Internships in

Immunity, Infection and Tolerance

Departments (LUMC):

- Hematology
- Immunohematology and blood transfusion
- Infectious diseases
- Medical microbiology
- Nephrology
- Parasitology
- Pediatrics
- Pulmonology
- Rheumatology
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Hematology

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Internship topic I: Identification of therapeutic TCR with selective reactivity to leukemic cells for clinical application

Contact persons: Dr. M.H.M. Heemskerk, m.h.m.heemskerk@lumc.nl

Background: Patients with hematological malignancies can be successfully treated with allogeneic hematopoietic stem cell transplantation (alloSCT). The desired Graft-versus-Leukemia (GvL) effect, however, is often accompanied with undesired reactivity to healthy non-hematopoietic tissues, a complication known as graft-versus-host disease (GvHD). To reduce the risk and severity of GvHD, donor T-cells can be depleted from the graft and re-administered later as donor lymphocyte infusion (DLI). DLI can induce long-lasting GvL responses, but GvHD remains a serious complication. Beneficial GvL reactivity is mediated by donor T-cells recognizing polymorphic peptide-HLA complexes on the malignant cells of the patient as foreign due to differences in single nucleotide polymorphisms (SNPs) between patient and donor. If these polymorphic peptide-HLA complexes are also expressed on healthy non-hematopoietic tissues, donor T-cells will mediate not only beneficial GvL, but also GvHD. One strategy to improve the balance between desired GvL and undesired GvHD is to administer donor T-cells that have been genetically engineered with T-cell receptors (TCRs) with desired reactivity to leukemic cells, but not against healthy non-hematopoietic tissues. At the department of Hematology, patients with hematological malignancies are treated with TCR gene therapy. TCRs with selective reactivity to leukemic cells are introduced into donor derived virus-specific T-cells, since virus-specific T-cells do not induce GvHD when administered early after alloSCT. Moreover, TCR-transduced donor derived T-cells for persistent viruses such as CMV and EBV may show improved survival and expansion in vivo upon triggering of their endogenous TCR.

Research question: The aim of this research project is to identify therapeutic T-cell receptors (TCR) with selective reactivity to leukemic cells and to improve the in vitro strategy to transfer TCRs into donor derived virus-specific T-cells for clinical application.

Laboratory skills: T-cell isolation, culture and cloning; cytokine ELISA; mRNA isolation; cDNA generation; PCR analysis; TCR isolation and cloning; retroviral transduction; FACS analysis and sorting.

Internship topic II: Identification of targets in cellular and humoral immune responses after allogeneic hematopoietic stem cell transplantation.

Contact persons: Dr. M. Griffioen, m.griffioen@lumc.nl

Background: Patients with hematological malignancies can be successfully treated with allogeneic hematopoietic stem cell transplantation (alloSCT). The desired Graft-versus-Leukemia (GvL) effect, however, is often accompanied with undesired reactivity towards healthy tissues, leading to
development of a complication known as Graft-versus-Host Disease (GvHD) and autoimmune diseases. It is known that donor derived T-cells play an important role in GvL by recognizing polymorphic peptide-HLA complexes on the malignant cells of the patient. These polymorphic peptide-HLA complexes as expressed on patient cells are recognized by donor T-cells as foreign due to differences in single nucleotide polymorphism (SNPs) between patient and donor. If these peptide-HLA complexes are also expressed on healthy non-hematopoietic tissues of the patient, donor T-cells induce not only GvL, but also GvHD. In addition to allo-reactive T-cells, antibodies are induced after alloSCT. In contrast to T-cells, the contribution of antibodies to development of GvL, GvHD and autoimmunity after alloSCT is largely unknown. Identification of (non)polymorphic targets for T-cells and antibodies will give relevant insight into the development (and interaction) of cellular and humoral immune responses in GvL, GvHD and autoimmunity after alloSCT. Moreover, identification of these structures is highly relevant for development of new T-cell therapies to improve the balance between beneficial GvL and undesired side effects after alloSCT.

Research question: The aim of this project is to identify (non)polymorphic targets for T-cells and antibodies after alloSCT.

Laboratory skills: T-cell isolation, cloning and culture; cytokine ELISA; Whole Genome Association scanning (WGAs); Human Protein Arrays; Luminex bead array; mRNA isolation; cDNA generation; PCR analysis; cDNA cloning; retroviral transduction; FACS analysis and sorting.

Internship topic III: In vitro generation of virus-specific and leukemia-reactive T-cells for clinical application.

Contact persons: Dr. I. Jedema, i.jedema@lumc.nl

Background: Patients with hematological malignancies can be successfully treated with allogeneic hematopoietic stem cell transplantation (alloSCT). The desired Graft-versus-Leukemia (GvL) effect, however, is often accompanied with undesired reactivity to healthy non-hematopoietic tissues of the patient, a complication known as graft-versus-host disease (GvHD). To reduce the risk and severity of GvHD, donor T-cells are depleted from the graft and re-administered later as donor lymphocyte infusion (DLI). T-cell depletion of the stem cell graft leads, however, to an increased risk for pathogenic infections in the early post-transplantation period as a result of delayed immune reconstitution. Administration of DLI after T-cell depleted alloSCT will improve immune reconstitution and can induce long-lasting GvL responses. Improved immune reconstitution will reduce the incidence of pathogenic infections due to the presence of donor T-cells recognizing viral antigens or other pathogens. Beneficial GvL is mediated by donor T-cells recognizing polymorphic peptide-HLA complexes on the malignant cells of the patient. These polymorphic peptide-HLA complexes as expressed on patient cells are recognized by donor T-cells as foreign due to differences in single nucleotide polymorphisms (SNPs) between patient and donor. At the department of Hematology, patients with hematological malignancies are treated with (donor derived) virus-specific T-cells and donor T-cells with specific reactivity to leukemic cells to improve the balance between beneficial GvL and undesired side effects after alloSCT.

Research question: The aim of this research project is to improve in vitro strategies to generate virus-specific and leukemia-reactive T-cells for clinical application.
**Laboratory skills:** T-cell isolation, cloning and culture; cytokine ELISA; cytotoxicity assays; multi-color FACS analysis and sorting.

**Internship topic IV: Identification of effector cells in acquired aplastic anemia before and after immune suppressive treatment with anti-thymocyte globulin**

Contact persons: Dr. C.J.M Halkes, c.j.m.halkes@lumc.nl

**Background:** Acquired Severe Aplastic Anemia (SAA) is characterized by immune-mediated destruction of hematopoietic stem cells causing pancytopenia. Without treatment the majority of patients die due to severe (mainly bacterial or fungal) infections or major bleedings. Several effector cells have been described to be involved in the pathogenesis of SAA among which oligoclonal CD8+ T-cells, clonal gamma delta T cell populations, NK-T cells and abnormal CD4+ T cells. The immune basis for most patients with SAA provides a rationale for immune suppressive therapy (IST), using anti-thymocyte globulin (ATG) leading to hematologic responses in up to 70% of patients. Hematological recovery is seen up to six months after ATG and is supposed to be caused by outgrowth of hematopoietic stem cells which are spared from primary immune mediated destruction. In order to identify the effector cells in the pathogenesis of SAA, we analyze the presence, disappearance and reappearance of different lymphocytes in the circulation and bone marrow of patients with SAA before, during and after treatment with ATG. By comparing the outcomes between responding and non-responding patients we will be able to determine whether the presence and disappearance of a specific cell population can predict clinical outcome. These cell populations will be sorted from patient material before ATG treatment and will be tested for their effector function against the presumed target cells in SAA, e.g. the hematopoietic stem cells, which will be collected from bone marrow before and after treatment with ATG. By identifying effector cell populations that are targeted by ATG in responding patients, we will be able to predict more precisely which patients respond to IST in order to design more specific, antibody-based treatment strategies for future use.

**Research question:** The aim of this research project is to identify the effector cells in SAA before and after immune suppressive treatment with ATG in order to predict which patients will respond to immune suppressive treatment and design more specific, antibody-based treatment strategies for future use.

**Laboratory skills:** Isolation and culture of effector cell populations; cytokine ELISA; cytotoxicity assays; multi-color FACS analysis and sorting.
Immunohematology and blood transfusion

General contact person of the department for internships: Prof. dr. Bart O. Roep, b.o.roep@lumc.nl

Internship topic I: Immunology of pregnancy

Contact person: Prof. dr. Frans Claas, F.H.J.Claas@lumc.nl

Background: During pregnancy the immune system of the mother has to tolerate the fetus, which is mismatched for the paternal HLA antigens. In case of an organ transplantation, this would result in immunological rejection of the graft, which is the reason why transplant recipients are treated with immunosuppressive drugs. In close collaboration with the department of Obstetrics, we study the immune regulation in normal and aberrant pregnancies. So far, we have observed that a lot of immune regulation takes place locally in the placenta. The immune regulation is not merely a feature of the woman. The potential father also contributes as immunoregulatory factors are present in semen.

Research questions: What are the differences in immune regulation in normal pregnancy versus preeclamptic pregnancies or recurrent abortions? How is the immune regulation in oocyte donation, where the fetus is completely HLA mismatched with the mother.

Laboratory skills: Cell isolation and cell cultures, detection of cytokine profiles by ELISA, characterization of immune cells by FACs.

Internship topic II: Specificity of autoreactive CD8 T-cells in Type 1 Diabetes

Contact person: Joana Abreu, j.abreu@lumc.nl

Background: Type 1 diabetes (T1D) is a chronic autoimmune disease in which the insulin-producing beta cells of the pancreas are selectively destroyed by the immune system. Beta-cells present self-peptides (islet-specific peptides) in the context of MHC class I, that are recognized by autoreactive CD8 T-cells. Increasing evidence suggests CD8 T-cells as important effectors in the cytotoxic process of beta-cell destruction. Discovery of the epitopes that are recognized by the autoreactive CD8 T-cells on the surface of beta-cells could not only provide new biomarkers to monitor disease progression and responses to therapeutic interventions, but also provide novel potential targets for therapy.

Research question: In this project we will investigate the specificity of autoreactive CD8 T-cells that were previously isolated from T1D patients. With this we aim to determine the epitopes recognized by these autoreactive T-cells. Furthermore, we will test the peripheral blood of T1D patients and healthy controls for the presence of autoreactive CD8 T-cells recognizing specific islet antigens. We will make use of MHC-tetramers, MHC molecules loaded with islet-specific peptides and tagged with a fluorochrome that can be detected by flow cytometry.
Laboratory skills: Cell culture, flow cytometry

Internship topic III: Investigating the capacity of small compounds to interfere with CD8 T-cell responses in Type 1 Diabetes

Contact person: Joana Abreu, j.abreu@lumc.nl

Background: Type 1 diabetes (T1D) is a chronic autoimmune disease for which no cure is available. There is an unmet medical need amongst patients, as insulin replacement therapy remains the only therapeutic option. Recent evidence indicates that autoreactive CD8 T-cells recognize islet-epitopes presented on the surface of beta-cells and promote beta-cell destruction in the pancreas. The interaction between CD8 T-cells and beta-cells occurs via the T-cell receptor (TCR) on the surface of CD8 T-cells and MHC class I molecules complexed with islet-peptides on the surface of beta-cells. The development of small compounds capable of interfering with this interaction could lead to a block in autoreactive T-cell activation, ultimately protecting beta-cells from destruction.

Research question: In this project we will investigate the capacity of small compounds to interfere with peptide binding to MHC-I molecules and/or CD8 T-cell recognition of target cells. We have used an in silico molecular docking program to screen a large chemical library (approximately 140,000 compounds screened) to define small compounds capable of occupying specific structural pockets in the HLA-A2 molecule. The top 45 scoring compounds have been selected and will now be tested in vitro in binding assays with HLA-A2 molecules and in functional T-cell assays.

Laboratory skills: Cell culture, Flow cytometry

Internship topic IV: Molecular mechanisms of immunoregulation by tolerogenic dendritic cells

Contact person: Dr. T. Nikolic, t.nikolic@lumc.nl

Background: Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by T-cell-mediated destruction of insulin-producing beta-cells in the pancreas. The incidence of T1DM in Europe has increased over the last decades, particularly in the 1-5-year age group. Since it carries a significant chronic disease burden, T1DM has become a major public health concern, emphasizing the urgent need for safe and effective intervention and prevention strategies. Dendritic cells (DCs) phagocytose and process antigens, which they bring to the attention of T-cells. The surface molecules expressed by DCs critically determine the type of T-cell response. We have designed and validated a protocol to generate stable tolerogenic DCs, which induce regulatory T-cells (Tregs) and in this way divert the destructive autoimmunity into immune protection.

Research question: Tolerogenic DCs induce antigen-specific Tregs, which modulate immune responses through linked suppression and infectious tolerance, a process in which one immune cells transfers tolerogenic capacities to other immune cells. The focus of this project is to investigate immunoregulatory molecules expressed by tolerogenic DCs, which are involved in the induction of
Tregs and infectious tolerance (e.g. CD25, CD52, costimulatory molecules of the B7-family, immunoregulatory cytokines and chemokines).

**Laboratory skills:** Cell culture: cultivating DCs, co-culture with T cells; Molecular techniques: analysis of gene expression data, RT-PCR; FACS analysis (phenotype, CFSE dilution assay)

**Internship topic V: Mesenchymal Stromal Cells (MSC) for tolerance induction in T1D**

Contact person: Dr. T. Nikolic, t.nikolic@lumc.nl

**Background:** Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by T-cell-mediated destruction of insulin-producing beta-cells in pancreas. The incidence of T1DM in Europe has increased over the last decades, particularly in the 1-5-year age group. Since it carries a significant chronic disease burden, T1DM has become a major public health concern, emphasizing the urgent need for safe and effective intervention and prevention strategies.

Mesenchymal Stromal Cells (MSCs) have immunosuppressive and regenerative capacity and are currently used for different clinical treatments. Beneficial effects of MSC transplantation in experimental models of T1D have been reported. MSCs work via secreted molecules but it is not clearly established whether MSCs present antigens to T cells directly and whether they induce regulatory T cells (Tregs).

**Research question:** Our aim is to test the potential of MSCs to reduce proinflammatory autoimmune reactivity against beta-cells originating antigens. We will test whether MSCs can act as APCs and present beta-cell antigens to CD4+ T cells and analyse the functionality of T cells stimulated by MSCs as determined by cytokine production (IFNg vs. IL-10 response). In addition, we will investigate whether HLA-DQ6 molecule, associated with genetic protection from T1D, supports tolerance induction by MSCs.

**Laboratory skills:** Cell culture: cultivating MSC, co-culture with T cells; FACS analysis (phenotype, CFSE dilution assay); Cytokine expression analyses: Luminex, ELISPOT

**Internship topic VI: The humoral immune response to renal allografts**

Contact person: Dr. Sebastiaan Heidt, s.heidt@lumc.nl

**Background:** Kidney transplantation is the treatment of choice for end-stage renal disease patients. Due to the huge diversity in tissue antigens, many kidney transplants are performed with some level of tissue antigen disparity. The immune system of the recipient will recognize these mismatched antigens as foreign, potentially leading to a detrimental immune response.

One of the cell types that is increasingly recognized to be an important player in both immune activation and tolerance in the setting of transplantation is the B cell. B cell are cells of the humoral immune system, which supply the body of antibodies as a natural defense mechanism to infections. Besides this, B cells can present antigen to activate T cells, and potentially can downregulate immune responses.
**Research questions:** What is the role of B cells in the rejection of kidney transplants? What are the interactions of B cells and T cells that lead to an alloimmune response? What are the kinetics of tissue-antigen specific B cells upon transplantation?

**Laboratory skills:** Cell isolation and cell cultures, ELISA, ELISpot, flow cytometry, qPCR

**Internship topic VII: Immunological response to steroid treatment in kidney transplantation**

Contact person: Dr. Michael Eikmans, m.eikmans@lumc.nl

**Background:** Kidney transplantation is the treatment of choice for end-stage renal disease patients. Most kidney transplants are performed with a considerable level of tissue antigen disparity between donor and recipient. Therefore, the transplanted patient needs to take medication to reduce the chance of rejection of the donor graft. Steroids are an important part of both the maintenance medication regime and anti-rejection therapy. Approximately 70% of patients with an acute rejection respond to steroid therapy, but the others are resistant and eventually need to be treated with more vigorous medication. We previously found that non-responsiveness can be partly predicted according to the composition of the immune infiltrate in the graft (biopsy) and to genetic polymorphisms (DNA) in steroid-metabolizing genes in the transplant recipient. We now like to incorporate analysis of blood cells as a possible less-invasive tool for identifying steroid-refractory rejection.

**Research aim and questions:** To set up a human cell culture system whereby cells are activated and treated with steroids. To establish parameters which form the basis of identifying steroid-response; these may be the extent of inhibition in cell proliferation upon treatment, the extent of inhibition of cytokine secretion into the supernatant, and dampening of transcriptional activity of immune markers. In this process it may be necessary to separate mononuclear fractions beforehand into T cells and monocytes, and test their response separately.

**Laboratory skills:** Cell isolation, cell cultures, proliferation assays, ELISA, flow cytometry, qPCR.
Infectious diseases

General contact persons of the department for internships: Dr. PH Nibbering, p.h.nibbering@lumc.nl; prof. THM Ottenhoff, t.h.m.ottenhoff@lumc.nl; Dr. LG Visser (clinical), l.g.visser@lumc.nl

Internship topic I: Discovery of new Mtb antigens that efficiently stimulate human T cells, and evaluate their TB vaccine potential

Contact person: Prof dr Tom H. M. Ottenhoff, t.h.m.ottenhoff@lumc.nl

Background: To dissect immunological and host-genetic mechanisms of protective and pathologic immunity to mycobacterial infections (as model diseases), in order to design more effective intervention strategies.

Research question: Discovery of new Mtb “latency” antigens that are expressed during latent infection, and on in vivo expressed antigens during in vivo lung infection. The second focus is on the study of newly identified, “alternative” T-cell subsets, notably: HLA-E restricted Mtb peptide specific CD8 T cells (including Tregs). This involves their specificity and dual functionality.

Laboratory skills: Mtb genome mining based discovery of antigens, epitope prediction bioinformatic tools, followed by functional validation in humans (T-cell recognition of recombinant proteins, peptides) in human in vitro models and in HLA transgenic mice, including a mouse TB challenge model.

Internship topic II: Dissecting T cell-macrophage interactions in intracellular infections

Contact person: Prof dr Tom H. M. Ottenhoff, t.h.m.ottenhoff@lumc.nl

Background: We have found new human cell biological targets (kinases, phosphatases, etc.) that can be targeted by new compounds to kill bacteria (antibiotics working on the host rather than the bacterium) (coll. NKI and Univ. Leiden).

Research question: Identification of new chemical compounds and corresponding targets that act on human host genes to improve infection control and The influence of functionally different macrophage subsets on: bacterial handling, antigen presentation and subsequent T cell fate (Tregs, T cell anergy etc).

Laboratory skills: siRNA, chemical genetics and functional assays in Salmonella and Mtb infection systems, using a wide array of technologies and collaborations.
Internship topic III: Larval therapy

Contact person: Dr. PH Nibbering, p.h.nibbering@lumc.nl

Background: Larval therapy, i.e. the application of larvae of the green bottle fly *Lucilia sericata* onto wounds, has been successfully used to treat severe and chronic wounds throughout history. In Europe, nowadays approximately 15,000 patients receive larval therapy annually. The larvae remove dead tissue effectively and their secretions also affect microbes, inflammatory processes, and wound healing.

Research question: To isolate and identify the components within larval secretions that can inhibit biofilm formation, complement activation and other inflammatory processes. To investigate possible systemic effects of local application of medicinal larvae.

Involved laboratory skills: Collection of secretions from medicinal maggots, Bradford assay for determination of the protein content of secretions, gel filtration and anion chromatography, biofilm assays using MRSA, complement assays, gel electrophoresis, cytokine assays, chemotaxis, FACS analysis.

Internship topic IV: Antimicrobial peptides and other alternatives to antibiotics

Contact person: Dr PH Nibbering; p.h.nibbering@lumc.nl

Background: Treating infections has become more complicated as antimicrobial resistance increases worldwide. Therefore, good antibiotic policies combined with new antimicrobial agents are urgently needed. Application of antimicrobial peptides is mentioned as an important alternative treatment strategy in fighting infections. Antimicrobial peptides have a wide range of biological activities ranging from direct killing of (anti-infective resistant) pathogens to modulation of immunity and other biological responses of the host. Antimicrobial peptides are produced by a variety of host cells that come in contact with pathogens. Interestingly, it is quite difficult for pathogens to develop resistance to antimicrobial peptides. In addition, we are currently testing the effects of plant molecules (herbal medicines) as an alternative to antibiotics as well as agents (of plant origin) that enhance the effects of current antibiotics, further referred to as sensitizers.

Research questions internship: Are antimicrobial peptides effective against infections that do not respond optimally to current treatments? What are the effects of antimicrobial peptides on cells of the immune system? Effect and mode of action of herbal medicines and sensitizers?

Laboratory skills: 3D-organotypic cultures, antimicrobial assays (in vitro killing assays, E-tests, biofilm assays, antimicrobial resistance), immunological assays (cytokine assays, DC-T cell subcultures, macrophage subsets, FACS, ELISA, immune-histology, multiplex assays), chromatography, qPCR.

Internship topic V: Biomarkers of protection in Tuberculosis and in metabolic co-morbidities affecting immunity to infection
Background: Vaccine development against tuberculosis is hampered by the highly persistent nature of the infectious pathogen, Mycobacterium tuberculosis (TB). Currently there are no correlates of protection identified that could help guiding vaccine development in TB and predict induction of protective immunity. Such correlates of protection are urgently needed, since they will accelerate selection and prioritisation of TB vaccine candidates.

Research question: We will identify relevant biomarkers in various clinical cohorts, including TB patients, BCG vaccinees and cohorts of TB patients suffering from co-morbidities, e.g. concomitant infections with HIV, malaria, worms or systemic diseases like type 2 diabetes. Biomarker profiling will be performed using advanced transcriptomic analysis (using dcRT-MLPA) as well as metabolomics. Biomarkers will be correlated to outcome of infection, i.e. disease or protection for disease. Moreover, basic immunological and genetic studies will be performed to unravel the nature and functional significance of identified biomarkers in host defence against infection TB. These include (already identified) molecules regulating basic T cell and macrophage interactions.

Laboratory skills: RNA isolation, dcRT-MLPA, Q-PCR, primary cell cultures, magnetic bead cell separation, multiparameter flow cytometry (LSR Fortessa), ELISA, infection experiments, siRNA gene knock down in human cells, data analysis

Internship topic VI: Development of diagnostic tools for leprosy

Background: Leprosy is a chronic granulomatous infection caused by Mycobacterium leprae. It particularly affects the skin and peripheral nerves and often results in severe, life-long disabilities and deformities. Host factors play a major role in controlling the outcome of infection: most individuals are naturally resistant to leprosy, but susceptible individuals can manifest completely different forms of the disease (TT/BT and LL/BL). Important questions thus arise including determining which genetic and/or environmental factors dictate these very different outcomes of infection and whether biomarker profiles can be derived that predict outcome of infection. This is particularly important in case of leprosy reactions (acute type 1/RR and type 2/ENL). These serious complications of leprosy are accompanied by strongly increased inflammatory and cell-mediated immune reactivity and are the major cause of irreversible nerve damage. There are no laboratory tests to predict or diagnose onset of leprosy reactions at an early stage. Application of recently developed technologies is essential to develop such tests. The ability to predict these immunopathological events will allow timely treatment, thereby preventing (further) disability.

Research questions:
1. Identify and verify differences in expression level of genes in leprosy patients’ blood.
2. Identify biomarkers that are specific for leprosy and measurable in field friendly blood-based assays.
**Laboratory skills**: T-cell isolation; cytokine ELISA; mRNA isolation; cDNA generation; PCR analysis; FACS analysis, gene expression profile analysis.
Medical microbiology

General contact persons for research internships:
- j.corver@lumc.nl (bacteriology)
- c.c.posthuma@lumc.nl (virology)

Internship topic I: Regulation of virulence in Clostridium difficile

Contact person: Jeroen Corver, j.corver@lumc.nl

Background: In the last decade, Clostridium difficile has emerged as an important gut pathogen. Symptoms of *C. difficile* infection can vary from mild diarrhoea to fulminant colitis with occasionally serious complications such as toxic megacolon and death. The symptoms of *C. difficile* are caused by two toxins, TcdA and TcdB. Before the expression of the toxins, *C. difficile* has to colonize the gut and survive the hostile environment in the host. The response to these stresses and the expression of the toxins is tightly regulated. Our group investigates these regulatory processes through molecular biology, biochemistry, microbiology, genomics and proteomics.

Research question: Several membrane embedded proteases are expected to be involved in the regulation of these processes. Our current focus is on the role of these proteases in the regulation of the stress response and virulence. In particular we are interested in the substrates of these proteases. In addition, we would like to know how these proteases are activated and which regulatory pathways are directed by the proteases.

Laboratory skills: Microbiology, genetics (genetic manipulation of *C. difficile*), molecular biology (cloning, protein expression), Protein analysis (W-blots, protein biochemistry), phenotypical assays (sporulation of *C. difficile*, determination of toxin titers on eukaryotic cells, binding of bacteria to eukaryotic cells).

Internship topic II: Clostridium difficile in the face of antibiotics

Contact person: Wiep Klaas Smits, w.k.smits@lumc.nl

Background: *Clostridium difficile* is the major cause of healthcare associated diarrhea. This is in part because antibiotics incite a dysbiosis of the gut, creating a favourable environment for the colonization and outgrowth of *C. difficile*. Ironically, the default treatment of *C. difficile* infections (CDI) is the administration of even more antibiotics. Resistance or tolerance to some of the commonly used antibiotics has already been reported, antibiotic pressure can result in an increase of resistant cells. There is an urgent need to develop new antimicrobials, and essential processes such as DNA replication, are promising targets.

Research question: There is very little information on how *C. difficile* responds to these antibiotics, especially when they are at sub-inhibitory levels. Moreover, the mechanisms by which *C. difficile* becomes resistance to antimicrobials are largely unknown. We want to understand in what way *C.
difficile physiology is changed when exposed to antibiotics, identify mechanisms that contribute to resistance, and use this information to characterize potential new compounds with antibiotic activity towards *C. difficile*. To specifically target DNA replication, we study this process in detail and will use the information to design screens for inhibitors of this process.

**Laboratory skills:** Recombinant DNA technology, culturing *Clostridium difficile* and other bacteria, antimicrobial susceptibility testing, genetic engineering, fluorescent microscopy, biochemistry.

**Internship topic III: Polyomavirus infection and the immunocompromized host**

Contact person: Mariet Feltkamp, m.c.w.feltkamp@lumc.nl

**Background:** Human polyomaviruses (HPyV), for instance BKV, JCV, MCV and TSV, frequently infect humans with seroprevalences up to 95% in the general population. Primary infection usually occurs asymptomatic after which a latent, lifelong infection sets in. In case of reduced immunity, for instance in the course of HIV infection or immunosuppression after solid organ transplantation, these viruses can reactivate and cause serious disease, ranging from nephropathy (BKV), encephalopathy (JCV) to skin disease and cancer (TSV and MCV). As a result, a substantial proportion of these patients die or lose their grafted organ.

**Research questions:** Which immunocompromized patients are particularly at risk of developing manifest HPyV disease, which pathogenic and oncogenic mechanisms are involved and which cellular pathways are disrupted?

**Laboratory skills:** PCR, RT-PCR, Transfection, Transduction, Northern Blot, Western Blot, Immunoprecipitation, Immunofluorescence, ELISA, Expression Profiling, Transformation Assay, Statistics, etc

**Internship topic IV: Antiviral innate immunity and immune evasion by +RNA viruses**

Contact person: Marjolein Kikkert, m.kikkert@lumc.nl

**Background:** We are studying the interactions of +RNA viruses (predominantly nidoviruses: SARS-CoV, MERS-CoV and non-human model viruses) with the innate immune system. Upon infection of the host cell, the innate immune system will recognize the foreign viral components and trigger a signalling cascade to suppress viral replication. Viruses have developed numerous ways to circumvent or inhibit these antiviral responses, in order to create a window-of-opportunity for establishing a productive infection. We are searching for novel innate immune responses targeted at +RNA viruses, and we are identifying and studying viral innate immune evasion activities.

**Research question:** Depending on the daily supervisor, students will be involved in subprojects answering questions such as:

- What are the cellular substrates of viral deubiquitinases with immune evasive function?
- How do viral components interact with innate immune factors?
- What innate immune factors target the membraneous viral replication complexes?
- Which novel innate immune evasive strategies do nidoviruses employ?
Laboratory skills: we routinely use mammalian cell culture, transfection techniques, Western Blotting, immunofluorescence analyses, confocal microscopy and electron microscopy (collaboration dept. MCB), qPCR, reporter assays, E.coli mediated protein expression, in vitro assays, siRNA mediated gene knockdown, CRISPr/Cas9 mediated gene knock-out, virus infections in cell lines and primary cell culture, and many more.

Internship topic V: Development of “next-generation” antiviral vaccines

Contact persons: Nadia Oreshkova, n.d.oreshkova@lumc.nl; Peter Bredenbeek, P.J.Bredenbeek@lumc.nl; Marjolein Kikkert, m.kikkert@lumc.nl

Background: Vaccines arguably are the most effective remedy against viral disease known to date. A well-known early example with a global impact is the vaccine against smallpox, the use of which resulted in eradication of one of the deadliest virus diseases in humans. Another example is the vaccine against rabies, which is the only anti-viral vaccine that can be used after infection and, provided its timely administration, is effective in preventing an otherwise lethal disease. Currently many vaccines are routinely used to prevent morbidity and mortality associated with dangerous (viral) pathogens.

Early vaccines against viruses were developed empirically, typically by multiple passaging of the virus through non-human hosts resulting in significant attenuation, which enabled use as a (modified live virus) vaccine. However, such attenuation is a random process, and therefore difficult to steer in a particular direction. Nowadays, the accumulation of fundamental knowledge about viruses, combined with the development of a broad spectrum of molecular biology techniques that allow the targeted manipulation of viral genomes, provide the opportunity to develop and adapt antiviral vaccines in a much more controlled fashion. Existing vaccines can for example be designed to express an antigen of another virus, or can be adapted to modulate the immune response into a desired direction.

Currently we are developing a vaccine platform that combines the principles of DNA vaccines with RNA viral vector vaccines. We aim at creating a single platform that can be tailor-designed for diverse pathogens, with our focus being (re-)emerging viral pathogens such as MERS-CoV.

Research questions:
- Can we develop a vaccine platform suitable for expression of diverse antigens of interest and which are the characteristics of such a platform?
- What are the immune responses against developed vaccine candidates in animal models (mice)?
- Which antigens of a virus most effectively elicit protective immunity?
- Can we remove immune evasive activity from a vaccine virus to increase its immunogenicity?

Laboratory skills: The projects in this topic include molecular biology techniques such as cloning, recombinant DNA techniques, in vitro synthesis of RNA, PCR; mammalian cell culturing and transfection/electroporation; evaluation of protein expression by immunofluorescence, western blotting, flow cytometry analysis, immunoprecipitation.

Evaluation of the immune response in animal models includes analysis of antibody responses (ELISA, virus neutralization assay) and T-cell responses (measuring of cytokine responses by ELIPOT, qPCR, intracellular staining and flow cytometry).
Internship topic VI: The quest for inhibitors of coronavirus and alphavirus replication

Contact persons: Clara Posthuma, c.c.posthuma@lumc.nl and Martijn van Hemert, m.j.van_hemert@lumc.nl

**Background:** Viral infections are a major cause of disease, with enormous costs in morbidity/mortality and economic losses worldwide. This is, for example, highlighted by the 2003 Severe Acute Respiratory Syndrome (SARS) outbreak, caused by the SARS-coronavirus (>8,000 people infected, 774 deaths), and the chikungunya virus outbreaks that have affected millions of people in Asia and Africa since 2005, and recently caused an explosive outbreak in the Caribbean. Only a few viral diseases can be prevented by vaccination and antiviral therapy is an essential additional instrument to control virus infections. At present, however, antiviral drugs have been developed against only few human pathogenic viruses.

Antiviral drugs can be directed towards functions of the virus itself (e.g. viral proteases or polymerases), but also host factors that are pivotal for virus replication may be targeted. In theory, targeting the virus directly may constitute a suitable antiviral strategy with limited side-effects. However, RNA viruses can rapidly develop drug resistance due to their intrinsically high mutation frequency. Thus, there is a growing interest in targeting specific host factors or pathways, which would reduce the likelihood of the development of drug resistance, as host genes are unlikely to mutate in response to therapy. Related viruses commonly use the same cellular resources for their replication, which also opens up the possibility for the development of broad-spectrum inhibitors.

Depending on the interest of the student and available lab space, a research project may focus on antiviral research on one of our favourite RNA virus groups: coronaviruses or alphaviruses, for which several biosafe model viruses and or replicon systems are available.

**Research questions internship:**
- Can we design/discover (broad-spectrum) antivirals against pathogenic RNA viruses?
- What is the mechanism of action by which such a compound blocks virus replication?

**Laboratory skills:** This study includes a broad range of techniques: cell culture, virology techniques (infections, plaque assays, qRT-PCR), molecular biology techniques like recombinant DNA techniques, RNA isolation and Northern blotting, *in vitro* RNA transcription, SDS-PAGE, Western blotting.
Nephrology

General contact person of the department for internships: Prof dr C van Kooten, C.van_Kooten@lumc.nl

Internship topic I: Innate immunity and Complement in ischemia/reperfusion injury

Contact person: Prof dr C van Kooten, C.van_Kooten@lumc.nl

Background: Ischemia/reperfusion is an early and inevitable process in organ transplantation, and the innate immune system has been implicated as a critical mediator of injury. In a combination of clinical, experimental and in vitro experiments we have established a new role for Mannan Binding Lectin (MBL), the initiator of the lectin pathway of complement. Recipients with low MBL show improved graft survival, whereas therapeutic inhibition of MBL in a rat model of IRI results in a complete protection against injury. Currently we are investigating the molecular mechanism of MBL-mediated injury, which appears to be independent of the complement activation. However, in vivo analysis showed that complement activation is occurring after IRI, but is a relatively late process. Most likely this represents a mechanisms for the clearance of dead and injured cells.

Research question: Investigate the mechanism of complement activation after experimental IRI and determine the role of complement in the removal of dead cells

Laboratory skills: Cell culture, immunohistochemistry, ELISA, Q-PCR, functional measurements of complement

Internship topic II: The role of dendritic cells in renal allograft rejection

Contact person: Dr Jurjen Ruben, J.M.Ruben@lumc.nl

Background: In view of the importance of local immune and inflammatory processes, we have developed immunohistochemical methods to characterize and quantify myeloid subsets in human renal biopsies (pre-transplant, protocol- and rejection biopsies). We were able to demonstrate that the composition of infiltrate has an impact on the long term function of transplanted organs. Moreover we found that during rejection, also plasmacytoid DC (pDC) were a major component of the infiltrate. pDC are the natural alpha-IFN producing cells and implicated in defence against viral infections. Since viral (re)-activation (CMV, BK) is a major complication in immunosuppressed individuals, we investigate the contribution of pDC in this process. In in vitro models with human cells we investigate how dying cells (with or without a viral infection) affect the function of professional APC. Moreover we try to establish a correlation between viral infection in the transplanted kidney and the presence of pDC/mDC.

Research question: Investigate the functional changes of human DC when exposed to viral-infected cells.
Laboratory skills: pDC and DC isolation and cell culture, FACS analysis, ELISA, Q-PCR, immunofluorescence

Internship topic III: Biomarkers of renal inflammation and chronic transplant failure

Contact person: Prof dr Cees van Kooten; C.van_Kooten@lumc.nl

Background: For balanced use of immunosuppressive agents, it is of critical importance to have biomarkers which allow a close monitoring of the allo-specific immunity as well as of the ingoing inflammatory and injury response. For renal transplantation it is believed that the urine might be a global, specific and easy accessible reflection of local immune and inflammatory processes. Therefore, we are exploiting the urine as a source of biomarkers, both on a hypothesis driven strategy (KIM-1, NGAL, complement factors, endothelial injury markers, ...) as well as using an unbiased strategy (metabolomics; in collaboration with the department of parasitology). For several patient cohorts we also have paired samples with serum and tissue available, which allows a more in depth analysis of the presence of the markers. Finally, our experimental models of ischemia reperfusion injury in mouse and rat provide to opportunity to perform detailed longitudinal kinetic experiments, to strengthen the functional role and causal relationships of these markers.

Research question: Investigate the regulation and expression of NGAL by human renal epithelial cells and relate this to measurements of NGAL and regulatory factors in urine and tissue

Laboratory skills: ELISA, immunohistochemistry, Q-PCR, cell culture
Parasitology

Internship topic I: Modulation of brown adipocyte metabolism by helminth-derived molecules

Contact person: Dr. Bruno Guigas, b.g.a.guigas@lumc.nl

**Background:** The prevalence of metabolic disorders has reached epidemic proportions worldwide, not only in our Western societies but also in most of the developing countries. Chronic low-grade inflammation associated with obesity is one of the major contributors to insulin resistance and impaired glucose/lipid metabolism in metabolic tissues, increasing the risk for developing type 2 diabetes. Helminths are endemic multicellular eukaryotic parasites, which elicit strong anti-inflammatory type-2 immune responses in both humans and animal models. We and others have recently reported that helminth infection and helminth-derived molecules (HDMs) can exert beneficial effects on whole-body glucose metabolism and insulin sensitivity in diet-induced obese mice, at least partly by promoting T helper cell 2 (Th2) response and alternatively-activated M2 macrophage polarization in adipose tissue. Interestingly, a mixture of HDMs [SEA: soluble egg antigens] and a single synthetic HDM-related LeX glycoconjugate (LNFPIII), were recently shown to improve insulin sensitivity and glucose tolerance in obese mice, at least partly by directly modulating lipid metabolism in hepatocytes. Furthermore, we have also recently found that some HDMs can inhibit gluconeogenesis in primary mouse hepatocytes and promote glucose uptake in 3T3-L1 adipocytes. Collectively, this suggests that HDMs can constitute a source of unique molecules for manipulating metabolic processes by directly targeting relevant metabolic cells via glycan-mediated interaction with specific receptors. Brown adipose tissue (BAT) is a metabolic organ that produces heat, notably in response to cold and dietary intake, through UCP1-mediated uncoupling of mitochondrial oxidative phosphorylation (OXPHOS). For long time, BAT was considered to be solely important in small mammals and young children, but the recent observation of functional BAT in adult humans shed a new light on this highly metabolic tissue. Indeed, owing to its capacity to dissipate energy and regulate both VLDL-triglyceride and glucose metabolism, BAT could be seen as a potential target for the treatment of obesity and metabolic disorders. Interestingly, we found that the beneficial effects of helminth infection on whole-body insulin sensitivity and glucose homeostasis in obese mice were associated with a significant decrease in BAT mass, a feature pointing toward increase BAT activity. Taken together, we hypothesize that single HDMs can promote brown adipocyte lipid and/or glucose metabolism and UCP1-mediated OXPHOS uncoupling.

**Research question:** Our aim is to investigate whether HDMs can modulate glucose/lipid metabolism and/or UCP1 expression in an *in vitro* model of differentiated brown adipocytes, and to elucidate the underlying molecular mechanism(s) involved.

**Laboratory skills:** Cell culture, Enzymatic and Radio-isotopic assays, qRT-PCR, Western Blot, Flow cytometry

Internship topic II: Glycan-induced immunity in schistosomiasis
Background: More than 200 million people worldwide suffer from schistosome infections. Schistosomes are parasitic helminths with a complex life cycle in which infectious larvae penetrate the human skin and develop to adult worms that can survive for years in the blood stream. Worm couples produce hundreds of eggs per day which contribute to transmission of the disease and immunopathology. A drug that kills adult worms is available, however, people in endemic areas become rapidly re-infected due to extremely slow development of natural immunity. Each life stage of the schistosome produces a variety of antigenic glycans as part of glycoproteins and glycolipids, and the infected host mounts antibody responses to many different glycan elements. Therefore, the parasite’s glycans are a potential target for the development of a prophylactic vaccine. We have developed a research line in which we study various aspects of antibody responses to parasite glycans by applying a glycan microarray technology to screen sera of naturally infected humans and experimentally infected animals. We use these arrays to relate responses to hundreds of different glycans to parameters like infection intensity, treatment, resistance to re-infestation, etc. By combining these immune-epidemiological data with \textit{in vitro} and \textit{in vivo} models for immunisation and vaccination we aim to identify protective glycan antigens that form the basis of an effective schistosomiasis vaccine.

Research question: The internship will address the question which of the anti-glycan antibodies observed in schistosome-infected hosts are contributing to protection, and which may have opposite effects. The student will use existing glycan arrays to study responses in existing human or animal serum cohorts. Glycan antibodies potentially related to protection on the basis of the (statistical) arrays analysis will be identified, and the molecular structure of their glycan target determined. The localisation and expression of the target across the schistosome life cycle will be studied. The most promising glycan antigens will be tested for their capacity to induce protective responses in the \textit{S. mansoni} immunisation and/or infection model in the mouse.

Laboratory skills: (fluorescence) microscopy/IFA, glycan array and ELISA assays, mass spectrometry, parasite and cell culture.

Internship topic III: Immunomodulation by helminth glycans

Contact persons: Dr. C.H. Hokke (c.h.hokke@lumc.nl) or Dr. N.N. Driessen (N.N.Driessen@lumc.nl)

Background: Parasitic helminths actively secrete and excrete immunomodulatory molecules which can regulate their hosts’ immune response in order to promote survival of the parasite and maintain a chronic infection. Many of these molecules are glycoconjugates of which the glycan portion can contribute in multiple ways to immune effects in the host. The glycan motifs of glycoproteins or glycolipids determine their route of uptake by a variety of host immune cells via carbohydrate-recognizing receptors, such as the C-type lectins. Specific glycan structures can also induce or modify intracellular signalling. Furthermore, glycans can evoke anti-glycan antibody responses. Identification of helminth-derived immunomodulatory glycans and glycoconjugates, the glycan-mediated pathways, lectin receptors and cellular mechanisms involved contribute to finding new ways to treat or prevent helminth infections as well as to the discovery of new immunomodulatory agents and pathways to treat hyper-inflammatory and auto-immune diseases.
Research question: The internship will address questions on the role of glycans in the interaction between the helminth’s antigens and its host immune system. Glycosylation profiles of individual molecules or mixtures of secreted antigens from medically important helminths such as schistosomes, liver flukes and filarial worms will be analysed. By combining different cell lines expressing one carbohydrate-recognizing lectin and flow cytometry, binding studies will be performed in order to assess which lectins bind to which helminth glycoproteins. In addition, the glycosylation of the helminth molecules can be modified enzymatically to be able to have a more detailed look on the exact glycan elements involved. Finally, the response of immune cells such as dendritic cells and macrophages towards helminth glycoproteins can be investigated by measuring cytokine production after co-incubation of the cells with the helminth antigens.

Laboratory skills: (a selection of) biochemistry and molecular biology tools and immunological assays, such as mass spectrometry, fluorescent labeling of helminth glycoproteins, cell culture, flow cytometry and ELISA.

Internship topic IV: Improving Genetically Attenuated Malaria Vaccines I: enhancing immunogenicity

Contact persons: Shahid Khan, Blandine Franke-Fayard and Chris Janse; B.Franke-Fayard@lumc.nl, S.M.Khan@lumc.nl, C.J.Janse@lumc.nl

Background: Malaria threatens the lives and livelihood of more than 50% of the world’s population, with around 300 million cases and a million deaths per year; it is one the world’s most important global health challenges. While vaccines remain the most cost effective methods of disease control and ultimately eradication, no licensed anti-malaria vaccine exists. It has been shown in studies with rodent and in experimental clinical studies with humans that immunization with live sporozoites attenuated by radiation can induce strong protective immunity. In rodent models of malaria similar protective immunity has been achieved by immunization using genetically modified sporozoites. These so called genetically attenuated parasites (GAP) have been generated by removing genes essential for liver stage development. The LMRG are experts in Plasmodium genetic modification, immunization and protection studies in both rodents AND humans malaria and have developed robust pre-clinical screening protocols to evaluate the suitability of GAPs for vaccination. Despite the rapid progress made in translating observations made in the rodents to now having a GAP ready for use in human, a number issue still remain with GAP vaccines in their current form. These include reducing the dose and number of immunizations of sporozoites to ensure complete protection, determining a clinically viable route administration and establishing that GAP-based immunity is strain (and even species) transcending. Therefore we wish to create a next, or second, generation GAP vaccine that can achieve the most comprehensive protection with the fewest parasites and doses that can be administered in a manner that is suitable for mass vaccination of children and infants in malaria endemic countries.

Research question: For next generation GAP vaccines, we would like to address several separate but related and critical areas of research (see this topic and topic IV)

- Can we make each attenuated sporozoites more potent immunologically by introducing genes that encode for example molecules that can acts as adjuvants (i.e. addition of TLR agonists into the parasites); thereby reducing the number of sporozoites required per immunizing dose? This research will require genetic modification of malaria parasites,
analysis of the fitness of genetically modified and attenuated parasites and analysing protective immunity by immunising mice with the genetically attenuated sporozoites

- Can we make GAPs more versatile and therefore cost effective by having them express vaccine antigens from multiple Plasmodium strains and species and, even, other pathogens?
- Studies performed on improved GAP potency in rodents will be used to inform the creation of equivalent enhanced human Plasmodium GAPs that can serve as a vaccine. This will require generating transgenic *P. falciparum* parasites that express adjuvants and/or multi-stage proteins; an activity that will require utilizing the latest CRISPR/Cas9 methodologies that have been developed in the Leiden Malaria Research Group.

This research will require genetic modification of malaria parasites, analysis of the fitness of genetically modified and attenuated parasites and analysing protective immunity by immunising mice with the genetically attenuated sporozoites.

**Laboratory skills:** Molecular Biology (standard and CRISPR/Cas9 based cloning/transfection, Southern, Northern and Western analyses), Cell Biology (in vivo imaging, cell culture, parasite culture, fluorescent microscopy), Immunology (immune responses to immunization in mice), Vaccinology (pre-clinical screening of GAP-based vaccines).

**Internship topic V: Improving Genetically Attenuated Malaria Vaccines II: identification of parasite proteins essential for inducing protective immunity**

Contact persons: Blandine Franke-Fayard, Shahid Khan and Chris Janse; B.Franke-Fayard@lumc.nl, S.M.Khan@lumc.nl, C.J.Janse@lumc.nl

**Background:** Malaria threatens the lives and livelihood of more than 50% of the world’s population, with around 300 million cases and a million deaths per year; it is one the world’s most important global health challenges. While vaccines remain the most cost effective methods of disease control and ultimately eradication, no licensed anti-malaria vaccine exists. It has been shown in studies with rodent and in experimental clinical studies with humans that immunization with live sporozoites attenuated by radiation can induce strong protective immunity. In rodent models of malaria similar protective immunity has been achieved by immunization using genetically modified sporozoites. These so called genetically attenuated parasites (GAP) have been generated by removing genes essential for liver stage development. The LMRG are experts in Plasmodium genetic modification, immunization and protection studies in both rodents AND humans malaria and have developed robust pre-clinical screening protocols to evaluate the suitability of GAPs for vaccination. Despite the rapid progress made in translating observations made in the rodents to now having a GAP ready for use in human, a number issue still remain with GAP vaccines in their current form. These include reducing the dose and number of immunizations of sporozoites to ensure complete protection, determining a clinically viable route administration and establishing that GAP-based immunity is strain (and even species) transcending. Therefore we wish to create a next, or second, generation GAP vaccine that can achieve the most comprehensive protection with the fewest parasites and doses that can be administered in a manner that is suitable for mass vaccination of children and infants in malaria endemic countries.

**Research question:** For next generation GAP vaccines, we would like to address several separate but related and critical areas of research (see also Parasitology internship topic VI)

- Can we identify proteins of the parasite that are exported into the hepatocytes, which may potentially good targets of CD8 T-cell based immunity
• The project will focus on characterizing genes/proteins expressed during liver stage development; by fluorescent tagging of proteins (transgenes) and generation of gene deletion parasites. This research will require genetic modification of malaria parasites, analysing the fitness of genetically modified parasites (transgenic and knock-out parasites) and by examining the location of tagged proteins.

**Laboratory skills:** Molecular Biology (cloning, Southern, Northern and Western analyses), Cell Biology (in vivo imaging, cell culture, parasite culture, fluorescent microscopy), Immunology (immune responses to immunization in mice), Vaccinology (pre-clinical screening of GAP-based vaccines).

Internship topic VI: Modulation of inflammation by malaria parasites: role of the skin and implications for immune responses and vaccination.*

* Together with the Leiden Malaria Research Group

Contact person: Dr. B. Franke-Fayard, B.Franke-Fayard@lumc.nl; Dr. Hermelijn H. Smits, h.h.smits@lumc.nl; Dr Meta Roestenberg, M.Roestenberg@lumc.nl

**Background:** Malaria remains one of the major health issues in much of the tropics and subtropics. The CDC estimates that there are 300-500 million cases of malaria each year that result in 800.000 to 1 million deaths, primarily children under 5 (http://www.cdc.gov/malaria/. The parasite exists within two hosts and has many morphologically and antigenically distinct forms. Malaria control is hampered by parasite drug resistance against all available anti-malarials and the lack of an effective vaccine. The most promising and advanced vaccine candidates in clinical development target the clinically silent sporozoite stage and liver forms of the parasite. Blocking parasite liver infection will not only prevent the initiation of the pathogenic blood stage but also prevent the development of the transmissible sexual stages of the malaria parasite. As such, these vaccines are a very powerful tool for malaria control. However, many of such vaccine candidates rely on the administration of live attenuated parasites. One of the bottlenecks in the development of an attenuated sporozoite malaria vaccine is the route and method of sporozoite administration. Intravenous immunization with sporozoites is effective in rodents and non-human primates, and being studied in humans, but is not yet used for licensed vaccines for infectious diseases. Intradermal and subcutaneous immunization procedures reduced protective efficacy, which both in rodents and humans, is associated with a decreased degree of parasite liver infection during immunization.

**Research question:** The objective of this study is to decipher and compare the immune responses derived from different routes of sporozoite administration (in rodent and human skin). These results will be translated into an alternative method for administration of attenuated sporozoites which can subsequently be tested in clinical trial. As such, it will provide a rational design for delivery of *Plasmodium falciparum* sporozoites and improve the efficacy of the human liver stage vaccination.

The aims of the project are:

• To determine the type of parasite – host cell interactions and immune responses induced against live-attenuated sporozoites in the skin and draining lymph nodes, comparing:
  - site of administration
- devices of parasite delivery
- use of adjuvants

- To establish correlation between parasite liver loads, immune interactions in the skin and (protective) immune responses against the liver stage of malaria.

**Involved laboratory skills:** Cell culture, flow cytometry, cell isolation from soft organ tissues, cell separations by magnetic beads, flow sorting, microscopy, in vivo imaging*, mouse handling*, dissecting lymph nodes*

*Note: If preferred, a sub-question of this project can be selected which does not require mouse handling or contact with animals

**Internship topic VII: Role of helminth molecules in the development of regulatory B cells**

**Contact person:** Dr. Hermelijn H. Smits, h.h.smits@lumc.nl

**Background:** The incidence of allergic diseases is strongly increased in industrialized countries during the past few decades and its prevalence is negatively associated with microbial exposure, such as having chronic helminth infections or living on a farm. It has been hypothesized that early microbial exposure is instrumental for the education of the immune system, leading to a fully developed regulatory arm and protection against allergic diseases. One of the cytokines that is central to this regulatory arm of the immune system is IL-10. IL-10 can be produced by regulatory T cells, but recent reports show that also B cells can produce IL-10. Initially, it has been demonstrated in experimental autoimmunity models that IL-10 producing B cells harbor a suppressive function leading to dampening of immune responses and were, therefore, called regulatory B (Breg) cells. Recent studies have shown that Breg cells indeed play a critical role and have clinical relevance in several autoimmune diseases and in allergic inflammation as well. Interestingly, helminth infections form an important trigger for the activation and/or induction of Breg cells in mice and these helminth-induced Breg cells could give protection against allergies. Different autoimmunity models have suggested that Toll-Like Receptor (TLR) ligands and/or B cell receptor (BCR) triggering in combination with CD40 ligand could drive to Breg development as well.

**Research question:** The aim is to investigate whether helminth molecules can drive the development of regulatory B cells, what pattern recognition receptors are targeted and what their functional capacities are.

**Laboratory skills:** Flowcytometry, (B and T) cell culture, PBMC isolation, ELISA/luminex, Cell separations by magnetic beads and flow sorting

**Internship topic VIII: Helminth infections and protection against allergic airway inflammation**

**Contact person:** Dr. Hermelijn H. Smits, h.h.smits@lumc.nl
Background: Helminth parasites have been shown to protect against allergic airway disease and asthma in both epidemiological and experimental studies. They are master regulators of the immune system and strongly suppress T-cell responses against themselves, but also to bystander antigens like allergens. Dendritic cells (DCs) are specialized in sensing environmental signals, including those from invading pathogens, and orchestrating appropriate immune responses or tolerance. New evidence is emerging that pathogens can influence the effector function of DCs and prime of regulatory responses, thereby inducing protection against inflammatory conditions, such as allergic asthma.

Research question: We aim to identify molecules isolated from schistosomes which protect in an allergic airway inflammation model and investigate how these molecules can influence DC function.

Involved laboratory skills: Flow cytometry, (dendritic and Treg) cell culture, cell isolation from soft organ tissues, cell separations by magnetic beads, flow sorting, mouse handling*, Lung lavage*, dissecting lymph nodes*

*Note: If preferred, a sub question of this project can be selected which does not require mouse handling or contact with animals

Internship topic IX: Immunometabolism of dendritic cells

Contact person: Dr. Bart Everts, b.everts@lumc.nl

Background: Dendritic cells (DCs) are key regulators of both immunity and tolerance by controlling activation and polarization of effector T helper cells (Th) and regulatory T cell responses (Treg). Therefore, there is a major focus on developing approaches to manipulate DC function for immunotherapy. It is well known that changes in cellular activation are coupled to profound changes in cellular metabolism. However, only recently the picture is emerging that manipulation of cellular metabolism can be used to shape immune responses. This field of immunometabolism is rapidly evolving as one of the new frontiers in science. Nonetheless, still little is known about the metabolic processes that support DC activation or about the metabolic requirements for DCs to drive Th1, Th2 or Treg responses.

Research question: This project aims to characterize the metabolic properties of in vitro cultured DCs with Th1, Th2 and Treg polarizing properties and to explore the role of DC metabolism in T cell-polarization.

Involved laboratory skills: Flow cytometry, ELISA, dendritic cell and T cell culture, molecular biology (real-time PCR, western blot), metabolic flux analysis.
Internship topic I: The influence of viral infections on NK cell repertoire after allogeneic hematopoietic stem cell transplantation

Contact persons: Drs. G. Lugthart, g.lugthart@lumc.nl; Dr. M. W. Schilham, M.W.Schilham@lumc.nl

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for children with high risk hematological diseases. After HSCT the immune system of the patient is usually severely compromised. Natural killer (NK) cells are the first lymphocytes to reach normal levels, and may play a pivotal role in defense against viral infections during the first few months after HSCT. The relationship between viral infections and NK cells will be studied after HSCT. Furthermore if NK cells are indeed playing a role in combating infections, they should be able to migrate towards sites of infection. At present, in contrast to T cells, no data are available on the kinetics of chemokine receptor expression and migration of NK cells during viral infections. To perform a thorough analysis of the correlation between HSCT parameters and immune reconstitution, a large and well documented patient cohort and corresponding biobank are available.

Research question: We aim to investigate in patients after HSCT the relationship between viral infections and NK cell reconstitution with a specific focus on chemokine receptors.

Laboratory skills: Human primary cell culture; FACS; Microscopy; Chromium release cytotoxicity assay; ELISA
Pulmonology

General contact person of the department for internships: Prof. dr. Pieter S. Hiemstra, p.s.hiemstra@lumc.nl

Internship topic I: Role of airway epithelial cells in chronic obstructive pulmonary disease (COPD) and asthma

Contact persons: Prof. dr. Pieter S. Hiemstra (p.s.hiemstra@lumc.nl)  
Dr. Anne van der Does (a.van_der_does@lumc.nl)

**Background:** Airway epithelial cells act as a first barrier against a large range of inhaled challenges, and play a central role in host defense against infection, inflammation, immunity, and tissue remodelling in the lung. Therefore these cells have been implicated in the pathogenesis and progression of a range of acute and chronic inflammatory and infectious lung diseases, including asthma and COPD. In various projects in the lab we study the function of these cells in asthma and COPD using advanced cell culture techniques of primary cells (including air-liquid interface culture). These studies include studying the proresolving role of microbiome-derived mediators and of omega-3 and -6 fatty acids, as well as the effect of vitamin D on epithelial host defense, including production of antimicrobial peptides and inflammatory mediators. To this end we use a variety of challenges relevant to asthma and COPD, including microbial exposure (bacterial and virus) and cigarette smoke, as well as co-cultures with e.g. macrophages, neutrophils and dendritic cells.

**Research question:** The various projects related to epithelial cell function in COPD and asthma have different individual research questions, all related to the central role of the airway epithelium in host defense and immunity in asthma and COPD.

**Laboratory skills:** Cell culture, ELISA, qPCR, Western blot, cellular imaging

Internship topic II: Modulation of allergic airway inflammation by microbial molecules

Contact person: Dr. Gerrit John-Schuster, G.John-Schuster@lumc.nl

**Background:** Both epidemiological and experimental studies have provided evidence that certain gastro-intestinal bacteria like *Helicobacter pylori* and helminth parasites may protect against allergic airway disease and asthma. They have been shown to regulate immune responses and induce T cell suppression against themselves, but also against bystander antigens such as allergens. In this project, a collaboration between the Department of Pulmonology and Parasitology [Dr. Hermelijn Smits], we aim to identify novel therapeutic approaches based on protective molecules from *H. pylori* and schistosomes. The protective capacities of selected molecules from *H. pylori* and
schistosomes and their mode of action will be assessed using experimental allergic airway inflammation induced in mice by exposure to house dust mite.

**Research question:** What is the protective capacity of selected molecules from *H. pylori* and schistosomes against experimental allergic airway inflammation in a mouse model of house dust mite exposure?

**Laboratory skills:** animal models (mice), FACS, immunohistochemistry, qPCR

**Internship topic III: Lung tissue repair in chronic obstructive pulmonary disease (COPD)**

Contact persons: Prof. dr. Pieter S. Hiemstra (*p.s.hiemstra@lumc.nl*)
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**Background:** Irreversible damage to lung injury and disrupted regeneration of damaged lung tissue are a major problem in chronic inflammatory lung diseases such as chronic obstructive pulmonary disease (COPD). Various treatment strategies are being explored, including stem cells, mesenchymal stromal cells and pharmacological modulation of endogenous repair. In two projects we are studying lung regeneration. In project 1 we are developing human alveolar epithelial and endothelial cells from human induced pluripotent stem cells (hiPSCs), with the aim to develop a lung-on-a-chip system mimicking the human alveolus. In project 2 we are studying disturbed Wnt-signalling in COPD, underlying causes for this disruption and options for correcting Wnt signalling to enhance repair.

**Research question:** Project 1: Can we develop lung alveolar epithelial and endothelial cells from hiPSCs for growth on flexible membranes for the ultimate development of a lung-on-a-chip?
Project 2: Why is Wnt-signalling disturbed in lung tissue in COPD, and can this be corrected to facilitate lung repair?

**Laboratory skills:** Cell culture, qPCR, Western blot, cellular imaging
Rheumatology

General contact person of the department for internships: Prof. dr. R.E.M. Toes, r.e.m.toes@lumc.nl

In general, the immune system is reacting against foreign intruders to maintain the integrity of the body. Therefore, it is required that it can distinguish self- from foreign antigens. However, in some cases, the immune system can also react to self-antigens to which it normally should not respond. Apparently, the balance between the activation of “regulatory” mechanisms, which normally prevent the arousal of unwanted immune responses, and “inflammatory” mechanism, which normally fight intruding micro-organisms, is distorted. Within the department of Rheumatology, the checkpoints and routes leading to (un)desired immune responses are studied in detail. Therefore, the department offers several internship possibilities in the field of (auto)immunity and immune regulation.

Internship topic I: The phenotype, magnitude and regulation of autoantibody-producing B cells in RA

Contact person: Prof Dr Rene Toes, R.E.M.Toes@lumc.nl

Background: Autoantibodies against citrullinated protein antigens (ACPA) are highly specific for rheumatoid arthritis (RA). Increasing experimental data indicate that ACPA could be involved in the pathogenesis of RA. In contrast to “conventional” (recall) antigens such as tetanus toxoid, ACPA-producing B cells are chronically and systemically exposed to antigen. Recently, we have obtained evidence that the ACPA B cell response differs from conventional antibody responses, as the majority of ACPA do not undergo avidity-maturation despite extensive class-switch recombination. These observations indicate that ACPA-producing B cells are not submitted to the same regulatory pathways as are conventional B cells, and that the biology and possibly molecular make-up and/or requirements for survival signals are thus distinct. Only very limited data are currently available describing the phenotype and regulation of ACPA-producing B cells and no information is present on ways to specifically inhibit these cells. So far, this is due to difficulties in growing and/or visualizing ACPA-producing B cells ex-vivo.

Research question: Recently, we successfully designed a system to culture ACPA-producing B cells from RA patients in vitro. The aim of this internship proposal is to examine the phenotype, magnitude and regulation of ACPA-producing B cells in RA.

Laboratory skills: We will sort B cells and B cell subpopulations isolated from patients with ACPA positive RA to high purity using specific markers and inhibit defined molecular signaling moieties to determine the phenotype and relevant regulatory mechanisms of ACPA-producing B cells. These studies will provide insight into the characteristics of ACPA-producing B cell responses and could lead to the identification of novel targets for the inhibition of autoantibody-producing B cells. The techniques we will use are cell culture, FACS, proliferation assay, suppression assay, human immunology, ELISA,
Internship topic II: The response modes of repeatedly stimulated mast cells

Contact person: Prof. Dr Rene Toes, R.E.M.Toes@lumc.nl

**Background:** Mast cells are well known for their ability to respond quickly and strongly to different stimuli and their role in, for example, allergies is well described. In allergic reactions, mast cells can respond immediately and even mediate anaphylactic shock through the abundant and swift release of a variety of mediators. However, the “natural” place of the mast cell in the immune system is, most likely, not found in mediating allergies reactions, but rather in the immune response against chronic infections with large parasites that cannot be “eaten” by immune cells. In this respect, it is tempting to speculate that, by analogy, mast cells also play a role in autoimmune diseases as these are also characterized by the chronic, abundant and persistent presence of (auto-) antigens that cannot be eradicated by the immune system, quite similar to situations found in, for example, worm infections. Indeed, mast cells have been implicated in several rheumatic conditions such as Rheumatoid Arthritis and it has been reported that they play a crucial role in arthritis in mouse models. Nonetheless, little to no information is currently available on the response of mast cells to chronic auto-immune stimuli such as found in the synovial compartment of patients with rheumatic diseases.

**Research question:** In the context of this project, we intend to study the response modes of mast cells that have- or have not been exposed repeatedly to different immune stimuli, as well as the regulation governing these response modes.

**Laboratory skills:** Cell culture, PCR, bio-informatic approaches, molecular biology and immunology

Internship topic III: Identification, localization, and function of oligosaccharides on autoantibodies

Contact person: Prof. Dr Rene Toes, R.E.M.Toes@lumc.nl

**Background:** Anti Citrullinated Protein Antibodies (ACPA) are highly specific for RA and are implicated in disease pathogenesis. Recently we discovered a unique property of these antibodies through the observations that ACPA are 10-20Kd larger as compared to other immunoglobulins. Our preliminary data further revealed that this increase in size is explained by hyperglycosylation of the Fab-fragment. This was not seen for other auto-antibodies or antibodies against recall antigens. We hypothesize that this hyperglycosylation is intimately involved in the pathogenic-potential of ACPA.

**Research question:** The goal of this project is to define the nature, precise location and biological relevance as well as to delineate the potential diagnostic value of ACPA-hyperglycosylation. These studies highlight an entirely novel aspect of ACPA biology and might have important implications for understanding ACPA pathogenicity. Therefore, they could reveal novel insights into the biological action mediated by auto-antibodies and identify a new biomarker for RA.

**Laboratory skills:** We intend to isolate ACPA and identify the nature and localization of the oligosaccharides by mass-spec. Likewise, the ability of ACPA to target specific sugar-binding lectins/siglecs will be analysed as well as their ability to activate immune cells through the Fab-linked sugar residues. Moreover, we intend to investigate the epidemiological aspects of ACPA Fab
glycosylation in different cohorts of (pre-)RA patients. The techniques we will use are laboratory skills: Glyco-analyses of proteins, biochemistry, protein purification and chemistry, SDS-page et cetera.

**Internship topic IV: The breach of tolerance and induction of immunity against self-proteins in arthritis**

Contact person: Prof. Dr Rene Toes, R.E.M.Toes@lumc.nl

**Background:** Rheumatoid Arthritis (RA) is characterized by heterogeneous patient populations that do not respond uniformly to therapeutics. The heterogeneous response to biologicals most likely exists because the specific biological pathway targeted by the drug may not be active in the particular patient subgroup. Currently, we are unable to determine biological pathways underlying RA pathogenesis that are involved in individual RA patients. Recently, our group identified a novel class of auto-antibodies in RA, directed towards carbamylated proteins (CarP). We demonstrated that the presence of anti-CarP antibodies predict the severity of joint damage in ACPA negative patients. Likewise, our data obtained last year demonstrated that the presence of anti-CarP antibodies in arthralgia patients predicts towards development of RA independent of ACPA. However, currently no information is available on biological pathways leading to anti-CarP antibody positive RA. Intriguingly, we recently discovered the emergence of anti-CarP immunity upon arthritis induction in animal models.

**Research question:** The goal of this study is to understand the breach of tolerance and induction of immunity against carbamylated proteins, and the relevance of anti-CarP immunity in arthritis.

**Laboratory skills:** Cell culture, T- and B-cell analyses, ELISA, ELIspot, diverse array of cellular immunology techniques.

**Internship topic V: The infrapatellar fat pad: a new player in osteoarthritis?**

Contact person: Andreea Ioan-Facsinay, PhD; a.ioan@lumc.nl

**Background:** Obesity is a risk factor for development and progression of Osteoarthritis. The biological pathways underlying this association are still incompletely understood. Recent research has shown that obesity is accompanied by inflammation in adipose tissue which leads to release of various inflammatory cytokines, adipokines, lipids from this tissue. These soluble mediators could influence systemic metabolism and could affect neighboring tissues. In previous research, we have investigated a rather neglected adipose tissue in the knee joint: the infrapatellar fat pad (IFP). We have found that this tissue becomes more inflammatory in obesity. Interestingly, we have shown that soluble mediators released by this tissue have immunomodulatory properties, being able to influence immune cells, such as T cells and macrophages. Our current research aims at understanding which soluble mediators mediate this effect and which molecular mechanisms are involved in the modulation. Several research questions are part of this line of research and the student will be able to choose between these.

**Research questions:**

1) How do fatty acids modulate the function of CD4+ T cells
2) How do fatty acids modulate the function of macrophages

**Involved laboratory skills:** Culturing cells and tissues under sterile conditions, FACS, ELISA, Western blot, Proliferation assays (CFSE dilution and tritium-thymidine incorporation)

**Internship topic VI: Pro-resolving lipid mediators in inflammatory arthritis**

Contact person: Andreea Ioan-Facsinay, PhD; a.ioan@lumc.nl

**Background:** Accumulating evidence suggests that inflammation plays a key role in the loss of joint homeostasis in osteoarthritis (OA) and rheumatoid arthritis (RA). Poly-unsaturated fatty acid metabolites such as lipoxins, resolvins, protectins and maresins are among the soluble factors generated during the resolution phase of inflammation. These have been described as potent anti-inflammatory and pro-resolving molecules in mouse models of acute inflammation. The role of specialized pro-resolving lipid mediators (SPM) in chronic inflammation is less understood, although some data indicate a possible beneficial role of SPM in arthritis, with resolvin D1 (RvD1) alleviating pain in a rat model of arthritis, and LXA4 having an anti-inflammatory function in a mouse model of arthritis. The role of SPM in OA/RA is yet unexplored. Likewise, the effect of these lipids on joint tissues such as synovial fibroblasts and resident macrophages, cartilage and bone is largely unknown, with only few reports indicating resolvin E1 (RvE1) to inhibit osteoclast differentiation in mice and LXA4 to have anti-inflammatory effects on synovial fibroblasts. The interaction of other SPM with various joint tissues is still unknown. This knowledge would be, however, highly relevant in estimating the possible therapeutic benefits of these potent anti-inflammatory/pro-resolving mediators in OA/RA.

Our current research aims at understanding what is the role of specialized pro-resolving lipid mediators in the disease process in Osteoarthritis and Rheumatoid Arthritis.

**Research question:** We are currently focusing on 3 main questions:

1) Are SPM present in the arthritic joint and how do they correlate with disease activity?
2) Which joint tissues can produce SPM and which joint tissues can be target of SPM?
3) Does treatment with SPM ameliorate disease in mouse models of Osteoarthritis and Rheumatoid Arthritis?

**Involved laboratory skills:** cell culture, FACS, Western Blot, ELISA, mouse handling, qPCR, LC/MS-MS (optional)

**Internship topic VII: Functional genomics and transcriptomics to understand mechanisms of inflammation**

Contact person: Fina Kurreeman, b.a.s.kurreeman@lumc.nl

**Background:** Inflammation is a key biological mechanism for maintaining the immunological integrity of higher organisms. While designed to protect the body from infections, uncontrolled chronic
inflammatory reactions are detrimental and represent the underlying cause of numerous socio-economically relevant disorders (e.g. rheumatoid arthritis (RA), inflammatory bowel disease, celiac disease, heart disease, alzheimers, etc). It is therefore not surprising that chronic inflammation has been termed the “silent killer” in the modern world and is responsible for large costs within the healthcare system. The mechanism behind chronic inflammatory processes is not well understood. One of the leading thoughts in the field is that chronic inflammation occurs when repetitive acute inflammation is not resolved properly. Our current research is centered around understanding the global changes that occur during this process in specific cell types of immune origin.

**Research questions:**

1. How do specific cells of the immune system respond to repeated stimulations and which pathways are dysregulated.
2. Of these molecules that seem to have a dysregulation, which mechanisms control these changes. One of the interesting questions in the field is are these sustained epigenetic changes.

**Laboratory skills:** Culturing cells and tissues under sterile conditions, FACS, ELISA, DNA/RNA isolation, qPCR, RNA sequencing, shRNA knockdown, Bioinformatics and Statistics
Internship abroad

Eurolife

LUMC is part of Eurolife. This is a network of eight prestigious European universities which aims to facilitate collaborative research, the exchange of researchers and research students and the creation of new research opportunities.

Eurolife universities are:

University of Edinburgh - College of Medicine and Veterinary Medicine
Leiden University Medical Center
Universitat de Barcelona
Trinity College Dublin - School of Medicine
Medical University of Innsbruck
University Medical Center Göttingen
University of Strasbourg
Karolinska Institutet

Gabon, Indonesia and Ghana

In collaboration with the department of Parasitology (LUMC), there are possibilities to perform an internship in Gabon, Indonesia or Ghana. Please contact Prof. Maria Yazdanbakhsh (m.yazdanbakhsh@lumc.nl).

For additional information, please visit the website of the profile area Immunity, Infection and Tolerance: https://www.lumc.nl/research/medical-research-profiles/immunity-infection-tolerance/