Origin versus context

Chapter 10

Impact

Expanding the understanding of MΦ phenotype in CVD and beyond

The studies presented in this thesis have added to our understanding of macrophage (MΦ) phenotype by demonstrating that the functional response of MΦ is not only influenced by environmental stimuli (Chapters 6 and 7) but also by their monocytic origin (Chapter 2 and 3) and by the growth factors which are driving monocyte-to-MΦ differentiation (Chapter 5). In this way, the findings in this thesis could eventually contribute to the development of novel therapeutic strategies employing targeted phenotypic modulation of MΦ. Unlike conventional approaches that target the complete MΦ pool or MΦ with a specific disease-related phenotype, such therapy would aim at interfering with a specific context or (systemic) environment to modulate MΦ function. Next to cardiovascular disease (CVD), such thorough understanding of MΦ phenotype and its regulation may also benefit flanking fields and accelerate the development of therapies for other diseases, as MΦ are implicated in a wide range of immune-driven diseases, including multiple sclerosis, rheumatoid arthritis, fibrosis, or cancer (1). Chapter 7 illustrates the relevance of this approach for immune-driven diseases by showing the prognosis-dependent differences in functional response of MΦ to the coronavirus disease 2019 (COVID-19) disease context. This thesis also illustrates the potential of the MacroScreen for assessing effects of novel leads on MΦ, as it represents a robust and cost-effective platform that can streamline functional measurements by combining multiple functional assays in a high-throughput approach. Thus, we expect that the MacroScreen platform could also be used for immunotoxicity screening, and to facilitate the discovery of novel drugs and of new insights into MΦ function in the context of disease.

A methodology makeover to combat MΦ mayhem

Besides the pursuit of discovery and new theories based thereon, progress in science also relies on the verification, falsification or questioning, and if opportune, refinement of established concepts and models (2). While revisiting previous findings can lead to innovation, failure to replicate them may indicate that the model's or concept's robustness has been overestimated (3). The systematic approach to science, the "scientific method", is challenged by the increasing failure and perpetual inability to independently reproduce previous findings, the so-called replication crisis (4, 5). Whereas general factors contributing to the replication crisis, such as the incomplete description of the methods used or the bias towards publishing positive results (6) apply to any scientific discipline, we have, in this thesis, pinpointed some pitfalls in methodologies and taxonomies commonly used in the monocyte/MΦ research field. Our study presented in Chapter 4 demonstrated that disregarding density could introduce bias as it is unclear whether observed effects are induced by the tested compound or treatment condition or are indirectly caused by effects on cell density. We have stressed the importance of density for MΦ function *in vitro* and argue for better awareness of cell density as a confounding factor in *in vitro* testing. Chapter 2 calls for an update of monocyte taxonomy, as our outline clearly shows that the current nomenclature inadequately captures monocyte heterogeneity, and is,

on top, inconsistently used in publications, which complicates the drawing of conclusions. Incorporating these findings into methodological considerations for future studies can help ensure their reproducibility.

Sharing is caring: Disseminating scientific outcome

Scientific progress is directly or indirectly enabled via financial support by the community, and requires the publication of research outcomes in academic journals to be available to other researchers and to ensure return of investment for society (7). Out of the 6 articles that comprise this thesis, 3 have been published in international, peer-reviewed journals, all of them open access. The RNA-sequencing (RNA-Seq) data we present in Chapter 5 will be made available to other researchers in the public database repository Gene Expression Omnibus (GEO). This will enable other scientists to use our data for verification, validation, and deeper analyses or meta-analyses. The findings of this thesis have also been presented at various national and international meetings, such as the 28th annual conference of the Scandinavian Society for Atherosclerosis Research (SSAR).

Turning knowledge into benefit: Implications for therapeutic strategies

Science also aims at bringing a benefit to society. In Chapter 6, we showed that the second-generation superparamagnetic iron oxide nanoparticle ferumoxytol seems to be safe for application in patients with CVD, whereas ferumoxtran administration increased apoptosis in human atherosclerotic plaques, which can considerably affect disease progression. Despite that the manufacturing of ferumoxtran has been discontinued, several clinical studies are recruiting participants for trials on its use in prostate cancer, head and neck squamous cell carcinoma, and aortic dissection, which involves mostly elderly patients that will suffer from moderate to advanced atherosclerosis. Our results in Chapter 6 demonstrate that thorough safety testing regarding atherosclerosis progression and plaque destabilisation are necessary before ferumoxtran can be considered safe for use in these patients. In Chapter 5, our findings challenge the prevailing dogma of granulocyte-macrophage colony-stimulating factor (GM-CSF) as the driving growth factor of MΦ phenotype in chronic inflammation, as we found macrophage colony-stimulating factor (M-CSF) to be dominant in atherosclerotic plaque. This also raises concern about GM-CSF-targeted interventions, such as in cancer therapy or cancer vaccines (7), as a concealed side effect could be plaque destabilisation by an enhancement of the M-CSF-driven MΦ phenotype. In Chapter 7, we have investigated the functional response of MΦ to the COVID-19 systemic disease environment which may help the development of MΦ-based therapies for acute COVID-19. Moreover, we have found certain disease-associated stimuli reflected in serum to sustain even 3 months after hospitalisation which could potentially help understand the development of post-acute sequelae of COVID-19 (PASC), more commonly referred to as Long COVID. However, larger, independent cohort studies will be needed to confirm our findings and to draw firm conclusions. Moreover, we have found phagocytosis as

a predictor of the need to be transferred to the intensive care unit (ICU). As the overload of ICU posed a burden on society during the peaks of the pandemic, this finding could help better categorise patients. Although COVID-19 is likely to become endemic, our results keep its relevance because the course of disease may still be severe for patients at risk, and the emergence of new variants and/or a decline in herd immunity might again lead to infection of large populations.

Conclusion

In conclusion, the findings presented in this thesis have increased our understanding of the (functional) phenotype of MΦ, and its dependence on origin, differentiation trajectory, and local context (density, disease), which could facilitate the development of MΦ-targeted therapies for CVD, COVID-19, and other MΦ-driven diseases. Moreover, we have identified potential pitfalls for the reproducibility of studies using *in vitro* MΦ models and point out approaches for improvement. Lastly, the MacroScreen platform used in this thesis could be used to further characterise the functional phenotype of MΦ or other cell types and in other disease contexts in the future.

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