Glucocorticoids; hub between infection-susceptibility and inflammation treatment

The current project will be conducted through collaboration between Technische Universität Dresden (TUD), the University of Zurich (UZH), and ETH Zurich. It is part of an International Research Training Group (IRTG3019) titled "**Metabolic and Endocrine Drivers of Infection Susceptibility**" comprising a total of 9 projects. Within this collaboration, students will have the opportunity to obtain a joint certificate from TUD and the universities in Zurich. The current project will take place in Dresden, with the option of an exchange to Zurich, under the supervision of Dr. Charlotte Steenblock.

Project 6: Glucocorticoids; hub between infection-susceptibility and inflammation treatment

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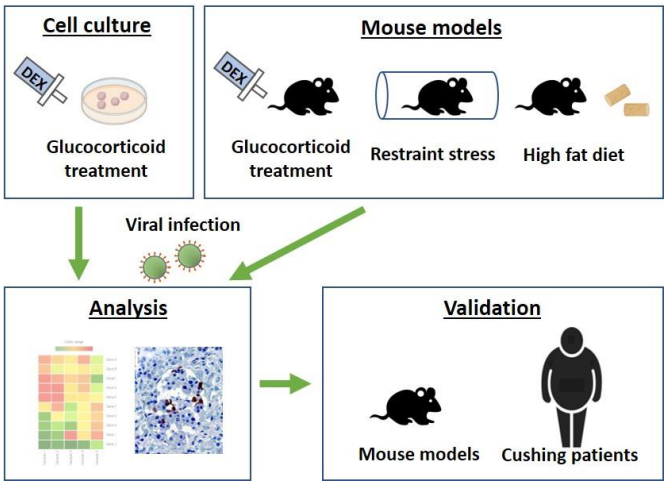
Background: Hypercortisolism (Cushing's syndrome) can be either endogenous, due to pituitary or adrenal tumours, or exogenous (iatrogenic) as a consequence of long-term exposure to excessive glucocorticoids. 3% of the population in western countries receive glucocorticoid treatment for chronic diseases, such as asthma or rheumatoid arthritis. In addition to a number of comorbidities, such as hypertension, diabetes, overweight, and myopathy, patients with Cushing's syndrome frequently suffer from multiple infections, including respiratory pathogens, that may lead to a fatal outcome if treatment is not initiated in time [1]. Upon immune challenge, the pro-inflammatory cytokines tumour necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6, and type I interferons (e.g. IFN-α/β) are released from a variety of cell types, including activated immune cells, vascular endothelial cells, fibroblasts, and neurons. Furthermore, the T cell cytokines IL-2 and IFN-γ (type II interferons) are important for mediating anti-viral defences. In addition to contributing to the progression of the immune response against viral infections, the cytokines can activate the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of adrenal glucocorticoids [2]. Oppositely, glucocorticoids exhibit negative feedback onto immune cells to suppress further synthesis and release of cytokines, thereby protecting the host from the detrimental consequences of an overactive immune response (e.g., tissue damage, autoimmunity, and septic shock) [3], as observed in the COVID-19 pandemic where glucocorticoids were used to treat COVID-19. This bidirectional relationship between the HPA axis and the immune system is not fully understood.

Aims: The overall goal is to elucidate the impact of glucocorticoids on infection susceptibility, thus evaluating why Cushing patients are more susceptible to infections. Consequently, the following aims will be addressed: 1) *In vitro* assessment of the impact of glucocorticoids on virus infections and innate immunity using cell culture models. 2) Assessment of the impact of chronic hypercortisolism on infection susceptibility in animal models. 3) Assessment of the effects on the immune system of chronic hypercortisolism in Cushing patients.

Approach: Aim 1 => We will use *in vitro* models to assess how endocrine disorders modulate the susceptibility to respiratory virus infections [4]. For this, we are going to infect primary differentiated human airway epithelial cells cultured at the air-liquid interface with important human respiratory viruses, such as influenza virus or SARS-CoV-2, and assess virus replication kinetics. Furthermore, these cells will be treated on their basolateral side with different concentrations of glucocorticoids before, during and after the infection. We will thus investigate how virus replication is affected by glucocorticoids, and monitor the innate host response of infected cells by looking at innate immune gene expression [5, 6]. Subsequent experiments will assess mechanisms and signalling pathways affected by glucocorticoids, using methods described previously such as specific gene depletion/knock-out techniques or pathway inhibitors [4, 7, 8]. It has recently been shown that steroids have differential effects on RIG-I and MDA-5 signalling, key sensors for different RNA viruses, which might indicate that steroids have different effects on different RNA viruses, although the underlying mechanisms of this specificity are unclear [9]. Therefore, it is critical to compare different viruses such as influenza virus and SARS-CoV-2 that are differentially sensed by these sensors.



Aim 2 => We will use different mouse models mimicking hypercortisolism. One will be injections of dexamethasone for 14 days as a well-defined model of exogenous Cushing's syndrome [10]. Chronic stress models resulting in increased endogenous corticosterone secretion that will be used in the current project are restraint stress and high-fat-diet models (e.g. 12 weeks of high-fat-diet) [11, 12]. These models will subsequently be exposed to viral infection (influenza virus H1N1). From these mouse models, we will isolate blood and tissue samples and investigate changes in the immune response and involved signalling pathways (systemic and tissue-specific) between the different groups before, during and after the infection.

Aim 3 => The Cushing registry will be used to confirm findings from aims 1 and 2. The registry consists of more than 110,000 collected samples, such as whole blood, plasma, fresh-frozen tumour samples, paraffin-embedded tumour samples, serum, urine, saliva, hair, and stool from patients with Cushing's syndrome. We will make usage of these samples for immunofluorescence staining, immunohistochemistry, western blotting, cytokine measurements, and quantitative PCR, among others.

Topics for the PhD studentships will be:

Ad i) Characterization of the effects of corticosteroid substitution on the immune system in cell culture models in response to viral infections. (Zurich)

Ad ii) Characterization of the effects of infection on the systemic immune response in animal models of hypercortisolism. (Dresden)

Ad iii) Characterization of the effects on the immune response in patients with Cushing's syndrome. (Dresden & Zurich)

Work to be performed in Dresden: The students will be taught how to work with animal models of chronic glucocorticoid excess and infections. Afterwards, students will isolate blood and organs from

the animals and use a range of molecular biology techniques for analysing the effects on different signalling pathways. Furthermore, the students will be trained in techniques of working with human tissues from Cushing patients.

Work to be performed in Zürich: In Zurich, the student will use *in vitro* infection models and analyse the antiviral response and virus replication in cells in response to glucocorticoids. The student will learn how to establish primary human airway epithelial cultures at the air-liquid interface, how to handle pathogens safely at containment levels 2 and 3, how to assess virus replication and innate immune responses. As the project progresses, there will be opportunities to develop greater skills in molecular cell biology to understand the mechanism of action of glucocorticoids on specific innate immune signalling pathways.

Added value through the collaboration between Dresden & Zurich: The Steenblock group has great experience in animal models of adrenal and metabolic diseases including also isolating, culturing and staining of cells isolated from endocrine organs. The Bornstein group has in-depth expertise in intercellular crosstalk in the endocrine system, with particular focus on the adrenal gland. The Beuschlein group has strong expertise in clinical and molecular characterization of various endocrine disorders, with particular focus on adrenal and pituitary gland tumors. The three groups are closely collaborating at characterizing the effect of metabolic stress on cells of the hypothalamus-pituitary-adrenocortical axis. The Hale group has strong expertise in assessment of immune response in relation to viral infectious diseases in cell culture models.

References

1. Braun LT, Vogel F, Reincke M. Long-term morbidity and mortality in patients with Cushing's syndrome. J Neuroendocrinol 2022; 34: e13113. DOI: 10.1111/jne.13113
2. Silverman MN, Pearce BD, Biron CA et al. Immune modulation of the hypothalamic-pituitary-adrenal (HPA) axis during viral infection. Viral Immunol 2005; 18: 41-78. DOI: 10.1089/vim.2005.18.41
3. Kanczkowski W, Sue M, Bornstein SR. The adrenal gland microenvironment in health, disease and during regeneration. Hormones (Athens) 2017; 16: 251-265. DOI: 10.14310/horm.2002.1744
4. Fernbach S, Spieler EE, Busnadiego I et al. Restriction factor screening identifies RABGAP1L-mediated disruption of endocytosis as a host antiviral defense. Cell Rep 2022; 38: 110549. DOI: 10.1016/j.celrep.2022.110549
5. Busnadiego I, Fernbach S, Pohl MO et al. Antiviral Activity of Type I, II, and III Interferons Counterbalances ACE2 Inducibility and Restricts SARS-CoV-2. mBio 2020; 11. DOI: 10.1128/mBio.01928-20
6. Pohl MO, Busnadiego I, Kufner V et al. SARS-CoV-2 variants reveal features critical for replication in primary human cells. PLoS Biol 2021; 19: e3001006. DOI: 10.1371/journal.pbio.3001006
7. Borold J, Eletto D, Busnadiego I et al. BRD9 is a druggable component of interferon-stimulated gene expression and antiviral activity. EMBO Rep 2021; 22: e52823. DOI: 10.15252/embr.202152823
8. Schmidt N, Domingues P, Golebiowski F et al. An influenza virus-triggered SUMO switch orchestrates co-opted endogenous retroviruses to stimulate host antiviral immunity. Proc Natl Acad Sci U S A 2019; 116: 17399-17408. DOI: 10.1073/pnas.1907031116
9. Singanayagam A, Glanville N, Girkin JL et al. Corticosteroid suppression of antiviral immunity increases bacterial loads and mucus production in COPD exacerbations. Nat Commun 2018; 9: 2229. DOI: 10.1038/s41467-018-04574-1

10. Finco I, Lerario AM, Hammer GD. Sonic Hedgehog and WNT Signaling Promote Adrenal Gland Regeneration in Male Mice. Endocrinology 2018; 159: 579-596. DOI: 10.1210/en.2017-03061
11. Steenblock C, Rubin de Celis MF, Delgadillo Silva LF et al. Isolation and characterization of adrenocortical progenitors involved in the adaptation to stress. Proc Natl Acad Sci U S A 2018; 115: 12997-13002. DOI: 10.1073/pnas.1814072115
12. Werdermann M, Berger I, Scriba LD et al. Insulin and obesity transform hypothalamic-pituitary-adrenal axis stemness and function in a hyperactive state. Mol Metab 2021; 43: 101112. DOI: 10.1016/j.molmet.2020.101112