Virus-host metabolic interactions: using metabolomics to probe oxidative stress, inflammation and systemic immunity

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Summary

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Curriculum Vitae

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Summary

The persisting pathogenicity of novel and old world viruses illustrates the capability of viruses to deploy evolutionary mechanisms to ensure their survival in a host by targeting cellular and immunological responses. The complex biochemistry and molecular biology which governs the interactions of viruses and hosts on the metabolic level has until present not received the attention it deserves, as the host's metabolism has been mostly overlooked as a possible disease determinant. Since the end of the 20th century, elucidation of viral engineering of host metabolic pathways for viral genome replication, viral maturation and progeny production and survival of infected cells has made great strides as introduced in **Chapter 1**. In this thesis, metabolomics complemented with transcriptomics and immunology was used to study the host-virus interaction on the metabolic level with a special emphasis on the metabolic role of inflammation and oxidative stress during the innate immune response against viral infection. We have chosen respiratory syncytial virus (RSV) and hepatitis B virus (HBV) as candidate viruses to study at a metabolic level their role in acute respiratory infection and chronic hepatitis B infection. Secondly we also investigated infant metabolic and immunological consequences of *in utero* exposure to antiretroviral intervention and human immunodeficiency virus (HIV).

The first research part of the thesis deals with metabolomics method development. **Chapter 2** describes the development of a method able to measure oxidative stress, nitrosative stress and inflammatory mediators in bio-fluids as well as tissue samples. This targeted metabolomics platform can analyse different lipid classes including: isoprostanes, prostaglandins, nitro-fatty acids, lysosphingolipids and lysophosphatidic acids metabolites. The method uses a liquid-liquid extraction sample-preparation followed by liquid chromatography and a mass spectrometric analysis. This method was optimised for different matrixes (serum as well as six organ tissues: heart, liver, lung, kidney, brain and spleen) to evaluate systemic as well as organ-specific (localised) metabolic responses. Applying this methodology too *in vitro* and animal models of viral infection will broaden our understanding of systemic and localised oxidative stress and inflammation experienced as part of the immune response on the metabolic level.

Chapter 3 entails the development of a β-glucuronides hydrolysis method for measuring oxidised lipids in urine. Urine is a non-invasive sample material containing the excreted metabolic compounds reflective of the system as a whole. Three different commercially available enzymes derived from *H. pomatia*, *E. coli* and bovine liver were tested in the development of a method to measure the total oxidised lipid profile. Subsequently, we developed and validated a bovine liver-GUS hydrolysing sample preparation method coupled with LC-MS/MS. This methodology allowed for specific and sensitive measurement of more than 70 oxidized lipids, biosynthesized from two non-enzymatic (ROS, RNS) and three enzymatic pathways (COX, LOX and P450). Thus we were able to establish a non-invasive host readout for inflammation and oxidative stress using urine.

The next part of the thesis focusses on *in vitro* based metabolomics work, using the A549 lung epithelial cell line and respiratory syncytial virus (RSV) as a model system. In **Chapter 4** the metabolic inflammatory response as induced by the innate immune response upon RSV infection was evaluated. The experimental design consisted of a three-day time course RSV infection of A549 cells. Metabolomics was used to elucidate the cellular oxylipin profile generated in response to RSV infection. The oxylipins are specialised signalling lipid mediators, playing an active role in the initiation, maintenance and resolution of inflammation. Complementary to this, a targeted transcriptomic approach focusing on the genes encoding for the oxylipin related enzymes and

receptors was also carried out and an integrative data analysis approach developed. A549 cells, upon RSV infection, displayed a strong pro-inflammatory response with upregulation of the cyclooxygenase (COX-II) pathway and reduced cytochrome P450 pathway in the first 24h. Concurrent alterations in the oxylipin response during RSV infection compared to both positive and negative controls were reflective of a cat and mouse game with host-virus co-evolution at the metabolic level.

In **Chapter 5** we seek to provide novel insights into the perturbed redox biology during an A549 *in vitro* RSV infection time course study. Through the use of metabolomics and targeted transcriptomics we explore the metabolic host-RSV relationship relating to redox biology, cellular anti-oxidant capacity and oxidative stress. We found increased levels of lipid peroxidation metabolites within 24 hours of infection revealing the capacity of RSV to induce oxidative stress in A549 cells upon infection. Furthermore, metabolomics revealed a compromised biogenic amine antioxidant capacity during RSV infection. The host appears not to be a passive bystander as we identified the upregulation of alternative antioxidant pathways resulting in increased levels of proline and spermidine. This study is a further proof of the exquisite ability of RSV to modulate host responses resulting in pathogenic mechanisms on the metabolic level.

Finally, this thesis deals with two patient based metabolomics studies. **Chapter 6** aims to characterise the altered metabolic state during the progression over the four clinical phases of a natural chronic Hepatitis B infection. Targeted metabolomics platforms were used to characterise the phospholipid, triglyceride, sphingomyelin, amino acid, acyl-carnitine, and signalling lipid profiles over the progressive nature of chronic hepatitis B, and to identify potential diagnostic disease severity markers and potential novel treatment options. We identified liver function – and liver injury related metabolic perturbations, which reflect the natural progression of the disease using serum of HBV infected patients. With the liver being the central organ in nutritional regulation and metabolism, it is not surprising that chronic HBV infections induce multiple metabolic alterations in host lipid metabolism via viral hijacking. These metabolite alterations might prove useful as markers of disease progression and severity. The presented study also provides many leads and insights to design follow-up studies and at the same time highlights the need of a complementary systems biology approach to better understand chronic HBV

Chapter 7 entails a study of the vulnerable growing population of *in utero* exposed HIV and combination-antiretroviral therapy (cART) infants. Targeted metabolomics platforms were used to measure the metabolite profile in cord blood obtained from infants who were exposed *in utero* to cART and born to HIV-infected women and from control infants born to healthy women. To determine the interaction between metabolites and immune responses we also quantified levels of classical cytokines and chemokines in the same cord blood samples and integrated the data with those generated by the metabolomics platforms. Our results demonstrate extensive metabolic dysregulation of several pathways in infants with direct in utero exposure to cART and indirect exposure to HIV, providing the underlying conditions for a pro-inflammatory milieu in the infants. More specifically, the lipid profile was significantly altered in cART-HIV-exposed infants, with increased triglyceride and decreased phospholipid species compared to control infants (without in utero HIV and cART exposure). Furthermore, we detected an increase in oxidized lipids, which are generated upon reactive oxygen species (ROS) production by dysregulated mitochondria. Finally, integration between metabolites and classical immunological markers revealed that alterations in lipid metabolites and lipid peroxidation products were associated with increased levels of pro-inflammatory lysophospholipids as well as the pro-inflammatory cytokines IL-1β and IP-10, respectively.

In conclusion, as illustrated in this thesis, metabolomics methodologies were developed to measure important signalling metabolites in a wide variety of biological matrixes. Subsequently, with these and earlier established metabolomics platforms, the host-virus interaction on the systemic metabolic level was characterised. The results have revealed the opportunistic nature of viruses in exploiting host metabolism to ensure their own survival. Concurrently, as the host is not a passive bystander, host metabolic pathways attempting to correct these viral induced imbalances were found.