17A is an important player in host defence towards local Gram-negative extracellular bacterial infections [29,101-107] and local *S. aureus* infections [108]. By inducing the production of the growth factors granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) from structural cells, granulopoiesis is stimulated and the proliferation of neutrophils is enhanced [109,110]. The cells are recruited to site of infection via IL-17A, which activates structural cells to produce CC-chemokines e.g. MCP-1 (also celled CCL2) and CXC-chemokines e.g. KC (also called CXCL1 and NAP-3), macrophage inflammatory protein-2α (MIP-2a) (also called CXCL2) and IL-8 (also called CXCL8) in humans [65,91,99,100,111-113]. IL-17A also induces the production of IL-6 and matrix metalloproteinases (MMP) such as MMP1, MMP3, MMP9 and MMP13 and cathepsin K and hence IL-17A is involved in bone turnover [113-116]. It has also been shown that IL-17A is involved in osteoclastogenesis by inducing RANKL in osteoblasts and synovicytes [117,118] (Figure 5).

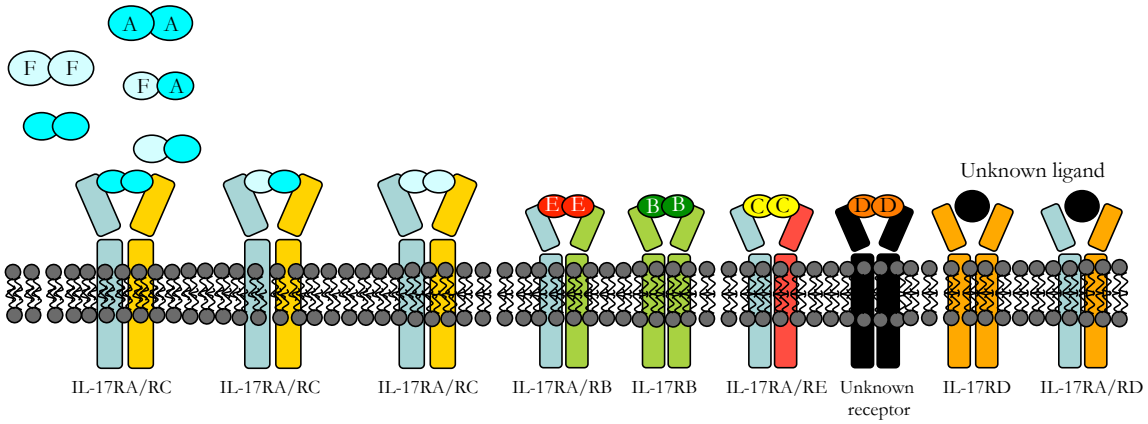


Figure 5. *IL-17 cytokines and their receptors.*

IL-17RA (grey) and IL-17RC (yellow) receptor complex binds IL-17A–IL-17F heterodimers and homodimers of IL-17A or IL-17F. In all cases the composition of the receptor complexes is largely unknown, though it is believed that IL-17RA and IL-17RC forms an IL-17RA–IL-17RC heterodimeric receptor complex. Adapted from Gaffen and Song *et al.* [119,120].

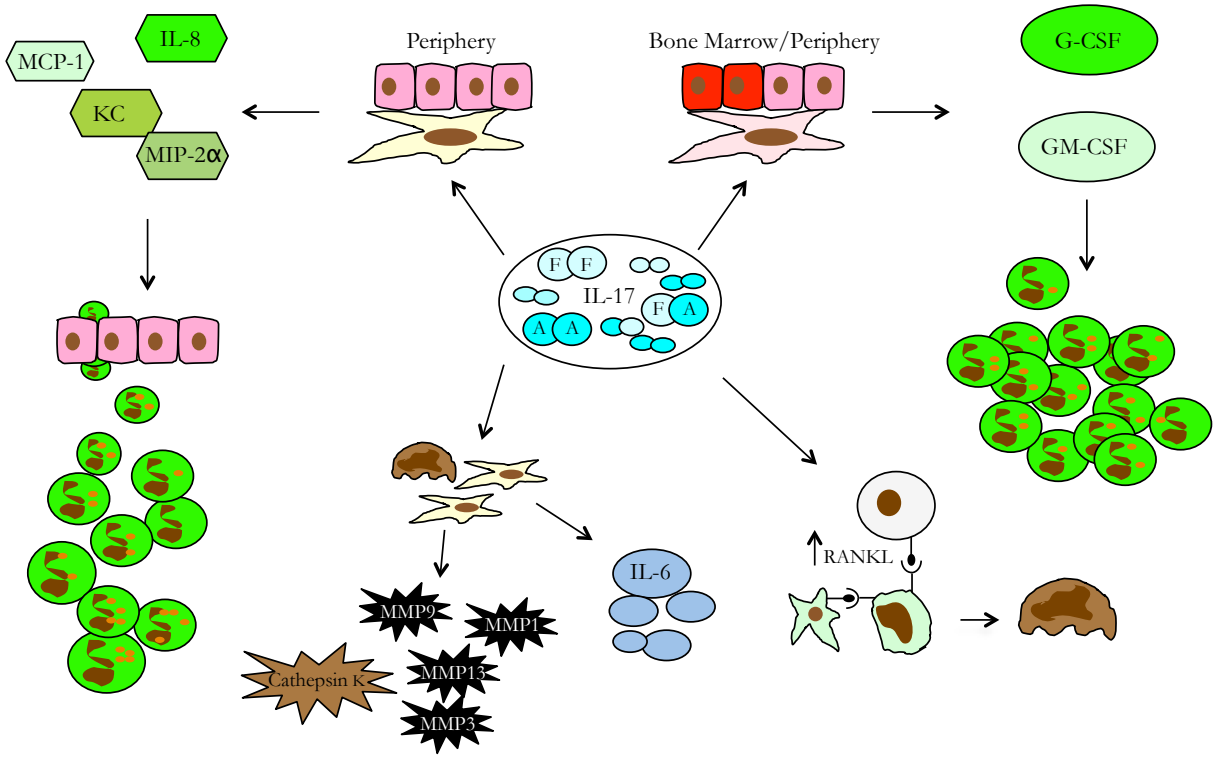


Figure 6. *Functions of IL-17A and IL-17F.*

Endothelial/epithelial cell, fibroblast, neutrophil, macrophage, osteoclast , stromal cell/osteoblast, synovicyte.

Interleukin-17A & Interleukin-17F

Within the IL-17 family, IL-17F is the cytokine that shares the greatest structural and functional homology with IL-17A [91,99]. IL-17F and IL-17A seems to share many biological effects, in particular with reference to the local mobilisation of neutrophils [121]. IL-17A and IL-17F have synergistic effects in combination with other cytokines; IL-17A in combination with TNF-α has been shown to give rise to a higher response with respect to many chemokines and cytokines than the combination IL-17F and TNF-α [122-124]. Both IL-17A and IL-17F exist as homodimers or as IL-17A–IL-17F heterodimers and these are believed to bind to an IL-17RA–IL-17RC heterodimeric receptor complex (Figure 5) [125-130]. IL-17A is mainly expressed by different kinds of T cells e.g. Th17, γδ T cells, NK T cells and $CD8^+$ cells, while IL-17F may be expressed by other cells in addition to these haematopoietic cells.

Interleukin-23

IL-23 is a cytokine produced by activated macrophages and dendritic cells [131-133]. The cytokine is a heterodimer, composed of p19 and p40, where p40 also is a subunit of IL-12. IL-23 was in the beginning thought to have a role in the differentiation of naïve T cells towards Th17 cells, but as it turned out that the IL-23R was not expressed on naïve T cells but on effector T cells [134], this seemed unlikely. Instead it was shown that T cells exposed to IL-6 and TGF-β induces the production of IL-21, which induces the expression of IL-23R in an autocrine loop [135]. Now its is generally believed that IL-23 is important for the expansion and stabilisation of the Th17 cells [57] and that IL-21 and IL-23 both are capable of inducing IL-17A production from Th17 cells (Figure 7) [135].

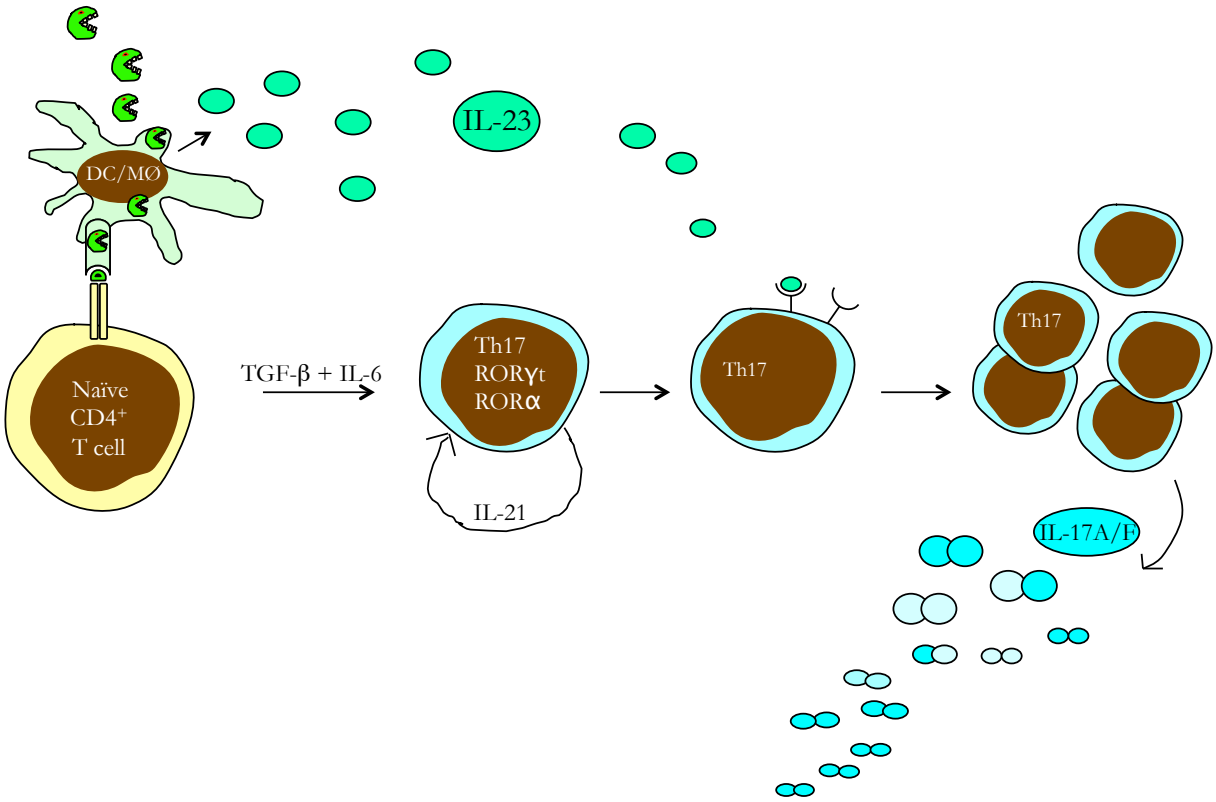


Figure 7. *Function of IL-23*

AIMS

I. To study the impact of Interleukin-15 in *Staphylococcus aureus* arthritis and sepsis.

II. To study the impact of Interleukin-17A in systemic and local *Staphylococcus aureus* arthritis.

III. To study the downregulation by Interleukin-17A on the upstream cytokine Interleukin-23 under both Gram-positive and Gram-negative conditions.

METHODS

For more detailed information of material and methods please refer to the individual papers (I-III).

Animals

Mice (I, II, III)

To investigate the role of specific cytokines in mice *in vivo* we use knockout mice. C57BL/6 wildtype (Scanbur, Sollentuna, Sweden) and IL-15 knockout [79] and IL-17A knockout [136] mice, both on a C67BL/6 background were used in order to investigate the impact of these cytokines in *S. aureus*–induced arthritis and sepsis (I, II). We also investigated the role of IL-17A in this model on a BALB/c background, where we used BALB/c wildtype and BALB/c IL-17A knockout mice [136] (II). Both male and female mice were used. To study the role of IL-17A on IL-23 release, in the model of sepsis induced pneumonia, male C57BL/6 wildtype and C67BL/6 IL-17A knockout mice were used while male BALB/c wildtype mice were used in the model of Gram-negative airway infection (III). Permission from the local Animal Research Ethics Committee, in accordance with national animal welfare legislation, was obtained for all the experiments.

Humans (III)

The study protocol was approved by the Ethics Committee in Gothenburg and has previously been used in a recently published study (45). Written informed consent was obtained from healthy volunteers recruited by advertisement. The investigated subjects were non-smoking and non-atopic (i.e. negative PhadiatopTM test) individuals without any regular medication, who constituted technical control subjects for a larger study (45). The subjects had normal ventilatory lung function, normal clinical status and electrocardiogram, in accordance with published study inclusion criteria (45). Bronchoscopy with lavage was also carried out at the Royal Liverpool and Broadgreen University Hospitals Trust, with similar and previously published (46) protocols and with the consent of local ethics committees. Volunteers recruited in Liverpool were all healthy, non-smoking adults, without any regular medication.

Bacterial inoculation in mice (I, II, III)

We have chosen to use the well described TSST-1 producing *S. aureus* strain LS-1 in our experiments which was originally isolated from a swollen joint of a mouse with spontaneous outbreak of arthritis [8]. After this finding extensive research has been done in this mouse model. The route of infection for the systemic model, intravenously, was chosen as haematogenously spreading of bacteria is the most common way of infection in human septic arthritis. The bacteria were injected into one of the tail veins. The number of inoculated bacteria is crucial for the outcome of the infection, e.g. a certain dose is tolerable and gives rise to arthritis whereas a higher dose causes sepsis (I) and increased mortality. Further, different strains of mice may react differently to the same amount of inoculated bacteria e.g. BALB/c (II) mice does not tolerate as high doses as C57BL/6 (I, II, III), and they were therefore inoculated with a lower dose of bacteria. To mimic a local infection of *S. aureus*, bacteria were inoculated directly into the knee joint, intraarticularly (II).

Anti–IL-15 antibody treatment (I)

To further investigate the effect of IL-15 in *S. aureus*–induced arthritis, wildtype C67BL/6 mice were injected intraperitoneally with monoclonal anti-mouse IL-15 antibody (aIL-15ab) or isotype control antibody starting 3 days post *S. aureus* infection. The antibodies were thereafter injected intraperitoneally at days 6, 10 and 13.

Local inflammation in the airways of mice (III)

The investigation of the impact of LPS on the immune response in the airway is examined using intranasal injection in BALB/c mice. LPS was administrated intranasally to induce Gram-negative airway stimulation.

Mice were treated with anti-mouse IL-17A monoclonal antibodies (aIL-17A ab) or the isotype control intraperitoneally. After 24 hours the mice were anaesthetised transiently and exposed to LPS intranasally. After yet another 24 hours the mice were sacrificed.

In another experiment the mice were anaesthetised transiently and exposed to recombinant mouse IL-17A protein (rmIL-17A) or the corresponding vehicle (phosphate buffered saline (PBS)) intranasally. The mice were sacrificed after 2 hours.

Bronchoalveolar lavage in mice & humans (III)

To evaluate the immunological response in the mouse lung after LPS and rmIL-17A protein exposure, bronchoalveolar lavage (BAL) was performed. For this a tracheotomy tube was inserted in the trachea and fluid (usually PBS) was used to wash the lungs in order to wash out the cells. The samples were centrifuged and the cell free BAL fluid and cell containing pellet were collected separately.

In human studies, BAL fluid was obtained from bronchoscopy with bilateral bronchoalveolar lavage on each subject. Briefly, during a first bronchoscopy, a balloon-tipped catheter was inserted through the bronchoscope, placed in a segmental bronchus, and inflated with air to occlude the segments chosen for challenge. Ten ml of PBS followed by 10 ml of air were then instilled into the bronchus segment. The bronchoscope was then retracted and the head end of the operating table was elevated with the subject in place for 1 hour, to minimise the spread of the instilled PBS. During a second bronchoscopy, the same protocol was followed but with the inclusion of BAL procedure (3*50ml of PBS) instead of PBS instillation. Endobronchial photographs were taken bilaterally on both occasions, to ensure that the BAL sampling was performed in the PBS exposed segment.

Culture of human cells of the monocyte-lineage (III)

In order to assess if IL-17A–induced inhibition of IL-23 is important also in humans, we used different types of human monocyte-lineage cells, monocytes, monocyte-derived macrophages and alveolar macrophages, to which recombinant human IL-17A (rhIL-17A) protein was added after LPS stimulation.

Peripheral blood mononuclear cells were harvested from venous blood of healthy human volunteers and collected by density centrifugation over a Ficoll gradient. Monocytes were then isolated using negative selection. To obtain monocyte-derived macrophages, monocytes were cultured in the presence of recombinant human GM-CSF protein during 5 days. Human alveolar macrophages were isolated from the other cells in BAL by adherence for 2 h.

Human monocytes, monocyte-derived macrophages and human alveolar macrophages were subsequently stimulated with LPS and rhIL-17A or the corresponding vehicle (supplemented medium) for 24 h. After 24 h of stimulation the supernatant was collected and centrifuged in order to get cell free medium. The macrophages that had adhered to the bottom of the culture

wells were lysed for quantitative (real-time) polymerase chain reaction (q-PCR) analysis (see below).

In another experiment, the human monocyte-derived macrophages were stimulated with LPS and rhIL-17A or its vehicle (supplemented medium), together with a Ras-related C3 botulinum toxin substrate 1 (Rac1) inhibitor at different concentrations.

Evaluations

Clinical evaluation (I, II, III)

All mice were followed individually and checked daily. Mice were graded blindly for clinical arthritis. This clinical evaluation includes finger/toe and ankle/wrist joints but not knees and elbows. The joints were inspected and arthritis was defined as visible erythema and or swelling. To evaluate the intensity of arthritis, a clinical scoring was carried out using a system where macroscopic inspection yielded a score of 0-3 points for each limb (0, neither swelling nor erythema; 1, mild swelling and/or erythema; 2, moderate swelling and erythema; 3, marked swelling and erythema). The total score was calculated by adding up all the scores within each animal tested. The overall condition of each mouse was also examined daily by assessing signs of systemic inflammation, i.e. weight change, reduced alertness and ruffled coat.

Histological evaluation (I, II)

In order to assess arthritis more thoroughly and to observe the arthritis in not only finger/toe and ankle/wrist joints but also in knees and elbows histological sections were made (I, II). Joints were fixated, decalcificated and paraffin embedded. Tissue sections from fore- and hind paws were sectioned, deparaffinised and stained with haematoxylin-eosin. The specimens were evaluated with regard to synovitis and bone/cartilage destruction. The degree of synovitis and destruction yielded a score from 0 to 3 in every joint.

Histochemical staining using Safranin-O was performed to determine the degree of cartilage destruction in hind paws (I). Safranin O is a molecule that binds to proteoglycan in the cartilage and therefore the staining intensity is proportional to the amount of cartilage [137]. The tissue sections were sectioned, deparaffinised and stained with Weigert's Iron haematoxylin prior to Safranin O staining with Fast Green counterstaining. The degree of cartilage destruction was evaluated from 0 to 3.

Immunohistochemical staining was also performed in order to evaluate the number of osteoclasts in the knee joints (I). Cathepsin K is a proteolytic enzyme expressed predominantly in osteoclasts and we therefore used a cathepsin K antibody [138]. Tissue sections from knees were sectioned, deparaffinised and an unlabeled rabbit anti-mouse serum containing cathepsin K antibody was used as a primary antibody and normal rabbit serum as a negative control. Biotin-labelled goat anti-rabbit was used as secondary antibody, and was followed by incubation with avidin-biotinylated enzyme complex. Thereafter the substrate was added and the osteoclasts became visible. All sections were counterstained with Mayer's haematoxylin. The number of osteoclasts per 0.1 mm^2 per mouse was counted.

Bacteriological evaluation (I, II, III)

S. aureus home mainly to the kidneys and joints but bacteria have also been found in other tissues such as lungs [139]. To evaluate bacterial clearance, bacterial cultures were performed from kidneys (I, II), lungs (III) and knee joints (II) and the number of colony forming units (CFU) were determined. In brief, both kidneys and lungs were aseptically removed, homogenised, serially diluted in PBS and spread on blood agar plates. The synovial membrane was aseptically removed and suspended in Luria-Bertani (LB) medium. After 24 h of incubation the solution was serially diluted in PBS and spread on blood agar plates. In addition, the synovial membrane was aseptically removed, mashed PBS and spread on blood agar plates. The number CFU was determined after 24 h of incubation at 37°C.

Evaluation of macrophage & neutrophil count (II, III)

In order determine the concentration of macrophages and neutrophils in BAL, cytospin slides were prepared (III). To determine the concentration of neutrophils in blood, smears of peripheral blood were prepared (II). The cells were stained using May Grünwald/Giemsa staining and counted manually. Total concentration of cells in BAL was counted manually in Bürkner chamber whereas the total concentration of leukocytes in blood were counted in cell counter.

Evaluation of MPO activity (II)

Myeloperoxidase (MPO) is a peroxidase most abundant in neutrophils. Measuring the MPO enzyme activity reflects the amount of neutrophils in the tissue. We measured MPO in synovial cells (II). The cells were lysed in lysis buffer and subsequently a peroxidase substrate was added to the samples. The absorbance of the colour change was measured at 450 nm.

Flow cytometric evaluation (II, III)

In the local arthritis model studying IL-17A impact on the disease (II) we wanted to see if the enhanced arthritis was due to a reduced infiltration of neutrophils and MHC II^+ cells. The synovial membrane was homogenised and the synovial cells were stained using a mouse anti-Gr-1 antibody and a mouse anti-I-A/I-E antibody.

To investigate if the resident macrophages in the lung can be responsible for IL-17A-induced inhibition of IL-23 (III) we wanted to evaluate if human alveolar macrophages express the receptor subunits IL-17RA and IL-17RC required for IL-17A signalling [125]. Using flow cytometry we investigated the presence of these receptors on the surface of human alveolar macrophages in BAL.

Cytokine evaluation (I, II, III)

To evaluate the amount of cytokines in blood (I, II) and in supernatants from various cells in mice and humans (III) we used enzyme-linked immunosorbent assay (ELISA) and cytometric bead assay (CBA). Blood was centrifuged and the serum was collected. Serum protein levels of IL-15 (I), IL-17A (II), IL-17F (II) and G-CSF (II) were measured by sandwich ELISA. Serum protein levels of IL-17A (I), IL-4 (I), IL-2 (I), IL-10 (I, II), TNF-α (I, II), IFN-γ (I, II), IL-6 (I, II), MCP-1 (II) and IL-12p70 (II) were measured using CBA kit. Supernatants were collected from BAL or various cell cultures and analysed using ELISA for IL-17A, IL-22, IL-23 (p19 and p40) and IL-12p70 content (III). For detection of the ELISA, the absorbance of the colour change was measured and cytokine detection using the CBA kit was detected using Fluorescence-activated cell sorting (FACS).

Evaluation of gene expression (II, III)

The gene expressions of IL-17F in lymph nodes (II) and of IL-23p19 and p40 in monocyte-derived macrophages and alveolar macrophages (III) were analysed using q-PCR. The cells were lysed with RLT buffer, mRNA was prepared and thereafter cDNA was synthesised. Primers and probes for IL-17F (II) and for IL-23p19 and IL-23p40 (III) were used. Each sample was run in duplicate. Mouse GAPD endogenous control (II) or human β-actin endogenous control (III) were used to normalise the data.

Statistical analysis

For all experiments, each donor contributed to one independent experiment. *n* represents the number of independent experiments and $p \leq 0.05$ was considered statistically significant. Data are expressed as median or mean [SEM] or mean [SD] as indicated.

Paper I & II

Statistical differences between independent groups were calculated using non-parametric Mann-Whitney U test or Fisher's exact probability test. Kaplan-Meier survival plots were prepared and the log-rank test was used for comparison between the two survival curves. Cytokine levels (I) were compared between wildtype and IL-15 knockout mice using a mixed model, including days after bacterial inoculation as continuous variable, genotype as fixed variable and cytokine level as dependent variable.

Paper III

Two-tailed student's t-test was used for statistical analysis of data from mouse BAL samples and human monocyte-derived macrophages and alveolar macrophages *in vitro* unless otherwise stated. The IL-23 concentrations in the cell culture medium of the human monocyte-derived macrophages and the alveolar macrophages were logarithmically transformed in order to normalise the baseline variance. The concentration-response data from Rac1-inhibitor treated human monocyte-derived macrophages *in vitro* were analysed by using repeated measures ANOVA.

The absence of IL-15 ameliorates S. aureus*–induced arthritis*

To investigate if IL-15 has any impact on systemic *S. aureus*–induced arthritis, *S. aureus* was inoculated intravenously into wildtype and IL-15 knockout mice. IL-15 knockout mice lost significantly less weight compared with wildtype mice after intravenous *S. aureus* inoculation, but no differences could be observed in mortality rate (Figure 8A & B). Clinical severity of arthritis and frequency of arthritis was significantly decreased in the IL-15 knockout mice compared with wildtype mice late during infection (Figure 8C & D).

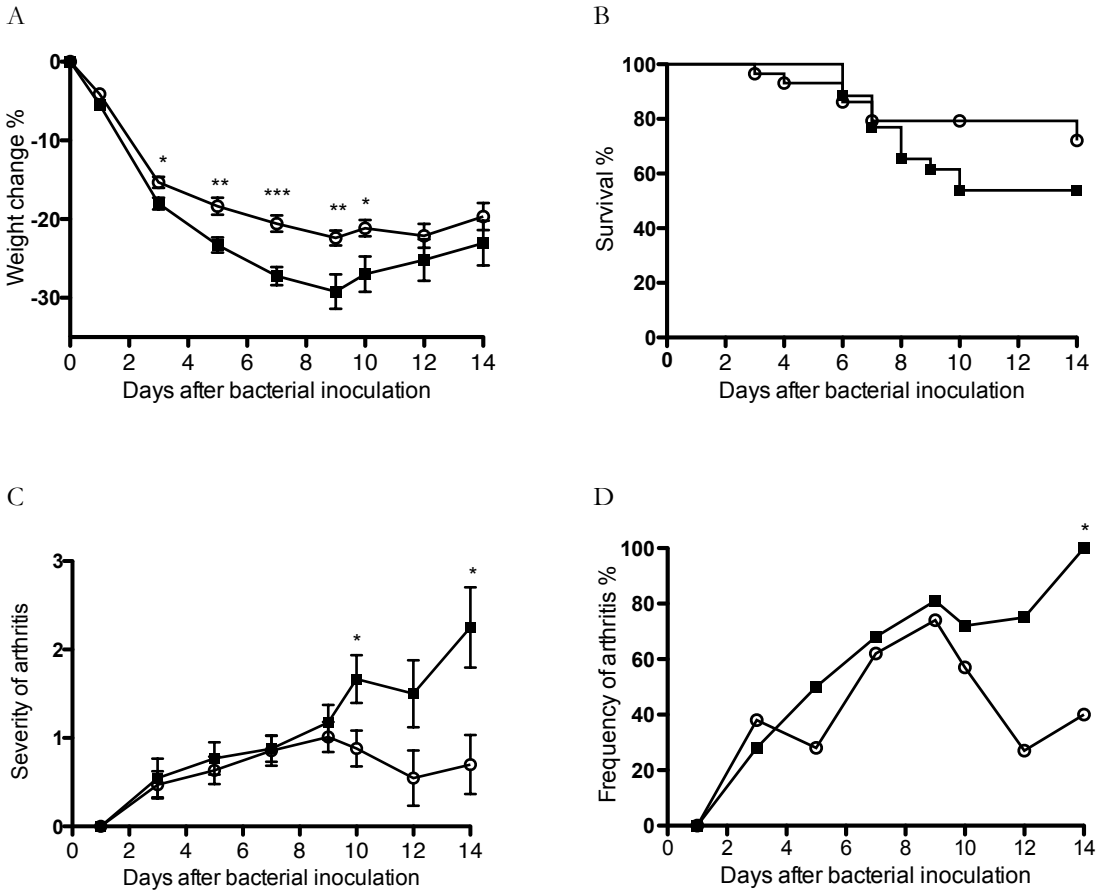


Figure 8. Weight change, survival, clinical severity of arthritis, together with frequency of arthritis in wildtype (■) and IL-15 knockout (○) mice after *S. aureus* intravenous inoculation.

The absence of IL-15 does not affect bacterial clearance

The host's ability to clear bacteria was measured per kidney pair at days 10 and 14 after bacterial inoculation, no significant differences were obtained between wildtype mice and knockout mice (Figure 9).

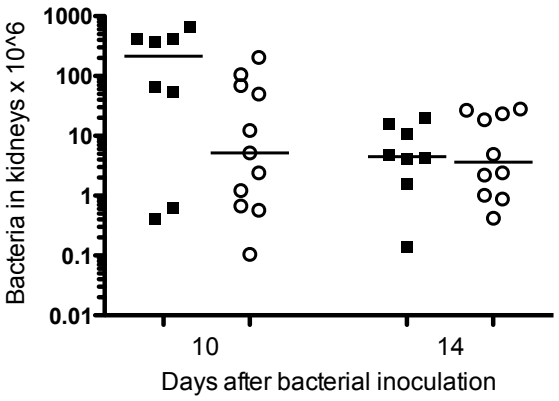


Figure 9. Amount of bacteria in kidneys after systemic *S. aureus* inoculation in wildtype (■) and IL-15 knockout (○) mice.

The absence of IL-15 leads to a reduction of cartilage destruction & number of osteoclasts

It has been shown that IL-15 affects the development of osteoclasts [87]. We wanted to investigate if this effect is the reason for reduced arthritis in the IL-15 knockout mice. Safranin O staining of cartilage showed significantly reduced cartilage destruction in the IL-15 knockout mice compared with wildtype mice at day 14 after *S. aureus* inoculation (Figure 10A). Also, cathepsin K staining of the joints revealed a significantly reduced number of osteoclasts in the IL-15 knockout mice compared with wildtype mice (Figure 10B).

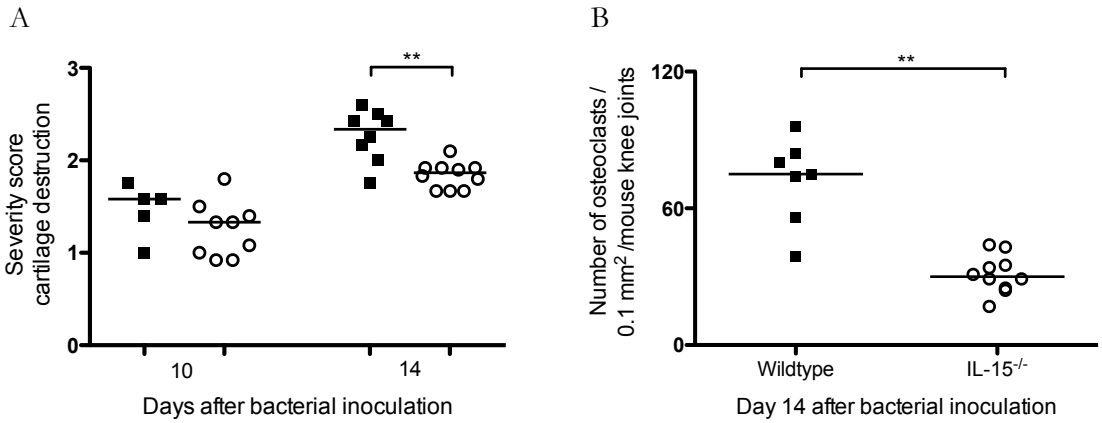


Figure 10. Safranin O and Cathepsin K staining in wildtype (■) and IL-15 knockout (○) mice after *S. aureus* intravenous inoculation.

IL-15 does not influence the outcome of S. aureus*–induced sepsis*

In order to investigate the importance of IL-15 during *S. aureus*–induced septic shock, a 10-fold higher (septic) dose of *S. aureus* was intravenously inoculated. Apart from a slight increase in weight loss at day 1 in the IL-15 knockout mice compared with wildtype mice (Figure 13A), no differences were observed in terms of mortality, severity or frequency of clinically assessed arthritis or bacterial clearance at any timepoints (Figure 13B-E).

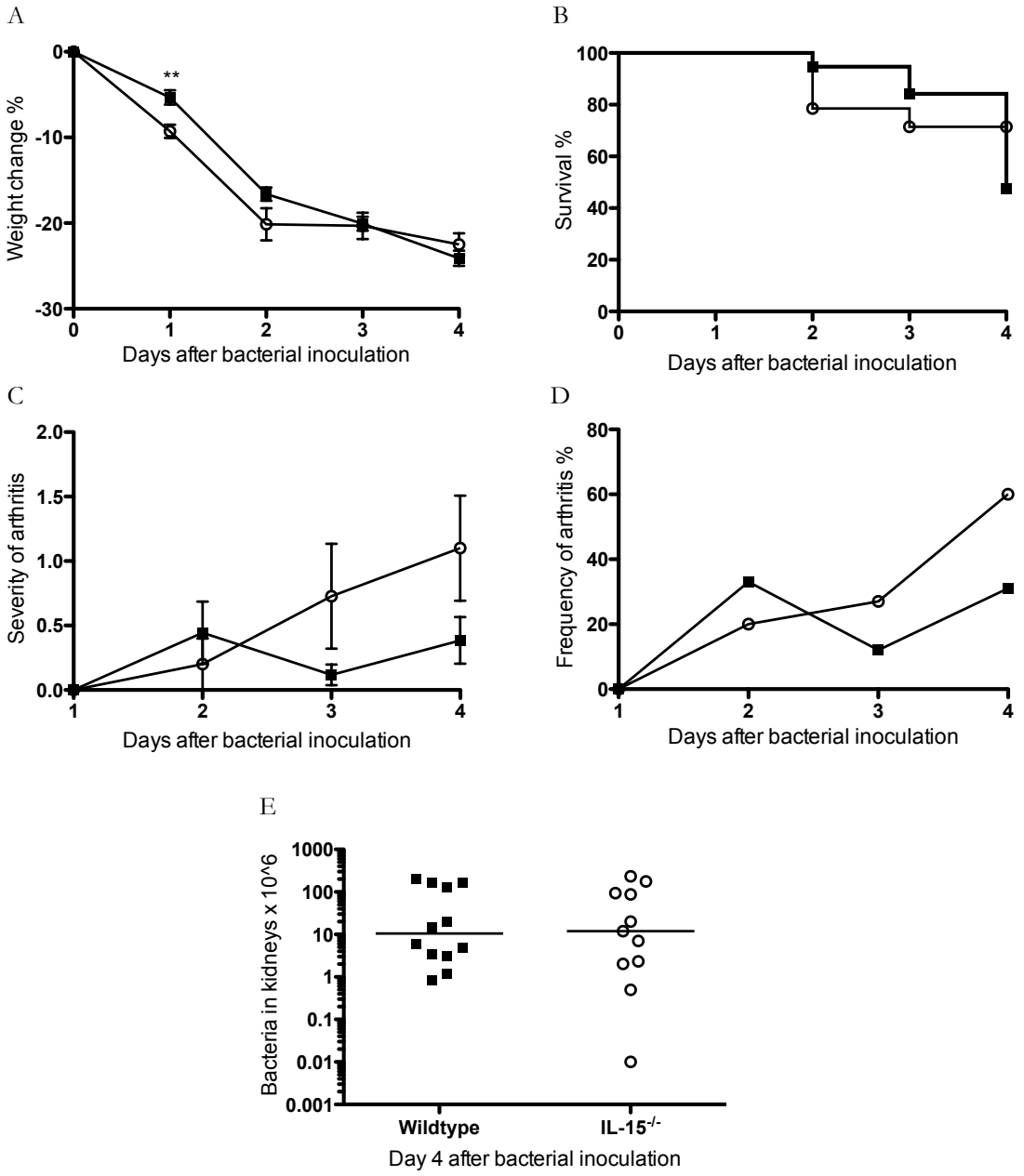


Figure 13. Weight change, survival, clinical severity of arthritis, frequency of arthritis together with amount of bacteria in kidneys in wildtype (■) and IL-15 knockout (○) mice after a septic dose of *S. aureus* intravenous inoculation.

Treatment with aIL-15ab ameliorates S. aureus*–induced arthritis*

To investigate if aIL-15ab treatment has any impact on systemic *S. aureus*–induced arthritis, *S. aureus* was inoculated intravenously into wildtype mice and 3 days later the mice were treated with aIL-15ab or its isotype control. Mice treated with aIL-15ab lost significantly less weight compared with mice treated with isotype control at day 14 after intravenous *S. aureus* inoculation (Figure 11A). Haematoxylin-eosin stained histological sections showed that mice treated with aIL-15ab had significantly reduced both severity of synovitis and bone/cartilage destruction compared with mice treated with isotype control antibodies at day 14 after intravenous inoculation of *S. aureus* (Figure 11B).

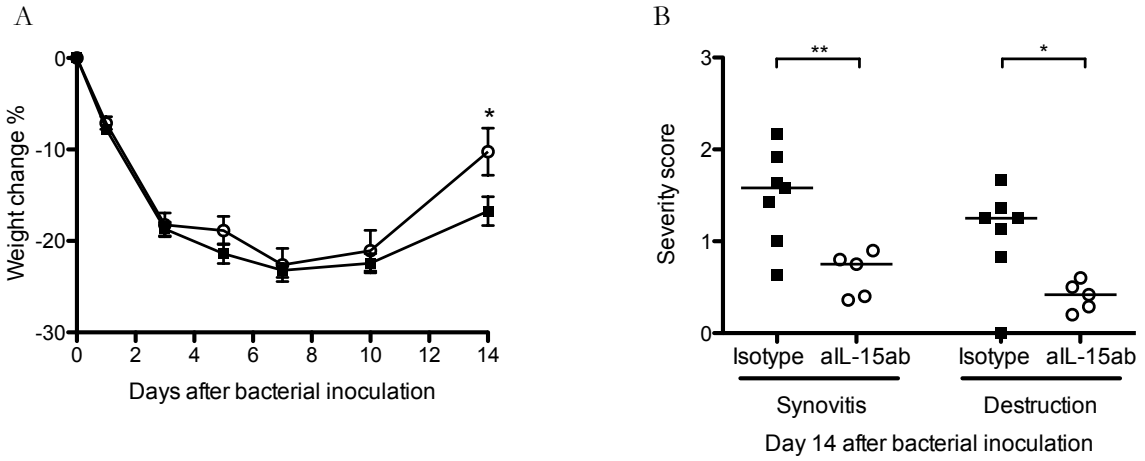


Figure 11. Weight change and histology of joints in wildtype mice treated with isotype control (■) or aIL-15ab (○) after intravenously inoculated with *S. aureus.*

Anti–IL-15ab treatment enhances bacterial clearance

Mice treated with aIL-15ab showed a significantly increased ability to clear bacteria from kidneys compared with mice treated with isotype control antibody (Figure 12).

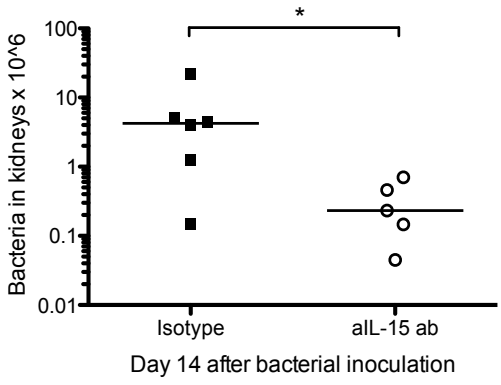
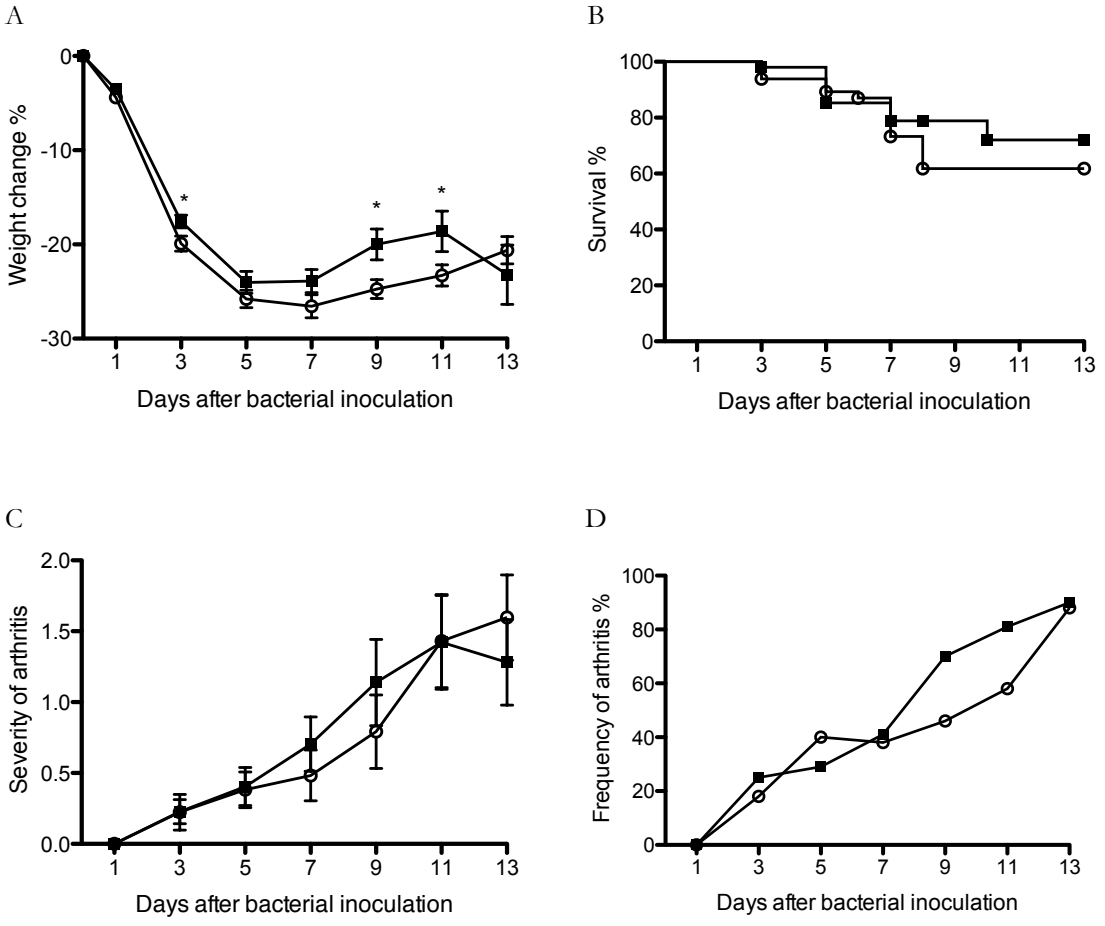


Figure 12. Amount of bacteria in kidneys after systemic *S. aureus* inoculation in mice treated with isotype control (■) or aIL-15ab (○).

IL-17A has a moderate impact on systemic S. aureus*–induced arthritis*

To evaluate if IL-17A have any impact on systemic *S. aureus* disease development, wildtype and IL-17A knockout mice were intravenously inoculated with *S. aureus*. Although the IL-17A knockout mice lost significantly more weight than wildtype mice at days 3, 9 and 11 after *S. aureus* inoculation, there were no differences in mortality (Figure 14A & B) or severity and frequency of clinically assessed arthritis (Figure 14C & D). However, histological sections from the C57BL/6 mice at days 13 showed that IL-17A knockout mice displayed significantly more erosions than wildtype mice (Figure 14E).



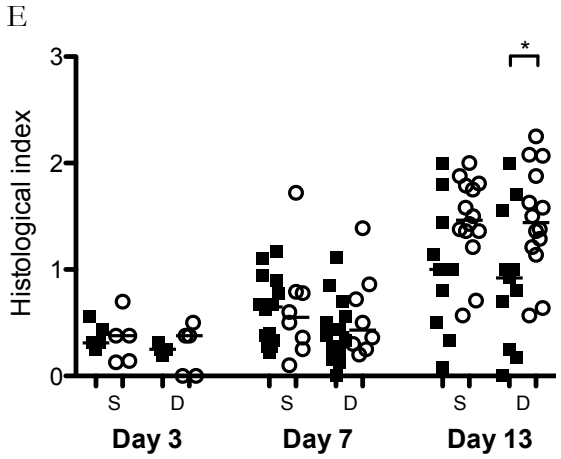


Figure 14. Weight change, survival, clinical severity of arthritis, frequency of arthritis and histology of joints in wildtype (■) and IL-17A knockout (○) mice after *S. aureus* intravenous inoculation. S: Synovitis, D: Bone/cartilage destruction.

The absence of IL-17A increases clearance of bacteria from kidneys early during systemic S. aureus *infection*

Bacterial clearance from kidneys was significantly higher in IL-17A knockout mice compared with wildtype mice at day 1 after *S. aureus* inoculation. At later timepoints, the bacterial clearance was similar in both groups of mice (Table 1).

Table 1. Amount of *S. aureus* from kidneys of wildtype and IL-17A knockout mice after intravenous inoculation.

	Wildtype (CFU)		IL-17A$^{-/-}$ (CFU)	
Day		n		n
1	31,400 ± 20,084	5	1200 ± 374*	5
3	1.7 ± 0.7 (x10^7)	5	1.1 ± 0.6 (x10^7)	5
7	9.3 ± 1.7 (x10^7)	19	12.7 ± 4.7(x10^7)	12
13	1.5 ± 0.5 (x10^7)	21	2.2 ± 0.6 (x10^7)	22

The absence of IL-17A leads to increased levels of IL-17F during systemic S. aureus *infection*

It has been shown that the absence of IL-17A leads to an increase in the levels of IL-17F [140]. Expression of IL-17F mRNA in lymph nodes was significantly higher in IL-17A knockout mice compared with wildtype mice both before and at day 13 after *S. aureus* inoculation (Figure 15A). These data were confirmed by significantly increased serum protein levels of IL-17F in IL-17A knockout mice at days 1, 7 and 13 after infection (Figure 15B).

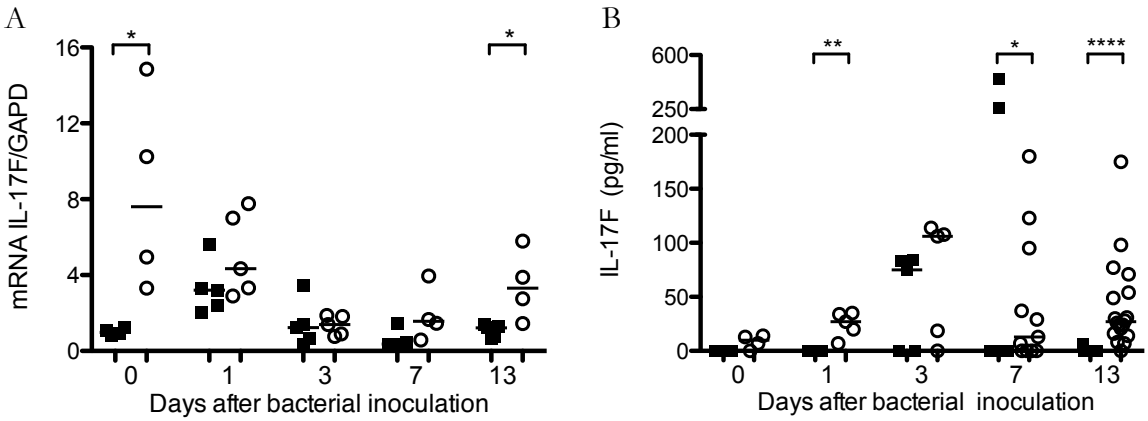


Figure 15. Levels of IL-17F mRNA in lymph nodes and serum protein levels of IL-17F in wildtype (■) and IL-17A knockout (○) mice before and after *S. aureus* intravenous inoculation.

The absence of IL-17A influences serum protein levels of IL-6 & IFN-γ during systemic S. aureus *infection*

S. aureus infection has profound effects on the immune response and many cytokines are upregulated during infection. We found differences in the levels of IL-6 and IFN-γ between wildtype and knockout mice. Serum protein levels of IL-6 were significantly decreased at day 13 after *S. aureus* inoculation (Figure 16A). In contrast, serum IFN-γ protein levels were significantly increased in IL-17A knockout mice compared with wildtype mice at day 1 (Figure 16B).

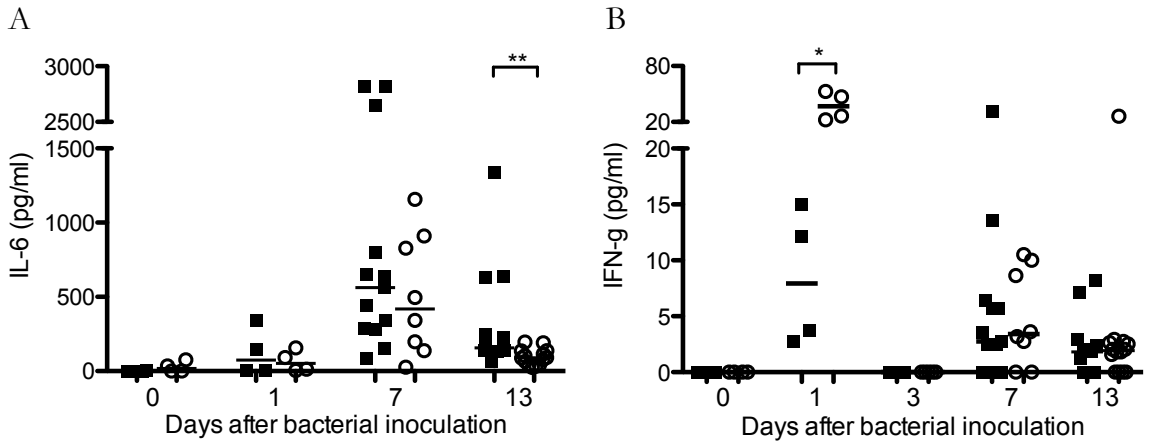


Figure 16. Levels of IL-6 and IFN-γ protein in serum in wildtype (■) and IL-17A knockout (○) mice before and after *S. aureus* intravenous inoculation.

IL-17A protects against local S. aureus*–induced arthritis*

To evaluate if IL-17A has any impact on local *S. aureus* infection, *S. aureus* was inoculated into the knee joint. IL-17A mice knockout showed significantly more severe synovitis and erosive arthritis compared with wildtype mice at day 3 (Figure 17A). Bacterial growth was found in 3 of 5 IL-17A knockout mice compared with 1 of 5 wildtype mice when the synovial membrane was directly spread on a blood agar plate. In addition, to determine the amount of bacteria per synovial membrane a broth culture of the synovial membranes was performed and the bacterial load was significantly increased in IL-17A knockout mice compared with wildtype mice at day 3 after intraarticular inoculation (Figure 17B). There was a clear trend towards decreased numbers of neutrophils in the synovial membranes from the IL-17A knockout mice compared with wildtype mice (Figure 17C), while the MPO activity was similar in both groups (Figure 17D).

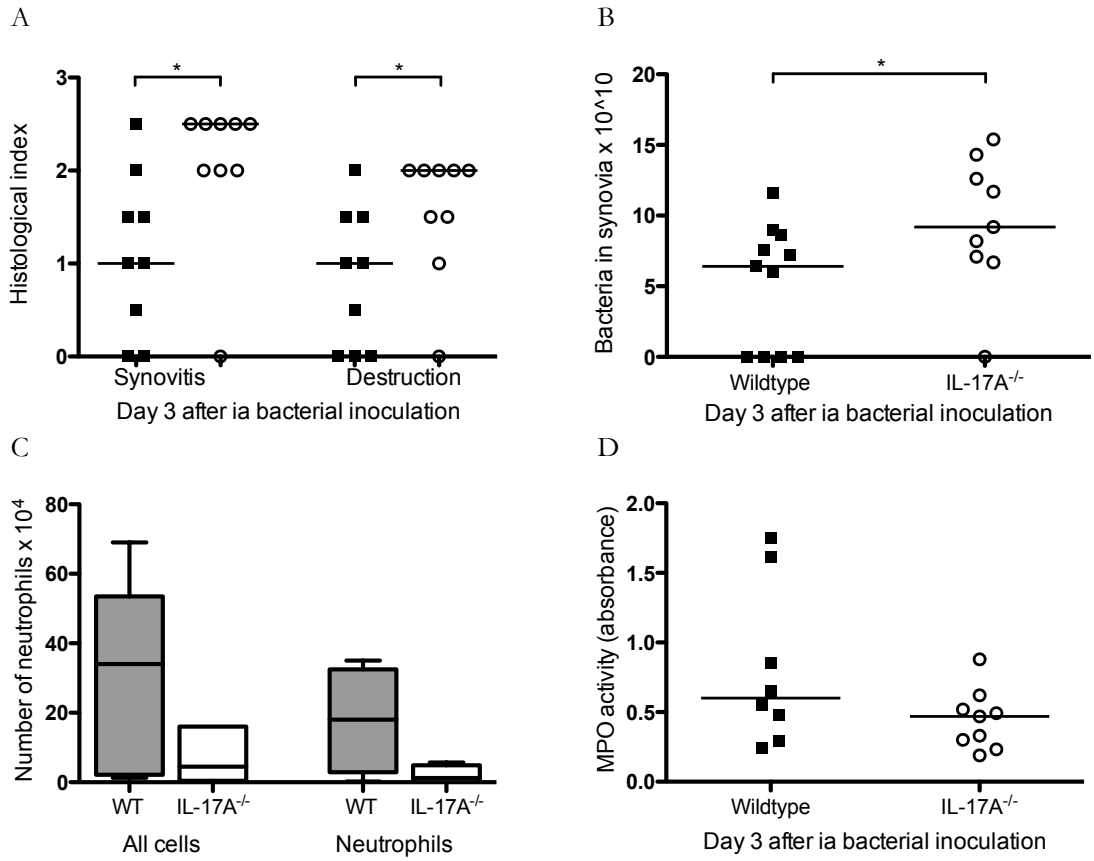


Figure 17. Histology of knees, amount of bacteria, number of neutrophils and MPO activity in the synovial membrane in wildtype (■) and IL-17A knockout (○) mice after local *S. aureus* intraarticular inoculation.

Local protein levels of IL-23 are elevated in IL-17A knockout mice after systemic inoculation of S. aureus

S. aureus was intravenously inoculated in C57BL/6 wildtype and IL-17A knockout mice as previously described. Subsequently the concentration of IL-23 protein in cell free BAL fluid was determined. We found that the concentration of IL-23 protein in cell free BAL fluid was significantly higher in IL-17A knockout mice compared with wildtype 24 h after but not before, inoculation of *S. aureus*. Also, the concentration of alveolar macrophages in BAL samples was higher in knockout mice compared with wildtype mice after *S. aureus* inoculation.

Local protein levels of IL-23 are elevated after aIL-17A ab treatment during Gram-negative airway stimulation

Mice were intranasally exposed to LPS, some mice were pre-treated with an aIL-17A ab or its isotype control intraperitoneally. Exposure to LPS alone caused a significant decrease in the protein levels of IL-23 in the cell free BAL fluid compared with vehicle control exposure. Further, exposure to LPS in combination with aIL-17A ab pre-treatment caused a significant increase in the concentration of IL-23 protein in the cell free BAL fluid compared with control mice. The concentration of alveolar macophages was significantly reduced in LPS exposed mice compared with vehicle control exposure. However, LPS exposure in combination with aIL-17A ab pre-treatment did not cause any differences in the concentration of alveolar macrophages in BAL samples compared with control mice. The concentration of neutrophils was significantly increased in BAL samples after LPS exposure compared both with vehicle control exposure and LPS exposure in combination with aIL-17A ab pre-treatment.

Local protein levels of IL-23 are reduced in mouse airways in vivo *after local administration of rmIL-17A*

After intranasal stimulation (2 h) with rmIL-17A protein only, mice displayed a significantly decreased concentration of IL-23 protein in cell free BAL fluid compared with the vehicle-stimulated control mice.

IL-17RA & IL-17RC are both expressed by human alveolar macrophages

After examination of cell surface expression of IL-17RA and IL-17RC on human alveolar macrophages, we observed the presence of both receptor units on a series of BAL samples collected from 8 human volunteers.

The production of IL-23 by human monocyte-lineage cells is reduced by rhIL-17A

Human monocytes, monocyte-derived macrophages and alveolar macrophages were stimulated with LPS in combination with rhIL-17A protein or vehicle, after which the protein concentration of IL-23 in the conditioned cell culture medium was measured. Conditioned medium from LPS stimulated human monocyte-derived macrophages and from LPS stimulated human alveolar macrophages contained detectable protein levels of IL-23 and addition of rhIL-17A protein decreased the concentration of IL-23 protein in the conditioned medium, compared with controls.

Rac1 inhibition did not affect the rhIL-17A-dependant IL-23 decrease in human

The inhibition of Rac1 caused a concentration dependant decrease in IL-23 concentration in the conditioned medium from the LPS and rhIL-17A stimulated human monocyte-derived macrophages. Importantly, there was also a similar trend in cells not stimulated with rhIL-17A protein.

Human macrophage p19 & p40 expression is not downregulated by rhIL-17A stimulation

Stimulation with LPS and rhIL-17 protein significantly increased the p19 mRNA expression in the human monocyte-derived macrophages compared with LPS and vehicle control stimulation. The same trend was found for the p40 mRNA expression in the monocyte-derived macrophages stimulated with LPS and rhIL-17A and in the human alveolar macrophages stimulated with LPS and rhIL-17A, with respect to both p19 and p40.

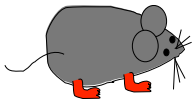
DISCUSSION

We have been investigating the cytokines IL-15 and IL-17A during the course of *S. aureus*–induced arthritis and sepsis in mice. Both cytokines are proinflammatory which, - although through different mechanisms - have effects on neutrophils and osteoclast. However, the cytokines seems to have different roles in *S. aureus*–induced arthritis.

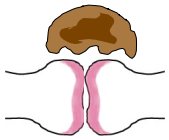
Paper 1

In this study we investigated the role of IL-15 in systemic *S. aureus*–induced arthritis.

I summarise the findings in <u>*S. aureus*–induced arthritis and sepsis in the IL-15 knockout mice.</u>

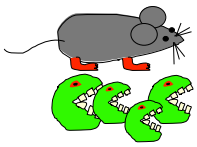


Mice lacking IL-15 lost significantly less weight throughout systemic *S. aureus*–induced arthritis and the severity and frequency of arthritis was decreased late during infection.



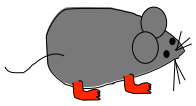
The decrease in arthritis in the IL-15 knockout mice coincided with a decrease in the number of osteoclasts and a decrease in cartilage destruction in the joints compared with wildtype mice.

The host's ability to clear bacteria was not affected in the absence of IL-15.

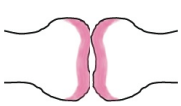


In *S. aureus*–induced sepsis, no differences were observed between wildtype and knockout mice with respect to weight loss, mortality, clinical arthritis and clearance of bacteria in the kidneys.

I summarise the findings in <u>*S. aureus*–induced arthritis the mice treated with aIL-15ab.</u>



Mice treated with aIL-15ab lost significantly less weight at day 14 after systemic *S. aureus*–induced infection compared with mice treated with isotype control.



Also, the aIL-15 treated mice displayed significantly reduced severity of synovitis and bone/cartilage destruction at day 14 compared with isotype control treated mice.

The reduced weight loss and arthritis coincided with an increased ability to clear bacteria in the mice treated with aIL-15ab.

Interleukin-15, Natural Killer cells & osteoclastogenesis

The differentiation of haematopoietic stem cells (HSC) to osteoclasts is dependent on two major factors, macrophage-colony stimulating factor (M-CSF) and RANKL [118,141]. First, differentiation of HSCs to common macrophage/osteoclast precursors and expansion of these cells is achived by M-CSF and second, the stimulation with RANKL leads to cell-cell fusion and formation of multinucleated osteoclasts. The induction of these mediators in osteoclastogenesis is regulated through various factors, e.g. proinflammatory cytokines, and lately it has been shown that IL-15 is one of them [87-89]. IL-15 induces RANKL expression in synovial fibroblasts in patents with RA [89]. In addition, IL-15 is capable of activating NK cells, which can express both RANKL and M-CSF and their production is further upregulated in the presence of IL-15 [90,142]. IL-15 is a critical survival factor for NK cells and the IL-15 or IL-15Ra knockout phenotype has a severe reduction or an absence of NK cells [79,143]. Depletion of NK cells in mice using an NK1.1 antibody aggravates the severity of arthritis after *S. aureus* inoculation, and IL-15 knockout mice (sometimes used as NK cell deficient mice) develop more severe *S. aureus*–induced lung infection [48,144]. Both these studies suggest a protective role for NK cells in *S. aureus* infections. The immunological mechanism involved in any of these studies have not been thoroughly investigated. In contrast, we observe that the lack of IL-15 ameliorates arthritis, probably mediated by a reduction in the number of osteoclasts in the joints from IL-15 knockout mice compared with wildtype mice. Giving that osteoclasts are of uttermost importance for the loss of juxta-articular bone in arthritis [145,146] it is plausible to believe that a fewer number of osteoclasts leads to reduced joint destruction.

Interleukin-15 & apoptosis

IL-15 is a potent survival factor as it inhibits proapoptotic factors such as Bax, and activates antiapoptosic factors such as Bcl-2 and Bcl-xL [76,147,148]. The capability of IL-15 to inhibit sepsis–induced apoptosis in lymphocytes as well as other immune cells might result in a defective immune response and poor survival in sepsis [83,148,149]. In arthritis, fibrosis is prominent due to excessive proliferation of fibroblasts, a response to excessive cytokine stimulation [150]. In our study we did not observe increased mortality rate in the mice lacking IL-15, however we did find a decreased severity of arthritis in these mice. As the antiapoptotic effects of IL-15 is not only applied on immune cells but also on other cells [76] we studied if enhanced apoptosis of synovial cells contributed to the reduced severity of arthritis. We performed *in situ* Terminal deoxynucleotidyl transferase dUTP Nick and Labelling (TUNEL) assay on frozen knee sections in IL-15 knockout mice and wildtype mice sacrificed at days 3, 7 and 10. Few TUNEL positive

cells were detected in the sections and no differences were found between the two groups (L. Henningsson, unpublished data).

Interleukin-6 & osteoclastogenesis

IL-6 is a pleiotropic cytokine and one of its main functions is to stimulate release of acute phase reactants from the liver [151,152]. The acute phase reactants and IL-6 itself promotes a profound immune response to *S. aureus* infections which on the one hand is needed for bacterial elimination [39,59], but on the other increases both morbidity and mortality. In addition, IL-6 is deeply involved in bone turnover [153]. It plays a prominent role in the formation of osteoclasts [154-157] e.g. by inducing the expression of RANKL from stromal cells [58,158] and hence contributes the upregulation of IL-6 to destruction of the joints in aseptic arthritis [56,159] and most probably also in septic arthritis. We observed increased serum levels of IL-6 in our IL-15 knockout mice which could contribute to the decreased weight loss and maintained bacterial clearance seen in this group. Interestingly, we did not observe any differences in serum levels of IL-6 in mice treated with aIL-15ab (where compensatory mechanisms probably are not established) while the synovitis and bone/cartilage destruction were reduced and the bacterial elimination increased. These findings suggest that it is the absence (IL-15 knockout) or reduced (aIL-15ab treatment) levels of IL-15 that contribute to the reduced joint destruction and not the increased levels of IL-6.

Interleukin-15 & bacterial clearance

The bacteria home to and reside in joints and kidneys. The clearance of bacteria is mainly dependent on phagocytic neutrophils and macrophages, and these cell types also contribute to joint destruction. Extracellular IL-15 has been reported to enhance neutrophil recruitment by the secretion of IL-8 in monocytes [86]. However, Orinska *et al.* found that intracellular IL-15 in mast cells reduced neutrophil recruitment to the site of infection and decreased bacterial clearance in in a CLP sepsis model [70]. In contrast to Orinska *et al.* [70], we did not observe any differences in our IL-15 knockout mice compared with wildtype mice with respect to bacterial clearance in the kidneys, neither in *S. aureus*–induced arthritis model nor in *S. aureus*–induced sepsis model. These differences are probably due to different infection models and different microbes, i.e. intravenous *S. aureus* (Gram-positive) infection *versus* local CLP (mixed but mainly Gram-negative) infection. Further, in the CLP model bacterial clearance is measured locally in the peritoneal lavage within 1-3 hours after infection, while in the *S. aureus* model, bacterial clearance is measured in a remote localisation (kidneys) several days after bacterial inoculation. Thus, the

reduced clinical and histological severity of arthritis and decrease in weight loss in the IL-15 knockout mice is not due to increased bacterial clearance. However when mice where treated with aIL-15ab, we observed a significantly enhanced bacterial clearance in the kidneys compared with mice treated with isotype control. It is possible that a total absence of IL-15 in the knockout mice has triggered compensatory mechanisms in these mice which has not taken place in mice treated with aIL-15ab, where IL-15 still is present, though in lower levels.

Interleukin-15 in S. aureus*–induced sepsis*

A 10 times higher dose of *S. aureus* leads to septic shock, which is the worst-case scenario and can lead to multiple organ failure and death. We investigated how the IL-15 knockout mice reacted after a 10-fold higher bacterial dose. We found that the IL-15 knockout mice did not exhibit any aggravation in mortality, bacterial clearance or arthritis during the course of infection. However, data with respect to the presence of IL-15 in sepsis are contradictory. We and Orinska *et al.* [70] did not observe any adverse effects in IL-15 knockout mice in sepsis models, while other studies have shown that administration of IL-15 increases survival in sepsis [83,148].

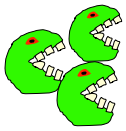
Paper II

In this study we investigated the importance of IL-17A in systemic and local *S. aureus*–induced arthritis.

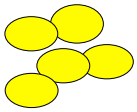
Summary of the findings in the IL-17A knockout mice injected systemically (intravenously) with *S. aureus*.

The absence of IL-17A had no marked impact on the overall mortality or on the clinical development of arthritis after systemic inoculation of *S. aureus*.

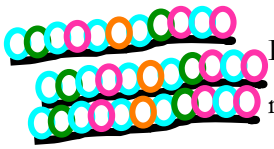
However, IL-17A knockout mice showed increased the bone/cartilage destruction in the joints at days 13 after *S. aureus* inoculation.



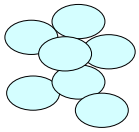
Clearance of bacteria in kidneys was significantly increased in the IL-17A knockout mice early during systemic infection.



The enhanced bacterial clearance coincided with increased serum levels of IFN-γ in IL-17A knockout mice.



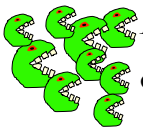
Expression of IL-17F mRNA was significantly higher in IL-17A knockout mice before and at day 13 after *S. aureus* inoculation.



Serum protein level of IL-17F was significantly increased in IL-17A knockout mice compared with wildtype mice before and after *S. aureus* inoculation.

Summary of the findings in the IL-17A knockout mice injected locally with *S. aureus*.

By contrast to systemic infection, local *S. aureus*–induced arthritis rapidly caused a significantly more severe synovitis and destructive arthritis in the IL-17A knockout mice compared with wildtype mice.



Also, locally impaired bacterial clearance was seen in the IL-17A knockout compared with wildtype mice.

Interleukin-17A & bacterial clearance…

…in local S. aureus *infection*

We show in our study that IL-17A knockout mice exhibit an increased severity of synovitis and bone/cartilage destruction in addition to impaired bacterial clearance 3 days after *S. aureus* inoculation into the knee joint. The reason for this is not completely clear but we did find a trend towards reduced neutrophil count and reduced MPO activity in the joints of IL-17A knockout mice compared with wildtype mice. Ishigame *et al.* showed that IL-17A and IL-17F double knockout mice developed local spontaneous mucocutaneous infections (impaired bacterial clearance) probably caused by *S. aureus*. These infections were not present in IL-17A or IL-17F single knockout mice [160]. The disagreement between our findings and those of Ishigame *et al.* could be due to different localisation of the infection and the fact that spontaneous mucocutanous infection might involve several bacterial strains and species.

…in systemic S. aureus *infection*

However, Ishigame *et al.* did not find any differences between the double knockout (IL-17A and F) and wildtype mice in systemic *S. aureus* infection in terms of survival and bacterial clearance measured at day 3 only [160] which is in line with our results using IL-17A single knockout compared with wildtype mice. IL-17A and IL-17F have similar biological functions e.g. neutrophil recruitment to the infected area and may compensate for each other. In our study, we found an upregulation of IL-17F probably minimising the effects on bacterial clearance. [161]. Further, bacterial clearance varies during the course of infection. Ishigame *et al.* [160] have measured bacterial clearance at day 3 only, while we have measured bacterial clearance at 4 different timepoints throughout a 13 days long experiment. Collectively our current as well as previously published data [108,160], suggest that IL-17A and F are involved in the host defence against *S. aureus* infection, but also that their relative importance depends on the site and duration of infection and whether it is local or systemic.

Interleukin-17A & Granulocyte-colony stimulating factor

The absence of IL-17A might result in changed serum levels of G-CSF and blood neutrophil count and hence affect the bacterial clearance [110,162]. These parameters were measured systemically in mice with systemic *S. aureus* infection. We did not observe any differences in serum levels of G-CSF or in blood neutrophil counts, but we did find increased serum levels of IL-17F in the IL-17A knockout mice compared with wildtype mice after systemic *S. aureus* infection. Both IL-17A and IL-17F are known to induce granulopoiesis through the production

of G-CSF [121] and these findings are compatible with IL-17F maintaining G-CSF levels in the absence of IL-17A.

Interleukin-17A & Interferon-γ

The role of IFN-γ in *S. aureus*–induced arthritis is complex, although it has been suggested that IFN-γ enhances bacterial clearance early during infection by increasing phagocytic activity in neutrophils and macrophages [61,163]. Also, it is known that IL-17A inhibits Th1 cells, and hence the production of IFN-γ [164]. Indeed, in the IL-17A knockout mice we found higher levels of IFN-γ in serum at day 1 after systemic bacterial inoculation, which coincided with an increased bacterial clearance in kidneys. Based upon this, we speculate that long-term elevated protein levels of IL-17F – as well as of IL-17A [164] – might have the capacity to downregulate the IFN-γ response during systemic infection.

Interleukin-17A & Interleukin-17F…

IL-17A and IL-17F have overlapping functions, as they use similar regulatory and signalling mechanisms and induce similar gene expression (IL-17F induces a somewhat weaker response) [123-125,165], e.g. IL-17A and IL-17F can both induce the expression of IL-6, IL-8 and G-CSF and both have a role in cartilage turnover [166]. However, they differ from each other with respect to their contribution in diseases e.g. in autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE), asthma and inflammatory bowel diseases [165,167] and infections [160, II] depending on the pathogen and its localisation. In some diseases IL-17F seems to be more prominent, while in others IL-17A predominate. On the one hand, IL-17F but not IL-17A has been implicated in asthma and airway inflammation in humans as elevated levels of IL-17F mRNA has been found in BAL from patients with asthma [169]. On the other hand, IL-17A seems to be more important than IL-17F in the pathogenesis of EAE [165]. In healthy mice, IL-17A is capable of inhibiting the production of IL-17F through an IL-17RA–dependent mechanism [140] which suggests that IL-17A and IL-17F could be functionally linked. In contrast, *Klebsiella pneumoniae* infections do not induce an increase in IL-17F levels in the absence of IL-17A [170]. The difference in IL-17A and IL-17F activity and function might be due to the fact that IL-17A and IL-17F can form both homo- and heterodimers and that these dimers (if they all bind the same receptor complex) may have different binding affinities for the RA/RC receptor complex [125,129].

…*in systemic* S. aureus *infection*

We found higher levels of both lymph node mRNA levels and serum protein levels of IL-17F in our systemic model of *S. aureus*–induced arthritis in the IL-17A knockout mice compared with wildtype mice. This is in line with previous studies where IL-17F levels are increased in healthy mice in the absence of IL-17A [140]. Thus, mice lacking IL-17A are not significantly affected by systemic *S. aureus* infection as IL-17F can functionally replace IL-17A and maintain host defence during systemic staphylococcal infection. However, in histological sections from IL-17A knockout mice we observed enhanced bone/cartilage destruction at days 13, which coincided with significantly enhanced levels of IL-17F serum protein in these mice. As we did not observe any differences in bacterial clearance, neutrophil count or G-CSF levels, the enhanced erosivity must be due to other factors and it seems as if IL-17F not completely can compensate for the loss of IL-17A.

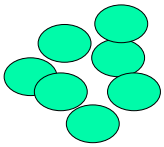
…*in local* S. aureus *infection*

In the local *S. aureus*–induced arthritis model, serum levels of IL-17F were not measured but no differences were found in synovial mRNA IL-17F levels. The IL-17A knockout mice exhibited increased severity of synovitis, bone/cartilage destruction and increased bacterial load compared with wildtype mice. We did also observe a tendency towards a reduced number of neutrophils in the joints of these mice. IL-17A is important in neutrophil recruitment to site of infection [112] and as we do not observe any increased levels of IL-17F, the absence of IL-17A and F signalling results in impared neutrophil recruitment, reduced bacterial clearance and enhanced arthritis.

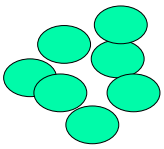
Paper III

In this study we investigated the role of IL-17A on its critical upstream regulator IL-23 in the airways.

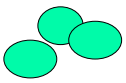
Summary of IL-23 findings in mice:



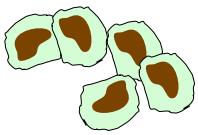
The IL-23 protein levels in BAL are significantly increased in IL-17A knockout mice compared with wildtype mice after intravenous *S. aureus* inoculation.



The IL-23 protein levels in BAL are significantly increased in mice pre-treated with aIL-17A ab and further exposed to LPS intranasally, compared with mice pre-treated with LPS and isotype control.

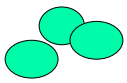


The IL-23 protein levels in BAL are significantly decreased in mice stimulated intranasally with rmIL-17A compared with vehicle control.

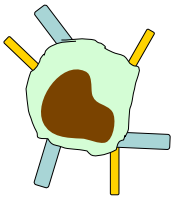


The changes in IL-23 protein levels are not due to alterations in the concentration of macrophages in BAL.

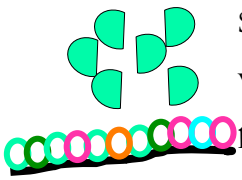
Summary of the IL-23 findings in humans:



Stimulation of human alveolar macrophages or monocyte derived macrophages with LPS and rhIL-17A decreased the IL-23 protein levels in supernatants compared with cells stimulated with LPS only.



We showed that IL-17 receptors A and C are present on human alveolar macrophages.



Stimulation of human alveolar macrophages or monocyte derived macrophages with LPS and rhIL-17A does not decrease the mRNA levels of subunits p19 or p40.

Interleukin-17A & related cytokines

Presently there are six IL-17 cytokines known, IL-17A-F [91] where IL-17A and IL-17F share the highest homology whereas IL-17E (IL-25) is most different from IL-17A [92]. It is well known that IL-17A and F bind as a homo-or- as a IL-17A/IL-17F heterodimer to IL-17RA and IL-17RC [125,127-129] whereas IL-17E (IL-25) has been reported to bind to a complex of IL-17RA and IL-17RB [171]. Our study suggests that IL-17A inhibits the production/release of IL-23 protein in the airways by acting on local macrophages at a post-translational level (as the mRNA levels are not altered) and this negative feedback mechanism protects the lung from excessive signalling via the IL-23 – IL-17(A) axis. The importance of IL-23 in autoimmune diseases has been recognised because of its role in expansion and activation of Th17 cells. IL-23p19 knockout mice are protected from inflammatory bone destruction and leaves IL-23 as a promising treatment target in RA [172,173]. Lately it has also been shown that IL-23 not only affects the Th17 cell dependent neutrophil recruitment but also the Th2 cell mediated eosinophil recruitment into the lung during airway inflammation [174,175]. Seeing IL-17A mainly as a pro-inflammatory cytokine, our findings might be contradictory however, lately several studies have been published suggesting that IL-17A has regulatory properties [176,177]. It has been shown that IL-17A can inhibit TNF-α induced chemokine production [178-181] and that IL-17A ameliorates allergic asthma and inflammatory bowel diseases [182-184]. Also IL-17E (IL-25), has regulatory properties [185] probably via downregulation of IL-23 [186,187]. Both IL-17A and IL-17E bind the signalling receptor unit IL-17RA [188] and hence it is possible that IL-17RA could mediate the downregulation of IL-23. As mentioned, IL-17A and IL-17F share many similar biological properties, but they also have separated functions [165]. Both cytokines signal through the IL-17RA subunit [125] but IL-17F binds the receptor with lower affinity [126] and its importance in IL-23 inhibition is unclear. The IL-10-like cytokine IL-22 is produced along with IL-17A by Th17 cells and has proinflammatory as well as regulatory properties [189-192] e.g. it is involved in bone destruction in aseptic arthritis [193] while it counteracts eosinophil mediated airway inflammation [190]. IL-17A, IL-17F and IL-22 are all expressed by Th17 cells and hence regulated by IL-23, whereas IL-17E (IL-25) is produced by Th2 cells and macrophages and hence regulated in a different manner [165,194-196].

How does Interleukin-17A inhibit Interleukin-23?

We have observed an increase in IL-23 protein levels in BAL in IL-17A knockout mice after intravenous *S. aureus* inoculation, an increase in IL-23 protein levels in BAL in mice pre-treated with aIL-17A ab intraperitoneally and then exposed to LPS intranasally and a decrease in IL-23

protein levels in BAL in mice exposed to rmIL-17A intranasally. Macrophages are the main source of IL-23 and changes in the IL-23 protein levels in BAL could be due to the concentration of macrophages in the airway. However, we have been able to show that the change in IL-23 protein levels not is due to a change in the concentration of alveolar macrophages as they remain similar in mice treated with aIL-17A ab and exposed to LPS compared with control mice. Further, mice stimulated intranasally with rmIL-17A produced significantly less IL-23 protein in BAL while the concentration of macrophages in BAL did not change. We have also shown that *in vitro* stimulation of a defined number of human alveolar macrophages with LPS and rhIL-17A decreased the IL-23 protein levels in the supernatant compared with cells stimulated with LPS only.

Previously, it has been shown on mRNA level that IL-17RA and IL-17RC are expressed in the mouse lung [197] and that both the subreceptors are expressed on circulating human monocytes [198]. Using flow cytometry of human macrophages from BAL we show that the receptor subunits required for IL-17A signalling, IL-17RA and IL-17RC are present on the cell surface and it is therefore likely that IL-17A perform its action on IL-23 by binding to these receptors.

Our findings so far suggest that IL-17A inhibits the production of IL-23. The question is on what level the regulation takes place. IL-23 is composed of the subunits p19 and p40 and a regulation on transcription level would lead to decreased mRNA levels of one or both components. However, we did not observe any decrease in mRNA expression of neither p19 nor p40 subunits in human alveolar macrophages or monocyte derived macrophages after stimulation with rhIL-17A and LPS. These data suggest that the negative feedback of IL-17A on IL-23 is mediated on a post-transcriptional level.

Rac1 is an intracellular protein involved in the signalling pathway downstream of IL-17RA and IL-17RC [199] and we wanted to investigate if it was involved in the upregulation of IL-23 [200,201]. It has been observed that IL-17A induces Rac1 activation [200], while LPS stimulation alone or in combination with a Rac1 inhibitor induce IL-23p19 mRNA expression, [201]. By adding a Rac1 inhibitor to LPS and IL-17A stimulated monocyte-derived macrophages, we wanted to investigate whether Rac1 was involved in the observed IL-17A–dependent downregulation of IL-23 in. In contrast to an expected increase in the levels of IL-23 we observed a decrease in the IL-23 levels irrespective of IL-17A stimulation. Thus, Rac1 signalling

is important for IL-23 production, however ligation of IL-17 receptors does not regulate the levels of IL-23 by a Rac1 dependent signalling pathway.

General discussion

Paper I:

In this study we present data which show that IL-15 contributes to arthritis in systemic *S. aureus*–induced arthritis. The severity of *S. aureus*–induced arthritis was decreased in IL-15 knockout mice compared with wildtype mice, probably due to a reduced production of osteoclasts. Treatment with aIL-15ab started at day 3 after bacterial inoculation also reduces the severity of arthritis. The host´s ability to clear bacteria is not negatively influenced in the absence of IL-15.

Paper II:

In this study we suggest that IL-17A is more important in local host defense than in systemic host defense against *S. aureus*-induced arthritis. The severity of systemic *S. aureus*–induced is increased late during the infection in IL-17A knockout mice. However, in early stage there are no differences between the groups with respect to arthritis development probably due to increased levels of IL-17F in the knockout mice. The host´s ability to clear bacteria is not negatively influenced in the absence of IL-17.

Paper III:

In this study we suggest that IL-17A decreases the extracellular concentration of its critical up-stream regulator IL-23 in the airways. The protein levels of IL-23 were increased in the absence and decreased in the presence of IL-17A in mice after stimulation with Gram-positive bacteria (*S. aureus*) or Gram-negative LPS. Also in human macrophages the protein levels of IL-23 were decreased in response to 17A stimulation.

IL-15, IL-17A and IL-23 has been studied in autoimmune diseases and also found in excess in the joints and serum in patients suffering from RA where they all seem to contribute to disease progression [89,114,202]. Inhibiting IL-17A in RA has shown good results while inhibition of IL-15 has been less successful [203-205]. A human monoclonal antibody against IL-23p40, inhibiting

both IL-12 and IL-23, has been successful in the treatment of psoriasis arthritis [173]. However, as their role in *S. aureus*–induced arthritis is unclear this has been the target of our research. *S. aureus*–induced arthritis is highly destructive and immunosuppression along with antibiotics is needed for optimal treatment. To find the perfect remedy, a deeper knowledge about the immune response to staphylococcal infection is required.

IL-15 has a multitude of functions; some studies have suggested that the presence of IL-15 enhances the expression of IL-17A from T cells [206] and stimulation of human Th17 cells with IL-15 results in cell proliferation [207]. In addition, it has been suggested that IL-15 could stimulate $CD4^+$ cells to IL-17 production during bacterial infection [208]. However, other studies state that IL-15 has no impact on the IL-17A levels [113]. In our experiments, we did not find any differences in IL-17A levels between wildtype and IL-15 knockout mice. Both IL-15 and IL-17A *per se* are important regulators of osteoclastogenesis via the upregulation of RANKL [89,117,209] and the production of bone degrading substances [116,210]. Further, they both contribute to formation of osteoclasts via the induction of IL-6, which in turn induces RANKL expression on stromal cells [113,157,158]. IL-8, also induced by both IL-15 and IL-17A [86,113], is a potent chemoattractant for e.g. neutrophils and produced mainly by macrophages and structural cells but also by neutrophils themselves. IL-8 also promotes osteoclast differentiation and activity both directly and indirectly via RANKL induction [211]. IL-8 induces osteoclast formation via activation of intracellular phospholipase D-1 (PLD), an activation pathway also implicated for IL-15 [89,212]. It is known that IL-17A and IFN-γ reciprocally inhibit each other and in contrast to IL-17A, IFN-γ is a potent inhibitor of osteoclastogenesis [117,213].

An effective immune response against an invader is important, but it is as important to limit the response in order to prohibit tissue damage. This requires a critical balance between pro-inflammatory and regulatory responses. Regulation is performed e.g. by the antiinflammatory cytokines IL-10 and TGF-β which possess potent immunosuppressive effects. Negative feedback mechanisms are also of importance in limiting the ongoing immune response. Although it has been shown that IL-17A and IL-17F are both upregulated in γδ T cells early after *S. aureus* infection in the skin [214], it is surprising that we observe a downregulation of IL-23 already 24 hours after *S. aureus* inoculation. The immune response should get downregulated at a later stage in order to get an early adequate response towards the bacteria reducing the bacterial load. IL-17A is important in the recruitment of neutrophils to site of infection, which is crucial for bacterial clearance [29,40]. Indeed, Verdrengh *et al.* [40] has shown that neutrophil count is

enhanced within the first 2 days after bacterial inoculation and neutrophil depletion leads to septic death within the first 3 days after bacterial inoculation.

We observe in paper III using the *S. aureus*-induced arthritis model that IL-17A inhibits the production/release of IL-23 24 h after bacterial inoculation. As we measured IL-23 levels in lung tissue only, the question is if the IL-23 levels also are downregulated at this early timepoint in other compartments of the mice. We do observe higher serum protein levels of IL-17F in the IL-17A knockout mice in paper II also at 24 hours after bacterial inoculation, which could indicate that IL-23 is not downregulated as efficiently by IL-17F as by IL-17A. However, decreased levels of IL-23 in other compartments might reduce the IL-17F levels, but as IL-17F is produced by many different cell types (not predominately by Th17 cells), its downregulation might not be suppressed sufficiently by IL-23. However, IL-17F levels in BAL were not measured in paper III, and it is not clear if IL-17F may have the same effect on IL-23 as IL-17A.

In summary, we have shown that both IL-15 and IL-17A are involved in *S. aureus*–induced arthritis. IL-15 leads to more destructive arthritis due to enhanced formation of osteoclasts, while IL-17A is essential especially for protection against local *S. aureus*–induced arthritis and contributes to bacterial clearance. Our data suggest that the use of IL-15 or IL-17A–inhibiting therapies do not increase the sensitivity to or progression of *S. aureus* infections. However, IL-15 but not IL-17A, could represent a promising treatment target along with antibiotics in *S. aureus*–induced arthritis. Furthermore, IL-17A may limit inflammation by suppressing its own production by inhibiting the release of IL-23. The effect of IL-17A on IL-23 levels is probably dependent on several factors in the microenvironement, such as cells in the vicinity, strength and type of stimuli and the levels of IL-17A.

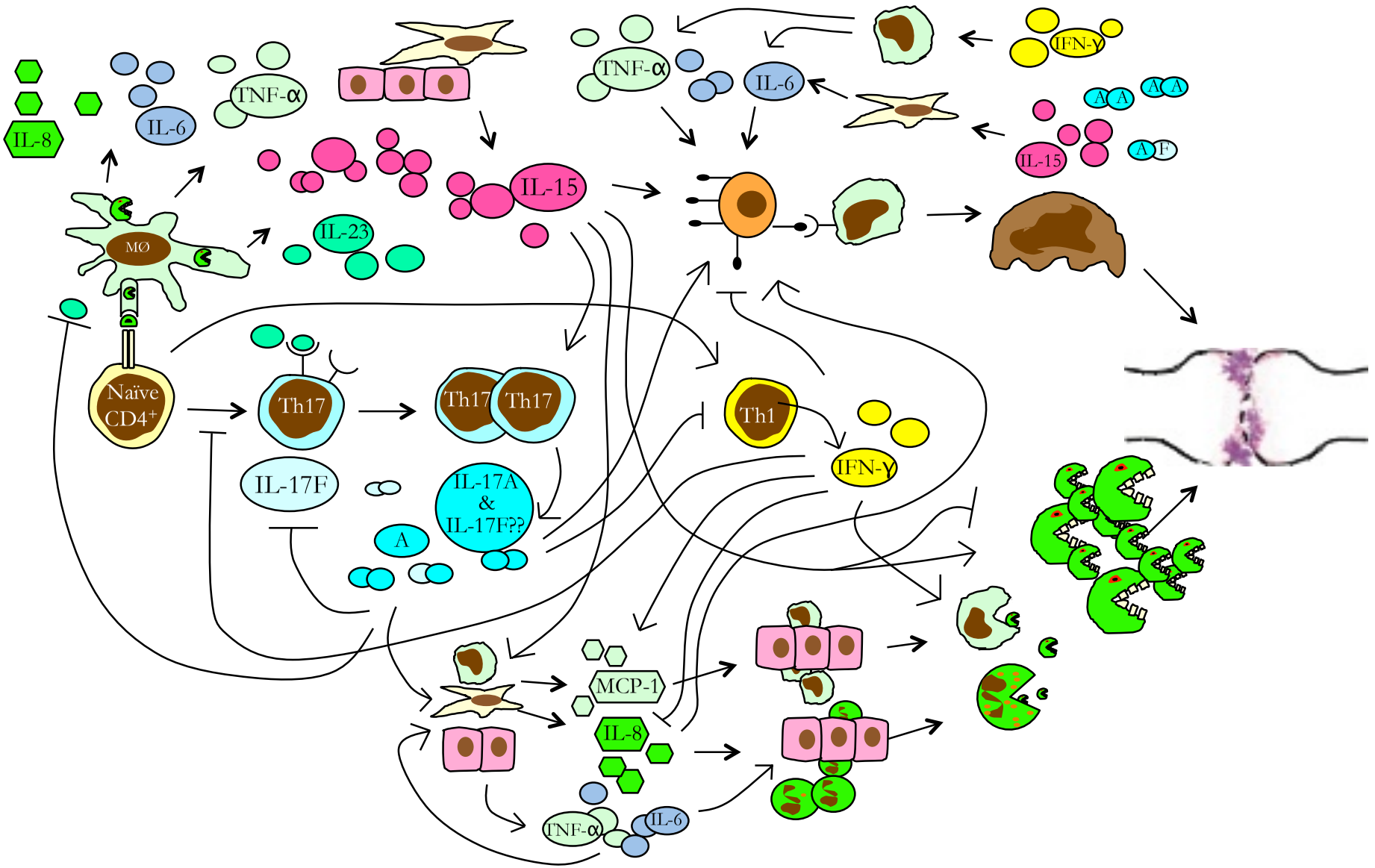


Figure 18. An overview of our findings in paper I, II and III in the relation with findings in previous work.

Bacteria, endothelial cell, fibroblast, neutrophil, phagocytic neutrophil, macrophage, phagocytic macrophage, osteoclast, RANKL expressing cell. The presence of an enhanced number of osteoclasts in wildtype mice inoculated with *S. aureus* leads to enhanced severity of arthritis compared with IL-15 knockout mice (I). Also, an increased bacterial load leads to enhanced severity of arthritis in 1) wildtype mice treated with isotype control compared with wildtype mice treated with aIL-15ab in systemic *S. aureus*¬induced arthritis (I) and 2) in wildtype mice after local *S. aureus* inoculation compared with IL-17A knockout mice (II). IL-17A seems to inhibit the production of IL-17F (II) and also has a regulatory role in a negative feedback loop inhibiting the production/release of its own inducer IL-23 (III).

FUTURE PROSPECTS

There are a lot more questions to be answered in order to be able to fully understand the results gained in this thesis. Here are a few thoughts that rose during the writing.

As treatment with aIL-15ab in mice during *S. aureus*–induced arthritis was very successful in reducing the severity of arthritis, continuing these studies is of great interest, in particular treatment combination using aIL-15ab and antibiotics, in both mice and men. IL-15 has a clear impact on osteoclastogenesis, although its precise role is not clear nor is its effect on general bone loss.

Although we and others suggest that IL-17A regulates the production and/or release of IL-17F, the interplay between these cytokines, the level of inhibition and the importance of this regulation is not clear. In addition, as IL-17A, is IL-17F also of importance in the pathogenesis of RA? We suggest that it is the upregulation of IL-17F that compensates for the loss of IL-17A. Is IL-17F of importance in other bacterial infections? Binding of these cytokines to its receptors is currently not known. Is the receptor composed of an IL-17RA/IL-17RC heterodimer complex or are additional receptor subunits involved? Or are even additional types of receptor subunits involved? What are the similarities/differences in IL-17A and IL-17F response and how are these differences achieved, as they seem to bind the same receptor?

We suggest that IL-17A inhibits the production of IL-23. The importance of this IL-17A–dependant inhibition of IL-23 is not clear. How important is this inhibition in infectious diseases? What role has IL-17F? Does also IL-17F affect the production of IL-23? Is IL-23 important in IL-17A and IL-17F production from other T cell subsets other than Th17 cells, such as γδ T cells, NK T cells and $CD8^+$ cells? Is the IL-17A–dependant inhibition of IL-23 impaired in autoimmune diseases? Is IL-17RA required for IL-23 inhibition or what receptor mechanism is involved?

POPULÄRVETENSKAPLIG SAMMANFATTNING

Ledinflammation som orsakas av bakterier är ett ovanligt men mycket allvarligt tillstånd eftersom bakterierna kan sprida sig till blodet och orsaka blodförgiftning, vilket i värsta fall kan leda till döden. En av de vanligaste bakterierna som orsakar ledinflammation framförallt hos personer som har ett försvagat immunförsvar, på grund av ålder eller immunnedsättande mediciner är den så kallade sjukhussjukebakterien, *Staphylococcus aureus (S. aureus)*. Dessa infektioner kan bli mycket allvarliga och kan ge bestående ledskador trots antibiotikabehandling. Bakterien tar sig in i lederna och har en tendens att stanna där. Vårt immunförsvar, som är helt avgörande för att vi skall kunna bli friska, försvarar oss mot bakterien men bakterien orsakar ett kraftigt immunsvar som också förstör ben och brosk i leden. Under en *S. aureus* infektion produceras flera signalmolekyler (cytokiner) som aktiverar immunsystemet (de är proinflammatoriska cytokiner) vilket leder till att vi kan döda bakterien och bli friska. Vi har i följande arbeten undersökt hur två proinflammatoriska cytokiner, interleukin (IL)-15 och IL-17A, påverkar *S. aureus*–orsakad ledinflammation i mus. Dessa cytokiner finns i överskott i lederna på personer med ledgångsreumatism och man vet att de är involverade i ledförstörelsen och man har även försökt behandla ledgångsreumatism med läkemedel som binder upp cytokinerna. Under en sådan behandling kan ett underskott av dessa cytokiner leda till att patienten blir mer mottaglig för *S. aureus*–orsakad ledinflammation. Detta ger oss två anledningar att studera IL-15 och IL-17 vid *S. aureus*–orsakad ledinflammation: 1) hur påverkar ett underskott av IL-15 och IL-17 känsligheten för infektionen? 2) Kan läkemedel mot IL-15 eller IL-17 vara ett bra tillägg till antibiotika vid *S. aureus*–orsakad ledinflammation? Vi har även studerat cytokinen IL-23 som är en cytokin som reglerar produktionen av IL-17A och alltså också är en proinflammatorisk cytokin.

Genom att använda möss som saknar generna för IL-15 och IL-17A och sedan jämföra sjukdomsförloppet med en mus som har generna för dessa cytokiner (vildtyps möss) har vi kunnat få en uppfattning av dessa cytokiners betydelse. Vi har injicerat bakterien antingen direkt in i blodet (systemisk infektion) eller direkt in i knäleden på musen (lokal infektion). Under sjukdomen har vi inspekterat musen efter tecken på artrit i lederna och vägt dem för att kontrollera deras allmäntillstånd. Vid försökets slut tar vi blod, leder och olika organ för att mäta cytokiner, bakterier och celler.

Delarbete I

I detta arbete ville vi klargöra vilken roll IL-15 har under *S. aureus*–inducerad artrit infektion. Vi upptäckte att möss som inte kunde uttrycka IL-15 uppvisade mycket mindre artrit än vad möss gjorde som uttryckte IL-15. Vi såg även att dessa möss som saknade IL-15 hade färre ben-nedbrytande celler i sina leder. Alltså verkar det som att IL-15 brist är skyddande under *S. aureus*–inducerad artrit infektion. Vi testade därför att behandla normala vildtyps möss med en antikropp mot IL-15 efter det att stafylokockerna sprutats in i blodet. Vi upptäckte då att även de möss som hade blivit behandlade med en IL-15 antikropp uppvisade mindre artrit i lederna. Slutsatsen på detta arbete är alltså att IL-15 främjar nedbrytning i lederna under *S. aureus*–inducerad artrit och att behandling med en IL-15 antikropp kan hämma denna lednedbrytning.

Delarbete II

I detta arbete ville vi klargöra vilken roll IL-17A har under *S. aureus*–inducerad artrit infektion. Vi fann att då bakterier injicerades direkt in i blodet (systemisk infektion) såg vi inga skillnader i artrit mellan möss som uttryckte IL-17A genen och vildtypsmöss förrän efter ca 14 dagar. Anledningen till detta var antagligen att de möss som inte uttryckte IL-17A uttryckte istället höga nivåer av IL-17F, en cytokin som är nära släkt med IL-17A och har nästan samma funktion. Däremot, när vi injicerade bakterier direkt in i knäleden på musen upptäckte vi att de möss som inte uttryckte IL-17A hade värre artrit i knäleden och även hade fler bakterier i leden. Dessa möss kunde alltså inte ta bort bakterien i leden på ett lika effektivt sätt som vildmössen kunde. IL-17A påverkade alltså förmågan att ta bort bakterier från leden. Slutsatsen på detta arbete är alltså att IL-17A behövs för att på ett effektivt sätt kunna eliminera bakterier från leden under en lokal infektion men att IL-17A inte är lika viktig under en systemisk infektion då IL-17F uppregleras och ersätter IL-17A.

Delarbete III

I detta arbete studerade vi hur IL-17A påverkade produktionen av IL-23. Normalt är IL-23 en cytokin som behövs för att vissa celler skall kunna producera IL-17A. Vi visar här att IL-17A kan även påverka produktionen och frisättningen av IL-23 genomen en negativ-feedback loop. Vi såg att IL-17A kunde reducera IL-23 nivåerna hos både mus och människa. Slutsatsen i detta arbete är att IL-17A kan verka antiinflammatoriskt genom att hämma frisättningen av IL-23.

Slutsats

Sammanfattningsvis har vi visat att både IL-15 och IL-17A är involverade i *S. aureus*–inducerad artrit men på olika sätt. IL-15 leder till mer destruktiv artrit på grund av att fler celler som bryter ned benet bildas medan IL-17A är nödvändig vid lokal *S. aureus*–inducerad artrit då den uppehåller en effektiv eliminering av bakterien. Användning av IL-15 eller IL-17A hämmande mediciner vid ledgångsreumatism borde alltså inte leda till en allvarligare infektion vid S. aureus–inducerad artrit. Vidare visar vi också att IL-17A kan begränsa inflammation genom att dämpa sin egen produktion genom att hämma frisättning av IL-23. Om IL-17A är pro- eller antiinflammatorisk beror i största sannolikhet på miljön, det vill säga vilken mängd och sorts bakterie, IL-17A nivåer och vilka celler som aktiveras i närheten.

ACKNOWLEDGMENTS

Ett superstort tack till min fantastiskt underbara handledare **Inger**. Att du är så entusiastisk och uppmuntrande hela tiden har gjort att jag överlevt dessa 4 åren. Hade det inte varit för det så hade det antagligen inte blivit någon avhandling. Hur det än går så är du alltid positiv och glad. Du är dessutom fantastiskt omtänksam och tar galet väl hand om mig!

Pernilla, du har lärt mig sååååå mycket och lyckats bra med att inte få mig att känna mig alltför knäpp i huet då jag fattar 0… och dessutom är det ju alltid fredag då vi labbar! Resten av lilla gruppen, **Tovan**, det har varit kul att labba och stolla med dig, **Yalda**, blev glad att det var du som kom till min räddning i somras! **Sara**, tack.

Soffeloffan, stolle! eller var det kanske tolle? tack för alla kuliga turer i bil, för att du går vilse på coop och för jag fick flytta in hos er, det var sjukt kul att dela rum med dig och ditt Spottans Hungry eyes, **Mattias**, det hade knappt varit lönt att gå till jubb om inte du varit där, **Li**, thanks for being my roommate and for one of the best dinners ever! **Annelojden**, du är så hjälpsam och uppmuntrande, tack för det och massa mer, **Angelojden**, du är sjukt kul kul, **Mia**, tack för Mexico, **Sofia**, **Martina**, **Alex**, **Annica**, **Emil**, **Karin**, **Gabriel**, **Andrey**, **Kuba**, …. och alla andra doktorander på avdelningen … Tack **Catharina** för all hjälp och för att du läste ramen, **Anna-Carin** och **Johan** och **Pernilla G** och **Petra** och **Ulrika** för bra grillning, **Elin S** för hjälp med peket, **Piotr** thanks for helping me with … stuff, allt-och dataexperten **Esbjörn**, facsexperten **Linda**, bakterieexperten **Elisabet J**, osteoclastexperten **Ulf**, labbexperterna **Ing-Marie** och **Malin** och **Inger N**, pappersexperterna **Eva**, **Harriet** och **Sofie,** ni är alla superfantastiska, tack för allt ni hjälpt mig med, och för att jag får komma och fråga 1000 frågor om och om igen, tack **Maggan**, för all hjälp i djurhuset och för att du är så himla bra, tack alla små **muslingar** …. och tack till alla andra på avdelningen och i huset.
Tack för ALLT **Mamma**, **Pappa**, **Syster**. **David** tack för pandaflickan, du är bäst!

REFERENSES

1. Tarkowski A. **Infection and musculoskeletal conditions: Infectious arthritis.** *Best Pract Res Clin Rheumatol* **2006**; 20(6):1029-1044.

2. Goldenberg DL. **Septic arthritis.** *Lancet* **1998**; 351(9097):197-202.

3. Waage A, Halstensen A, Espevik T. **Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease.** *Lancet* **1987**; 1(8529):355-357.

4. Odio CM, Ramirez T, Arias G, Abdelnour A, Hidalgo I, Herrera ML, Bolanos W, Alpizar J, Alvarez P. **Double blind, randomized, placebo-controlled study of dexamethasone therapy for hematogenous septic arthritis in children.** *The Pediatric infectious disease journal* **2003**; 22(10):883-888.

5. Sakiniene E, Bremell T, Tarkowski A. **Addition of corticosteroids to antibiotic treatment ameliorates the course of experimental Staphylococcus aureus arthritis.** *Arthritis and rheumatism* **1996**; 39(9):1596-1605.

6. Fei Y, Wang W, Kwiecinski J, Josefsson E, Pullerits R, Jonsson IM, Magnusson M, Jin T. **The combination of a tumor necrosis factor inhibitor and antibiotic alleviates staphylococcal arthritis and sepsis in mice.** *The Journal of infectious diseases* **2011**; 204(3):348-357.

7. Remick DG. **Pathophysiology of sepsis.** *The American journal of pathology* **2007**; 170(5):1435-1444.

8. Bremell T, Lange S, Svensson L, Jennische E, Grondahl K, Carlsten H, Tarkowski A. **Outbreak of spontaneous staphylococcal arthritis and osteitis in mice.** *Arthritis Rheum* **1990**; 33(11):1739-1744.

9. Aguilar B, Amorena B, Iturralde M. **Effect of slime on adherence of Staphylococcus aureus isolated from bovine and ovine mastitis.** *Vet Microbiol* **2001**; 78(2):183-191.

10. Mathur T, Singhal S, Khan S, Upadhyay D, Fatma T, Rattan A. **Adverse effect of staphylococci slime on in vitro activity of glycopeptides.** *Jpn J Infect Dis* **2005**; 58(6):353-357.

11. Souli M, Giamarellou H. **Effects of slime produced by clinical isolates of coagulase-negative staphylococci on activities of various antimicrobial agents.** *Antimicrobial agents and chemotherapy* **1998**; 42(4):939-941.

12. Arbeit RD, Karakawa WW, Vann WF, Robbins JB. **Predominance of two newly described capsular polysaccharide types among clinical isolates of Staphylococcus aureus.** *Diagnostic microbiology and infectious disease* **1984**; 2(2):85-91.

13. Verbrugh HA, Peterson PK, Nguyen BY, Sisson SP, Kim Y. **Opsonization of encapsulated Staphylococcus aureus: the role of specific antibody and complement.** *Journal of immunology* **1982**; 129(4):1681-1687.

14. Karakawa WW, Sutton A, Schneerson R, Karpas A, Vann WF. **Capsular antibodies induce type-specific phagocytosis of capsulated Staphylococcus aureus by human polymorphonuclear leukocytes.** *Infection and immunity* **1988**; 56(5):1090-1095.

15. Nilsson IM, Lee JC, Bremell T, Ryden C, Tarkowski A. **The role of staphylococcal polysaccharide microcapsule expression in septicemia and septic arthritis.** *Infect Immun* **1997**; 65(10):4216-4221.

16. Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ. **Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2.** *J Biol Chem* **1999**; 274(25):17406-17409.

17. Wang JE, Jorgensen PF, Almlof M, Thiemermann C, Foster SJ, Aasen AO, Solberg R. **Peptidoglycan and lipoteichoic acid from Staphylococcus aureus induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model.** *Infect Immun* **2000**; 68(7):3965-3970.

18. Palmqvist N, Foster T, Tarkowski A, Josefsson E. **Protein A is a virulence factor in Staphylococcus aureus arthritis and septic death.** *Microbial pathogenesis* **2002**; 33(5):239-249.

19. Xu Y, Rivas JM, Brown EL, Liang X, Hook M. **Virulence potential of the staphylococcal adhesin CNA in experimental arthritis is determined by its affinity for collagen.** *J Infect Dis* **2004**; 189(12):2323-2333.

20. Patti JM, Bremell T, Krajewska-Pietrasik D, Abdelnour A, Tarkowski A, Ryden C, Hook M. **The Staphylococcus aureus collagen adhesin is a virulence determinant in experimental septic arthritis.** *Infect Immun* **1994**; 62(1):152-161.

21. McDevitt D, Francois P, Vaudaux P, Foster TJ. **Molecular characterization of the clumping factor (fibrinogen receptor) of Staphylococcus aureus.** *Mol Microbiol* **1994**; 11(2):237-248.

22. Que YA, Francois P, Haefliger JA, Entenza JM, Vaudaux P, Moreillon P. **Reassessing the role of Staphylococcus aureus clumping factor and fibronectin-binding protein by expression in Lactococcus lactis.** *Infect Immun* **2001**; 69(10):6296-6302.

23. Nilsson IM, Hartford O, Foster T, Tarkowski A. **Alpha-toxin and gamma-toxin jointly promote Staphylococcus aureus virulence in murine septic arthritis.** *Infect Immun* **1999**; 67(3):1045-1049.

24. Abdelnour A, Bremell T, Holmdahl R, Tarkowski A. **Clonal expansion of T lymphocytes causes arthritis and mortality in mice infected with toxic shock syndrome toxin-1-producing staphylococci.** *European journal of immunology* **1994**; 24(5):1161-1166.

25. Zhao YX, Abdelnour A, Kalland T, Tarkowski A. **Overexpression of the T-cell receptor V beta 3 in transgenic mice increases mortality during infection by enterotoxin A-producing Staphylococcus aureus.** *Infection and immunity* **1995**; 63(11):4463-4469.

26. Lowy FD. **Staphylococcus aureus infections.** *N Engl J Med* **1998**; 339(8):520-532.

27. Barnes PJ. **Immunology of asthma and chronic obstructive pulmonary disease.** *Nature reviews Immunology* **2008**; 8(3):183-192.

28. Alcorn JF, Crowe CR, Kolls JK. **TH17 cells in asthma and COPD.** *Annual review of physiology* **2010**; 72(495-516.

29. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE *et al.* **Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense.** *J Exp Med* **2001**; 194(4):519-527.

30. Wu Q, Martin RJ, Rino JG, Breed R, Torres RM, Chu HW. **IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory Mycoplasma pneumoniae infection.** *Microbes Infect* **2007**; 9(1):78-86.

31. Leon CG, Tory R, Jia J, Sivak O, Wasan KM. **Discovery and development of toll-like receptor 4 (TLR4) antagonists: a new paradigm for treating sepsis and other diseases.** *Pharmaceutical research* **2008**; 25(8):1751-1761.

32. Casey LC, Balk RA, Bone RC. **Plasma cytokine and endotoxin levels correlate with survival in patients with the sepsis syndrome.** *Annals of internal medicine* **1993**; 119(8):771-778.

33. Cunnion KM, Zhang HM, Frank MM. **Availability of complement bound to Staphylococcus aureus to interact with membrane complement receptors influences efficiency of phagocytosis.** *Infection and immunity* **2003**; 71(2):656-662.

34. Cunnion KM, Lee JC, Frank MM. **Capsule production and growth phase influence binding of complement to Staphylococcus aureus.** *Infection and immunity* **2001**; 69(11):6796-6803.

35. Cunnion KM, Benjamin DK, Jr., Hester CG, Frank MM. **Role of complement receptors 1 and 2 (CD35 and CD21), C3, C4, and C5 in survival by mice of Staphylococcus aureus bacteremia.** *The Journal of laboratory and clinical medicine* **2004**; 143(6):358-365.

36. von Kockritz-Blickwede M, Konrad S, Foster S, Gessner JE, Medina E. **Protective role of complement C5a in an experimental model of Staphylococcus aureus bacteremia.** *Journal of innate immunity* **2009**; 2(1):87-92.

37. Palmqvist N, Patti JM, Tarkowski A, Josefsson E. **Expression of staphylococcal clumping factor A impedes macrophage phagocytosis.** *Microbes and infection / Institut Pasteur* **2004**; 6(2):188-195.

38. Hair PS, Echague CG, Sholl AM, Watkins JA, Geoghegan JA, Foster TJ, Cunnion KM. **Clumping factor A interaction with complement factor I increases C3b cleavage on the bacterial surface of Staphylococcus aureus and decreases complement-mediated phagocytosis.** *Infection and immunity* **2010**; 78(4):1717-1727.

39. Bremell T, Abdelnour A, Tarkowski A. **Histopathological and serological progression of experimental Staphylococcus aureus arthritis.** *Infect Immun* **1992**; 60(7):2976-2985.

40. Verdrengh M, Tarkowski A. **Role of neutrophils in experimental septicemia and septic arthritis induced by Staphylococcus aureus.** *Infect Immun* **1997**; 65(7):2517-2521.

41. Verdrengh M, Tarkowski A. **Role of macrophages in Staphylococcus aureus-induced arthritis and sepsis.** *Arthritis Rheum* **2000**; 43(10):2276-2282.

42. Klebanoff SJ, Vadas MA, Harlan JM, Sparks LH, Gamble JR, Agosti JM, Waltersdorph AM. **Stimulation of neutrophils by tumor necrosis factor.** *Journal of immunology* **1986**; 136(11):4220-4225.

43. Klimiuk PA, Sierakowski S, Domyslawska I, Fiedorczyk M, Chwiecko J. **Reduction of soluble adhesion molecules (sICAM-1, sVCAM-1, and sE-selectin) and vascular endothelial growth factor levels in serum of rheumatoid arthritis patients following multiple intravenous infusions of infliximab.** *Archivum immunologiae et therapiae experimentalis* **2004**; 52(1):36-42.

44. Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W. **Tumor necrosis factor alpha (TNF-alpha)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55.** *The Journal of experimental medicine* **1993**; 177(5):1277-1286.

45. Feldmann M, Maini RN. **Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned?** *Annual review of immunology* **2001**; 19(163-196.

46. Gamble JR, Harlan JM, Klebanoff SJ, Vadas MA. **Stimulation of the adherence of neutrophils to umbilical vein endothelium by human recombinant tumor necrosis factor.** *Proceedings of the National Academy of Sciences of the United States of America* **1985**; 82(24):8667-8671.

47. Nathan CF. **Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes.** *The Journal of clinical investigation* **1987**; 80(6):1550-1560.

48. Nilsson N, Bremell T, Tarkowski A, Carlsten H. **Protective role of NK1.1+ cells in experimental Staphylococcus aureus arthritis.** *Clin Exp Immunol* **1999**; 117(1):63-69.

49. Abdelnour A, Bremell T, Holmdahl R, Tarkowski A. **Role of T lymphocytes in experimental Staphylococcus aureus arthritis.** *Scand J Immunol* **1994**; 39(4):403-408.

50. Bremell T, Lange S, Holmdahl R, Ryden C, Hansson GK, Tarkowski A. **Immunopathological features of rat Staphylococcus aureus arthritis.** *Infection and immunity* **1994**; 62(6):2334-2344.

51. Abdelnour A, Bremell T, Tarkowski A. **Toxic shock syndrome toxin 1 contributes to the arthritogenicity of Staphylococcus aureus.** *The Journal of infectious diseases* **1994**; 170(1):94-99.

52. Gjertsson I, Hultgren OH, Stenson M, Holmdahl R, Tarkowski A. **Are B lymphocytes of importance in severe Staphylococcus aureus infections?** *Infect Immun* **2000**; 68(5):2431-2434.

53. Megyeri K, Mandi Y, Degre M, Rosztoczy I. **Induction of cytokine production by different Staphylococcal strains.** *Cytokine* **2002**; 19(4):206-212.

54. Tufano MA, Cipollaro de l'Ero G, Ianniello R, Galdiero M, Galdiero F. **Protein A and other surface components of Staphylococcus aureus stimulate production of IL-1 alpha, IL-4, IL-6, TNF and IFN-gamma.** *Eur Cytokine Netw* **1991**; 2(5):361-366.

55. Islander U, Andersson A, Lindberg E, Adlerberth I, Wold AE, Rudin A. **Superantigenic Staphylococcus aureus stimulates production of interleukin-17 from memory but not naive T cells.** *Infect Immun* **2009**; 78(1):381-386.

56. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. **The pro- and anti-inflammatory properties of the cytokine interleukin-6.** *Biochimica et biophysica acta* **2011**; 1813(5):878-888.

57. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. **TGFbeta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells.** *Immunity* **2006**; 24(2):179-189.

58. Palmqvist P, Persson E, Conaway HH, Lerner UH. **IL-6, leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-kappa B ligand, osteoprotegerin, and receptor activator of NF-kappa B in mouse calvariae.** *J Immunol* **2002**; 169(6):3353-3362.

59. Zhou Q, Gu CQ, Hu XY, Wang DH, Li XM, Zhou SQ, Cheng GF. **Role of interleukin-6 in the pathogenesis of an avian model of Staphylococcus aureus arthritis.** *Poult Sci* **2007**; 86(6):1245-1250.

60. Hultgren OH, Stenson M, Tarkowski A. **Role of IL-12 in Staphylococcus aureus-triggered arthritis and sepsis.** *Arthritis research* **2001**; 3(1):41-47.

61. Zhao YX, Nilsson IM, Tarkowski A. **The dual role of interferon-gamma in experimental Staphylococcus aureus septicaemia versus arthritis.** *Immunology* **1998**; 93(1):80-85.

62. Lee TH, Lee GW, Ziff EB, Vilcek J. **Isolation and characterization of eight tumor necrosis factor-induced gene sequences from human fibroblasts.** *Molecular and cellular biology* **1990**; 10(5):1982-1988.

63. Robson RL, McLoughlin RM, Witowski J, Loetscher P, Wilkinson TS, Jones SA, Topley N. **Differential regulation of chemokine production in human peritoneal mesothelial cells: IFN-gamma controls neutrophil migration across the mesothelium in vitro and in vivo.** *Journal of immunology* **2001**; 167(2):1028-1038.

64. Borgmann S, Bayer A, Konig W, Ambrosch A, Kraus J. **Contrasting effects of long-term treatment with IFN-gamma in endothelial cells: increase in IL-6 secretion versus decrease in IL-8 secretion, NF-kappa B, and AP-1 activation.** *Endothelium : journal of endothelial cell research* **2002**; 9(3):173-178.

65. Andoh A, Takaya H, Makino J, Sato H, Bamba S, Araki Y, Hata K, Shimada M, Okuno T, Fujiyama Y, Bamba T. **Cooperation of interleukin-17 and interferon-gamma on chemokine secretion in human fetal intestinal epithelial cells.** *Clinical and experimental immunology* **2001**; 125(1):56-63.

66. Furst DE. **Development of TNF inhibitor therapies for the treatment of rheumatoid arthritis.** *Clinical and experimental rheumatology* **2010**; 28(3 Suppl 59):S5-12.

67. Hultgren O, Eugster HP, Sedgwick JD, Korner H, Tarkowski A. **TNF/lymphotoxin-alpha double-mutant mice resist septic arthritis but display increased mortality in response to Staphylococcus aureus.** *Journal of immunology* **1998**; 161(11):5937-5942.

68. Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, Beers C, Richardson J, Schoenborn MA, Ahdieh M, et al. **Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor.** *Science* **1994**; 264(5161):965-968.

69. Waldmann TA. **The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design.** *Nat Rev Immunol* **2006**; 6(8):595-601.

70. Orinska Z, Maurer M, Mirghomizadeh F, Bulanova E, Metz M, Nashkevich N, Schiemann F, Schulmistrat J, Budagian V, Giron-Michel J, Brandt E *et al.* **IL-15 constrains mast cell-dependent antibacterial defenses by suppressing chymase activities.** *Nat Med* **2007**; 13(8):927-934.

71. Tagaya Y, Kurys G, Thies TA, Losi JM, Azimi N, Hanover JA, Bamford RN, Waldmann TA. **Generation of secretable and nonsecretable interleukin 15 isoforms through alternate usage of signal peptides.** *Proc Natl Acad Sci U S A* **1997**; 94(26):14444-14449.

72. Gaggero A, Azzarone B, Andrei C, Mishal Z, Meazza R, Zappia E, Rubartelli A, Ferrini S. **Differential intracellular trafficking, secretion and endosomal localization of two IL-15 isoforms.** *Eur J Immunol* **1999**; 29(4):1265-1274.

73. Rubinstein MP, Kovar M, Purton JF, Cho JH, Boyman O, Surh CD, Sprent J. **Converting IL-15 to a superagonist by binding to soluble IL-15R{alpha}.** *Proceedings of the National Academy of Sciences of the United States of America* **2006**; 103(24):9166-9171.

74. Inoue S, Unsinger J, Davis CG, Muenzer JT, Ferguson TA, Chang K, Osborne DF, Clark AT, Coopersmith CM, McDunn JE, Hotchkiss RS. **IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis.** *J Immunol* **2009**; 184(3):1401-1409.

75. Zheng X, Wang Y, Wei H, Ling B, Sun R, Tian Z. **Bcl-xL is associated with the anti-apoptotic effect of IL-15 on the survival of CD56(dim) natural killer cells.** *Molecular immunology* **2008**; 45(9):2559-2569.

76. Bulfone-Paus S, Ungureanu D, Pohl T, Lindner G, Paus R, Ruckert R, Krause H, Kunzendorf U. **Interleukin-15 protects from lethal apoptosis in vivo.** *Nat Med* **1997**; 3(10):1124-1128.

77. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. **Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15.** *Immunity* **1998**; 8(5):591-599.

78. Dubois SP, Waldmann TA, Muller JR. **Survival adjustment of mature dendritic cells by IL-15.** *Proceedings of the National Academy of Sciences of the United States of America* **2005**; 102(24):8662-8667.

79. Kennedy MK, Glaccum M, Brown SN, Butz EA, Viney JL, Embers M, Matsuki N, Charrier K, Sedger L, Willis CR, Brasel K *et al.* **Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice.** *J Exp Med* **2000**; 191(5):771-780.

80. Lucas M, Schachterle W, Oberle K, Aichele P, Diefenbach A. **Dendritic cells prime natural killer cells by trans-presenting interleukin 15.** *Immunity* **2007**; 26(4):503-517.

81. Dubsky P, Saito H, Leogier M, Dantin C, Connolly JE, Banchereau J, Palucka AK. **IL-15-induced human DC efficiently prime melanoma-specific naive CD8+ T cells to differentiate into CTL.** *European journal of immunology* **2007**; 37(6):1678-1690.

82. Flamand L, Stefanescu I, Menezes J. **Human herpesvirus-6 enhances natural killer cell cytotoxicity via IL-15.** *The Journal of clinical investigation* **1996**; 97(6):1373-1381.

83. Hiromatsu T, Yajima T, Matsuguchi T, Nishimura H, Wajjwalku W, Arai T, Nimura Y, Yoshikai Y. **Overexpression of interleukin-15 protects against Escherichia coli-induced shock accompanied by inhibition of tumor necrosis factor-alpha-induced apoptosis.** *The Journal of infectious diseases* **2003**; 187(9):1442-1451.

84. Maeurer MJ, Trinder P, Hommel G, Walter W, Freitag K, Atkins D, Storkel S. **Interleukin-7 or interleukin-15 enhances survival of Mycobacterium tuberculosis-infected mice.** *Infection and immunity* **2000**; 68(5):2962-2970.

85. Di Sabatino A, Calarota SA, Vidali F, Macdonald TT, Corazza GR. **Role of IL-15 in immune-mediated and infectious diseases.** *Cytokine & growth factor reviews* **2011**; 22(1):19-33.

86. Badolato R, Ponzi AN, Millesimo M, Notarangelo LD, Musso T. **Interleukin-15 (IL-15) induces IL-8 and monocyte chemotactic protein 1 production in human monocytes.** *Blood* **1997**; 90(7):2804-2809.

87. Ogata Y, Kukita A, Kukita T, Komine M, Miyahara A, Miyazaki S, Kohashi O. **A novel role of IL-15 in the development of osteoclasts: inability to replace its activity with IL-2.** *J Immunol* **1999**; 162(5):2754-2760.

88. Djaafar S, Pierroz DD, Chicheportiche R, Zheng XX, Ferrari SL, Ferrari-Lacraz S. **Inhibition of T cell-dependent and RANKL-dependent osteoclastogenic processes associated with high levels of bone mass in interleukin-15 receptor-deficient mice.** *Arthritis Rheum* 62(11):3300-3310.

89. Park MK, Her YM, Cho ML, Oh HJ, Park EM, Kwok SK, Ju JH, Park KS, Min DS, Kim HY, Park SH. **IL-15 promotes osteoclastogenesis via the PLD pathway in rheumatoid arthritis.** *Immunology letters* **2011**; 139(1-2):42-51.

90. Soderstrom K, Stein E, Colmenero P, Purath U, Muller-Ladner U, de Matos CT, Tarner IH, Robinson WH, Engleman EG. **Natural killer cells trigger osteoclastogenesis and bone destruction in arthritis.** *Proc Natl Acad Sci U S A* **2010**; 107(29):13028-13033.

91. Kolls JK, Linden A. **Interleukin-17 family members and inflammation.** *Immunity* **2004**; 21(4):467-476.

92. Iwakura Y, Ishigame H, Saijo S, Nakae S. **Functional specialization of interleukin-17 family members.** *Immunity* **2011**; 34(2):149-162.

93. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM. **Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3.** *The Journal of experimental medicine* **2003**; 198(12):1875-1886.

94. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. **Transforming growth factor-beta induces development of the T(H)17 lineage.** *Nature* **2006**; 441(7090):231-234.

95. Ivanov, II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. **The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells.** *Cell* **2006**; 126(6):1121-1133.

96. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS *et al.* **T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma.** *Immunity* **2008**; 28(1):29-39.

97. Kimura A, Naka T, Kishimoto T. **IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells.** *Proceedings of the National Academy of Sciences of the United States of America* **2007**; 104(29):12099-12104.

98. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, Weaver CT. **Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages.** *Nat Immunol* **2005**; 6(11):1123-1132.

99. Gaffen SL. **An overview of IL-17 function and signaling.** *Cytokine* **2008**; 43(3):402-407.

100. Ivanov S, Linden A. **Interleukin-17 as a drug target in human disease.** *Trends Pharmacol Sci* **2009**; 30(2):95-103.

101. Dubin PJ, Kolls JK. **IL-23 mediates inflammatory responses to mucoid Pseudomonas aeruginosa lung infection in mice.** *Am J Physiol Lung Cell Mol Physiol* **2007**; 292(2):L519-528.

102. Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, Odden AR, Shellito JE, Bagby GJ, Nelson S, Kolls JK. **Divergent roles of IL-23 and IL-12 in host defense against Klebsiella pneumoniae.** *J Exp Med* **2005**; 202(6):761-769.

103. Higgins SC, Jarnicki AG, Lavelle EC, Mills KH. **TLR4 mediates vaccine-induced protective cellular immunity to Bordetella pertussis: role of IL-17-producing T cells.** *J Immunol* **2006**; 177(11):7980-7989.

104. Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. **Microbial lipopeptides induce the production of IL-17 in Th cells.** *J Immunol* **2000**; 165(11):6107-6115.

105. Luzza F, Parrello T, Monteleone G, Sebkova L, Romano M, Zarrilli R, Imeneo M, Pallone F. **Up-regulation of IL-17 is associated with bioactive IL-8 expression in**

Helicobacter pylori-infected human gastric mucosa. *J Immunol* **2000**; 165(9):5332-5337.

106. Shibata K, Yamada H, Hara H, Kishihara K, Yoshikai Y. **Resident Vdelta1+ gammadelta T cells control early infiltration of neutrophils after Escherichia coli infection via IL-17 production.** *J Immunol* **2007**; 178(7):4466-4472.

107. Yu JJ, Ruddy MJ, Wong GC, Sfintescu C, Baker PJ, Smith JB, Evans RT, Gaffen SL. **An essential role for IL-17 in preventing pathogen-initiated bone destruction: recruitment of neutrophils to inflamed bone requires IL-17 receptor-dependent signals.** *Blood* **2007**; 109(9):3794-3802.

108. Ma CS, Chew GYJ, Simpson N, Priyadarshi A, Wong M, Grimbacher B, Fulcher DA, Tangye SG, Cook MC. **Deficiency of Th17 cells in hyper IgE syndrome due to mutations in STAT3.** *The Journal of experimental medicine* **2008**; 205(7):1551-1557.

109. Laan M, Prause O, Miyamoto M, Sjostrand M, Hytonen AM, Kaneko T, Lotvall J, Linden A. **A role of GM-CSF in the accumulation of neutrophils in the airways caused by IL-17 and TNF-alpha.** *The European respiratory journal : official journal of the European Society for Clinical Respiratory Physiology* **2003**; 21(3):387-393.

110. Schwarzenberger P, Huang W, Ye P, Oliver P, Manuel M, Zhang Z, Bagby G, Nelson S, Kolls JK. **Requirement of endogenous stem cell factor and granulocyte-colony-stimulating factor for IL-17-mediated granulopoiesis.** *Journal of immunology* **2000**; 164(9):4783-4789.

111. Laan M, Cui ZH, Hoshino H, Lotvall J, Sjostrand M, Gruenert DC, Skoogh BE, Linden A. **Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways.** *J Immunol* **1999**; 162(4):2347-2352.

112. Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE *et al.* **Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense.** *The Journal of experimental medicine* **2001**; 194(4):519-527.

113. Hwang SY, Kim JY, Kim KW, Park MK, Moon Y, Kim WU, Kim HY. **IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kappaB- and PI3-kinase/Akt-dependent pathways.** *Arthritis research & therapy* **2004**; 6(2):R120-128.

114. Agarwal S, Misra R, Aggarwal A. **Interleukin 17 levels are increased in juvenile idiopathic arthritis synovial fluid and induce synovial fibroblasts to produce proinflammatory cytokines and matrix metalloproteinases.** *The Journal of rheumatology* **2008**; 35(3):515-519.

115. Sylvester J, Liacini A, Li WQ, Zafarullah M. **Interleukin-17 signal transduction pathways implicated in inducing matrix metalloproteinase-3, -13 and aggrecanase-1 genes in articular chondrocytes.** *Cellular signalling* **2004**; 16(4):469-476.

116. Zhang F, Tanaka H, Kawato T, Kitami S, Nakai K, Motohashi M, Suzuki N, Wang CL, Ochiai K, Isokawa K, Maeno M. **Interleukin-17A induces cathepsin K and MMP-9**

expression in osteoclasts via celecoxib-blocked prostaglandin E2 in osteoblasts. *Biochimie* **2011**; 93(2):296-305.

117. Lubberts E, van den Bersselaar L, Oppers-Walgreen B, Schwarzenberger P, Coenen-de Roo CJ, Kolls JK, Joosten LA, van den Berg WB. **IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-kappa B ligand/osteoprotegerin balance.** *Journal of immunology* **2003**; 170(5):2655-2662.

118. Nakashima T, Takayanagi H. **Osteoclasts and the immune system.** *Journal of bone and mineral metabolism* **2009**; 27(5):519-529.

119. Gaffen SL. **Structure and signalling in the IL-17 receptor family.** *Nature reviews Immunology* **2009**; 9(8):556-567.

120. Song X, Zhu S, Shi P, Liu Y, Shi Y, Levin SD, Qian Y. **IL-17RE is the functional receptor for IL-17C and mediates mucosal immunity to infection with intestinal pathogens.** *Nature immunology* **2011**.

121. Tan W, Huang W, Gu X, Zhong Q, Liu B, Schwarzenberger P. **IL-17F/IL-17R interaction stimulates granulopoiesis in mice.** *Exp Hematol* **2008**; 36(11):1417-1427.

122. Hot A, Zrioual S, Toh ML, Lenief V, Miossec P. **IL-17A- versus IL-17F-induced intracellular signal transduction pathways and modulation by IL-17RA and IL-17RC RNA interference in rheumatoid synoviocytes.** *Annals of the rheumatic diseases* **2011**; 70(2):341-348.

123. Hot A, Miossec P. **Effects of interleukin (IL)-17A and IL-17F in human rheumatoid arthritis synoviocytes.** *Annals of the rheumatic diseases* **2011**; 70(5):727-732.

124. Zrioual S, Ecochard R, Tournadre A, Lenief V, Cazalis MA, Miossec P. **Genome-wide comparison between IL-17A- and IL-17F-induced effects in human rheumatoid arthritis synoviocytes.** *Journal of immunology* **2009**; 182(5):3112-3120.

125. Wright JF, Bennett F, Li B, Brooks J, Luxenberg DP, Whitters MJ, Tomkinson KN, Fitz LJ, Wolfman NM, Collins M, Dunussi-Joannopoulos K *et al.* **The human IL-17F/IL-17A heterodimeric cytokine signals through the IL-17RA/IL-17RC receptor complex.** *J Immunol* **2008**; 181(4):2799-2805.

126. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, Collins M, Goldman SJ, Dunussi-Joannopoulos K, Williams CM, Wright JF, Fouser LA. **An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment.** *Journal of immunology* **2007**; 179(11):7791-7799.

127. McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, Steele C, Finder JD, Pilewski JM, Carreno BM, Goldman SJ, Pirhonen J *et al.* **Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis.** *Journal of immunology* **2005**; 175(1):404-412.

128. Hu Y, Ota N, Peng I, Refino CJ, Danilenko DM, Caplazi P, Ouyang W. **IL-17RC is required for IL-17A- and IL-17F-dependent signaling and the pathogenesis of experimental autoimmune encephalomyelitis.** *Journal of immunology* **2010**; 184(8):4307-4316.

129. Ely LK, Fischer S, Garcia KC. **Structural basis of receptor sharing by interleukin 17 cytokines.** *Nature immunology* **2009**; 10(12):1245-1251.

130. Toy D, Kugler D, Wolfson M, Vanden Bos T, Gurgel J, Derry J, Tocker J, Peschon J. **Cutting edge: interleukin 17 signals through a heteromeric receptor complex.** *Journal of immunology* **2006**; 177(1):36-39.

131. Smits HH, van Beelen AJ, Hessle C, Westland R, de Jong E, Soeteman E, Wold A, Wierenga EA, Kapsenberg ML. **Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development.** *European journal of immunology* **2004**; 34(5):1371-1380.

132. Hunter CA. **New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions.** *Nature reviews Immunology* **2005**; 5(7):521-531.

133. Meeks KD, Sieve AN, Kolls JK, Ghilardi N, Berg RE. **IL-23 is required for protection against systemic infection with Listeria monocytogenes.** *Journal of immunology* **2009**; 183(12):8026-8034.

134. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W *et al.* **A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R.** *Journal of immunology* **2002**; 168(11):5699-5708.

135. Zhou L, Ivanov, II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. **IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways.** *Nature immunology* **2007**; 8(9):967-974.

136. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, Sekikawa K, Asano M, Iwakura Y. **Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses.** *Immunity* **2002**; 17(3):375-387.

137. Camplejohn KL, Allard SA. **Limitations of safranin 'O' staining in proteoglycan-depleted cartilage demonstrated with monoclonal antibodies.** *Histochemistry* **1988**; 89(2):185-188.

138. Zenger S, Hollberg K, Ljusberg J, Norgard M, Ek-Rylander B, Kiviranta R, Andersson G. **Proteolytic processing and polarized secretion of tartrate-resistant acid phosphatase is altered in a subpopulation of metaphyseal osteoclasts in cathepsin K-deficient mice.** *Bone* **2007**; 41(5):820-832.

139. Bremell T, Lange S, Yacoub A, Ryden C, Tarkowski A. **Experimental Staphylococcus aureus arthritis in mice.** *Infect Immun* **1991**; 59(8):2615-2623.

140. von Vietinghoff S, Ley K. **IL-17A controls IL-17F production and maintains blood neutrophil counts in mice.** *J Immunol* **2009**; 183(2):865-873.

141. Teitelbaum SL. **Bone resorption by osteoclasts.** *Science* **2000**; 289(5484):1504-1508.

142. Carson WE, Giri JG, Lindemann MJ, Linett ML, Ahdieh M, Paxton R, Anderson D, Eisenmann J, Grabstein K, Caligiuri MA. **Interleukin (IL) 15 is a novel cytokine that activates human natural killer cells via components of the IL-2 receptor.** *The Journal of experimental medicine* **1994**; 180(4):1395-1403.

143. Lodolce JP, Boone DL, Chai S, Swain RE, Dassopoulos T, Trettin S, Ma A. **IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation.** *Immunity* **1998**; 9(5):669-676.

144. Small CL, McCormick S, Gill N, Kugathasan K, Santosuosso M, Donaldson N, Heinrichs DE, Ashkar A, Xing Z. **NK cells play a critical protective role in host defense against acute extracellular Staphylococcus aureus bacterial infection in the lung.** *Journal of immunology* **2008**; 180(8):5558-5568.

145. Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM. **TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis.** *Am J Pathol* **2001**; 159(5):1689-1699.

146. Gravallese EM, Harada Y, Wang JT, Gorn AH, Thornhill TS, Goldring SR. **Identification of cell types responsible for bone resorption in rheumatoid arthritis and juvenile rheumatoid arthritis.** *Am J Pathol* **1998**; 152(4):943-951.

147. Oh S, Perera LP, Terabe M, Ni L, Waldmann TA, Berzofsky JA. **IL-15 as a mediator of CD4+ help for CD8+ T cell longevity and avoidance of TRAIL-mediated apoptosis.** *Proceedings of the National Academy of Sciences of the United States of America* **2008**; 105(13):5201-5206.

148. Inoue S, Unsinger J, Davis CG, Muenzer JT, Ferguson TA, Chang K, Osborne DF, Clark AT, Coopersmith CM, McDunn JE, Hotchkiss RS. **IL-15 prevents apoptosis, reverses innate and adaptive immune dysfunction, and improves survival in sepsis.** *Journal of immunology* **2010**; 184(3):1401-1409.

149. Hotchkiss RS, Dunne WM, Swanson PE, Davis CG, Tinsley KW, Chang KC, Buchman TG, Karl IE. **Role of apoptosis in Pseudomonas aeruginosa pneumonia.** *Science* **2001**; 294(5548):1783.

150. Szekanecz Z, Koch AE. **Update on synovitis.** *Current rheumatology reports* **2001**; 3(1):53-63.

151. Kopf M, Baumann H, Freer G, Freudenberg M, Lamers M, Kishimoto T, Zinkernagel R, Bluethmann H, Kohler G. **Impaired immune and acute-phase responses in interleukin-6-deficient mice.** *Nature* **1994**; 368(6469):339-342.

152. Assier E, Boissier MC, Dayer JM. **Interleukin-6: from identification of the cytokine to development of targeted treatments.** *Joint, bone, spine : revue du rhumatisme* **2010**; 77(6):532-536.

153. Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Costantini F. **Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion.** *The EMBO journal* **1994**; 13(5):1189-1196.

154. Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC. **Increased osteoclast development after estrogen loss: mediation by interleukin-6.** *Science* **1992**; 257(5066):88-91.

155. Wong GG, Witek-Giannotti JS, Temple PA, Kriz R, Ferenz C, Hewick RM, Clark SC, Ikebuchi K, Ogawa M. **Stimulation of murine hemopoietic colony formation by human IL-6.** *Journal of immunology* **1988**; 140(9):3040-3044.

156. Kurihara N, Civin C, Roodman GD. **Osteotropic factor responsiveness of highly purified populations of early and late precursors for human multinucleated cells expressing the osteoclast phenotype.** *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research* **1991**; 6(3):257-261.

157. Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Koishihara Y, Ohsugi Y, Kumaki K, Taga T, et al. **Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6.** *Proceedings of the National Academy of Sciences of the United States of America* **1993**; 90(24):11924-11928.

158. Hashizume M, Hayakawa N, Mihara M. **IL-6 trans-signalling directly induces RANKL on fibroblast-like synovial cells and is involved in RANKL induction by TNF-alpha and IL-17.** *Rheumatology* **2008**; 47(11):1635-1640.

159. Tanaka T, Ogata A, Narazaki M. **Tocilizumab for the treatment of rheumatoid arthritis.** *Expert Rev Clin Immunol* **2010**; 6(6):843-854.

160. Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, Fujikado N, Tanahashi Y, Akitsu A, Kotaki H, Sudo K *et al.* **Differential roles of interleukin-17A and -17F in host defense against mucoepithelial bacterial infection and allergic responses.** *Immunity* **2009**; 30(1):108-119.

161. Tan W, Huang W, Gu X, Zhong Q, Liu B, Schwarzenberger P. **IL-17F/IL-17R interaction stimulates granulopoiesis in mice.** *Experimental hematology* **2008**; 36(11):1417-1427.

162. von Vietinghoff S, Ley K. **Homeostatic regulation of blood neutrophil counts.** *Journal of immunology (Baltimore, Md : 1950)* **2008**; 181(8):5183-5188.

163. Zhao YX, Tarkowski A. **Impact of interferon-gamma receptor deficiency on experimental Staphylococcus aureus septicemia and arthritis.** *J Immunol* **1995**; 155(12):5736-5742.

164. Toh ML, Kawashima M, Zrioual S, Hot A, Miossec P. **IL-17 inhibits human Th1 differentiation through IL-12R beta 2 downregulation.** *Cytokine* **2009**; 48(3):226-230.

165. Yang XO, Chang SH, Park H, Nurieva R, Shah B, Acero L, Wang YH, Schluns KS, Broaddus RR, Zhu Z, Dong C. **Regulation of inflammatory responses by IL-17F.** *The Journal of experimental medicine* **2008**; 205(5):1063-1075.

166. Hymowitz SG, Filvaroff EH, Yin JP, Lee J, Cai L, Risser P, Maruoka M, Mao W, Foster J, Kelley RF, Pan G *et al.* **IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding.** *EMBO J* **2001**; 20(19):5332-5341.

167. Chang SH, Dong C. **IL-17F: regulation, signaling and function in inflammation.** *Cytokine* **2009**; 46(1):7-11.

II. Henningsson L, Jirholt P, Lindholm C, Eneljung T, Silverpil E, Iwakura Y, Linden A, Gjertsson I. **Interleukin-17A during local and systemic Staphylococcus aureus-induced arthritis in mice.** *Infection and immunity* **2010**; 78(9):3783-3790.

169. Kawaguchi M, Onuchic LF, Li XD, Essayan DM, Schroeder J, Xiao HQ, Liu MC, Krishnaswamy G, Germino G, Huang SK. **Identification of a novel cytokine, ML-1,**

and its expression in subjects with asthma. *Journal of immunology* **2001**; 167(8):4430-4435.

170. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S *et al.* **IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia.** *Nature medicine* **2008**; 14(3):275-281.

171. Chang SH, Dong C. **Signaling of interleukin-17 family cytokines in immunity and inflammation.** *Cellular signalling* **2011**; 23(7):1069-1075.

172. Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, Tanaka S, Kodama T, Akira S, Iwakura Y, Cua DJ *et al.* **Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction.** *The Journal of experimental medicine* **2006**; 203(12):2673-2682.

173. Duvallet E, Semerano L, Assier E, Falgarone G, Boissier MC. **Interleukin-23: A key cytokine in inflammatory diseases.** *Annals of medicine* **2011**; 43(7):503-511.

174. Wakashin H, Hirose K, Iwamoto I, Nakajima H. **Role of IL-23-Th17 cell axis in allergic airway inflammation.** *International archives of allergy and immunology* **2009**; 149 Suppl 1(108-112.

175. Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, Saito Y, Hatano M, Tokuhisa T, Iwakura Y, Puccetti P *et al.* **IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice.** *American journal of respiratory and critical care medicine* **2008**; 178(10):1023-1032.

176. Otani K, Watanabe T, Tanigawa T, Okazaki H, Yamagami H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Arakawa T. **Anti-inflammatory effects of IL-17A on Helicobacter pylori-induced gastritis.** *Biochemical and biophysical research communications* **2009**; 382(2):252-258.

177. Ke Y, Liu K, Huang GQ, Cui Y, Kaplan HJ, Shao H, Sun D. **Anti-inflammatory role of IL-17 in experimental autoimmune uveitis.** *Journal of immunology* **2009**; 182(5):3183-3190.

178. Silverman MD, Zamora DO, Pan Y, Texeira PV, Baek SH, Planck SR, Rosenbaum JT. **Constitutive and inflammatory mediator-regulated fractalkine expression in human ocular tissues and cultured cells.** *Investigative ophthalmology & visual science* **2003**; 44(4):1608-1615.

179. Albanesi C, Cavani A, Girolomoni G. **IL-17 is produced by nickel-specific T lymphocytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effects with IFN-gamma and TNF-alpha.** *Journal of immunology* **1999**; 162(1):494-502.

180. Maertzdorf J, Osterhaus AD, Verjans GM. **IL-17 expression in human herpetic stromal keratitis: modulatory effects on chemokine production by corneal fibroblasts.** *Journal of immunology* **2002**; 169(10):5897-5903.

181. Kanda N, Koike S, Watanabe S. **IL-17 suppresses TNF-alpha-induced CCL27 production through induction of COX-2 in human keratinocytes.** *The Journal of allergy and clinical immunology* **2005**; 116(5):1144-1150.

182. O'Connor W, Jr., Kamanaka M, Booth CJ, Town T, Nakae S, Iwakura Y, Kolls JK, Flavell RA. **A protective function for interleukin 17A in T cell-mediated intestinal inflammation.** *Nature immunology* **2009**; 10(6):603-609.

183. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, Quesniaux V, Fossiez F, Ryffel B, Schnyder B. **Interleukin-17 is a negative regulator of established allergic asthma.** *The Journal of experimental medicine* **2006**; 203(12):2715-2725.

184. Ogawa A, Andoh A, Araki Y, Bamba T, Fujiyama Y. **Neutralization of interleukin-17 aggravates dextran sulfate sodium-induced colitis in mice.** *Clinical immunology* **2004**; 110(1):55-62.

185. Caruso R, Stolfi C, De Nitto D, Pallone F, Monteleone G. **The dual role of interleukin-25 in the control of immune-mediated pathologies.** *Current molecular medicine* **2011**; 11(1):26-30.

186. Zaph C, Du Y, Saenz SA, Nair MG, Perrigoue JG, Taylor BC, Troy AE, Kobuley DE, Kastelein RA, Cua DJ, Yu Y *et al.* **Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine.** *The Journal of experimental medicine* **2008**; 205(10):2191-2198.

187. Kleinschek MA, Owyang AM, Joyce-Shaikh B, Langrish CL, Chen Y, Gorman DM, Blumenschein WM, McClanahan T, Brombacher F, Hurst SD, Kastelein RA *et al.* **IL-25 regulates Th17 function in autoimmune inflammation.** *The Journal of experimental medicine* **2007**; 204(1):161-170.

188. Rickel EA, Siegel LA, Yoon BR, Rottman JB, Kugler DG, Swart DA, Anders PM, Tocker JE, Comeau MR, Budelsky AL. **Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities.** *Journal of immunology* **2008**; 181(6):4299-4310.

189. Besnard AG, Sabat R, Dumoutier L, Renauld JC, Willart M, Lambrecht B, Teixeira MM, Charron S, Fick L, Erard F, Warszawska K *et al.* **Dual Role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A.** *American journal of respiratory and critical care medicine* **2011**; 183(9):1153-1163.

190. Takahashi K, Hirose K, Kawashima S, Niwa Y, Wakashin H, Iwata A, Tokoyoda K, Renauld JC, Iwamoto I, Nakayama T, Nakajima H. **IL-22 attenuates IL-25 production by lung epithelial cells and inhibits antigen-induced eosinophilic airway inflammation.** *The Journal of allergy and clinical immunology* **2011**.

191. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, Ouyang W. **Interleukin-22, a T(H)17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis.** *Nature* **2007**; 445(7128):648-651.

192. Sonnenberg GF, Nair MG, Kirn TJ, Zaph C, Fouser LA, Artis D. **Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A.** *The Journal of experimental medicine* **2010**; 207(6):1293-1305.

193. Marijnissen RJ, Koenders MI, Smeets RL, Stappers MH, Nickerson-Nutter C, Joosten LA, Boots AM, van den Berg WB. **Increased expression of interleukin-22 by synovial Th17 cells during late stages of murine experimental arthritis is controlled by**

interleukin-1 and enhances bone degradation. *Arthritis and rheumatism* **2011**; 63(10):2939-2948.

194. Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, Heppner FL, Renauld JC, Becher B. **IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis.** *Journal of immunology* **2007**; 179(12):8098-8104.

195. Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, Muchamuel T *et al.* **IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo.** *Immunity* **2001**; 15(6):985-995.

196. Kang CM, Jang AS, Ahn MH, Shin JA, Kim JH, Choi YS, Rhim TY, Park CS. **Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles.** *American journal of respiratory cell and molecular biology* **2005**; 33(3):290-296.

197. Lin Y, Ritchea S, Logar A, Slight S, Messmer M, Rangel-Moreno J, Guglani L, Alcorn JF, Strawbridge H, Park SM, Onishi R *et al.* **Interleukin-17 is required for T helper 1 cell immunity and host resistance to the intracellular pathogen Francisella tularensis.** *Immunity* **2009**; 31(5):799-810.

198. Shahrara S, Pickens SR, Dorfleutner A, Pope RM. **IL-17 induces monocyte migration in rheumatoid arthritis.** *Journal of immunology* **2009**; 182(6):3884-3891.

199. Tybulewicz VL, Henderson RB. **Rho family GTPases and their regulators in lymphocytes.** *Nature reviews Immunology* **2009**; 9(9):630-644.

200. Huang H, Kim HJ, Chang EJ, Lee ZH, Hwang SJ, Kim HM, Lee Y, Kim HH. **IL-17 stimulates the proliferation and differentiation of human mesenchymal stem cells: implications for bone remodeling.** *Cell death and differentiation* **2009**; 16(10):1332-1343.

201. Utsugi M, Dobashi K, Ishizuka T, Kawata T, Hisada T, Shimizu Y, Ono A, Mori M. **Rac1 negatively regulates lipopolysaccharide-induced IL-23 p19 expression in human macrophages and dendritic cells and NF-kappaB p65 trans activation plays a novel role.** *Journal of immunology* **2006**; 177(7):4550-4557.

202. Kageyama Y, Kobayashi H, Kato N. **Infliximab treatment reduces the serum levels of interleukin-23 in patients with rheumatoid arthritis.** *Modern rheumatology / the Japan Rheumatism Association* **2009**; 19(6):657-662.

203. Genovese MC, Van den Bosch F, Roberson SA, Bojin S, Biagini IM, Ryan P, Sloan-Lancaster J. **LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: A phase I randomized, double-blind, placebo-controlled, proof-of-concept study.** *Arthritis Rheum* 62(4):929-939.

204. Baslund B, Tvede N, Danneskiold-Samsoe B, Larsson P, Panayi G, Petersen J, Petersen LJ, Beurskens FJ, Schuurman J, van de Winkel JG, Parren PW *et al.* **Targeting interleukin-15 in patients with rheumatoid arthritis: a proof-of-concept study.** *Arthritis Rheum* **2005**; 52(9):2686-2692.

205. Gabay C, McInnes IB. **The biological and clinical importance of the 'new generation' cytokines in rheumatic diseases.** *Arthritis Res Ther* **2009**; 11(3):230.

206. Halvorsen EH, Stronen E, Hammer HB, Goll GL, Sollid LM, Molberg O. **Interleukin-15 induces interleukin-17 production by synovial T cell lines from patients with rheumatoid arthritis.** *Scandinavian journal of immunology* **2011**; 73(3):243-249.

207. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, Parente E, Fili L, Ferri S, Frosali F, Giudici F *et al.* **Phenotypic and functional features of human Th17 cells.** *The Journal of experimental medicine* **2007**; 204(8):1849-1861.

208. Ferretti S, Bonneau O, Dubois GR, Jones CE, Trifilieff A. **IL-17, produced by lymphocytes and neutrophils, is necessary for lipopolysaccharide-induced airway neutrophilia: IL-15 as a possible trigger.** *Journal of immunology* **2003**; 170(4):2106-2112.

209. Djaafar S, Pierroz DD, Chicheportiche R, Zheng XX, Ferrari SL, Ferrari-Lacraz S. **Inhibition of T cell-dependent and RANKL-dependent osteoclastogenic processes associated with high levels of bone mass in interleukin-15 receptor-deficient mice.** *Arthritis and rheumatism* **2010**; 62(11):3300-3310.

210. Constantinescu CS, Grygar C, Kappos L, Leppert D. **Interleukin 15 stimulates production of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 by human peripheral blood mononuclear cells.** *Cytokine* **2001**; 13(4):244-247.

211. Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ. **Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease.** *Bone* **2003**; 33(1):28-37.

212. Hsu YL, Hung JY, Ko YC, Hung CH, Huang MS, Kuo PL. **Phospholipase D signaling pathway is involved in lung cancer-derived IL-8 increased osteoclastogenesis.** *Carcinogenesis* **2010**; 31(4):587-596.

213. Kohara H, Kitaura H, Fujimura Y, Yoshimatsu M, Morita Y, Eguchi T, Masuyama R, Yoshida N. **IFN-gamma directly inhibits TNF-alpha-induced osteoclastogenesis in vitro and in vivo and induces apoptosis mediated by Fas/Fas ligand interactions.** *Immunology letters* **2011**; 137(1-2):53-61.

214. Cho JS, Pietras EM, Garcia NC, Ramos RI, Farzam DM, Monroe HR, Magorien JE, Blauvelt A, Kolls JK, Cheung AL, Cheng G *et al.* **IL-17 is essential for host defense against cutaneous Staphylococcus aureus infection in mice.** *The Journal of clinical investigation* **2010**; 120(5):1762-1773.