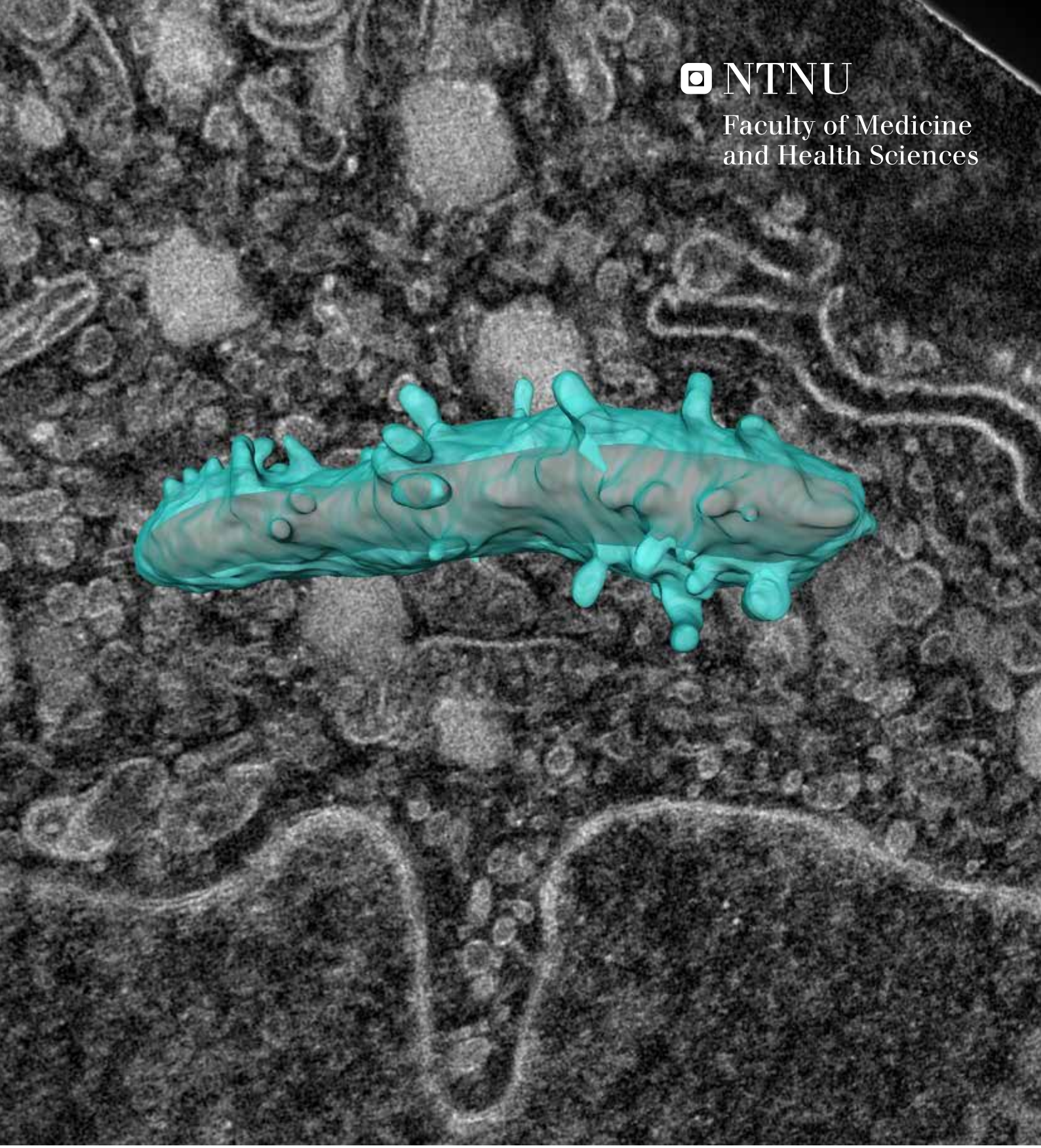

NTNU
Faculty of Medicine
and Health Sciences

CEMIR
Centre of Molecular Inflammation Research

Annual Report 2016

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Cover photo made by Marianne Beckwith:
A macrophage infected with *Mycobacterium avium* was imaged in 3D using a Focused Ion Beam/Scanning Electron Microscope (FIB/SEM) at NTNU Nanolab. A series of some hundred images was acquired with the FIB/SEM, and a 3D model of a bacterium (grey) inside of its phagosome (cyan) was reconstructed from the stack of images. One representative SEM image from the stack is shown in black and white in the background, depicting a range of intracellular organelles. The phagosome surrounding the bacterium is loose, and seems to be in contact/fusing with other endosomal compartments of the cell.

Highlights from the first four years

The vision of CEMIR is to find out how sensors in the innate immune system initiate and regulate inflammatory responses. This new knowledge will be used in disease models to identify new therapeutic targets and diagnostic tools for inflammatory diseases.

CEMIR was established as a Centre of Excellence January 1, 2013. During the first years the main priority was to establish a unified research group in which multidisciplinary collaboration was encouraged and stimulated. In June 2014, all CEMIR research activities were moved to the new Knowledge Centre at Øya Campus in Trondheim which hosts first-class laboratories with state of the art cellular imaging instruments. In October 2015, we opened a new BSL3-laboratory, offering the highest level of security for research on viruses and bacteria in Norway. The new lab contains an advanced Leica SP8 confocal microscope making it possible to study immune cells infected with viable mycobacteria and HIV virus. CEMIR has access to unique infrastructure and has now grown to be a vibrant and dynamic centre with 64 scientific staff members, 11 engineers, 15 students and one administrative coordinator.

CEMIR has established close collaboration with excellent researchers from other institutions by recruiting six outstanding scientists in the fields of cell biology and innate immunity from universities in Boston, Los Angeles, Bonn and Oslo (Drs Fitzgerald, Lien, Underhill, Latz, Mollnes and Stenmark). They have contributed extensively to the research programme at the Centre, and also supervised our PhD students and post docs. Two PhD students and three post docs have spent extended periods in their labs.

We have improved and strengthen the scientific quality and scope of our centre by recruiting two new group leaders in 2015. The positions were announced as Group leaders with startup-package. We were searching for persons with excellent scientific background to complement and strengthen existing CEMIR research groups and improve the scientific expertise further, especially within the fields of proteomics, bioinformatics and models of inflammatory diseases. Two qualified persons were accepted for the positions. Richard Kandasamy started in December 2015, and Menno Oudhoff joined CEMIR in March 2016. The two new group leaders have already managed to obtain substantial external funding to their research projects.

The scientific activities at CEMIR have proceeded with very good progress. CEMIR researchers have published more than 200 articles since 2013, several in high quality journals like Nature, Nature Immunology, Autophagy, PNAS and J Immunol. Seventeen PhD students have completed their theses at the centre since 2013.

A major highlight happened May 30th – June 2nd 2016 at the Knowledge Centre, Trondheim, when CEMIR arranged an international conference: Conference on Molecular Mechanisms of Inflammation. Twentyfive outstanding international researchers were invited to give presentations, and this gave a unique opportunity to expand our insight into processes of inflammatory disorders. The conference attracted 200 participants from all over the world and this arrangement was a fantastic success where we were able to make CEMIR visible for the international scientific community in this important field of research.

CEMIR RESEARCH THEMES

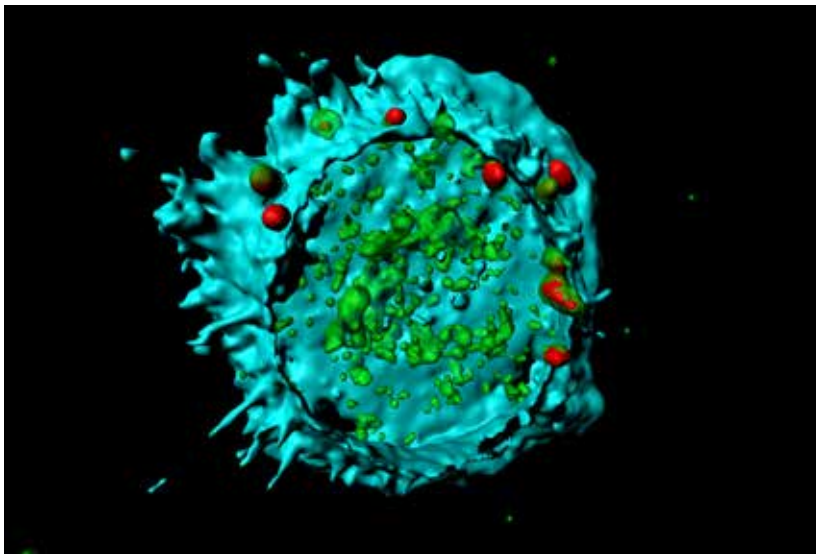
Inflammatory Responses Induced by Bacteria – Identification of Vesicle Transport Components and Signalling Pathways that regulate Interferon Responses from Endosomes

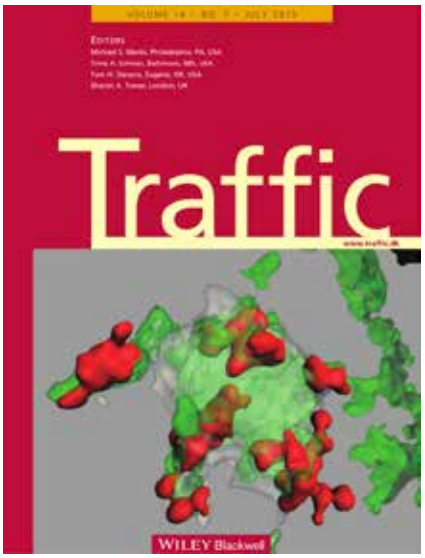
Theme Manager:
Professor Terje Espevik

In the presence of systemic infection, microbial pathogens induce strong inflammatory- and coagulation activation, leading to sepsis and septic shock. Also, an anti-inflammatory response is induced during sepsis that can contribute to secondary infections. Severe bacterial infections may lead to high amounts of type I IFNs that can result in production of immunosuppressive molecules increasing the risk for secondary infections. The main aim of this theme is to find new principles of Toll-like receptor (TLR) signalling resulting in type I interferons from endosomes and phagosomes. A second aim is to find ways to inhibit inflammatory responses by targeting TLRs and the complement system.

MAIN ACTIVITIES IN 2013–2016

The induction of type I IFNs by various types of bacteria in different immune cells has gained increased attention in recent years. The impact of type I IFNs on bacterial infections is not clear and spans from immune stimulation to immune suppression. However, several reports have suggested that induction type I IFN contributes to the progression of septic shock. Lipopolysaccharide (LPS) from Gram-negative bacteria is recognized by TLR4 and activates two distinct signalling pathways. One of the pathways needs the sorting adapter protein TRAM, and the signalling adapter TRIF for inducing IFN-β. We have shown that LPS induces an immobile fraction of TLR4 in punctuates structures in the plasma membrane containing CD14/LPS and clathrin. The small GTPase Rab11a drives TRAM into the endocytic recycling compartment (ERC) and onto endosomes. These data suggest that Rab11a regulates TLR4 mediated IFN-β production through its ability to transport TRAM from Golgi to ERC and further onto endosomes where it interacts with TLR4 (Husebye and Espevik et al., *Traffic*, 2015).





Left: A 3D reconstruction of a human macrophage phaocytosing *E.coli* (red). TRAM (green) is recruited to phagosomes. Cyan represents actin filaments. Right: In July 2015 a publication by Husebye and Espevik et al. was displayed on the front page of *Traffic*. Figure: Harald Husebye

We have previously found that Rab11a controls trafficking of TRAM to *E. coli* phagosomes which is required for the IFN-β response (Husebye and Espevik et al., *Immunity* 2010). In 2013-16 we have aimed to find the molecular mechanisms for how Rab11a mediates this TRAM recruitment to phagosomes containing gram negative bacteria. Rab11a transports vesicular cargo through interaction with Rab11 effector molecules. One important Rab11 effector protein is Rab11 family interacting protein 2 (FIP2) that recruits the actin motor Myo5b and mediates transports of cargo along actin filaments. investigated the location and mobility of TRAM towards the *E.coli* phagosome and how the Rab11a effector molecule, FIP2, regulates TRAM trafficking. We have found that FIP2 plays a critical role in the transport of TRAM to phagosomes and in the induction of IFN-β by *E.coli* in human primary macrophages. Moreover, we have demonstrated that FIP2 binds to TRAM outside of the TIR domain. This is an observation that may have essential functional consequences in the regulation of responses to gram-negative bacteria. A manuscript describing these data will be submitted in 2017.

Another potential TRAM interacting protein is CD150, which belongs to the signaling lymphocyte activation molecule family (SLAMF) receptors. These receptors modulate a wide range of functions, such as myeloid cell and lymphocyte development, and T- and B cell responses to microbes and parasites. Moreover, CD150 serves as microbial sensors and can control phagosomal maturation. To our surprise, we have found that CD150 regulates macrophage responses to gram-negative bacteria through interaction with TRAM. These findings point to new drug targets for controlling inflammatory reactions that occur during severe bacterial infections. A patent and a manuscript describing these data will be submitted in 2017.

Toll-like receptor 2 is a primary pattern recognition receptor for sensing gram positive bacteria such as *S. aureus*. TLR2 is activated by lipoproteins and are though to mediate signalling through the MyD88 dependent pathway. We have found a new role for TRAM and TRIF also in TLR2 regulation and signalling. The findings broaden our understanding of how Toll/interleukin-1 receptor adaptor proteins may participate in signalling downstream from TLR2 (Lien, Espevik et al., *J. Biol. Chem*. 2015).

One of the aims of this theme has been to examine the role of TLR2 and other pattern recognition receptors for *S. aureus*–induced IFN-β production in human primary monocytes and monocyte-derived macrophages. Unexpectedly, we found that TLR2 activation could suppress the *S. aureus*–induced production of IFN-β. In contrast, induction of IFN-β was triggered by *S. aureus* RNA, which activated a TLR8– IRF5 signaling axis in a TGF-β–activated kinase 1 (TAK1)– and IkB kinase (IKK)β–dependent fashion. In this study, we establish TLR8 as a second MyD88-dependent pattern recognition receptor of *S. aureus* in human primary monocytes and human and showed that it is essential for the induction of IFN-β production by whole bacteria via a IKKβ–IRF5 activation pathway. We also demonstrated a cross-regulatory function of TLR2 in TLR8–IRF5 signaling. This mechanism may be important for the sensing of infection with *S. aureus* and possibly other pyogenic bacteria, thus providing new possible targets for pharmacological immunomodulation in conditions such as Gram-positive sepsis (Stenvik, Espevik et al., *J. Immunol.* 2015).

Another focus of this theme has been to inhibit inflammatory responses by targeting TLRs and the complement system. Through a close collaboration with the Mollnes group we have constructed and characterized a chimeric recombinant anti-human CD14 IgG2/4 CD14 with minimal ability to activate complement and bind to FcγRs. The original anti CD14 hybridoma (18D11) was generated in the laboratory of Espevik. The Mollnes group has previously found that the combination of 18D11 and complement inhibition is a particular potent attenuator of bacterial inflammatory responses in human whole blood. The recombinant 18D11 chimeric antibody would be particularly valuable tools for future in vivo studies to explore the combined tool inhibition of CD14 and complement as a therapeutic approach for inflammatory diseases (Mollnes, Espevik et al., *J. Immunol.* 2013). The potency of CD14 and complement factor 5 inhibition has been demonstrated in a cecal ligation and puncture- induced sepsis model in mice and in pigs where this treatment significantly reduces the cytokine responses and mortality. Thus, the combined inhibition of the complement and TLR pathway represents a most promising therapeutic approach to improve outcomes for patients with polymicrobial sepsis (Mollnes, Espevik et al., *J. Immunol.* 2014; *Crit. Care* 2015). Moreover, whole bacteria–induced inflammation was inhibited more efficiently by anti-CD14 than by the TLR4 antagonist eritoran, particularly when combined with complement inhibition (Mollnes, Espevik et al., *J. Immunol.* 2016). CD14 is a known coreceptor with several of the TLRs, including TLR4 and TLR2. Thus, we also wanted to examine if a combined CD14 and complement inhibition was more effective than each treatment regime alone on *S.aureus* induced responses in human whole blood. We found that disrupting upstream recognition by inhibiting complement and CD14 efficiently attenuated *S. aureus*–induced inflammation and might be a promising treatment also in Gram-positive sepsis (Mollnes, Espevik et al., *J. Immunol.* 2014, and reviewed in *J. Leukoc.* Biol. 2016).

We have published 12 papers within this theme and one PhD student has graduated, and one more will graduate in 2017. The major scientific results are summarized below:

MAIN ACHIEVEMENTS IN 2013–2016

- Generated recombinant anti-porcine and anti-human CD14 Abs endowed with the IgG2/IgG4 hybrid Fc region. These Abs are unique tools for future studies of CD14 inhibition using porcine in vivo models, and pave the way for human therapy with CD14 inhibition, preferentially in combination with complement inhibition (Mollnes, Espevik et al., *J. Immunol.* 2013).
- Published data suggesting that specific blockade of CD14 and complement factor C5 represents a promising new therapeutic strategy for treatment of polymicrobial sepsis (Mollnes, Espevik et al., *J. Immunol.* 2014).
- Published data demonstrating that inhibiting complement and CD14 efficiently attenuated Staphylococcus aureus – induced inflammation (Mollnes, Espevik, *J. Immunol.* 2014).
- Published data showing that Rab11a regulates TLR4 mediated IFN-β production through its ability to transport TRAM

form Golgi to ERC and further onto endosomes (Husebye Espevik et al., *Traffic* 2015).
- Demonstrated a physiological role of TLR8 in the sensing of entire *S. aureus* in human primary phagocytes, including the induction of IFN-β and IL-12 production via a TAK1 -: IKKβ -: IRF5 pathway that can be inhibited by TLR2 signaling (Stenvik, Espevik et al., *J. Immunol.* 2015).
- Published data showing that combined inhibition of complement factor C5 and CD14 significantly improved survival, hemodynamic parameters and inflammation in a blinded, randomized trial of porcine polymicrobial sepsis (Mollnes, Espevik et al., *Crit. Care* 2015).
- Published data demonstrating that human endothelial cell activation by *E. coli* and *S. aureus* is mediated by TNF and IL-1β secondarily to activation of C5 and CD14 in whole blood. Thus, both upstream combined targeting of C5 and CD14 and downstream combined targeting of TNF and IL-1β appear to be promising foci to modulate EC activation in inflammation (Mollnes, Espevik et al., *J. Immunol* 2015).
- Published results showing a novel function of TRAM and TRIF in TLR2-mediated signal transduction (Lien, Espevik et al., *J. Biol. Chem.* 2015).
- Demonstrated that whole bacteria-induced inflammation was inhibited more efficiently by anti-CD14 than by the TLR4 antagonist eritoran, particularly when combined with complement inhibition. The data suggest that combined CD14 and complement inhibition may prove a promising treatment strategy for bacterial sepsis (Mollnes, Espevik et al., *J. Infect. Dis.* 2016).
- Established that TLR8 is a major sensor for Group B Streptococci in human macrophages.
- Produced experimental data showing that CD150 plays an essential role in regulating TLR4-mediated TRIF-dependent signaling from gram-negative bacteria phagosomes in human macrophages. This makes CD150 a potential target for modulation of TLR4-dependent type I IFN expression.

AMBITIONS FOR 2017

- To identify the mechanisms and functional consequences behind cross talks between TLR8 and TLR2 signalling in monocytes and macrophages.
- To understand the detailed role of TRAM and Rab11FIP2 in regulating phagocytosis and *E.coli*-induced cytokine responses.
- To establish the role of CD150 in regulating TLR4 and TRAM trafficking in host responses to *E.coli*.
- To reveal new components that regulate trafficking of TLR9 from endoplasmic reticulum to the endolysosomes.
- To identify CD14 and complement activation in the type I IFN response induced by gram- positive and gram-negative bacteria.
- Assess the role of endosomal TLR8 as target for inhibiting responses to Gram-positive bacteria

The Molecular Basis for Inflammasome Activation

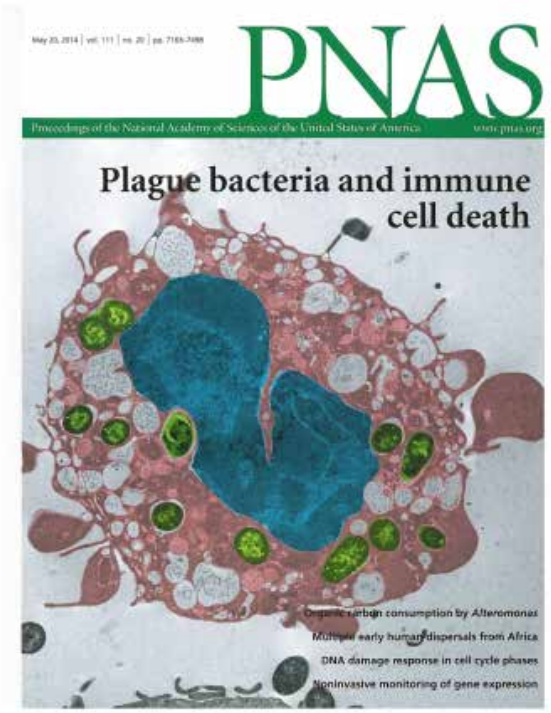
Theme Manager:
Professor Egil Lien

Inflammasomes are multi-molecular complexes that process pro-caspase-1 into the active enzyme. Caspase-1 mediates maturation of pro-forms of cytokines IL-1β and IL-18 into active forms. These cytokines play key roles in the host defenses towards a number of infections, but can also be harmful in some inflammatory disorders. The work in this theme is focused on describing mechanisms leading to inflammasome activation, and to study implications on infectious and non-infectious inflammation.

MAIN ACTIVITIES 2013–2016

During this period we have investigated roles of new components in inflammasome activation and inhibition. One goal has been evaluation of signalling pathways of innate immunity utilized by vaccine adjuvants. We have shown that vaccine adjuvants containing the TLR4 ligand MPLA and saponins such as QS-21, found in licensed and exploratory vaccines against malaria, hepatitis, HIV-1 and cancer, activate inflammasomes and IL-1β/IL-18 release via NLRP3 (Lien et al., *J Biol Chem.* 2016). Thus, QS-21 was identified as a molecularly defined saponin vaccine adjuvant activating the NLRP3 inflammasome. However, cell death induction associated with QS-21 is independent on NLRP3 and caspase-1, and hence not pyroptosis. In vivo role of the NLRP3 inflammasome in the protective responses are at present unclear, in fact, some vaccination regimen may be inhibited by NLRP3.

In separate paradigm-changing studies, we have identified free extracellular inflammasome complexes containing the adapter ASC that are released from dying cells. These complexes have by themselves inflammatory properties, and human and mouse tissue inflammation has been associated with accumulation of these free inflammasome aggregates. Additionally, autoantibodies against these complexes can be found during human autoimmune disease. Furthermore, the ASC complexes can be phagocytosed and trigger nucleation and the formation of bigger "prion"-like structures with additional soluble ASC molecules, promoting IL-1β cleavage (Latz, Espevik et al., *Nat Immunol* 2015).



In 2014 a publication by Lien et al. was displayed on the front page of *PNAS*.

A key tool in further investigations into mechanisms of activation and evasion of bacterially induced inflammation has been infections with Yersinia bacteria, but we have also used other bacteria such as Salmonella, and components from a variety of infectious agents. A major focus has been to identify and characterize molecular aspects of a new RIP1/caspase-8 pathway leading to bacterially induced IL-1β/IL-18 release, caspase-1 cleavage and cell death, thus providing evidence for a strong link between inflammasome activation and members of apoptotic machinery (Lien et al., *PNAS* 2014). This pathway is triggered by components of the bacterial type III secretion system (T3SS), which is a central factor for regulation of inflammation by Gram-negative bacteria, and is important for resistance to infection.

The T3SS contains a needle bridging bacteria and host cells, and allows translocation of bacterial effector molecules into the host cell cytoplasm, setting the stage for disruption of various signalling pathways necessary for the host to fight the infection (Lien et al., *J Leuk Biol.* 2016). The caspase-8 pathway, a non-canonical activation pathway for caspase-1 and IL-1β, is triggered by the effector YopJ. Further studies of this topic has been to describe aspects of how bacteria block innate immune responses by using their T3SS, and a new com-

plexity in terms of involvement of new signalling pathways has been shown (Lien, *J Biol Chem.* 2016). We have identified a bacterial effector molecule (YopM) which can inhibit activation of Pyrin inflammasomes, and visualized the complexity in how bacteria can both activate and inhibit certain pathways. As an example, T3SS from Yersinia bacteria can activate inflammasomes via caspase-8, NLRP3, NLRC4 and Pyrin, and contain specific inhibitors of two of these pathways (Lien et al., *Plos Path.* 2016). YopM is the first microbial inhibitor found to specifically inhibit Pyrin inflammasomes.

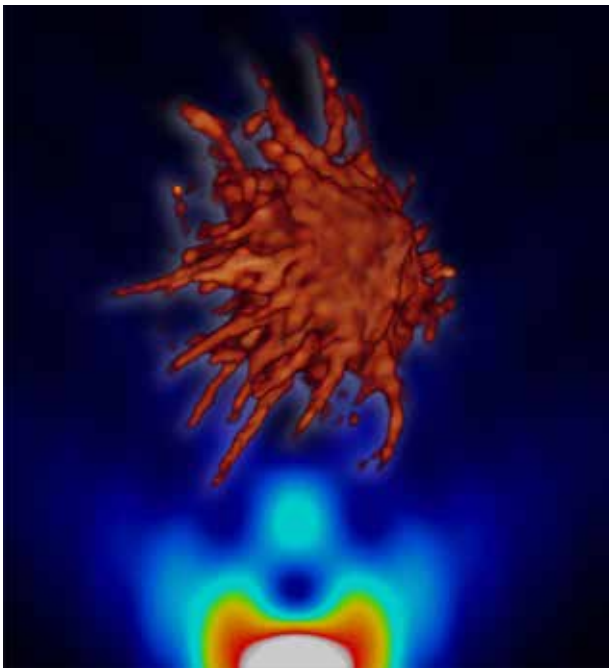
Overall, the activities under this theme have characterized several new players in regulation of inflammation and inflammasomes, both on the host and on the microbial side. One PhD student and one post doc have spent extended time periods in the Lien Fitzgerald lab.

MAJOR ACHIEVEMENTS IN 2013-2016

- Showed that IL-18, NLRP12 and NLRP3 regulate host defenses in the lungs during bacterial infection.
- Completed and published a study on the roles of caspase-8 and RIP kinases inflammasome activation, NF-κB activation and cell death in response to bacterial infection (Lien et al., *PNAS* 2014).
- Identified a novel inflammatory role of ASC inflammasome complexes that are released into the extracellular environment, suggesting a systemic signalling role of these complexes (Latz, Espevik et al., *Nat Immunol* 2015)
- Showed that vaccine adjuvants containing QS-21 saponin activate NLRP3 inflammasomes (Lien et al., *J Biol Chem.* 2016, 1123)
- Completed and published a study demonstrating the first bacterial effector molecule that can inhibit Pyrin inflammasomes (Lien et al., *Plos Path.* 2016)
- Characterized new complexity in how bacteria containing type III secretion systems can manipulate multiple inflammasome pathways leading to IL-1β and IL-18 release Lien et al., *J Biol Chem.* 2016, 9894)

AMBITIONS FOR 2017

- Ascertain further roles for RIP/caspase-8 signaling in host inflammatory responses to bacterial and parasitic infection
- Characterize newly identified (using CRISPR screens) host signaling components regulating inflammasomes following bacterial exposure
- Characterize roles of newly identified (using RNAseq) non-coding genes in modifying bacterially induced cell death, activation of inflammasomes and NF-kB
- Complete work on inflammasomes and metabolic syndrome-associated inflammation
- Continue studies of mechanisms of activation and inhibition of Pyrin inflammasomes during disease



The image shows a 3D volumetric representation of a live ASC speck inflammasome, consisting of 31 optical sections acquired on a Confocal Laser Scanning Microscope (CLSM) and deconvolved in Huygens Professional. Figure: Bjørnar Sporsheim

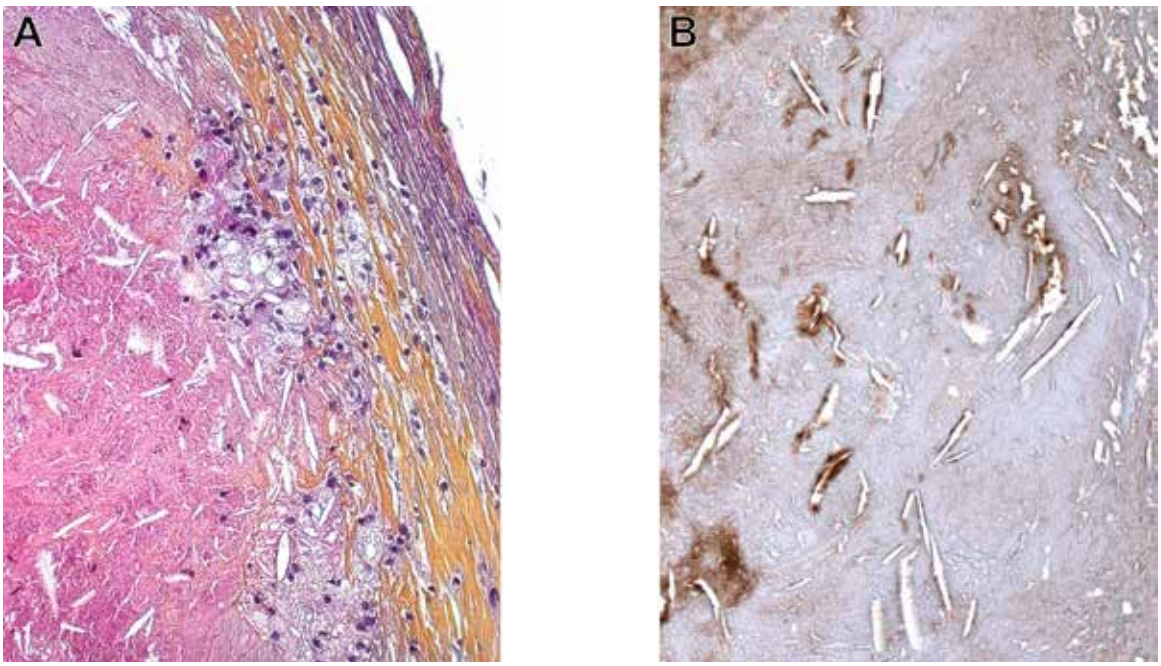
Inflammatory Responses induced by Cholesterol

Theme Manager:
Professor Jan Kristian Damaas

Despite ongoing advances in the prevention and treatment of atherosclerosis, cardiovascular disease remains the leading cause of death worldwide. Atherosclerosis is an inflammatory disease linked to elevated blood cholesterol concentrations. Cholesterol is a lipid that is an essential structural component of cellular membranes as well as a precursor of numerous endogenous signaling molecules that regulate the interface between metabolism and inflammation. Recently, aggregated, crystalline cholesterol (CC) has been recognized as a prominent driver of atherogenic inflammation. The main aim of this theme is to uncover the mechanisms by which CC induce inflammatory responses and to explore novel treatment strategies in atherosclerosis.

MAIN ACTIVITIES IN 2013–2016

High cholesterol levels in blood constitute the most prominent risk factor for atherosclerotic coronary artery disease. We have previously found that cholesterol itself can cause inflammation in its crystalline form by activating the NLRP3 inflammasome (Espevik, Latz, et al., *Nature*, 2010). In this paper, we found that priming is needed for NLRP3 activation. In subsequent papers in this theme we have explored the mechanisms by which CC prime monocytes and induce inflammatory responses. First, we found that CC induced a robust complement activation in human serum revealed by activation products from the alternative and terminal pathways (Samstad OE, Niyonzima N, Damås JK, Mollnes TE, Espevik T, et al. *J Immunol*, 2014). In this paper we also found that CC induced cytokine release in whole blood, which was efficiently attenuated by complement inhibitors. Interestingly, combined C5a and TNF potently primed CC-induced IL-1β release in peripheral mononuclear cells (PBMC) and monocytes by increasing IL-1β transcription. In a following paper, we hypothesized that the pattern recognition molecules (PRMs) from the lectin pathway could bind CC and function as an upstream innate inflammatory signal in the pathophysiology of atherosclerosis. Our main finding was that the lectin pathway was activated on CC by binding of ficolin-2 and mannose-binding lectin (MBL), resulting in activation and deposition of complement activation products. MBL and ficolin-2 were present in human carotid plaques, and binding of MBL to CC was confirmed in vivo by immunohistochemistry, showing localization of MBL around



A: Cholesterol crystal clefts in a human atherosclerotic plaque. B: Mannose binding lectin accumulates around crystal clefts suggesting involvement of the lectin pathway of complement activation. Figure: Bjørnar Sporsheim and Terje Espevik

CC clefts (Bakke SS, Niyonzima N, Mollnes TE, Espevik T, et al. *J Immunol*, 2016). In another paper, we have also examined the effect of CC on human umbilical vein endothelial cells. In this paper we found that CC caused a marked and dose-dependent increase in the adhesion molecules E-selectin and ICAM-1 on the surface of the endothelial cells after incubation with whole blood (Niyonzima N, Espevik T, Mollnes TE, et al. *Immunobiology*, 2014). Notably, complement inhibitors at the C3 and C5 levels markedly reduced the whole blood induced endothelial cell activation and abolished TNF release, and the TNF inhibitor infliximab reduced endothelial activation to background levels. In this theme, we have also sought to determine the effect of reconstituted HDL (rHDL) on CC-induced inflammation in a human whole blood model. HDL exhibits cardioprotective and anti-inflammatory properties thought to explain its inverse correlation to cardiovascular risk. Our data these findings by showing that rHDL bound to CC and inhibited the CC-induced complement activation as measured by soluble terminal C5b-9 formation and C3c deposition on the CC surface. rHDL also attenuated the amount of CC-induced complement receptor 3 (CD11b/CD18) expression on monocytes and granulocytes, as well as reactive oxygen species generation(Niyonzima N, Samstad OE, Damås, JK, Mollnes TE, Espevik T, et al. *J Immunol*, 2015). Our study suggests that rHDL may have a beneficial role in controlling the CC-induced inflammatory responses by inhibiting complement deposition on the crystals. Finally, we have explored the effect of CC in clinical samples, including both whole blood collected from patients with stable and unstable angina as well as in plaques from patients with carotid atherosclerosis. These findings we present in a paper that will be submitted in 2017 (Niyonzima N, Bakke SS, Damås JK, Mollnes TE, Espevik T, et al., *Manuscript*, 2017).

Because cholesterol accumulation and deposition of CC trigger a complex inflammatory response, we have also tested the efficacy of the cyclic oligosaccharide 2-hydroxypropyl-β-cyclodextrin (CD), a compound that increases cholesterol solubility in preventing and reversing atherosclerosis. We showed that CD treatment of murine atherosclerosis reduced atherosclerotic plaque size and CC load and promoted plaque regression even with a continued cholesterol-rich diet (Bakke SS, Espevik T, Latz et al. *Sci Transl Med.* 2016). Mechanistically, we show that CD increased oxysterol production in both macrophages and human atherosclerotic plaques and promoted liver X receptor (LXR)-mediated transcriptional reprogramming to improve cholesterol efflux and exert anti-inflammatory effects. In the period 2013-2016), we also explored how CD may attenuate CC-induced inflammation. We have performed several experiments showing that this substance also may alleviate CC-induced inflammation and complement activation. The manuscript is ready for submission (Bakke SS, Aune M, Latz E, Damås JK, Mollnes TE, Espevik T, et al., *Manuscript*, 2017). Because CD treatment in humans is safe and CD beneficially affects key mechanisms of atherogenesis, our findings support that CD maybe used clinically to prevent or treat human atherosclerosis. Accordingly, based on these studies, we have developed a protocol together with Department of Cardiology, Rikshospitalet, for studying the effect of CD in patients with clinically evident atherosclerosis. We have described our findings on the CC and the involvement of complement in a recent review article (Niyonzima, Mollnes, Espevik et al., *Mol Immunol* 2016).

In parallel to mechanistic studies, we have also investigated the clinical aspects of inflammation in atherosclerosis. First, we examined the association of Single Nucleotide Polymorphisms (SNPs) in genes with the incidence of myocardial infarction in a nested case-control study among participants of the second survey of the HUNT Study. The study population included 1624 cases and 4087 age- and sex-matched controls. We found that the NEIL3 SNP rs12645561, the TT genotype was associated with increased risk of myocardial infarction both in the genotypic test and in the recessive genetic model (Damås JK et al., *DNA repair*, 2015). We have also examined serum/plasma markers as independent predictors for cardiovascular disease. We have published a paper on CXC16 as an independent predictor for future cardiovascular events (Damås JK, et al, *Atherosclerosis*, 2016). We have also submitted a paper on extracellular matrix (ECM) markers (Damås JK, et al. *Submitted manuscript*, 2017) and a paper on monocyte/macrophage and T cell activation markers (Damås JK, et al. *Submitted manuscript*, 2017). Finally, we have examined the effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction (NSTEMI). This was a double-blind, randomized, placebo-controlled phase 2 trial showing that tocilizumab attenuated the inflammatory response and primarily PCI-related troponin T release in NSTEMI patients (Espevik T, Damås JK, et al. *Eur Heart J,* 2016). Based on this study, a new multi-center study on the effect of tocilizumab in patients with ST-elevation infarction will start to include patients in 2017.

Altogether, in this theme we have published 10 papers and 3 reviews, and have 2 manuscripts ready for submission. Two PhD students have graduated in this period. One post doc is currently in the lab of Claudia Kemper at NIH. The major scientific results are summarized below:

MAJOR ACHIEVEMENTS IN 2013–2016

- Published data showing that CC induced a robust complement activation in human serum revealed by activation products from the alternative and terminal pathways (Samstad OE, Niyonzima N, Damås JK, Latz E, Mollnes TE, Espevik T, et al. *J Immunol,* 2014).
- Published data showing that CC causes a marked and dose-dependent increase in the adhesion molecules E-selectin and ICAM-1 on the surface of the endothelial cells after incubation with whole blood (Niyonzima N, Espevik T, Mollnes TE, et al. *Immunobiology*, 2014).
- Demonstrated that rHDL binds to CC and inhibits the CC-induced complement activation as measured by soluble terminal C5b-9 formation and C3c deposition on the CC surface (Niyonzima N, Samstad OE, Damås JK, Latz E, Mollnes TE, Espevik T, et al. *J Immunol*, 2015).
- Published data showing that the NEIL3 SNP rs12645561, the TT genotype is associated with increased risk of myocardial infarction (Damås JK et al., *DNA repair*, 2015).
- Published data demonstrating that the lectin pathway is activated on CC by binding of ficolin-2 and mannose-binding lectin (MBL) in vitro, resulting in activation and deposition of complement activation products. (Bakke SS, Niyonzima N, Mollnes TE, Espevik T, et al. *J Immunol*, 2016).

- Published a paper showing that CXC16 as an independent predictor for future cardiovascular events in the HUNT-population (Damås JK, et al, *Atherosclerosis*, 2016).
- Published data showing that CD treatment of murine atherosclerosis reduced atherosclerotic plaque size and CC load and promoted plaque regression even with a continued cholesterol-rich diet (Bakke SS, Espevik T, Latz et al. *Sci Transl Med.* 2016).
- Published data from a double-blind, randomized, placebo-controlled phase 2 trial showing that tocilizumab attenuated the inflammatory response and primarily PCI-related troponin T release in NSTEMI patients (Espevik T, Damås JK, et al. *Eur Heart J,* 2016).
- Demonstrated that CD may alleviate CC-induced inflammation and complement activation. (Bakke SS, Aune M, Latz E, Damås JK, Mollnes TE, Espevik T, et al., *Manuscript*, 2017).
- Demonstrated that CC induce complement-dependent inflammation in clinical samples, including both whole blood and in plaques from patients with carotid atherosclerosis. (Niyonzima N, Bakke SS, Latz E, Damås JK, Mollnes TE, Espevik T, et al., *Manuscript*, 2017).

AMBITIONS FOR 2017

- To analyze the involvement of the lectin pathway in CC-induced complement activation and coagulation.
- To study the role of CC on intracellular complement in macrophages for NLRP3 activation and IL-1β release.
- To study the function of macrophage survival factor CD5L in the uptake of cholesterol by macrophages and on cell death prior to transformation into foam cells.
- To study CD5L as a predictor for cardiovascular events in the HUNT population.
- To perform studies of the efficacy of inhibiting complement and CD14 in myocardial infarction models.
- To perform sub-studies on serum/plasma samples from the clinical studies and to explore these findings in experimental models.
- To perform the study the effect of CD in patients with clinically evident atherosclerosis together with Department of Cardiology, Rikshospitalet.

Infection, Inflammation & Autophagy

Theme Manager:
Professor Trude Helen Flo

Cells frequently experience stress resulting in activation of PRR responses and induction of autophagy, a process that is essential for cellular homeostasis and, if defective, leads to disorders like degenerative diseases, cancers, infections, inflammation and cardiovascular disease. We aim to define novel relations between oxidative stress, signalling through PRRs and autophagy in inflammatory diseases, including mycobacterial and HIV infections where the focus is molecular host defence mechanisms involved in immunity to mycobacteria and HIV, and virulence strategies employed by these pathogens to parasitize host cells.

MAIN ACTIVITIES 2013–2016

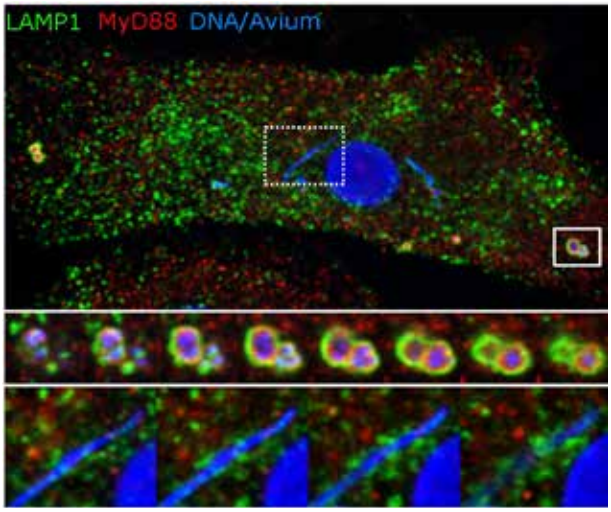
One of the highlights of this theme has been the discovery that Keap1 is a negative regulator of inflammatory responses in human primary macrophages, facilitating intracellular growth of *M. avium*. The work was published in *PNAS* in 2015 (Awuh et al) and it suggests Keap1 as a general regulator of inflammation. These studies are now extended to include septic shock. We have also identified an association of disturbed acylcarnitine metabolism, immune dysregulation and disease progression in HIV-infected patients including increased susceptibility to *M. avium* infection (Waagsbø et al, *Eur J Clin Invest* 2016). Another important activity has been the finalization of our BSL3 facility and training of personnel, and now our research projects include live-cell confocal imaging and drug screening of pathogenic *M. tuberculosis* and HIV.

A reliable method has been established for correlative imaging of macrophages infected with mycobacteria at an ultra-high resolution and in 3D using Focused Ion Beam/Scanning Electron Microscopy tomography together with confocal fluorescence microscopy (Beckwith et al, *Plos One* 2015). The method is further developed and used to visualize membranes and compartments as mycobacteria traffic within macrophages, and in studies of trans-infection of HIV in T-cells. We aim to delineate the spatiotemporal dynamics of mycobacterial and HIV recognition and inflammatory signalling in macrophages and T-cells, respectively, in particular from which compartment signalling is initiated and if endosomal TLRs are involved. We have findings that *M. avium* hides in a compartment from where no inflammatory signalling is generated. Targeting this compartment, preventing its formation or forcing Mav out of it should be of therapeutic value as host-directed therapy to face the challenge with antimicrobial resistance. To better characterize it we will isolate, sort and subject Mav phagosomes to proteomic analyses of mycobacterial and host proteins. Similarly, in collaboration with the new CEMIR group leader, R Kandasamy, we are conducting CRISPR/Cas9 knockout screens on HIV-infected T-cells for discovery of genes restricting or facilitating viral growth.

We have created a useful protein expression tools for mycobacteria (Dragset et al, *Plos One* 2015). In our quest for understanding iron metabolism and in particular the type VII secretion system ESX-3 (Siegrist et al, *MBio* 2014), we discovered how a chemical compound inhibits growth of mycobacteria by working as an intracellular iron chelator (Dragset et al, *Antimicrob Agents Chemother* 2015). In 2016 and as part of a Joint Programming Initiative on Antimicrobial Resistance consortium we have established a screen for novel antimycobacterial compounds targeting ESX-3. We also continue to investigate how *M. tuberculosis* secreted proteins manipulate host systems, since secreted proteins are more accessible drug targets than intracellular mycobacterial proteins.

Autophagy is a core process to maintain cellular homeostasis during starvation and protein folding stress. Cancer cells may face both starvation and increased damage of proteins due to elevated levels of reactive oxygen species. Autophagy is very difficult to quantify in tumor biopsies but the major pathway regulating autophagy in cancer cells, the PI3K-Akt-mTOR-ULK pathway, can be monitored. We developed an approach that allowed quantification of the activity in this pathway in tumour sections using near infrared fluorescence after immunostaining of active Akt (Moestue et al, *Breast Cancer Res* 2013). After targeting the PI3K pathway with chemical inhibitors we could quantify the drug responses and identify changes in tumour metabolism by MR (Moestue et al, Breast *Cancer Res* 2013, Cebulla et al, *Br J Cancer* 2015). We could also use the immunostaining of tumour PI3K signalling in the stratification of responses to therapy in breast cancer patients (Flågend et al, *J Steroid Biochem Mol Bio* 2016).

Autophagy is in principle a cell survival pathway with disease preventive potential and we have shown that n-3 polyunsaturated fatty acids (PUFAs) induce autophagy in non-transformed retinal epithelial cells (Johansson et al, *Autophagy* 2015). On the other side, transformed epithelial cells with low basal autophagy and reduced ability to induce the process are sensitive to physiologic concentrations of PUFAs (Pettersen et al, *Free Radic Biol Med* 2016). PUFAs also induce oxidative stress and increase autophagy in macrophages from mouse and humans resulting in tolerance for pro-inflammatory stimuli with decreased CXCL10 (IP10) observed both in vitro and in patients. The NRF2-Keap1 axis seems central in these lipid-induced changes in inflammatory signaling in the macrophages.



Confocal images showing that the MyD88 signaling adaptor is recruited to LAMP1 positive phagolysosomes harboring partially degraded *M. avium*, but not to LAMP1 negative compartments harboring dividing M. avium (lower images) in human primary macrophages. Figure: Alexandre Gidon

We also found that autophagy is an important survival mechanism in myeloma cells treated with irreversible proteasome inhibitors (Starheim et al., *Blood Cancer J* 2016). Surprisingly, the survival of myeloma cells in the presence of reversible proteasome inhibitors rather depend on a high level of endogenous antioxidants (Baranowska et al, *Oncotarget* 2016). We have also been involved in testing the bioactivity of novel ATP mimicking tyrosine kinase inhibitors (Kaspersen et al, *Eur J Pharm Sci* 2014; Bugge S et al, *Eur J Med Chem* 2014). During this work we have serendipitously found novel compounds that inhibit the mCSF1R tyrosine kinase activity with high potency and good selectivity and want to exploit these novel compounds in the formation of tumor associated macrophages and metastasis.

3 PhD students have graduated from this theme, 4 more will graduate in 2017.

MAJOR ACHIEVEMENTS IN 2013–2016

- Established the role of Lipocalin 2 in urinary tract infection
- Established the role of Keap1 as a negative regulator of inflammatory signalling in *M. avium* infected primary human macrophages
- Established that TLR7 and 8 recognize mycobacterial and HIV RNA and induce inflammatory responses from macrophage and T-cell intracellular compartments
- Established correlative imaging of infected macrophages at an ultra-high resolution and in 3D using FIB-SEM tomography together with confocal fluorescence microscopy
- Established a method for benzoic acid-inducible gene expression in mycobacteria
- Established that a novel antimycobacterial compound acts as an iron chelator and identified novel proteins involved in iron metabolism in *M. tuberculosis*
- Established dynamics of *M. avium* infection in mice and infection models and routines for working with HIV and Mtb in new BSL3 labs
- Identified intra-patient mutations in *M. avium* and established phenotypic differences between the strains
- Established an assay for protein secretion by the *M. tuberculosis* ESX-3 secretion system (for use in drug screens targeting ESX-3)
- Established the role of lipid metabolism in HIV disease susceptibility and progression
- Established that n-3 polyunsaturated fatty acids protect retinal epithelial cells from harmful stress by inducing autophagy and oxidative stress defences
- Established that the moderate oxidative stress induced by physiologic concentrations of omega-3 fatty acids is toxic to cancer cells with downregulated autophagy
- Established that n-3 polyunsaturated fatty acids in a lipid selective manner dampen LPS induced pro-inflammatory signalling in macrophages
- Established that myeloma cells use different resistance mechanisms towards reversible and irreversible proteasome inhibitors
- Founded a basis for identification of novel ATP-competitors targeting macrophage activation
- Developed an approach to quantify PI3K signaling in tumor sections obtained prior to and after targeted therapy and correlated the changes with effects on tumor metabolism using MR.

AMBITIONS FOR 2017

- Elucidate the spatiotemporal relation of *M. avium* and Mtb trafficking, compartmentalized inflammatory signalling and cell death of host macrophages.
- Elucidate the implications of PRR activation of T-cells in HIV infection and identify HIV restrictive and permissive factors in T-cells by CRISPR screening.
- Elucidate if Keap1 is a negative regulator of inflammation in sepsis.
- Elucidate on mycobacterial iron metabolism and intramacrophage virulence using *M. smegmatis*, *M. avium* and Mtb mutants
- Elucidate a role for the ESX-3 secretion system in survival of mycobacteria and identify small molecule inhibitors of ESX-3 secretion
- Based on exom- and transcriptome sequencing of a breast cancer model, identify factors secreted from the cancer cells that instruct the immune system to aid in aggressive cancer development
- Elucidate how cancer cells secrete compounds that induce autophagy locally and systemically in cancer cachexia
- Unravel the difference in resistance towards reversible and irreversible proteasome inhibitors in myelomatosis
- Evaluate novel ATP mimicking compounds for their CSF1R inhibiting activity in macrophages

Inflammation underlying Preeclampsia and Atherosclerosis

Theme Manager: Professor Ann-Charlotte Iversen

Inflammation plays a key role in cardiovascular disease, a major cause of illness and death worldwide, with gender-specific manifestations. Pregnancy is a natural state of low-grade inflammation and a stress test to the cardiovascular system. Women with preeclampsia have doubled risk for later cardiovascular disease and develop atherosclerosis-like lesions in uterine wall arteries during pregnancy, suggesting shared underlying mechanisms for these vascular diseases. Inflammatory mediators like oxidized lipoproteins and cholesterol crystals are implicated, but with largely unknown molecular action. We hypothesize that inflammatory danger response by pattern recognition receptors (PRRs) is central to preeclampsia pathogenesis and the gender-specificity of cardiovascular disease. In this theme we aim to define PRR-initiated inflammation underlying preeclampsia and determine the relation to later cardiovascular disease.

MAIN ACTIVITIES 2013–2016

The first site of maternal fetal interaction occurs when fetal trophoblasts from the fertilized egg invade the uterine wall in early pregnancy. Maternal spiral arteries are remodeled to wide high-capacity blood vessels and the invading trophoblasts both line the arteries and interact with maternal macrophages in the uterine wall. In preeclampsia, trophoblast invasion and spiral artery remodeling is insufficient and atherotic lesions are formed, narrowing the spiral arteries and reducing blood flow to the growing fetus. In uterine wall arteries, we focus on characterizing the atherotic lesions with foam cells in addition to the trophoblast interaction with maternal macrophages, and these sites are being morphologically defined with focus on cholesterol accumulation and PRR mediated inflammation.

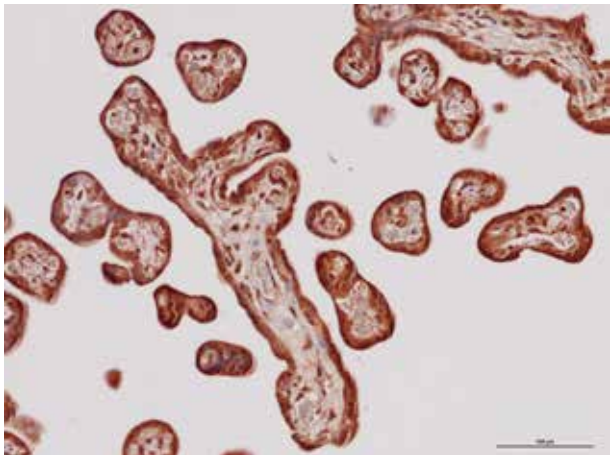
The second maternal fetal interaction site is the trophoblasts layer called the syncytium that covers all fetal tissue in the placenta. In preeclampsia, harmful placental inflammation proceeds the hypertensive disease development in the mother and immune activation in the trophoblast syncytium is crucial. Earlier studies, mostly in trophoblast cell lines, have not pointed to trophoblasts as immune active cells and focus has been set on maternal immune activity for decades. We have revealed that primary first trimester trophoblasts are indeed potent immune cells responding broadly to PRR activation (Tangerås, Iversen et al., *J. Reprod. Immunol*. 2014), while the commonly used trophoblast cell lines are lacking this immune activity (Gierman, Iversen et al., *Placenta* 2015), thus explaining the somewhat misleading literature and underlining the crucial trophoblast role. A central role for PRR-mediated inflammation in the trophoblast syncytium of normal and preeclamptic placentas is currently being confirmed by identification of mechanisms involving inflammasome NLRP3, TLR2, TLR3 and TLR4.

Completion and diagnostic validation of our unique Preeclampsia Biobank has given the basis for an extensive quantitative immunohistochemical analysis comparing placental inflammatory mechanisms in normal and preeclamptic pregnancies. PRR mechanisms identified in patient samples are being functionally assessed by PRR-activation studies in placental cells and explant biopsies.

Metabolomic profiling is a sensitive method for overall assessment of disease state in biofluids and tissues. Preeclampsia, gestational hypertension and fetal growth restriction are heterogenous diseases with variable involvement of placental inflammation and dysfunction, and we lack biomarkers for both prediction and causal disease classification. We have established the methodology for metabolomics profiling of serum, urine (Austdal, Iversen et al., *Plos One* 2014) and placental biopsies (Austdal, Iversen et al., *Placenta* 2015) in pregnant women, and identified novel early predictive biomarkers and insight to the pathogenesis of these diseases (Austdal, Iversen et al., *Int. J. Mol. Sci. 2015*). Metabolomic profiling is being further developed for causal classification of the placental disease component of preeclampsia and fetal growth restriction. This classification will be important for the targeted study of inflammatory mechanisms we are undertaking.

Serum cytokine profiling is another novel tool for assessing the overall inflammatory state of early disease development. We have used cytokine profiling and cluster analysis for identification of differences in inflammatory status before onset of clinical signs in women later developing hypertensive pregnancy disorders (Tangerås, Iversen et al., *ATVB* 2015). Women later developing gestational hypertension showed a distinct inflammatory cytokine pattern compared to women later developing preeclampsia, pointing to separate etiology for these disorders. Cytokine profiling is currently used to elucidate other inflammatory pregnancy disorders.

Novel maternal and fetal preeclampsia risk genes are currently being revealed in the largest meta-analysis of GWAS data in preeclampsia, performed in the EU FP7 project InterPregGen where we participate with a cohort of normal and



Placenta of a preeclamptic pregnancy. Fetal structures in the placenta are sourrounded by the specialized syncytium layer protecting fetal cells from maternal blood. The syncytium in preeclampsia strongly expresses IL-1β as indicated by positive IHC staining (brown). Figure: Guro Stødle

preeclamptic women from the HUNT Study (Morgan, Iversen et al. *Norwegian J. Epidemiol*. 2014). In a separate approach based on the HUNT pregnancy cohort we have identified the first pleiotropic preeclampsia risk gene; the antihypertensive MTHFR gene polymorphism rs17367504-G (Thomsen, Iversen et al., *J. Hypertension* 2016). Furthermore, in families with increased occurrence of preeclampsia, we have identified that other diseases were also heritable, including chronic hypertension, severity of cardiovascular disease, pulmonary disease and fetal growth restriction (Thomsen, Iversen et al., *J. Hypertension* 2015).

Overall, this work has added further evidence to the importance of PRR-mediated inflammation in fetal trophoblasts of the placenta in preeclampsia development, and led to discovery of underlying inflammatory mechanisms, genetic risk factors and novel predictive tools for hypertensive pregnancy disorders. We have published 13 papers within this theme 5A and five PhD students have graduated. The major scientific results are summarized below:

MAJOR ACHIEVEMENTS IN 2013–2016

- Established serum cytokine profiling in early pregnancy as a novel tool for identifying differences in inflammatory status before onset of clinical signs in women later developing hypertensive pregnancy disorders (Tangerås, Iversen et al., *ATVB* 2015).
- Identified that metabolomic profiling of maternal serum in early pregnancy can predict later development of hypertensive pregnancy disorders (Austdal, Iversen et al., *Int. J. Mol. Sci. 2015*).
- Identified that metabolomic profiling of placental tissue is a novel and sensitive tool for characterization of the placental disease in preeclampsia (Austdal, Iversen et al., *Placenta* 2015).
- Discovered broad PRR mediated inflammation by primary trophoblasts, defining an inflammatory role for trophoblasts in placental development in normal pregnancy and in development of preeclampsia (Tangerås, Iversen et al., *J. Reprod. Immunol.* 2014).
- Revealed that several widely-used trophoblast cell lines do not possess the strong inflammatory capacity of primary first trimester trophoblast, shedding new light on the importance of trophoblast PRR-mediated inflammation in pregnancy (Gierman, Iversen et al., *Placenta* 2015).
- Revealed shared inheritable phenotypes between preeclampsia and cardiovascular disease in a Preeclampsia Family Study (Thomsen, Iversen et al., *J. Hypertension* 2015).
- Identified the first maternal genetic risk factors for preeclampsia as shared cardiovascular risk genes (Thomsen, Iversen et al., *J. Hypertension* 2016).

AMBITIONS FOR 2017

- Identify PRR mechanisms and cholesterol accumulation during pregnancy in placental villi, adipose tissue, and in the uterine wall at sites of macrophage and trophoblast interaction and atherotic lesions, relevant for development of preeclampsia.
- Identify shared risk genes and risk traits for subgroups of preeclampsia and cardiovascular disease and mortality.
- Establish novel causal classification of the placental disease in preeclampsia based on metabolomic and transcriptomic profiling.
- Expanded collection of pregnancy- and obesity-related biobanks for translational inflammation studies.
- Establish animal models for spiral artery atherosis and placental inflammation for functional testing of findings from translational analysis of patient samples.

Inflammatory Bowel Disease

Theme Manager:
Professor Arne Kristian Sandvik

Inflammatory bowel disease (IBD) is a major clinical problem, with approximately 2 mill Europeans chronically affected by either ulcerative colitis or Crohn's disease. Current hypotheses on etiology and pathogenesis focus on dysfunctional inflammatory pathways including PRRs and autophagy, with presently 204 susceptibility gene loci identified. Hence, we hypothesize that IBD results from an inappropriate inflammatory response to intestinal microbes and endogenous molecules in genetically susceptible hosts. The main aim of this theme is to understand central mechanisms for mucosal homeostasis, how this is disrupted in active disease and subsequently restored in remission.

MAIN ACTIVITIES 2013–2016

The research group has, as planned, focused on studying IBD disease mechanisms in patient material. A basic resource is the IBD biobank which has been expanded to >700 individuals by late 2016, and includes a wide array of biological samples per individual in addition to detailed disease phenotyping. Hypothesis generation has developed from whole-biopsy transcriptomes, to studying global gene expression in the epithelial monolayer by microdissection and RNASeq. Mechanistic studies have mostly been done in standard cell lines from colorectal cancer, which have shown to be useful although these cells' malignant phenotypes may introduce bias in studies on intercellular signaling. In the search for better models, the group has successfully generated colonic organoids from IBD patients and healthy individuals, which are now being used for mechanistic studies on the IBD inflammatory processes. In late 2016 small intestinal organoids, intended for specific studies on Crohn's disease, are in an advanced stage of development.

A survey of the HUNT population biobank has identified approximately 800 IBD patients, the majority of which are among 58.000 SNP genotyped HUNT participants. The process of genotyping our IBD biobank is ongoing, and eQTL analyses combining genomic variation and the RNASeq derived mucosal transcriptome in a subcohort underway. A group member was awarded a Young Research Talents 4-year grant from RCN for this project.

A number of specific research projects have examined the roles of TLR3, and the CCL20/CCR6 axis in IBD. Both its clinical utility as an IBD biomarker, and the pathobiology of NGAL/LCN2 have been and are still being studied. The relation between the neuroendocrine system and inflammation has been extensively studied in human material, animal models and *ex vivo* systems, in particular changes in mucosal serotonin turnover and fibrosis which is highly relevant for Crohn's disease.

Other projects have emerged during the last year, with members of the group working on specific aspects of the interaction between microbes and the epithelial layer of the small and large intestine in IBD; and on how the intestinal epithelial cells interact with γ-δ lymphocytes. Our broad research activity has created international interest, and positioned us to establish formal collaboration with outstanding research groups both in the USA and Europe. A dataset with microbiome data from IBD patients was generated in collaboration with Professor Underhill at Cedars-Sinai in Los Angeles and is now being analysed. In early 2017 a researcher from the group will visit the Department of Immunobiology at Yale School of Medicine for a collaborative project, and a postdoc position for IBD research shared between NTNU and Yale has been advertised.

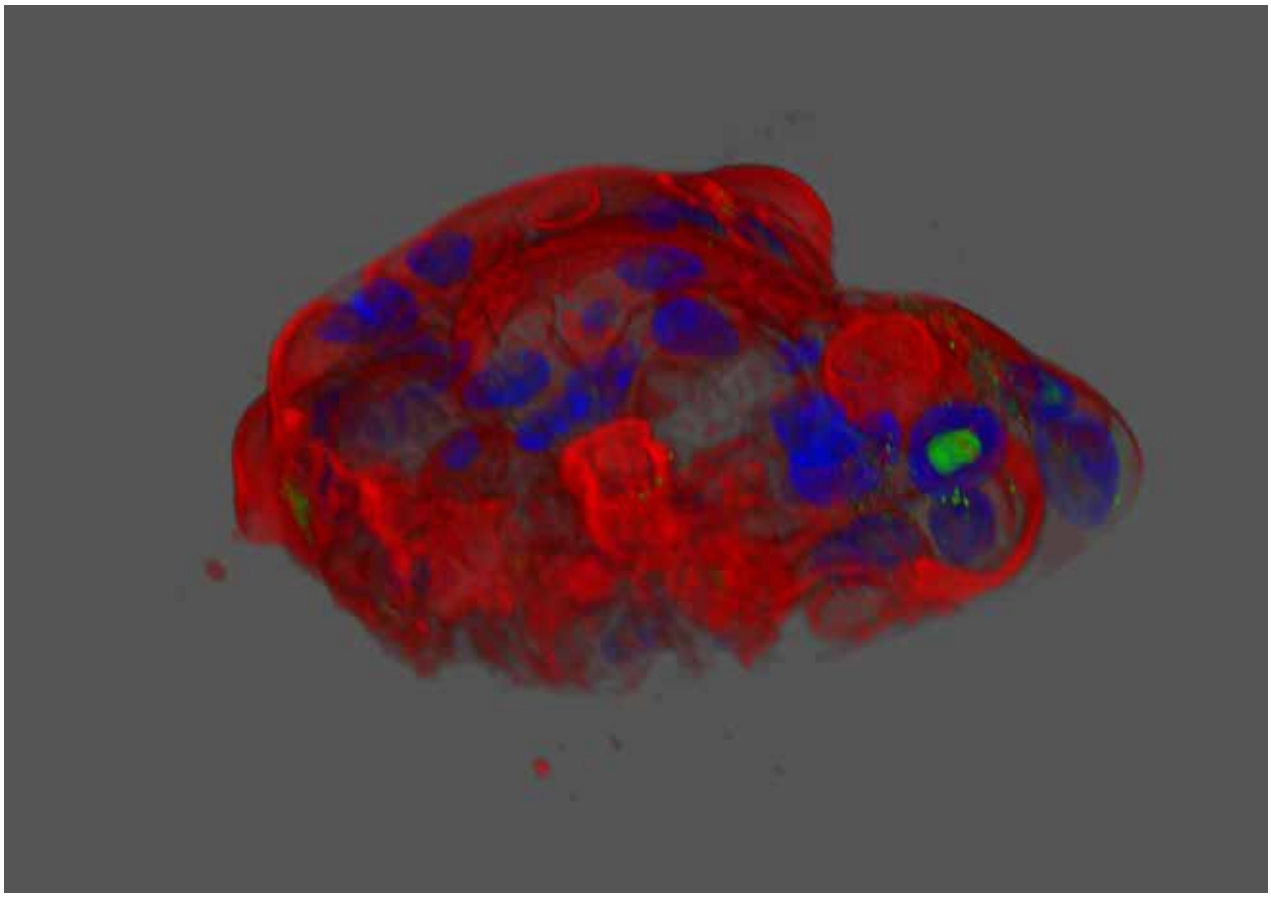
The IBD research group had one PhD defense in 2013 and one in 2014, and has published a total of 12 research papers in the period 2013-2016.

MAJOR ACHIEVEMENTS IN 2013–2016

- Established one of the largest and best controlled IBD biobanks in the world, and created a link to the HUNT population study/biobank for future epidemiological and genetic studies on IBD.
- Developed methods for RNASeq analysis of microdissected intestinal epithelium, and created colonic organoids for studies of disease mechanisms in IBD.
- Identified TLR3 mediated inflammatory responses through e.g. CXCL10, complement factor B and NGAL/LCN2 as disease mechanisms in IBD. Østvik AE et al., *Inflammatory Bowel Diseases 2013, J Clin Exp Immunol 2013, Inflammatory Bowel Diseases 2014.* Skovdahl et al., *PloS One 2015*
- Established NGAL/LCN2 as a promising biomarker for IBD in clinical practice. Thorsvik S et al., *J Gastroenterol Hepatol 2016*

AMBITIONS FOR 2017

- Establish permanent cultures of small intestinal epithelial organoids from healthy and IBD endoscopic biopsies, and start mechanistic studies on these related to hypotheses generated from the transcriptome and protein network analyses
- Finish SNP genotyping of our IBD cohort, and merge this with the HUNT population biobank genotyping results, including the IBD subpopulation in HUNT. Utilize the results together with existing transcriptome data, in an eQTL analysis
- Penetrate the biological role of NGAL beyond its anti-bacterial effect, especially with respect to proliferation and repair.
- Further activate the collaboration with Immunobiology/ Yale, through one mutual postdoc and one visiting researcher from CEMIR, to maximally utilize our biobank material and the institutions' complementary skills.

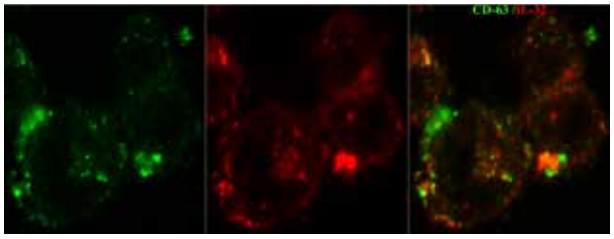


Immunofluorescence staining of a differentiated patient derived colonoid showing nuclei (blue), F-actin (red) and a few proliferating cells (Ki67;green.) A volumetric visualization of a Z-stack consisting of 29 images, using Leica SP8 STED 3X confocal microscope and Leica Las X 3D software Figure: Ingunn Bakke and Bjørnar Sporsheim

Bone Destruction caused by Cancer and Inflammation

Theme Manager:
Professor Therese Standal

Cancer and inflammatory diseases can induce changes in normal bone homeostasis resulting in pain and increased fracture risk. Destruction of bone is common in cancers like multiple myeloma and breast- and prostate cancer metastasizing to bone, in inflammatory diseases such as inflammatory bowel disorder and in certain autoimmune diseases such as rheumatoid arthritis (RA). The main aim of this theme is to reveal underlying mechanisms for bone loss associated with cancer and inflammation.



Multiple myeloma cells stained for IL-32 (red) and CD63 (green). Figure: Muhammad Zahoor

MAIN ACTIVITIES IN 2013–2016

During these years we have studied different aspects of the effect of inflammation, inflammatory signals and cancer cell-derived factors on bone-degrading osteoclasts, bone-forming osteoblasts and their precursors. We studied how caspase-8 downstream of TLR-TRIF- signaling modulates the expression of pro- and anti-inflammatory cytokines from human bone marrow-derived mesenchymal stromal cells (Moen et al *Immun Inflamm Dis. 2016)*. We also addressed the importance of GDF15, a member of the TGFβ superfamily of cytokines, for the bone disease of multiple myeloma. GDF15 was elevated in patients with osteolytic bone disease compared with patients without bone disease, and we showed that GDF15 promotes osteoclast differentiation and at the same time inhibits osteoblast differentiation (Westhrin et al *Haematologica 2015)*. We identified inflammation-related genes relevant for myeloma bone destruction by screening bone marrow RNA isolated from myeloma patients with and without bone disease using Nanostring technology. We are currently studying in more detail some of these factors, in particular the role of certain adipokines and IL-32. We have identified IL-32 as a novel cytokine produced by malignant plasma cells in response to hypoxia, and demonstrated that recombinant IL-32 can promote osteoclast differentiation both *in vitro* and *in vivo*. We also found that IL-32 is secreted on extracellular vesicles (EV), and that EVs obtained from myeloma cells expressing IL-32 promote osteoclast differentiation *in vitro* and *in vivo* (Zahoor et al, manuscript in preparation). We are now investigating how IL-32 is recruited to the vesicles, and which intracellular signals are regulating the expression and release of IL-32 from myeloma cells. We have generated several IL-32 knock out cell lines using Crispr/CAS9, which we will be useful to further explore the role of IL-32 in multiple myeloma disease progression. We established a humanized mouse model for multiple myeloma her in Trondheim and have performed pre-clinical trials using the model. The first results from these studies will be published in 2017. During the time period the theme leader Therese Standal worked as a visiting scientist at St. Vincent's Institute in Melbourne, Australia, on a project aiming to understand the role of gp130 in osteocytes for bone formation (Standal et al *J Endocrinol. 2014,* Johnson, Standal et al *J Bone Miner Res. 2014*). One of the PhD students was in Prof. Anton Marten's laboratory in Utrecht, the Netherlands, to learn the novel mouse model of multiple myeloma. One post doctor stayed in Prof. Franscesco Dazzi's laboratory at King's College to study how mesenchymal stem cells influence immune cells. In total three PhD theses were completed.

MAJOR ACHIEVEMENTS IN 2013–2016

- Established a humanized mouse model for multiple myeloma in Trondheim
- Performed the first pre-clinical drug testing in the human-mouse hybrid model
- Demonstrated that caspase-8 downstream of TLR-TRIF may modulate bone marrow stromal cells into gaining a pro-inflammatory phenotype (Moen et al *Immun Inflamm Dis. 2016*).
- Demonstrated that GDF15 might play a role in myeloma bone disease (Westhrin et al, *Haematologica 2015*).
- Identified IL-32 on extracellular vesicles obtained from myeloma cells and that the vesicles potently stimulate osteoclast differentiation in an IL-32-dependent manner.

AMBITIONS FOR 2017

- To continue our studies on the effect of inflammatory signals and hypoxic/ER-stress on mesenchymal stromal cell function, osteoblast - and osteoclast differentiation.
- To identify and characterize endogenous PRR ligands in bone marrow samples obtained from myeloma patients.
- To continue our studies on the role of IL-32 in multiple myeloma disease progression and mechanisms for IL-32 induction and secretion.
- To complete our study on the effect of AAV-BMP4 gene therapy for multiple myeloma in mice

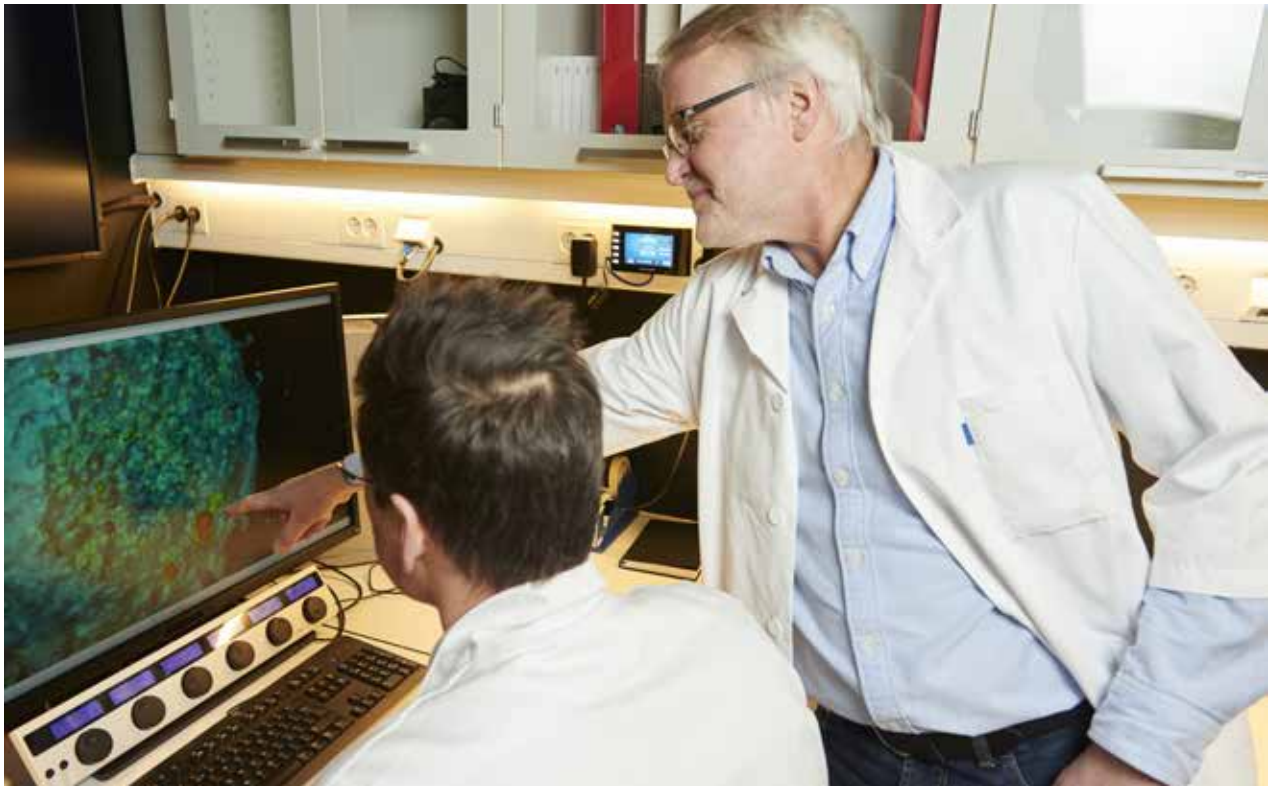
CEMIR RESEARCH GROUPS

The Inflammation Research Group

The strategy of the Inflammation Research Group is to study the cellular and molecular mechanisms that inflammasomes, Toll-like receptors (TLRs) and the complement system are using to mount sterile and non-sterile inflammatory responses. The group has a long track record and has made several significant contributions within innate immunity and host defence over the last 25 years. Currently, we have a focus on mechanisms involved in trafficking of TLRs and their adaptor molecules between intracellular compartments where TLR signalling is taking place. This project aims to increase our understanding of how Gram-negative - and Gram-positive bacteria are able to induce inflammatory signalling from different cellular compartments, and identify new targets for treatment of severe inflammatory conditions. Moreover, we work on inflammatory responses that occur during the development of atherosclerosis. The aim of this project is to identify the detailed molecular mechanisms of how cholesterol crystals activate the complement system and immune cells. The goal is to design effective therapeutic agents to diagnose and treat atherosclerosis.

The Inflammation Research Group has a strong interest in applying and developing molecular and cellular imaging techniques for use in the CEMIR projects. The group leader is also scientific leader for the Imaging Core Facility at NTNU (http://www.ntnu.edu/dmf/cmic). This core facility has recently acquired the most recent state of the art 3X STED super-resolution laser confocal microscope with a PicoQuant single molecule detection upgrade, and a TIRF microscope. Both these instruments have been installed in the new CEMIR laboratories.

The research group is led by professor Terje Espevik and currently consists of 23 persons including 6 PhD students, 7 post docs, 6 research scientists and 4 staff engineers. Within CEMIR we collaborate with the Mycobacteria/HIV group and CEMIR adjunct prof. H Stenmark on trafficking and compartmentalized PRR sensing of bacterial infections, the Systems inflammation group on CRISPR screens and knockouts, and the Inflammatory bowel disease group on the role of TLRs and complement in IBD. The group closely collaborates with CEMIR adjunct profs. E Lien, K Fitzgerald and H Stenmark on TLR signalling, trafficking and inflammasome activation, and with the Pregnancy group and CEMIR adjunct profs. E Latz and TE Mollnes on cholesterol crystal induced inflammation in cardiovascular disease. The latter theme includes national partners from Oslo University Hospital (B Halvorsen, P Aukrust and A Yndestad) and international partners (C Kemper, NIH, P Garred, University of Copenhagen, and J Lambris, University of Pennsylvania). We are also actively collaborating with G Teti, University of Messina, on the role of TLR8 in Streptococcal infections, and M McCaffrey, University of Cork, on Rab11-FIP2 in phagocytosis of Gram-negative bacteria.

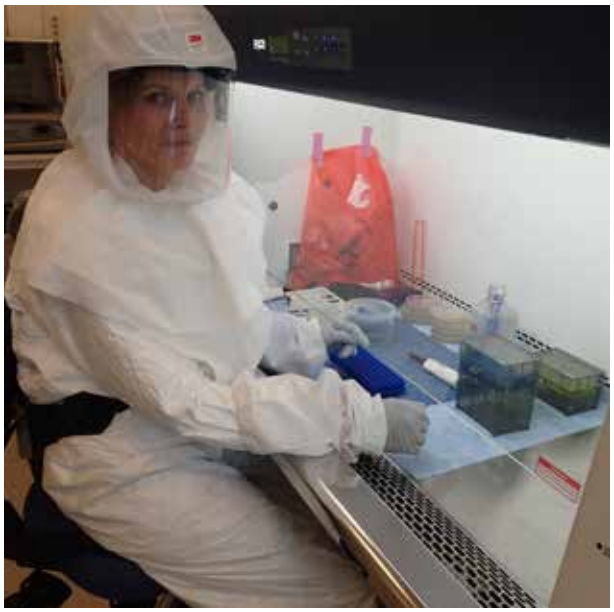


The Research Group on Molecular Mechanisms of Mycobacterial and HIV Infections

Mycobacteria and HIV can cause life-long infections and pose a global health challenge. Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), kills about 1.8 million people each year and the prevalence of non-tuberculous mycobacterial infections caused by *M. avium* is increasing in individuals who are immunocompromised due to underlying disease or use of immunosuppressant drugs. Mycobacterial infections require long treatment with antibiotics, and drug resistant strains are emerging.

Our primary research focus is the molecular host defence mechanisms involved in immunity to mycobacterial pathogens and virulence strategies employed by mycobacteria to parasitize host cells. Intracellular trafficking, compartmentalized pattern recognition receptor signalling and nutrient metabolism are central for survival and attractive targets for drug development, and are currently investigated in our lab both in the host and in the pathogen.

There has been an increase in TB following the HIV epidemic: HIV increases the risk for active TB and one third of HIV deaths are from TB. We are studying innate properties of the T-cell responses to HIV and mycobacteria. T-cells express PRRs and respond to microbial ligands with cytokine production. The significance of this in HIV disease is currently not understood and something we are interested in. In collaboration with the Systems Inflammation group we also do CRISPR-screens to reveal host factors central for HIV defence and virulence. We believe our basic research strategy may contribute to revealing new therapeutic targets and adjunct host-directed therapies, as well as in vaccine development.



The research group is led by professor Trude H. Flo and includes two senior research scientists, five post docs, four PhD students, two medical research students, two staff engineers and master students. We have developed expertise, methods and tools to study HIV, mycobacteria and the host innate and adaptive immune defences both in vitro in human primary cells and in vivo in mice. We have strains of Mtb, *M. avium* and *M. smegmatis* available with fluorescence and firefly luciferase, and we have a confocal microscope in our BSL-3 facility for live imaging of Mtb and HIV infections. Transposon mutant libraries with more than 150 000 mutants in *M. smegmatis*, *M. avium* and Mtb are available.

We are mainly focused on CEMIR theme 4 but collaborate closely with the Systems inflammation group on CRISPR screens of HIV-infected T-cells, the Autophagy group on regulation of inflammatory signalling and autophagy, the Inflammatory bowel disease group on lipocalin 2 in IBD, and the Inflammation group and CEMIR adjunct profs. H Stenmark and D Underhill on compartmentalized signalling in bacterial infections.

Nationally, K Tasken (NCMM UiO) and M Trøseid (OUS) are collaborators on inflammation in HIV. We use Focused Ion-Beam Scanning Electron Microscopy at NTNU Nanolab to perform high resolution and correlative imaging of Mtb and HIV infected cells in 3D, and collaborate with P Sikorski (NTNU), P Peters (Maastricht University), M Niederweis (University of Alabama) and M Lerm (University of Linkøping). Other international collaborators are E Rubin (Harvard School of Public Health), W Bitter (VU University Medical Center) and R Strong (Institut Pasteur) on mycobacterial secretion systems, and N Reiling (Research Center Borstel) on mycobacterial phagosome isolation.

The Autophagy and Oxidative Stress Defence Group

Autophagy is a principal catabolic route needed for the lysosomal degradation of damaged or excessive intracellular components. The autophagy activity is tightly regulated to match the need of the cell, tissue and organism and is crucial for cells and organisms to survive starvation and cell damage. Since we identified the molecular mechanisms for selective autophagy, the focus has been on how this self-degradation system is regulated by external and internal signals in normal physiology and how this may change in disease and in response to treatment.

We have studied the interplay between the oxidative stress defence and autophagy, particularly in relation to responses towards n-3 poly-unsaturated fatty acids in normal and transformed epithelial cells as well as in isolated macrophages and in patients. Lately we have also studied how the autophagy and oxidative stress defence is used as survival and resistance pathways in myeloma cells in response to proteasome inhibitors used in the clinic. Furthermore, we investigate how autophagy is regulated in a growing tumour as a starvation response locally and systemically to induce tissue wasting in cancer patients. The communication between cancer cells and normal cells recruited into the tumour is further studied using a mouse model of breast cancer. Here we particularly aim to identify proteins secreted from the cancer cells that contribute to cancer cell survival and tumour progression in nutrient and oxygen poor conditions.

The group is led by professor Geir Bjørkøy and consists of two post-doctor fellows, three PhD students, one and a half staff engineers, master and bachelor students. The group has established several cellular models of both normal and cancerous cells and uses complementary methods including mRNA and protein analyses, imaging, flow cytometry and metabolic analyses. In addition, we have implemented next generation sequencing and gene editing to decode how autophagy and oxidative stress defence may be controlled normally and deregulated in aggressive cancers in mouse models. The group collaborates with other groups at CEMIR on regulation of inflammatory signalling and autophagy (Mycobacteria/HIV group, Inflammation group) and autophagy in multiple myeloma (Bone disease group). We are also involved in several collaborative projects on autophagy in cancer at NTNU/St Olavs hospital (S Mostue, A Bofin, L Thommesen, S Kaasa, E Sundby) and other national institutions (T Johansen, UiT, PE Lønning and A Molven, UiB, R Skotheim, OUS). International collaborators include the groups of Dr. Carsten Jacobi (Novartis, Basel) on cancer cachexia, K. Kaarniranta (Univ of Kuopio) on protein aggregates in neurodegeneration, and David Hume (Univ of Edinburgh) and Peter ten Dijke (Leiden University) on macrophages and aberrant signalling in cancer metastasis.

The Research Group of Inflammation in Pregnancy

In preeclampsia extensive atherotic lesions develop in the uterine wall arteries and these closely resemble atherosclerotic lesions, but a causal role in preeclampsia has not yet been established. The Research Group of Inflammation in Pregnancy holds a unique collection of decidual tissues containing atherotic lesions and focus on revealing the inflammatory processes in atherosis and how this influence placental development and eventually preeclampsia. Lessons are learned from the central role of cholesterol crystals and PRR activation in atherosclerosis development and CVD. In addition the central role of the fetal throphoblasts in harmful placental inflammation in preeclampsia is focus for molecular inflammation studies.

The research group is led by professor Ann-Charlotte Iversen. In 2016 the group counted 10 persons; one post doc, three PhD students, three MD PhD students, one Master student, and one staff engineer. We work closely with other researchers at CEMIR and the core facility CMIC, and are particularly linked to themes focusing on the molecular studies of lipids and cholesterol crystals and activation of inflammasomes, TLR2 and TLR4 (Inflammation group). Several pregnancy-based biobanks and an obesity biobank are collected and administered by the research group providing unique materials for the molecular inflammation analyses.

The broad research approach involving molecular studies, biobanking, metabolomics, epidemiology and genetics is made possible by a strong collaboration between clinical departments and basic researchers in different disciplines both nationally and internationally. Central collaborators include professors L Bjørge at Haukeland University Hospital, G Acharya at the University Hospital of Northern Norway, E Vanky and B Kulseng at St Olavs Hospital, and T Bathen at NTNU. Professor C Hedrick at La Jolla Institute for Allergy and Immunology in San Diego hosted Ann-Charlotte Iversen as Visiting Scientist in 2014-2015 for study of mice models of atherosclerosis and pregnancy complications. The Research Group is partner in a large 12-partner EU 7FP project InterPregGen coordinated by professor L Morgan at University of Nottingham, unravelling genetic risk factors for preeclampsia in relation to cardiovascular risk traits, in the world's largest pregnancy based cohort collaboration for genetic studies, and the research group at CEMIR is involved with a pregnancy cohort from the HUNT Study and responsible for functional placental risk gene analysis.

The Inflammatory Bowel Disease Research Group

The inflammatory bowel diseases (IBD) research group studies disease mechanisms in IBD, with patients and clinical biobanks as central resources. The ultimate aim is to use knowledge of IBD pathobiology to improve diagnostics and prognostics, and for optimization of treatment and drug discovery. The IBD projects concentrate on understanding how mucosal homeostasis is disrupted in IBD. Hypotheses are generated by e.g. transcriptome analysis of biobank material from precisely characterized IBD patients; and tested in a wide array of animal and in vitro models. When relevant, projects are done in close collaboration with other CEMIR groups working with e.g. innate immune mechanisms (Inflammation group and Mycobacteria group), mucosal repair and proliferation (Regeneration group), and genome editing of elements of the inflammatory pathways (Systems inflammation group).

The IBD group is led by professor and gastroenterologist Arne Sandvik and is closely connected with clinical medicine, also through combined university/hospital positions. The group moreover collaborates with clinicians in 7 different hospitals in the Central Norway Health Region, and regional hospital staff is involved in translational research projects. The group is cross-disciplinary, and includes cell biologists and molecular biologists in addition to clinicians. One of the two IBD scientists is also the scientific head of the faculty Genomics Core Facility (high-throughput genomics and transcriptomics), and is experienced within transcriptome analysis and bioinformatics. The group has access to excellent animal experimental facilities, and is among few in the world doing routine colonoscopy on rat and mouse IBD models.

An international network has been established with formal collaborative agreements, and includes Immunobiology at Yale University, CT USA (profs. NW Palm and RA Flavell) on microbiota and epithelial pathobiology, Cedars-Sinai Hospital, LA USA (D Underhill) on fungal microbiota, and Institute of Health Research (FISABIO), Valencia Spain, (JC Andreu-Ballester) on γ–δ lymphocytes in IBD.

The Bone Disease Group

Loss of bone is a common feature of different inflammatory diseases as well as for cancers metastasizing to or located within bone. Multiple myeloma is a cancer of plasma cells, located within the bone marrow. The bone disease of multiple myeloma is highly aggressive, and is the cause of pain and reduced quality of life for the myeloma patients. Hypoxic and ER stress and a low grade, chronic inflammation characterizes the myeloma bone marrow. Our research is centered on identifying inflammatory factors present in the bone marrow microenvironment that influence differentiation or activation of bone cells. The underlying hypothesis is that the causes of bone loss associated with inflammatory diseases and cancer might be common.

The Bone Disease Group is lead by professor Therese Standal and currently consists of three post doctors, two PhD students and one technician. We profit from a close collaboration with clinicians and researchers at the multiple myeloma group headed by A Sundan at NTNU. In close collaboration with the Hematology Department at St. Olavs Hospital and the Regional Biobank we have access to well characterized samples from myeloma patients. We also benefit from collaboration with the Nordic Myeloma Study Group, in particular with Niels Abildgaard at Odense University Hospital in Denmark. In collaboration with Anton Martens at the VU University Medical Center, Amsterdam, we have established a mouse model for multiple myeloma here in Trondheim. This model allows for a reconstruction of a human hematopoietic environment in scaffolds that are subsequently implanted in immune compromised mice. This model has given us new opportunities in terms of in vivo experiments. The acquirement of a uCT machine at the animal facility as well as a collaborative effort together with the osteoporosis group at NTNU (headed by professor Unni Syversen) to establish a bone histomorphometry laboratory has further strengthen our opportunities in terms of bone quality assessments. Other central collaborators are F Dazzi (King's College London) on the effect of mesenchymal stem cells on immune cells and F Bosch (Universitat Autonoma, Barcelona) on BMP-4 gene therapy for multiple myeloma in mice.

The Research Group on Cellular and Molecular Mechanisms in Regeneration

Inflammation or infection causes various degrees of damage to tissue that impairs functionality of affected organs. Upon damage, appropriate wound healing and regenerative responses are of the utmost importance to regain organ function and prevent chronic inflammation. Our research group is interested in both the cellular and molecular mechanisms that trigger and execute regenerative processes. Commonly, there is interplay between various cell types, each giving and receiving cues that together orchestrate an optimal response. In addition, there are biomechanical cues such as tissue stiffness that can modulate these responses. Our group combines (bio)-chemical and cell biological tools with in vivo mouse and ex vivo organoid model systems to study these processes.

Our group currently has two related research programs. The first research program studies the control of intestinal epithelial cell proliferation and differentiation during regeneration and tumorigenesis. This program focuses on the molecular control of stem cells in the intestine, which are the cells that fuel the cellular component of repair, but are also the initiating cells for cancer. This involves the study of several signaling pathways such as Hippo and Wnt. The second program studies the role of non-histone lysine methylation in various cellular responses. Lysine methylation has been mainly studied in its role as a histone modification to control gene expression. However, an emerging research field now also studies lysine methylation in its role as a modifier of cellular signaling.

The group joined CEMIR in March 2016, is led by Menno Oudhoff and further consists of 2 Postdocs, 1 PhD student, 1 staff engineer, and 1 MSc student. We are actively collaborating within CEMIR on intestinal organoids and inflammatory bowel diseases (IBD research group) and internationally with C Arrowsmith (University of Toronto, Canada) on epigenetic probe library and inhibitors; M Altelaar (Utrecht University, Holland) on mass spectrometry; T Sato (Keio University, Tokyo) on human organoids; C Zaph (Monash University, Melbourne) on Hippo/Wnt signaling in the gut; G Perona-Wright (University of Glasgow, UK) on helminth infection models and F Rossi (UBC, Vancouver, Canada) on stem cell signalling.

The Systems Inflammation Research Group

Infectious diseases and inflammatory disorders are major contributors to the global burden of disease, thereby having a huge socio-economic impact. Decades of research have unravelled the arsenal of mechanisms by which the host immune system detects an invading microbe and elicit an innate immune response ensuring clearance of the pathogen while exerting a minimal damage to the host. Any failure in this chain could lead to chronic diseases such as atherosclerosis; as well as devastating conditions like sepsis and sepsis-induced death. It is critical for the host to restore homeostasis and resolve the inflammation upon microbial infections through a collective and meticulous coordination of a number of controlled molecular events such as chromatin remodelling, transcription, translation and post-translational modifications (PTMs) in addition to metabolic reprogramming. The systems inflammation research group aims to specifically study the role of two major PTMs – phosphorylation and ubiquitinome; and metabolic reprogramming; in antiviral signaling and inflammation using state-of-the-art systems-level approaches using mass spectrometry-based proteomics and metabolomics. Additionally, we employ CRISPR/Cas9-based genome-wide/targeted genetic screens to identify key regulators; upon viral infection such as HIV or Influenza and other inflammatory stimuli. We believe that our basic research-focused systems-level approaches would yield deeper and broader understanding of inflammatory signaling which will have enormous translational potential.

The research group led by Richard K. Kandasamy currently includes 1 PhD student, 1 post-doc and 1 staff engineer. We work in close collaboration with CEMIR research groups on genome editing of elements of inflammatory pathways (Inflammation group and IBD group), CRISPR screens of HIV-infected T-cells (Mycobacteria/HIV group). Other collaborators at NTNU are P Bruheim (Department of Biotechnology), G Slupphaug (NTNU Proteomics Core) and M Anthonsen (Dep. of Laboratory Medicine, Children's and Women's Health) Our international collaborators include G Superti-Furga (Center for Molecular Medicine, Vienna, Austria), R Linding (University of Copenhagen, Copenhagen, Denmark), K Prasad (YU-IOB Center for Systems Biology and Molecular Medicine, Yenepoya University, Mangalore, India), M-S Kim (Kyung Hee University, Seoul, South Korea) and A Pandey (Johns Hopkins University, Baltimore, USA). We are also partnering a H2020 MCSA-ITN application led by R Lang and F Alexander (University hospital Erlangen, Germany) on dual-specific phosphatases.

RESEARCH COLLABORATION ACROSS RESEARCH GROUPS IN THE CENTRE

– scientific results of collaborations in the centre so far and collaborative projects

There is a wide range of collaborations across research groups at CEMIR that involve specific research projects as well as methods and model systems.

Systems-, Inflammation-, IBD-, Autophagy- and Mycobacteria groups: The Systems research group has introduced the CRISPR/Cas9 knockout technology in the centre and is currently collaborating on gene editing with the Inflammation - (knock out of TRAM and complement factor C5 in human macrophages) and the Mycobacteria groups (knockout screens on HIV-infected T-cells for discovery of genes restricting or facilitating viral growth). In addition, the Systems group are providing essential knowledge and tools in proteomics, genomics and bioinformatics used by the above-mentioned research groups. Papers in preparation.

Inflammation- and Mycobacteria groups are collaborating on the role of TLR7/8 in sensing bacterial infection *(S. aureus and Mycobacteria)*. Papers: Awuh et al, *PNAS* 2015; Bergstrøm et al., *J Immunol.* 2015.

IBD-, Inflammation- and Mycobacteria groups are collaborating on the involvement of TLR3, complement activation, and lipocalin 2 in inflammatory bowel disease. Papers: Østvik et al, *Inflamm Bowel Dis.* 2014; Østvik et al, *Clin Exp Immunol.* 2013; Granlund et al, *Plos One* 2013, Østvik et al., *Inflamm Bowel Dis.* 2013; Thorsvik et al, *J Gastroenterol Hepatol.* 2017

Inflammation- and Pregnancy groups are collaborating on the involvement of cholesterol crystals as inducers of inflammation in pre-eclampsia. No papers yet.

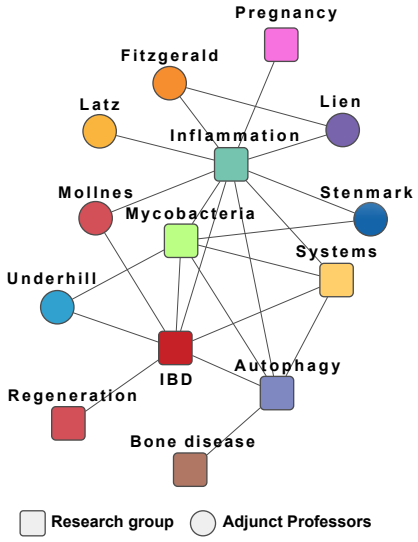
Mycobacteria-, Inflammation- and Autophagy groups are working together on regulation of inflammatory signalling and autophagy by n-3 polyunsaturated fatty acids. Papers: Awuh et al, *PNAS* 2015; Mildenberger et al, manuscript in review for *Autophagy*.

Authophagy- and Bone Disease groups are collaborating on the role of autophagy and NFR2 in the resistance towards proteasome inhibitors used in the clinic to treat multiple myeloma. Papers: Starheim et al, *Blood Cancer Journal* 2016; Baranowska et al. *Oncotarget* 2016.

Regeneration- and IBD groups are collaborating on studies using human organoids. No papers yet.

RESEARCH COLLABORATIONS WITH CEMIR'S ADJUNCT PROFESSORS:

Harald Stenmark is collaborating with the Inflammation group on the role of Rab11-FIP2 and TRAM in regulating host responses against bacteria, with the Mycobacteria group on mycobacterial phagosome escape, and the Authophagy group on FYCO1 tools and cytokine secretion. A post doc from CEMIR has spent 1 year in the Stenmark lab. No papers yet.



Tom Eirik Mollnes collaborates with the Inflammation- and IBD groups on the role of complement and CD14 in host responses against bacteria; activation of complement system by cholesterol crystals in relation to atherosclerosis; and the involvement of complement activation in IBD. 21 co-publications since 2013. Espevik has been a co-supervisor for two PhD candidates in the Mollnes lab. One patent application.

Egil Lien and Kate Fitzgerald have collaborations with the Inflammation group on the role of the adaptor molecules TRAM and TRIF in TLR2 signaling; mechanisms and consequences of NLRP3 activation; and role of lncRNAs in regulating inflammatory responses. One post doc and a PhD student from CEMIR have spent more than 1.5 years in their labs. Papers: Nilsen NJ., et al, *J Biol Chem.* 2015; Sandanger et al., *Cardiovasc Res.* 2013.

Eicke Latz collaborates with the Inflammation group on mechanisms behind cholesterol crystals induced inflammation, and strategies to inhibit these responses; and the role of extracellular ASC specks in propagating inflammation. A postdoc from CEMIR has spent close to one year in the Latz lab on a collaborative project (cyclodextrin). Papers: Zimmer S. et al., *Sci Transl Med.* 2016; Niyonzima et al., *J Immunol.* 2015; Franklin et al., Nat Immunol. 2014; Samstad et al., *J Immunol.* 2014.

David Underhill collaborates with the Mycobacteria group on host responses to mycobacteria killed by antibiotics with different modes of action. Co-supervisor for Signe Åsberg who has spent six months in the Underhill lab. No papers yet.

INTERNATIONAL COLLABORATION

An academic exchange agreement has been established between CEMIR and the ImmunoSensation, Bonn Cluster of Excellence, University of Bonn, Germany (http://www.immunosensation.de/home.html). The field of collaboration includes any programs offered at either institution that is desirable and reasonable for the development of cooperation between the two centres, and includes all CEMIR groups.

The CEMIR groups have extensive international collaboration that has led to important scientific results and co-authorships:

INFLAMMATION GROUP:

Claudia Kemper, NIH, USA. Intracellular complement factor 5 and control of inflammasome-activation in macrophages. One CEMIR post doc in the Kemper lab.

Peter Garred, University of Copenhagen. Mechanisms of cholesterol crystal-induced complement activation (lectin pathway). Pilely et al, *J Immunol*. 2016, Niyonzima et al, *Mol Immunol.* 2016; Hovland et al., *Atherosclerosis* 2015; Lappegård et al., *Mol Immuno*. 2014.

Guiseppe Teti, University of Messina. The role of TLR8 in Group B Streptococci infections and responses. Two PhD students from the Teti lab has worked for 1 year each at CEMIR. Co-author manuscript in preparation.

Mary McCaffrey, University of Cork. The role of Rab11-FIP2 in transporting TRAM to the phagposome containing Gram negative bacteria. Co-author manuscript in preparation.

John Lambris, University of Pennsylvania. Inhibition of complement activation at the C3 level. Gustavsen et al., *J Infect Dis.* 2016; Samstad et al., *J Immunol.2014*; Rokstad et al., *Biomaterials* 2013.

Cornelis Terhorst, Harvard Medical School, Role of CD150 in macrophage killing of bacteria. Co-manuscript submitted.

MYCOBACTERIA GROUP:

Eric Rubin, Harvard School of Public Health on mycobacterial secretion systems and in general on methods, reagents and tools for research on mycobacteria. Two CEMIR scientists have had research stays in the Rubin lab. Dragset et al., *PLoS ONE* 2015; Dragset et al, *Antimicrob Agents Chemother.* 2015; Siegrist et al, *MBio* 2014.

The Mycobacteria group (Steigedal) is a partner in the EU project **Joint Programming Initiative on Antimicrobial Resistance**. The focus is on new intervention strategies for tuberculosis: blocking multiple essential targets.

Norbert Reiling, Research Center Borstel on isolation and proteomic characterization of mycobacterial phagosomes.

Michael Niederweis, University of Alabama, Peter Peters, Maastricht University and Maria Lerm, Linkøping University are collaborators on mechanisms of *M tuberculosis*-induced cell death.

Thomas Hawn, Univ Washington on the role of Lipocalin 2 in urinary tract infection, and in general on the role of TLRs in divers infectious diseases of global importance. Steigedal et al., *J Immunol*. 2014.

IBD GROUP:

Noah W Palm and Richard A Flavell, Department of Immunobiology, Yale University School of Medicine, USA. Shared postdoc Yale/NTNU is being advertised. Additional scheduled 4-6 mo stay at Yale by IBD/CEMIR researcher first in early 2017 with subsequent visits as appropriate. Project agreement within microbiota and epithelial pathobiology.

Juan Carlos Andreu-Ballester, Fundación para el Fomento de la Investigación Sanitaria y Biomedica de la Comunidad Valenciana (FISABIO), Valencia, Spain. Collaborative project on γ-δ lymphocytes in IBD ongoing. Planned stay in Valencia by CEMIR postdoc during 2017. Provisionally accepted review article on γ-δ lymphocytes in IBD is under revision.

SYSTEMS GROUP:

Giulio Superti-Furga, Center for Molecular Medicine, Vienna, Austria. Application of interaction proteomics and CRISPR/Cas9-based genetic screens to find host factors of viruses.

Keshava Prasad, Institute of Bioinformatics, Bangalore, India; Center for Systems Biology, Yenepoya University, Mangalore, India; and Akhilesh Pandey, Johns Hopkins University, Baltimore, USA. Mass spectrometry-based identification of novel genes and splice variants in inflammatory and antiviral signaling.

Min-Sik Kim, Kyung Hee University, Seoul, South Korea; Rune Linding, University of Copenhagen, Denmark; and Akhilesh Pandey, Johns Hopkins University. Decoding the phosphorylation dynamics and kinome networks underlying Toll-like receptor signaling.

AUTOPHAGY GROUP:

Dr. Carsten Jacobi, Novartis, Basel. Work together on cancer cachexia in human and mouse models.

K. Kaarniranta, University of Kuopio. Collaborate on protein aggregates in neurodegeneration/AMD. Johansson et al, *Autophagy 2015*.

Peter ten Dijke, Leiden University. The role of GREM1/SMAD signaling in metastasis.

David Hume, Univ of Edinburgh. – The role of CSF1R macrophages in metastasis.

BONE DISEASE GROUP:

Anton Martens and Richard Groen, University Medical Center, Amsterdam: collaboration on the human-mouse hybrid myeloma model.

Fatima Bosch, Center of Animal Biotechnology and Gene Therapy, Universitat Autònoma Barcelona: collaboration on BMP-4 gene therapy for multiple myeloma in mice (provides AAV-vectors).

Niels Abildgaard, Odense University Hospital: collaboration on access to patient material from multiple myeloma.

Franscesco Dazzi, King's College, London: collaboration on studies of the effect of mesenchymal stem cells on immune cells (one post doctor stayed two months at King's college in 2016).

REGENERATION GROUP:

Cheryl Arrowsmith, Uni. Of Toronto, Canada. Collaboration on supply and potential modification of epigenetic probe library and inhibitors. Oudhoff et al. *Dev Cell* 2016

Maarten Altelaar, University Utrecht, The Netherlands. Collaboration on measurements of protein methylation and interactions by MS.

Toshiro Sato, Keio University, Tokyo, Japan. Collaboration on human organoid models. Oudhoff et al. *Dev Cell* 2016.

Colby Zaph, Monash University, Melbourne, Australia. SETD7 and Hippo/Yap and Wnt signalling in the gut. Oudhoff et al. *Dev Cell & PLoS Pathogens* 2016.

Georgia Perona-Wright, University of Glasgow, UK. Helmint infection models (*H. polygyrus bakeri).* Oudhoff et al. *PLoS Pathogens* 2016.

Fabio Rossi, University of British Columbia, Vancouver, Canada, The role of SETD7 in muscle stem cells. Manuscript under review for *Cell Stem Cell*

PRE-ECLAMPSIA GROUP:

Eric Moses, University of Western Australia. Collaboration on Genetics and bioinformatics in pre-eclapsia. Løset et al, *Pregnancy Hypertension* 2014

Linda Morgan, University of Nottingham, and Ralph McGinnis, Sanger Institute. Collaboration on genetics in pre-eclampsia and cardiovascular disease. McGinnis, Iversen et al, Submitted to *Nature Genetics.*

Successful International Conference on **Molecular Mechanisms of Inflammation**

May 30th – June 2nd, 2016, Trondheim, Norway

From May 30th – June 2nd , 2016, CEMIR organized an international conference on molecular mechanisms of inflammation in Trondheim. With a particular emphasis on the regulation of inflammation in sterile and infectious diseases, the conference brought together experts from basic and clinical inflammation research and provided significant insight into common underlying processes of inflammatory disorders that can be translated to clinical settings.

The presentations covered cutting edge highlights on cellular trafficking mechanisms and compartmentalized signaling, systems inflammation, novel mechanisms of innate immunity recognition and prevention, mechanism of acute and chronic inflammation, and metabolic reprogramming in inflammatory responses.

The conference attracted 200 participants from all over the world and was a fantastic success making CEMIR visible for the international scientific community in this important field of research.

INVITED CONFERENCE SPEAKERS WERE:

Alan Aderem
Julie Blander
Petr Broz
Terje Espevik
Kate Fitzgerald
Richard Flavell
Trude Helen Flo
Douglas Golenbock
Göran Hansson
Paul Herzog
Harald Husebye
Jonathan Kagan
Richard Kandasamy
Claudia Kemper
Egil Lien
Tom Eirik Mollnes
Kim Newton
Luke O'Neill
Alexander Poltorak
Felix Randow
Alan Sher
Harald Stenmark
Lynda Stuart
David Underhill
Stephanie Vogel

← CEMIR director Terje Espevik opened the conference May 30th, 2016

→ The conference venue was Kunnskapssenteret, NTNU/ St.Olavs Hospital, Trondheim

RESEARCHER TRAINING AND EDUCATIONAL ACTIVITIES

PHD COURSES

CEMIR organizes three PhD courses yearly. The courses are part of NTNU's PhD programme in Molecular Medicine and open for PhD students nationally and internationally that are interested in molecular inflammation:

"Receptor Signaling and Trafficking" is an advanced course that describes the most commonly used methods for studying receptor signaling and discusses cell signaling downstream of the most important receptor classes. CEMIR affiliated Professors Harald Stenmark and Kate Fitzgerald are responsible for this course.

"Molecular Mechanism of Inflammation" gives an overview of mechanisms and signaling pathways involved in inflammatory processes, mainly connected to activities at CEMIR. This is inflammation related to infections, but also sterile inflammation. CEMIR affiliated Professors Egil Lien and Tom Eirik Mollnes are responsible for this course.

"Advanced Cellular Imaging Techniques" focuses on light microscopy and electron microscopy and students will learn about molecular imaging techniques with a focus on imaging innate immune cell activation. The course will provide the theoretical background for diverse imaging techniques that can be applied to study innate immune activation. CEMIR affiliated Professors Eicke Latz and David Underhill are responsible for this course.

MASTER OF SCIENCE IN MOLECULAR MEDICINE

NTNU offers an International Master of Science (MSc) programme in Molecular Medicine. The purpose of the MSc programme is to develop knowledge and skills in cellular and molecular biology.

CEMIR offers master's thesis projects for the MSc in Molecular Medicine and researchers affiliated with CEMIR also contribute with lectures and seminars.

MEDICAL STUDENT RESEARCH PROGRAMME

The Medical Student Research Programme (MSRP) is a national research education and grant scheme for medical students who wish to carry out research. These students are interested in medical research and willing and motivated to do research besides their regular studies. CEMIR offers supervision for MSRP students doing course work and thesis.

CEMIR PhD DISSERTATIONS 2013–2016 (2 MEN, 15 WOMEN)

2013

ATLE VAN BEELEN GRANLUND April 11

Colonic mucosal gene expression in inflammatory bowel disease – From whole genome to REG gene family expression

JANE ATESOH AWUH April 22

Autophagy as a survival mechanism or a cause of disease

MARTE SINGSÅS DRAGSET Dec 11

Riding the Ferrous Wheel: Study and Identification of Genes Involved in Mycobacterial Iron Acquisition

2014

SIV HELEN MOEN Jan 29

Influence of inflammation and cancer on mesenchymal stem cell function

MARI LØSET Feb 7

Genetic Predisposition to Pre-eclampsia -Genetic Association Studies on Population-Based Cohorts and Transcriptional Studies on Decidua Basalis Tissue

ANN ELISABETH ØSTVIK April 24

Innate immune responses in colonic mucosa during inflammatory bowel disease – effects of TLR3 signaling

BERIT H. GRANDAUNET June 19

Bone loss in rheumatoid arthritis: Possible roles for hepatocyte growth factor, syndecan-1 and dickkopf-1

2015

EIVIND OTTERSEN SAMSTAD Feb 6

Molecular Mechanisms of NLRP3 Inflammasome activation by Crystalline Material

LIV CECILIE V. THOMSEN March 13

Preeclampsia: Specific genetic risk factors and shared predisposition with cardiovascular disease

NATHALIE NIYONZIMA April 10

Role of the complement system in inflammatory responses to cholesterol crystals

MARITA WESTHRIN June 18

Beauty and the Beast - The Multifaceted Potential of Mesenchymal Stem Cells in Bone Health and Disease

KRISTIN M. STRAND June 22

Markers of placental insufficiency: etiology and the risk of cerebral palsy – Population-based studies of preeclampsia, low birth weight, and abnormal placental weight

MARIE AUSTDAL Sep 21

Biomarkers for prediction and characterization of preeclampsia using magnetic resonance metabolomics

LINE TANGERÅS Oct 16

Toll-like receptors and inflammation in pregnancy

IDA JOHANSSON Dec 17

A dual role of autophagy in disease prevention and drug resistance

2016

KRISTINE PETTERSEN March 11

Autophagy as a survival mechanism or a cause of disease

MARIT BUGGE April 12

Toll-Like Receptor 3 Signaling in Intestinal Epithelial Cells

LABORATORY FACILITIES

The CEMIR laboratories are located in the Knowledge Centre and the Gastro Centre at the Øya Campus of St.Olavs Hospital/NTNU. New laboratories opened in the Knowledge Centre in June 2014, and in October 2015 we opened a new Biosafety Level Three (BSL-3) laboratory, offering the highest level of security for research on viruses and bacteria in Norway. CEMIR hosts first-class laboratories with state-of-the-art equipment for performing research on cells, tissues and microorganisms:

- high resolution STED confocal microscope
- total internal reflection fluorescence (TIRF) microscope
- live cell- and spinning disk confocal microscopes
- image flow cytometer
- cell sorter
- a confocal microscope installed in a biosafety level 3 facility

The BSL-3 lab contains an advanced Leica SP8 confocal microscope making it possible to study infections in immune cells with viable *Mycobacterium tuberculosis* and HIV virus.

CEMIR personnel wearing protective equipment while working in the BSL-3 laboratory.

Leica SP8 Confocal microscope in the BSL3-laboratory

Zeiss TIRF microscope

General laboratory

CEMIR-USE OF THE IMAGING CORE FACILITY

Researchers and students at CEMIR have access to a multitude of different imaging techniques, for live cell studies as well as imaging of fixed cells and tissue preparations. These instruments are all part of the Cellular and Molecular Imaging Core Facility, CMIC, at Faculty of Medicine, NTNU, http://www.ntnu.edu/mh/cmic. The CEMIR director is the scientific leader for this core facility. CMIC is closely integrated with the CEMIR's laboratories and its research groups, and CEMIR scientists are the biggest user group of CMIC. The core facility offers several services: standard confocal microscopy and live cell imaging, fully automated high-throughput image acquisition, image visualization and image processing, immune histochemistry and electron microscopy.

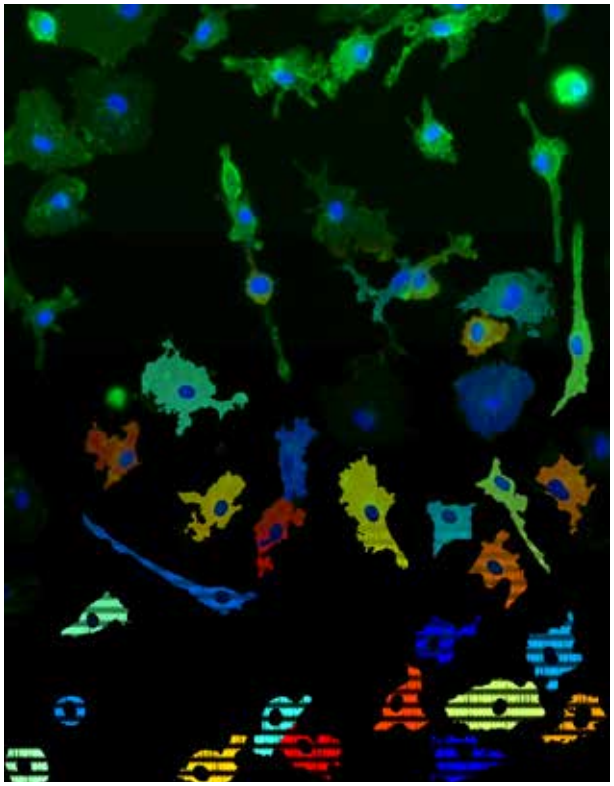
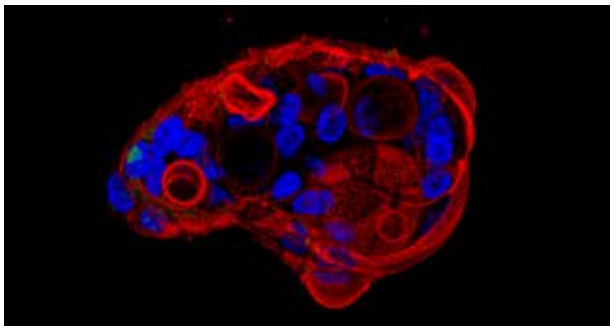
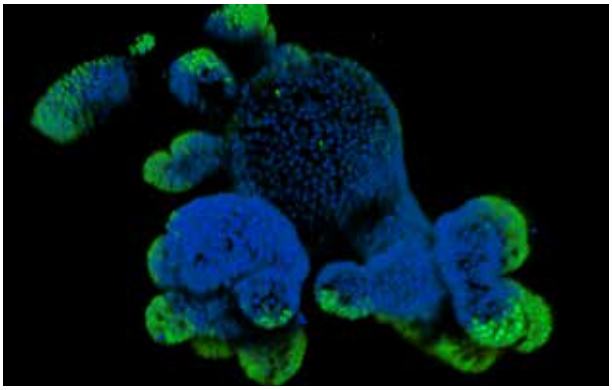


Illustration of the process of high content image analysis from a microscopy image, via recognition algorithms to pure numeric values. Scan^R analysis software, CellProfiler and GNU R has been used. Figure: Jenny Ostrop and Korbinian Bösl

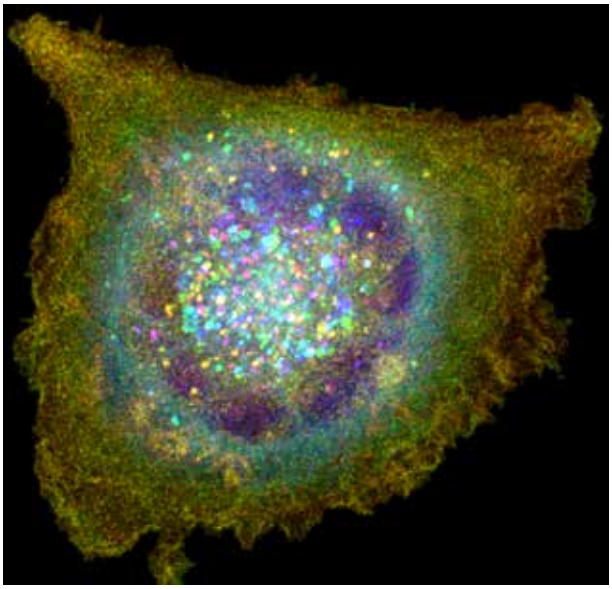


Immunofluorescence staining of a differentiated patient derived colonoid showing nuclei (blue), F-actin (red) and a few proliferating cells (Ki67; green). The image is a volumetric visualization of a Z-stack consisting of 29 images, acquired on a Leica SP8 confocal microscope. Figure: Ingunn Bakke and Bjørnar Sporsheim

In 2014 a super resolution light microscope and a total internal reflection fluorescence microscope were installed at CMIC and placed in the CEMIR laboratories. In 2016 CMIC purchased a PicoQuant single molecule detection (SMD) upgrade for our Leica SP8 STED 3X super-resolution microscope. This add-on is in particular useful for studying molecular interactions in cells. In addition, CEMIR researchers are using Focused-Ion Beam Scanning Electron Microscopy (FIB-SEM) in collaboration with the NTNU Nanolab. A new 3-D serial block face scanning electron microscope has also recently been added to the instrument park.



3D visualization of nuclei (blue) and wild type LSD1 (green) expressed in mouse organoid from the first part of the small intestine. Acquired on a Zeiss LSM 510 META and visualized using Amira. Figure: Rosalie Zwiggelaar and Bjørnar Sporsheim



Confocal microscopy of a live U373 cell expressing TRL2 mNeon Green. The image is a depth coded maximum projection consisting of around 25 optical slices, acquired on a Leica SP8 confocal microscope. Figure: Kai Beckwith

COLLABORATION WITH CLINICAL DEPARTMENTS

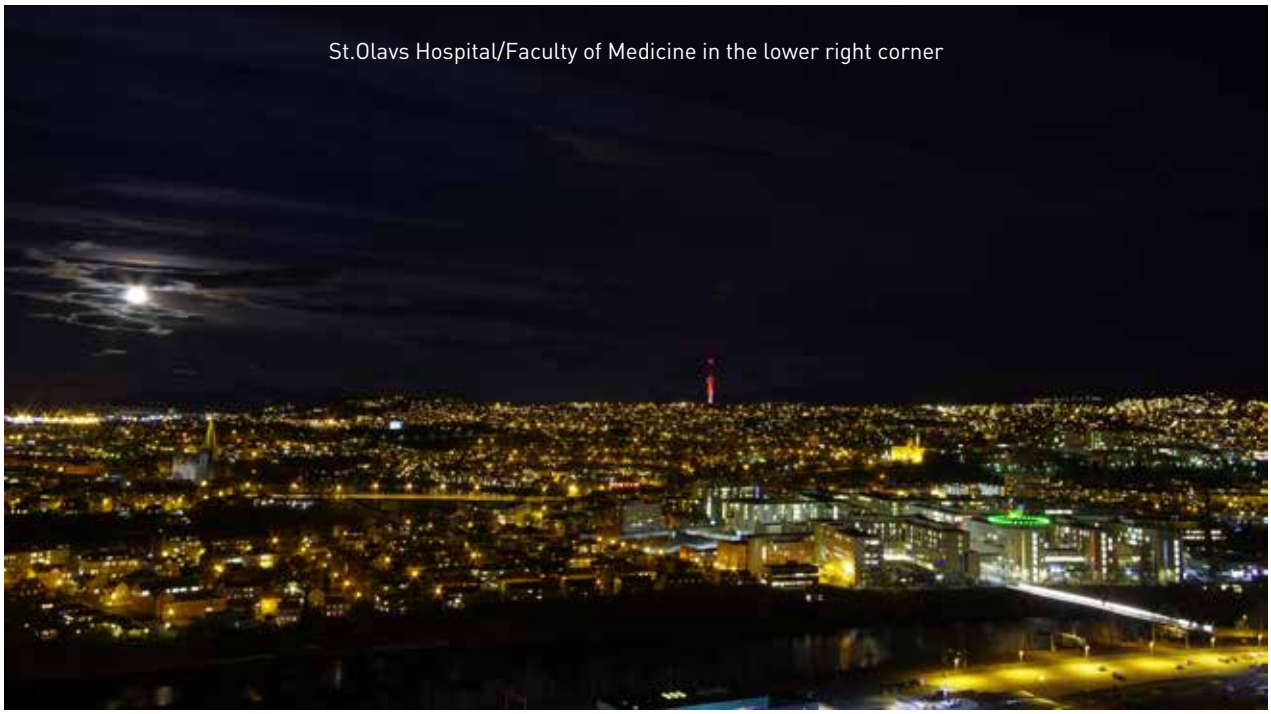
Chronic inflammatory processes play an important role in the pathophysiological process in diseases such as atherosclerosis, diabetes, rheumatoid arthritis (RA), inflammatory bowel disease (IBD) and various neurological disorders. During the last years several inflammatory mediators have been identified as novel treatment targets in disorders such as in RA and IBD, and medications blocking or modulating these targets have been very successful. The vision of CEMIR is to lay the foundation for identifying new therapeutic targets and in developing new diagnostic tools for inflammatory diseases through research in molecular innate immune responses.

A close collaboration with the clinical departments is crucial for addressing our goal. CEMIR benefits from a close integration between NTNU and St.Olav's Hospital and the location of both institutions at Øya Campus. Several of our staff members are employed both in the clinic and the university. This close integration between CEMIR and St.Olav's Hospital has also been important in building up several biobanks with clinical specimens from patients with diseases such as coronary artery disease (CAD), IBD, preeclampsia and multiple myeloma.

Several CEMIR papers from 2013-2016 includes analyses using biobank samples. These studies have demonstrated the clinical relevance of results generated in more experimental systems. Many of these patients were also previously included in the HUNT cohort which during 2015 was fully SNP genotyped with genome variability data from around 60.000 individuals. Several studies investigating genetic polymorphisms and plasma levels of cytokines as biomarkers for disease have been performed or are ongoing, hoping to identify novel diagnostic and prognostic tools for chronic inflammatory disorders such as CAD and IBD.

In 2016 we have published a placebo-controlled, randomized double-blinded study in patients with acute coronary syndrome. In this study we explored the effect of toculizumab, a blocking monoclonal anti-IL-6 receptor antibody, on inflammatory processes related to atherosclerosis and myocardial damage. The study was performed in collaboration with the Institute for Internal Medicine, University in Oslo and the Departments of Cardiology at St. Olav's Hospital and Oslo University Hospital (OUS) Rikshospitalet. This study is the first to demonstrate that IL-6 inhibition is safe and beneficial in CAD patients.

Among other clinically oriented projects, extensive studies have been done on biomarkers for IBD. In particular, fecal neutrophil gelatinase-associated lipocalin (NGAL) has performed well as a marker for inflammation in patients with ulcerative colitis and Crohn's disease. Studies uniting clinical and basal groups in CEMIR will further examine the basal mechanisms behind NGAL regulation, and explore the potential of this antimicrobial protein to modulate mucosal inflammation. This type of studies, reaching from bedside to laboratory with real-life material from diseased individuals, is done only in a few places world-wide. This shows that the interaction between CEMIR and the clinical departments is active, leading to novel findings with clinical potential.



St.Olavs Hospital/Faculty of Medicine in the lower right corner

CEMIR OUTREACH ACTIVITY 2013-2016

At CEMIR we aim to make the public aware of and understand our research on inflammation, and how our research can contribute to the development of new treatments and diagnostic tools. We are involved in many outreach activities.

NORWEGIAN ANNUAL SCIENCE WEEK

Researchers from CEMIR organized a **"TEDDY BEAR HOSPITAL"** together with NTNUs medical students where they did surgery and vaccinated teddy bears. Forskningstorget 2014 and 2016.

RESEARCHER'S FRIDAY 2016, Byscenen September 30th: New technology – a better life? Bjørnar Sporsheim talked about how advanced microscopes is used to look into living cells.

RESEARCHER'S NIGHT 2015, Byscenen September 25th: Fornuft og mageføelelser. Trude Helen Flo participated in talk-show.

FORSKER GRAND PRIX 2014. Byscenen December 20th: PhD candidate Marianne Beckwith participated in the science communication competition.

CEMIR IS ALSO VISIBLE AT:

- Blogging: #NTNUmedicine blog
- Exhibition at The Medical Museum at NTNU/St.Olavs Hospital
- The CEMIR website: www.ntnu.edu/cemir
- Prestasjonsklyngen (prof. Trude Helen Flo was invited to give a talk at Prestasjonsklyngen in May 2014)
- Kunnskapsbyen /DKNVS, Vitenskapsmuseet Trondheim
  (Prof. Trude Helen Flo talked on Inflammation in 2013 and Vaccination in2016)
- Guided tours in CEMIR's lab for invited guests or prominent visitors to NTNU

MEDIA HIGHLIGHTS

ABC nyheter13.04.16, NRK 14.04.16 og Wall Street Journal 06.04.16: Ny behandling av åreforkalkning med sukkerstoff/Drug Rare Disorder Linked to Reducing Cholesterol Plaque.

NRK Norgesglasset 30.05.16, Forskning.no 31.05.16, Gemini 30.05.16: Banebrytende om svangerskapsforgiftning (new findings on eclampsia)

Adresseavisen August 10th, 2015: fant protein som hindrer immunforsvaret å løpe løpsk.

Adresseavisen March 13th, 2013: Krigercellene gir ny viten.

Adresseavisen November 12th, 2014: Prøver å finne «farlige» graviditeter.

TV

CEMIR researchers are visible at various TV shows and educational programs like Kunnskapskanalen, Newton and Schrödinger's Katt.

Picture: CEMIR researchers talked about the new CEMIR BSL3-lab on the TV show Schrödingers Katt in March 2016.

VISIT FROM MINISTER OF HEALTH AND CARE SERVICES BENT HØIE

FEBRUARY 21ST, 2016

Tour in the CEMIR lab with NTNU rector Gunnar Bovim and Cemir Professor Trude Helen Flo. The researchers discussed the importance of high quality research to combat antibiotic resistance.

CEMIR INTERNATIONAL CONFERENCE ON MOLECULAR INFLAMMATION

MAY 30TH – JUNE 2ND, 2016

CEMIR hosted this successful international conference in 2016, with 200 delegates from 20 different countries.

BY NTNUMEDICINE | JUNE 8, 2016 - 15:34 ↓ Jump to Comments

Successful international conference on molecular inflammation in Trondheim

May 30th – June 2nd CEMIR organized an international conference on mechanisms of molecular inflammation. The venue was Kunnskapssenteret at NTNU/ St. Olavs Hospital.

CEMIR director Terje Espevik opened the conference. Photo: Ann-Charlotte Iversen

200 delegates from 20 different countries participated and made this conference an important arena to foster further innovative research on molecular mechanisms and regulation of inflammation. The conference brought together scientists from basic and clinical research and provided significant insight into common underlying processes of inflammatory disorders that can be translated to clinical settings.

INNOVATION AND PATENTS

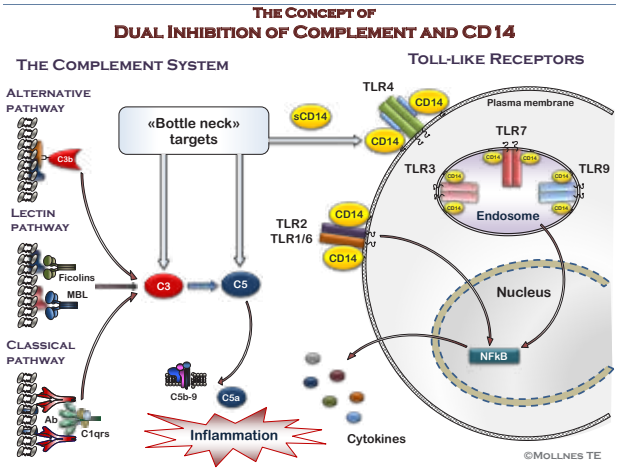
THE CONCEPT OF DOUBLE-BLOCKADE OF COMPLEMENT AND CD14 TO ATTENUATE INFLAMMATION.

Microbial as well as sterile inflammation is initiated by pattern recognition. This is the initial and most upstream event for the inflammatory response, which subsequently leads to activation of a broad inflammatory network with release of innumerable of mediators. Using specific complement inhibitors of the central components C3 and C5 we observed that certain branches of inflammation was substantially inhibited, including granulocyte activation with surface receptor up-regulation and oxidative burst, whereas other mediators including a number of cytokines were less complement dependent. CD14 is a co-receptor for several of the Toll-like receptor molecules, in particular TLR4 and TLR2 and thus could be another key target for inhibition. Using specific antibodies to block CD14 we documented a marked reduction in a broad panel of cytokines and monocyte-mediated responses, differential from the complement-dependent responses.

Based on these observations we combined complement inhibitors (C3 or C5) with anti-CD14 and found these to be crucial "bottle-neck" molecules which virtually abolished the whole inflammatory response when inhibited in combination. This was shown for both exogenous danger signals like Gram-negative and Gram-positive bacteria *in vitro* (human) and *in vivo* (pigs and baboons), for polymicrobial sepsis in mice and pigs, and for endogenous danger like meconium, which is sterile and induces a serious inflammation in newborns. In a whole genome array we documented that 70% of all Gram-negative bacterial induced genes (a total of >2000) were reversed by an average of >80% signal by combined inhibition of C3 and CD14. Thus, blocking of two "bottle-neck" molecules (C3 or C5 of complement) and CD14, at the very first step of danger recognition might be a potent therapeutic strategy to attenuate undesired inflammation occurring in a number of pathophysiological states leading to different disease conditions. Eritoran (E5564) is a specific inhibitor of the TLR4-MD4 complex. Although promising results were initially observed in human sepsis, the study was closed in phase III due to lack of improved survival. Importantly, we recently found that anti-CD14 was more efficient in inhibiting bacterial-induced inflammation than eritoran, in particular for the monocytes, and that the combined inhibition of CD14 and complement was substantially more efficient than eritoran, supporting the broad-acting role for CD14 and complement in the innate immune response.

The principle of double-blockage of complement and CD14 to attenuate inflammation was proposed, and has been driven by one of the CEMIR researchers (TE Mollnes). Moreover, CEMIR researchers at NTNU (T Espevik et al) have developed the anti-CD14 antibody 18D11 that is effective in the combined treatment and currently is under production as a recombinant humanized antibody for therapeutic use. Three patents have been posted related to this scientific project. A formal collaboration contract has been made with a company producing a C5 inhibitor for clinical use. The vision is to test this principle in clinical therapeutic settings in collaboration with Inven2 (the TTO at University of Oslo), and NTNU Technology Transfer AS. The project with the CEMIR affiliated professor Mollnes received grants from the BIOTEK program from The Research Council of Norway for the period 2015–2017 counting 7,063,000 NOK.

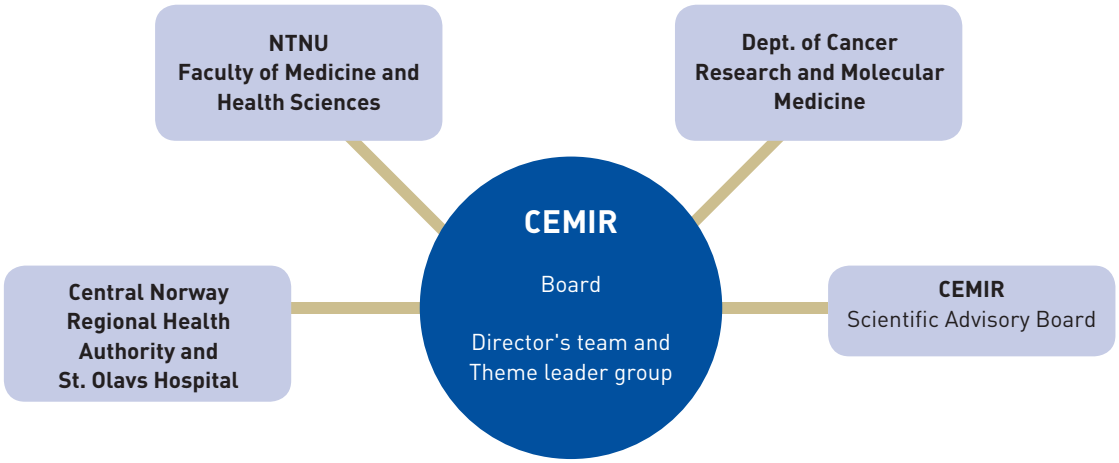
The figure below illustrates the principle of the dual blockade and the main hypothesis of the research in the Mollnes research group.



The "double-blockade" of bottleneck recognition molecules at innate immune recognition. An upstream approach for inhibition of inflammation achieved by targeting the key complement molecules C3 or C5, and the CD14 molecule of the TLR family is proposed. Activation through all initial complement pathways converge at C3 and C5, and blocking of the bottle neck molecule C5 inhibits formation of the potent anaphylatoxin C5a, which is a main contributor in the pathogenesis of a number of disease conditions. Since CD14 serves as co-receptor for several of the TLRs, including the important TLR4 and TLR2, it might be regarded as a bottleneck molecule in the TLR family. Combined inhibition of these molecules will reduce the downstream inflammatory response substantially.

ABOUT CEMIR

ORGANISATION CHART



CEMIR is closely connected to the host department, Department of Cancer Research and Molecular Medicine at the Faculty of Medicine and Health Science, NTNU. Agreement documents regulate the cooperation with our partners. The Centre management reports to the CEMIR board.

From the start in 2013 CEMIR had two main partners that contribute by performing research activity and providing financing: Sør-Trøndelag University College (HiST) and The Central Norway Regional Health Authority/St.Olavs Hospital. From January 2016 NTNU and HiST merged, and the research group from HiST became an internal NTNU collaborator. The fruitful collaboration continues after the merge and the new Faculty of Technology (Former HiST) continues to be represented in the CEMIR Board.

The Centre also has 6 international researchers employed as adjunct professors.

The Centre activities integrate various research themes and unite research groups across disciplines to break new grounds in inflammation research.

THE CEMIR BOARD

The authority of the board is to ensure that the intensions and terms of contract described in the contract are fulfilled within the time frameworks. The board oversee that cooperation proceeds smoothly between the Centre, the host institution and the consortium participants.

The board has members from NTNU and St.Olavs Hospital:

Prof. Bjørn Gustafsson
Dean, Faculty of Medicine, NTNU

Prof. Magne Børset
Head of Dep. of Cancer Research and Molecular Medicine, NTNU

Prof. Terje Meisler
Dean, Faculty of Technology, NTNU

Prof. Petter Aadahl
Research director, St. Olavs Hospital

Prof. Anne Borg
Dean, Faculty of Natural Sciences and Technology, NTNU

CEMIR SCIENTIFIC ADVISORY BOARD (SAB)

SAB members give valuable input to our Centre by reviewing the scientific progress of the Centre and giving guidance to future research directions. SAB-meetings has been held in Trondheim in September 2014 and May 2016. SAB had discussions with the CEMIR management and researchers during their visits, and gave constructive and useful feedback to the CEMIR management. During their visits all members of CEMIR's Scientific Advisory Board held presentations at the annual CEMIR seminar on inflammation and at the CEMIR international conference May 30-June 2 2016.

SAB has five members:
Professor Douglas Golenbock
University of Massachusetts Medical School

Professor Alan Aderem
Seattle Biomedical Research Institute

Professor Göran Hansson
Karolinska Institutet

Professor Stefanie Vogel,
University of Maryland medical Center

Professor Lynda Stuart,
B & M Gates Foundation

An extract from the summary of SAB's latest report (June 2016):
Encouraging, very good job and excellent progress in general. Responsive to ALL of the 2014 comments. Great trajectory, on the rise and moving in the right direction.

Their goal should be a world leading center for basic innate immunity and consider some, but a limited and focused, applications to human biology. The comparators on the global scale are UMass and to a lesser extent Yale. In this regard they are one of the few dedicated centers that has a strong focus on innate. They have a critical mass that can be developed further.

SAB members Douglas Golenbock, Alan Aderem, Göran Hansson and Stefanie Vogel outside CEMIR, Kunnskapssenteret, NTNU. Photo: K. Håland

GUEST LECTURES ORGANIZED BY CEMIR

CEMIR invites a number of guest lecturers every year. These lectures are open for all and is a great opportunity for the centre members as well as other researchers at Faculty of Medicine and Health Science and St. Olav's Hospital to get scientific insight from excellent researchers from other universities.

In the period 2013-2016 CEMIR had the pleasure of hosting 24 guest lecturers:

Edward Miao, Department of Microbiology and Immunology at University of North Carolina at Chapel Hill: *Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis.*

Bente Halvorsen, Oslo University Hospital: *"Atherosclerosis - from mice to men"*

Bruce Beutler, University of Texas: *"Real time identification of mutations that cause phenotype"*.

Felix Randow from the MRC laboratory of molecular biology in UK: *"Autophagy in host-pathogen interactions"*

Joerg Köhl, University of Lübeck: «Cross-talk between complement and IgG Fc receptors in autoimmunity».

Guttorm Haraldsen, University of Oslo: *"Notch signalling in vascular quiescence and inflammation»*,

Claudia Kemper, Kings College London: *"New tricks for an Old Dog: unexpected roles for complement in basic cellular processes"*,

Sanjay Ram, University of Massachusetts: *"Harnessing the sialylation machinery of Neisseria gonorrhoeae to design novel immunotherapeutics against multidrug-resistant gonorrhea"*.

Paul Kaufman, University of Massachusetts: "Two subjects: Higher-order genome interactions at the human nucleolus; new approaches to combatting fungal pathogens"

Alan Aderem, Seattle Biomedical Research Institute, USA: *"A Systems approach to dissecting Immunity"*

Terje Johansen, University of Tromsø: "Molecular Basis for Selective Autophagy"

Göran Hansson, Karolinska Institute, Sweden: *"Immune-metabolic crosstalk - role in atherosclerosis"*

Stefanie Vogel, Univ. of Maryland Medical Center, USA: *"Novel host-oriented treatments of viral respiratory infections"*

Roland Brosch, Pasteur Institute Paris: *"The ESX-1 secretion system of mycobacteria and its impact on virulence and immune responses"*

Douglas Golenbock, Univ. of Massachusetts Medical School, USA: *"Innate Immunity in Malaria"*

Bjørn Naume, Oslo University Hospital: "*Tumor cell dissemination in early breast cancer. Detection and potential clinical use of occult tumor cell detection"*

Tone Tønjum, Oslo University Hospital: *"Genome repair in Neisseria menigitidis"*

Andrei Medvedev, University of Connecticut Health Center, USA*: "TLR Signaling and Tolerance: Molecular Regulation"*

Giuseppe Teti, University of Messina, Italy: *Caspase-1-independent mechanisms of interleuchin-1 beta production during group B strep infection*

Tom Eirik Mollnes, Oslo University Hospital: *"Bride and groom in systemic inflammation – the bells ring for complement and Toll in cooperation"*

Eicke Latz, University of Bonn: "*Western diet induced reprogramming of bone marrow stem cells"*

Andrea Wolf, Cedars-Sinai Hospital, Los Angeles, USA: *"Innate immune sensing of bacterial peptidoglycan by hexokinase"*

Harald Stenmark, Oslo University Hospital: *"Regulation of membrane dynamics and signalling by ESCRT proteins"*

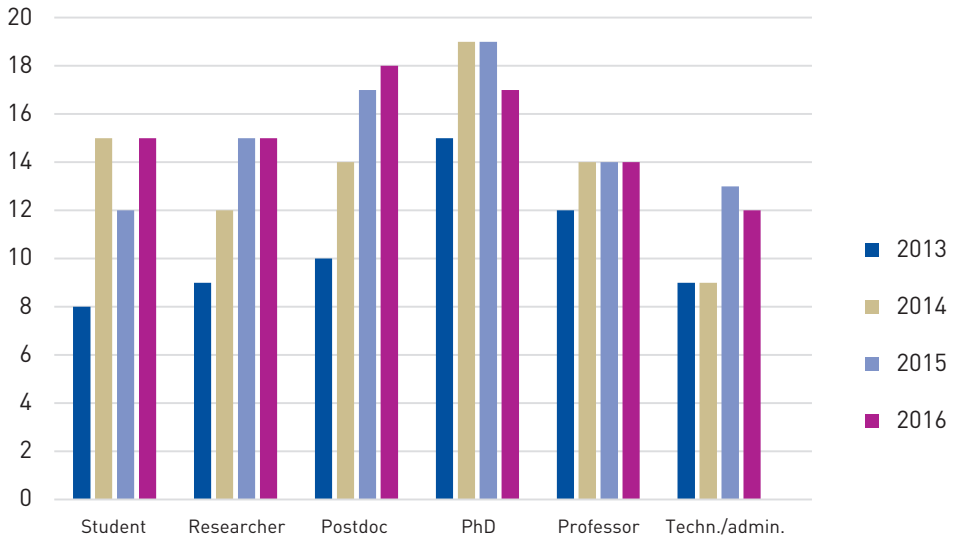
Kate Fitzgerald, Univ. of Massachusetts, USA: *"Long non-coding RNAs"*

Egil Lien, Univ. of Massachusetts, USA: *"Regulation of innate immune responses to bacteria: new players"*

Guest lecturer Professor Bruce Beutler and CEMIR Professor Trude Helen Flo, Trondheim March 2015

CEMIR STAFF AND STUDENTS

CEMIR was established as a Centre of Excellence January 1, 2013. We have emphasized the importance of establishing a unified research group in which multidisciplinary research cooperation is encouraged and stimulated. By the end of 2016 64 scientific staff members, 11 technicians, 15 students and an administrative coordinator were associated with the Centre.



Name		Position	Nationality	Research Group
Agliano	Federica	Guest researacher	Italy	Inflammation
Andersen	Sonja	Staff engineer	Norway	Autophagy
Aune	Marie Hjelmseth	Postdoctor	Norway	Inflammation
Awuh	Jane	Postdoctor	Cameroon	Mycobacteria
Bakke	Siril Skaret	Postdoctor	Norway	Inflammation
Beckwith	Kai	Postdoctor	Norway	Mycobacteria
Beckwith	Marianne Sandvold	PhD candidate	Norway	Mycobacteria
Bergstrøm	Bjarte	Postdoctor	Norway	Inflammation
Bjørkøy	Geir	Professor	Norway	Autophagy
Bözl	Korbinian Michael	PhD candidate	Germany	System Inflammation
Boyartchuk	Victor	Researcher	Ukraine	Inflammation
Bugge	Marit	Postdoctor	Norway	Inflammation
Damaas	Jan K	Professor	Norway	Inflammation
Dragset	Marte Singsås	Postdoctor	Norway	Mycobacteria
Egeberg	Kjartan	Staff engineer	Norway	Inflammation
Ehrnstrøm	Birgitta	PhD candidate	Sweden	Inflammation
Espevik	Terje	Professor	Norway	Inflammation
Fitzgerald	Kate	Professor II	USA	
Flo	Trude Helen	Professor	Norway	Mycobacteria
Giambelluca	Miriam	Postdoctor	Argentina	System Inflammation
Gidon	Alexandre	Postdoctor	France	Mycobacteria
Gierman	Lobke	Postdoctor	Netherlands	Pregnancy
Ginbot	Zekarias	Researcher	Eritrea	Mycobacteria
Granlund	Atle Van Beelen	Postdoctor	Norway	IBD
Grøvdal	Lene Melsæther	Researcher	Norway	Inflammation
Haug	Markus	Researcher	Norway	Mycobacteria
Husebye	Harald	Researcher	Norway	Inflammation
Håland	Kari	Head of administration	Norway	
Ibrahim	Hany	PhD candidate	Egypt	Mycobacteria
Iversen	Ann-Charlotte	Professor	Norway	Pregnancy
Johansson	Ida	Postdoctor	Norway	Autophagy
Kandasamy	Richard Kumaran	Associate Professor	India	System Inflammation
Kannan	Nisha	PhD candidate	India	Mycobacteria
Kojen	June Frengen	Staff engineer	Norway	Inflammation
Kovcic	Vlado	PhD candidate	Serbia	Bone disease
Latz	Eicke	Professor II	Germany	
Lien	Egil	Professor II	Norway	
Louet	Claire	Staff engineer	France	Mycobacteria
Marstad	Anne	Staff engineer	Norway	Mycobacteria
Mildenberger	Jennifer	PhD candidate	Germany	Autophagy
Moharrami	Neda Nejati	PhD candidate	Iran	Inflammation
Mollnes	Tom Eirik	Professor II	Norway	
Mundal	Siv Boon	PhD candidate	Norway	Pregnancy
Mærk	Mali	Postdoctor	Norway	Mycobacteria

Neckmann	Ulrike	PhD candidate	Germany	Autophagy
Nilsen	Nadra	Researcher	Norway	Inflammation
Niynzima	Nathalie	Postdoctor	Norway	Inflammation
Nonstad	Unni	Staff engineer	Norway	Inflammation
Ostrop	Jenny	Postdoctor	Germany	Regeneration
Oudhoff	Menno	Researcher	Netherlands	Regeneration
Paulsen	Julie	PhD candidate	Norway	Inflammation
Pettersen	Kristine	Postdoctor	Norway	Autophagy
Richard	Gabriel	Staff engineer	India	System Inflammation
Rokstad	Anne Mari	Researcher	Norway	Inflammation
Ryan	Liv	Staff engineer	Norway	Inflammation
Samstad	Eivind	Researcher	Norway	Inflammation
Sandvik	Arne	Professor	Norway	IBD
Serrre	Ignacio Katalan	Postdoctor	Spain	IBD
Silva	Gabriela Brettas	PhD candidate	Brazil	Pregnancy
Skei	Bente	Staff engineer	Norway	Pregnancy
Skjesol	Astrid	Researcher	Norway	Inflammation
Standal	Therese	Professor	Norway	Bone disease
Starheim	Kristian K.	Researcher	Norway	Inflammation
Steigedal	Magnus	Researcher	Norway	Mycobacteria
Steinkjer	Bjørg	Staff engineer	Norway	Inflammation
Stenmark	Harald	Professor II	Norway	
Stenvik	Jørgen	Researcher	Norway	Inflammation
Strand	Trine Aakvik	Staff engineer	Norway	Mycobacteria
Stødle	Guro	PhD candidate	Norway	Pregnancy
Sundan	Anders	Professor	Norway	Bone disease
Thorsvik	Silje	PhD candidate	Norway	IBD
Underhill	David	Professor II	USA	
Vik	Randi	Staff engineer	Norway	Inflammation
Wolowczyk	Camilla	PhD candidate	Norway	Autophagy
Yurchenko	Mariia	Postdoctor	Ukraine	Inflammation
Zahoor	Muhammad	Postdoctor	Pakistan	Bone disease
Zwiggelaar	Rosalie	PhD candidate	Netherlands	Regeneration
Ørning	Mathias Pontus	PhD candidate	Norway	Inflammation
Østvik	Ann Elisabeth	Researcher	Norway	IBD
Åsberg	Signe	PhD candidate	Norway	Mycobacteria

SCIENTIFIC RESULTS 2013–2016

The scientific activities at CEMIR have proceeded with very good progress since 2013. CEMIR researchers have published more than 200 articles, several in high quality journals like *Nature, Nature Immunology, Autophagy, PNAS* and *J Immunol.*

	2013	2014	2015	2016	Total
Articles published in scientific journals	27	40	77	61	205
Dissertations	3	4	8	2	17
Conference/meetings contributions	48	46	39	51	184
Dissemination	18	17	10	17	62

IMPORTANT CEMIR PUBLICATIONS FROM THE FIRST CENTER PERIOD ARE:

2016:

Atianand, Maninjay K; Hu, Wenqian; Satpathy, Ansuman T; Shen, Ying; Ricci, Emiliano P; Alvarez-Dominguez, Juan R; Bhatta, Ankit;Schattgen, Stefan A; McGowan, Jason D; Blin, Juliana; Braun, Joerg E; Gandhi, Pallavi; Moore, Melissa J; Chang, Howard Y.; Lodish, Harvey F; Caffrey, Daniel R; Fitzgerald, Katherine A..
A Long Noncoding RNA lincRNA-EPS Acts as a Transcriptional Brake to Restrain Inflammation. *Cell* 2016 Volum 165, side 1672-1685.

Awuh, Jane Atesoh; Flo, Trude Helen.
Molecular basis of mycobacterial survival in macrophages. *Cellular and Molecular Life Sciences (CMLS)* 2016, side 1-24.

Baranowska, Katarzyna Anna; Misund, Kristine; Starheim, Kristian K.; Holien, Toril; Johansson, Ida; Darvekar, Sagar; Buene, Glenn; Waage, Anders; Bjørkøy, Geir; Sundan, Anders.
Hydroxychloroquine potentiates carfilzomib toxicity towards myeloma cells. *OncoTarget* 2016 Volum 7, side 70845-70856.

Franklin, Bernardo S.; Mangan, Matthew S.; Latz, Eicke.
Crystal Formation in Inflammation. *Annual Review of Immunology* 2016 Volum 34, side 173-202.

Gustavsen, Alice; Nymo, Stig Haugset; Landsem, Anne; Christiansen, Dorte; Ryan, Liv; Husebye, Harald; Lau, Corinna; Pischke, Søren Erik; Lambris, John D.; Espevik, Terje; Mollnes, Tom Eirik.
Combined inhibition of complement and CD14 attenuates bacteria-induced inflammation in human whole blood more efficiently than antagonizing the toll-like receptor 4-MD2 complex. *Journal of Infectious Diseases* 2016 Volum 214, side 140-150.

Kleveland, Ola; Kunszt, Gabor; Bratlie, Marte; Ueland, Thor; Broch, Kaspar; Holte, Espen; Michelsen, Annika; Bendz, Bjørn; Amundsen, Brage H.; Espevik, Terje; Aakhus, Svend; Damås, Jan Kristian; Aukrust, Pål; Wiseth, Rune; Gullestad, Lars.
Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-STelevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial. *European Heart Journal* 2016 Volum 37, side 2406-2413.

Niyonzima, Nathalie; Halvorsen, Bente; Sporsheim, Bjørnar; Garred, Peter; Aukrust, Pål; Mollnes, Tom Eirik; Espevik, Terje.
Complement activation by cholesterol crystals triggers a subsequent cytokine response. *Molecular Immunology* 2016

Nymo, Stig Haugset; Gustavsen, Alice; Nilsson, Per; Lau, Corinna; Espevik, Terje; Mollnes, Tom Eirik.
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Personnel and indirect costs	52 919
Equipment	352
Other operating costs	18 675
Total expenditures	**71 946**

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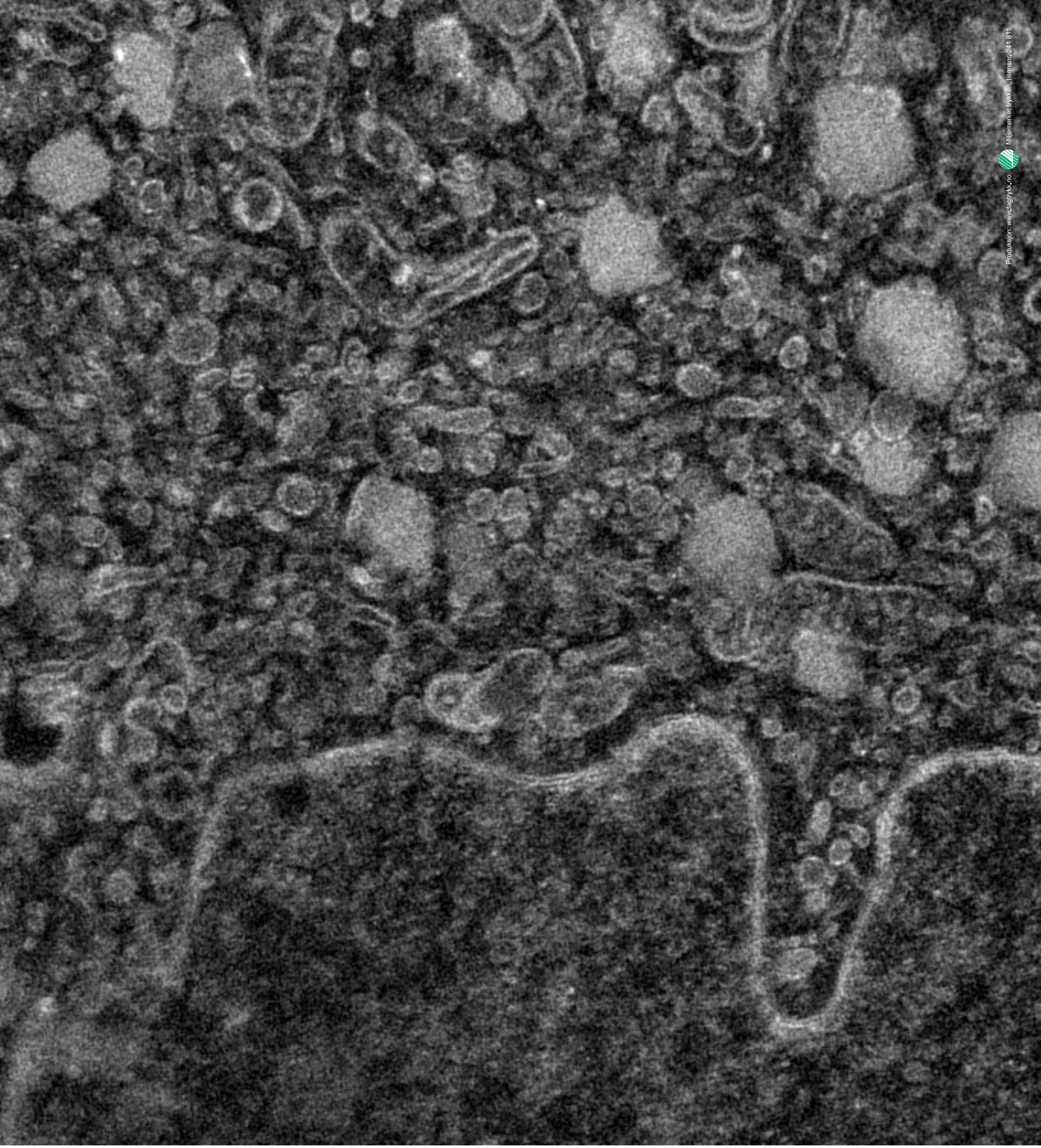

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