# PhD project No. 19, Prof. Grimbacher

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| **Scientific Area** | Immune-related diseases |
| **Three project titles** | A) GENETIC ENGINEERING OF BACTERIA TO COMBAT INFLAMMATORY BOWEL DISEASE<br>B) UNDERLYING MECHANISMS IN IMMUNODEFICIENCY AND IMMUNE DYSREGULATION SYNDROMES DUE TO ACTIN CYTOSKELETON ABNORMALITIES<br>C) DEVELOPMENT OF THE GENETIC IMMUNOLOGY ADVISOR PLATFORM: GEMMA-P |
| **Host country** | Germany |
| **Supervisor, institution** | Bodo Grimbacher, Medical Center - University of Freiburg, Germany |
| **Co-Supervisor, institution** | A), B) Sophie Jung, Straßbourg<br>C) tbd |
| **Mentor, institution** | A), B) and C) tbd |
| **Secondment institution** | A) and B) University of Strasbourg, France |

**Short description of the supervisor's lab with introduction to the topic**

https://www.uniklinik-freiburg.de/cci/forschung/bodo-grimbacher.html

**Topic description, including techniques to be used**

**Project A)** Immune homeostasis is critical for the integrity of the mucosal surface. In case of imbalances, gut inflammation is the consequence. Imbalances can either be caused by invading pathogens such as salmonella in case of food poisoning, or alternatively, by an impaired mucosal immune system, allowing the growth of pathogenic bacteria. One example are chronic inflammatory bowel diseases (IBD) such as Crohn's disease or Ulcerative colitis in which a defective interaction between an impaired host immune system and the colonizing microbiome leads to chronic bowel inflammation (PMID: 24560869). One reason for an impaired host immune is the lack of regulatory T cells or the lack of interleukin 10 (IL10) (PMID: 19890111; 20934598; 22236434). Replacing IL10 in IBD has been tried, but oral administration leads to an immediate degradation in the acidic stomach/duodenum and systemic administration of IL10 by subcutaneous injections had unwanted side effects (PMID: 9883011: 7729636).

In this project we will replace IL10 on the mucosal surface by genetically engineered lactobacilli which will produce and secrete human IL10 on the mucosal surfaces. Certain orally administered lactobacilli pass the gastrointestinal tract without long-term colonization and without side effects.

We plan to stably transfect lactobacilli to produce and secrete murine IL10. We will test their anti-inflammatory potency in an IL10-deficient mouse model of inflammatory bowel disease (PMID: 7554464). We plan to show that bacterial (lactobacilli)-delivered IL10 is capable of replacing the lack of macrophage or regulatory T cell-derived IL10, curing the gut inflammation in IL10-deficient mice. In a second and third set of experiments, the established and similar bacterial systems will be used to express other immune regulatory effector molecules on mucosal surfaces such has human cytokines and chemokines in order to support and strengthen the mucosal immune system in other medical conditions, hopefully without any side effects.

**Project B)** The actin cytoskeleton plays a pivotal role in many aspects of the immune response, ranging from immune cell development and migration, to inter and intracellular signalling. Its importance is reflected by the growing list of a complex subgroup of primary immunodeficiencies (PIDs) called "actinopathies" that are caused by deleterious mutations in genes involved in actin cytoskeleton regulation (Sprenkeler et al. J Innate Immunol 2020; Tangye et al. Immunol Cell Biol 2019). In addition to immunodeficiency, patients with actinopathies frequently present multiple autoimmune manifestations, which have been associated with defective migration and adhesion of B and T cells, as well as altered B/T-cell receptor signalling and immune synapse formation. Therefore, a better understanding of the actinopathies is crucial for the development of targeted

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therapy to improve patients' quality of life and life expectancy. In this project, we aim: (1) to identify and functionally validate novel actinopathies-causing mutations in PID patients from Freiburg and Strasbourg cohorts, in particular those presenting associated autoimmune manifestations. Indeed, our Genetic Unit is specialized in next generation sequencing including targeted gene panels and whole exome sequencing. (2) to develop common laboratory tools for the precise characterization of actinopathies (e.g., time-lapse imaging and confocal microscopy for the evaluation of cell migration and immune synapse formation). Both laboratories have access to microscopy core facilities with various imaging technologies. (3) to characterize B and T cell cytoskeleton-related defects observed in two different PIDs that are currently being studied in our laboratories, as well as in the newly identified actinopathies. This work will provide mechanistic insight into the pathogenesis of actinopathies but also a better understanding of normal actin regulation in immune cells.

**Project C)** The rapid advancement of NGS technologies and its exponential cost drop over the last decade has led to a vast accumulation of sequencing data in large .fastq/.bam files. Generally, these are bioinformatically processed into variant calling files (.vcf) and eventually into spreadsheets containing SNV, Indel and/or CNV annotations, which are then not stored in a uniform and centralized manner. Moreover, result interpretation and reporting pose a challenge to clinical immunologists. Therefore, we designed an SQL-based database intended to overcome the data and metadata storage challenge, which facilitates combined data analysis and research, result interpretation, and reporting.
Our database (GEMMA-DB) currently stores and integrates clinical, laboratory values, experimental and NGS data (whole exome, targeted gene panels, and 16s-rRNA). These datasets are combined with curated data extracted from the scientific literature, public resources (e.g. from Ensembl, NCBI or OMIM), and standard and hierarchical terminology from different biological and medical ontologies, such as the HPO, GO, ORDO, ICD10, NCIT, MESH, among others. GEMMA-DB currently contains over 3,700 families with more than 6,800 individuals, including around 800 unaffected subjects and over 1,600 subjects who have been reported in the literature. Genetic data is available for more than 2,800 individuals, ~2000 of which were sequenced using targeted gene panels and ~800 were exome sequenced. 16s rRNA-seq data is available for 867 samples from 629 different individuals. All these datasets only use 10 Gb of space.
In order to facilitate user interaction with the database, we developed an internal prototype web application (written in PHP, HTML, CSS, and JQuery). This user-friendly interface (combined with our database) has significantly improved work efficiency in regard to data management and storage, analysis and reporting, data curation and result interpretation, and patient registry. However, this web application is only a prototype and a more sophisticated platform is needed in order to perform advanced analysis and attract different types of users, but also to allow access to external collaborators.
Our group is now seeking for a highly motivated PhD candidate with a background in Computer Science or (Bio)Informatics and interest or knowledge in biology, genetics and/or immunology. The successful applicant will be required to develop the above-mentioned platform, which should be preferentially written in Python and web languages.

**Recommended applicant's training (technical expertise and knowledge)**

Techniques: see project description above
Knowledge: Msc in Biology, Molecular Medicine, or Computer Sciences, Programming.

**Maximum two relevant publications**

Sprenkeler et al., 2020, J Innate Immunol
Tangye et al., 2019 Immunol Cell Biol

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# Ethics description

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| **1. Humans** | |
| This research involves human participants. | YES ☒ / NO ☐ |
| This research involves physical interventions on the study participants. | YES ☐ / NO ☒ |
| **2. Human Cells /Tissues** | |
| This research involves human cells or tissues, such as blood. | YES ☒ / NO ☐ |
| **3. Personal Data** | |
| This research involves personal data collection and/or processing. | YES ☒ / NO ☐ |
| This research involves further processing of previously collected personal data (secondary use). | YES ☒ / NO ☐ |
| **4. Animals** | |
| This research involves animals, such as mice. | YES ☒ / NO ☐ |

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