Monitoring the immune responses to vaccination and pertussis: bordetella pertussis and beyond

Diks, A.M.

Summary

Our immune system consists out of several complementary layers which all play an important role in defending our body from pathogens and tissue damage. In **Chapter 1**, I explain the immune system and how immune monitoring can be a valuable source of information about the immune status of an individual or a group, both when the immune system is in homeostasis and when an active response is ongoing. An example of an active response is the response triggered/elicited by vaccination. Here, immune monitoring can give us information about the efficacy of applied vaccination/immunization method, or it can give information about the functioning of the immune system in one or more individuals. The importance of immune monitoring is well-recognized and various efforts are made to harmonize or, preferably, standardize the laboratory protocols used in immune monitoring studies.

Standardization in (multi-centre) clinical trials is very important to obtain data that is reliable and comparable in-between study locations. Therefore, we first evaluated the influence of various pre- and post-analytical procedures on the quality of the samples used for immunophenotyping of leukocyte subsets in blood. In **Chapter 2.1**, we assessed the impact of delayed sample processing and acquisition, storage temperature and the impact of fixatives on the quantity and distribution of leukocyte subsets. Based on our findings, we formulated a set of guidelines to improve reliability and reproducibility of data regarding immune cell quantity and phenotype.

Data analysis is part of a post-analytical procedure that requires standardization. Therefore, we evaluated the impact of delayed sample processing on the performance of automated (database-driven) analysis in **Chapter 2.2**. We showed that automated analysis is superior to manual analysis regarding reproducibility and robustness. However, expert revision of database-driven analysed files remains necessary in samples with numerical alterations and aberrant B- and T-cell maturation and/or marker expression profiles.

The knowledge gathered in **Chapter 2** was utilized in several clinical trials where we aimed to dissect the cellular immune responses following controlled pertussis infection or vaccination. These clinical trials were initiated locally at the LUMC or within the IMI-2 PERISCOPE consortium and their major outcomes are summarized in **Chapter 3**.

In **Chapter 3.1**, we investigated kinetics of >250 circulating immune cell subsets after an acellular pertussis (aP) booster vaccination. We observed the kinetics of circulating immune cells at various timepoints post-vaccination and found that the most prominent changes occur in the plasma cell compartment; especially (IgG1) plasma cells showed a homogenous expansion and maturation at day 7 post-vaccination. Consequently, in **Chapter 3.2** we focused on kinetics in the B-cell compartment in different age cohorts of vaccinees, and found that irres-

pective of age and the related primary vaccination background, the expansion and maturation of (primarily) IgG1 plasma cells was the most prominent feature of an immune response initiated by aP booster vaccination. Despite these similarities, specific differences in plasma cell responses appeared to be associated with age (primarily IgG1, IgG3 and IgA1 responses in adults, and IgG1, IgG3 and IgG4 responses in children) and primary vaccination background (stronger plasma cell responses in those originally primed with wP). The observed difference may be explained by a better protection induced by wP vaccination, or by the fact that older people have most likely encountered Bp multiple times in life, and thus probably had several 'natural boosters'. In this chapter, we also found that the flow cytometry data corroborated ELISpot data, and both techniques were complementary to each other.

Conclusions drawn from flow cytometry data can not only be supplemented by techniques such as ELISpot and antibody serology, but also by more exploratory transcriptome techniques such as B-cell receptor (BCR) repertoire analysis. Therefore, we further extended our studies on the immune response after aP vaccination in **Chapter 3.3**. Here, we investigated the BCR repertoire and clonal relatedness of plasma cells from different time points by means of single cell sequencing techniques. We observed that the BCR repertoire in plasma cells at day (d) 5, 7, 10, and 14 after vaccination differed from the repertoire at baseline, such as high usage of IGHG1 in expanded clones, increased class-switching events and somatic hypermutation (SHM) patterns, fitting with positive selection of CDR3 sequences over time. In this chapter, we confirmed that the distribution of plasma cell subclasses was highly comparable between the flow cytometry and sequencing data. Moreover, we built a bioinformatic query tool to help searching for BCRs specific to individual vaccine components.

As diverse cell populations and timepoints can be of importance in different models, we compared the cellular kinetics (within innate immune cells, T cells, and B cells) between an aP booster vaccination and a bacterial challenge with *Bordetella pertussis* (strain B1917) in **Chapter 3.4**. We found that the expansion and maturation of plasma cells was less prominent and more delayed in time in the bacterial challenge model, as well as more heterogenous with respect to the isotypes of the expanding plasma cells. Using the data gathered in **Chapter 3,** we identified informative timepoints and cell populations that could be monitored in future vaccination and human challenge studies. Especially prominent for non-colonized participants were the early expansion of (CD36-) non-classical monocytes at d1, natural killer cells (d3), follicular T helper cells (d1-d3) and plasma cells (d3). These populations may be interesting for future monitoring. This data emphasizes the importance of pilot studies (for timepoint finding) and highlights differences in the generated immune response upon vaccination (which has a precise dosage and timing) or controlled infection (which has a more variable exposure in antigen dosage and time, due to the process of colonization and infection through mucosal layers). As current pertussis vaccines do not protect against transmission, unraveling the immune response associated with protecti-

on against infection may help the design of more effective vaccines against pertussis.

Finally, in **Chapter 4**, we set out to use immune monitoring to evaluate the immune system in homeostasis. We investigated the leukocyte composition and function in a group of individuals carrying a specific genetic variant in the *PLCG2* gene (p.P522R). This variant is associated with cognitively healthy aging and longevity. Although it is known that the PLCγ2 protein is involved in signaling downstream of cellular receptors such as the B-cell receptor and the Fc-receptor, the impact of p.P522R on the human immune system remains unknown. We performed an in-depth analysis on the quantity and functionality of the circulating immune cells in (older) adults and supplemented this with the evaluation of and active immune response: the SARS-CoV-2 vaccination responses in a subset of these individuals. Although no differences were observed in the response to SARS-CoV-2 vaccination, carriers of the genetic variant tended to show less signs of immunosenescence compared to age-matched non-carriers.

In this thesis, I evaluated pre- and post-analytical procedures critical for reproducibility of clinical studies and applied them to investigate immune responses to *Bordetella pertussis* and to characterize a polymorphism in healthy older adults. By doing so, I (1) created a set of guidelines for sample handling in clinical trials, (2) showed differences in immune responses to vaccination against *Bordetella pertussis* versus controlled natural infection, and (3) described possible implications of carrying the p.P522R *PLCG2* variant on the human immune system. In **Chapter 5**, I discuss the potential implications of these findings and indicate what next steps can be made in the process of improving pertussis vaccines and in the process of dissecting the impact of the *PLCG2* P522R genetic variant on the immune system and evaluate its potential as a therapeutic target.