Summary and discussion

SUMMARY AND DISCUSSION

The content of this thesis is based on research that was conducted at the travel and vaccination clinic at Leiden University Medical Centre. It covers a variety of topics relating to travel medicine and immunity. This final chapter starts by discussing methodological aspects of the various studies. Next it discusses the results of the studies on travel medicine and finally the chapters pertaining to immunity and vaccination.

METHODOLOGICAL ISSUES

Based on the method that was used, the chapters in this thesis can be categorised as either experimental studies (i.e. trials) (chapters 6, 7, 8 and 9) or non-experimental studies (i.e. observational studies) (chapters 1, 2, 3, 4, 5, 10 and 11). Experimental studies are defined as prospective follow-up studies in which the exposure to a determinant is manipulated and assigned at random, thus creating an index- and a reference group.[1] The purpose of random allocation is to create groups that differ only randomly at the time of allocation with regard to subsequent occurrence of the study outcome.[2] The goals of the study, rather than the subject's needs, determine the exposure assignment, so as to prevent 'confounding by indication'.[2] Furthermore, for ethical reasons, the treatment possibilities in an experimental study must be equally acceptable given current knowledge.[2] This is called the principal of equipoise.[3-9] Therefore in many trials, the reference group is treated with an active comparator as opposed to a placebo. For ethical reasons and for methodological reasons, it is essential that people who are assigned to a reference group are exposed to the best available active comparator. When feasible, clinical trials should attempt to employ blinding with respect to treatment assignment. This prevents certain biases that could affect assignment, compliance, treatment or assessment.[2] In an experimental study, the sample size depends on pre-defined assumptions and variables: (i) an assumption about the incidence of the primary endpoint, (ii) an assumption about the difference in the effect of treatment between the index- and the reference group., (iii) the magnitude of the type I error that is deemed acceptable (α) (i.e. asserting something that is absent) and (iv), the magnitude of the type II error that is deemed acceptable (β) (i.e. failing to assert what is present). Table 1 summarizes the methodology of the experimental studies that are described in chapters 6 and 7 of this thesis. Chapters 8 and 9 were open label, non-randomized phase I exploratory trials.

Non-experimental studies differ from experimental studies in that randomization is not used to assign treatments.[10] The researcher is an observer rather than an agent who assigns interventions.[2] There are four main types of non-experimental studies: cohort studies,

STUDY	STUDY PHASE	TRIAL TYPE	PRIMARY ENDPOINT	EXPERIMENTAL TREATMENT	STANDARD TREATMENT	EQUIPOISE	QUALITY OF STANDARD TREATMENT
VACCINE FOR TRAVELLERS' DIARRHOEA	3	SUPERIORITY TRIAL	INCIDENCE PROPORTION FOR ALL CAUSE TRAVELLERS' DIARRHOEA	CVD 103-HGR LIVE ORAL CHOLERA VACCINE	PLACEBO	😊	😊
POLIO VACCINE	3	NON-INFERIORITY TRIAL	DIFFERENCES IN THE LOG_2 GMC	1/5TH DOSE INTRADERMAL JET INJECTOR	FULL-DOSE INTRA-MUSCULAR NEEDLE	😊	😊

STUDY	RANDOMIZATION	CONCEALMENT OF ALLOCATION	PRE-DEFINED EXPECTED TREATMENT EFFECT	OBSERVED TREATMENT EFFECT	PRE-DEFINED SAMPLE SIZE	ATTAINED SAMPLE SIZE	PRE-DEFINED TYPE I ERROR	PRE-DEFINED TYPE II ERROR
VACCINE FOR TRAVELLERS' DIARRHOEA	COMPUTER GENERATED LIST	DOUBLE-BLIND	PROTECTION RATE ≥ 50%	PROTECTION RATE 0%	100 PER TREATMENT ARM	65 PER TREATMENT ARM	0.025 (ONE-SIDED)	0.1 (ONE-SIDED)
POLIO VACCINE	SEALED ENVELOPES NUMBERED IN RANDOM ORDER	OPEN-LABEL	LOWER END OF 95% CI FOR THE DIFFERENCE IN THE LOG_2 GMC LESS THAN -1	LOWER END OF 95% CI FOR THE DIFFERENCE IN THE LOG_2 GMC MORE THAN -1	30 PER TREATMENT ARM	30 PER TREATMENT ARM	0.025 (ONE-SIDED)	0.2 (ONE-SIDED)

GMC: GEOMETRIC MEAN CONCENTRATION. PV1: POLIOVIRUS TYPE 1, PV2: POLIOVIRUS TYPE 2, PV3: POLIOVIRUS TYPE 3.

TABLE 1

Summary of methodological aspects of the experimental studies that are described in chapters 6 and 7 of this thesis.

TABLE 2 (page 140)

Summary of methodological aspects of the non-experimental studies that are described in chapters 1, 2, 3, 4, 5, 10 and 11 of this thesis.

in which all subjects in a source population are classified according to their exposure status and followed over time to ascertain disease incidence; case control studies, in which investigators compare exposures between subjects with a particular disease outcome (cases) and people without that outcome (controls); cross-sectional studies, in which one ascertains exposure and disease status at a particular time; and ecological studies, in which the units of observation are groups of people.[2,11] The objective of an epidemiological study is to obtain a valid and precise estimate of the frequency of a disease or of the effect of an exposure on the occurrence of a disease in the source population of the study. Often, a further objective is to obtain an estimate that is generalizable to relevant target populations.[2] Errors in estimation may occur due to random- or systematic errors and are of influence on the internal validity of a study. Violations of internal validity can be classified into three categories: confounding, selection bias and information bias. Confounding produces relations that are factually right, but that cannot be interpreted causally because some underlying, unaccounted for factor is associated with both exposure and outcome.[11] Bias is a systematic deviation of a study's result from a true value. Typically, it is introduced during the design or implementation of a study and cannot be remedied later. Bias arises from flawed information or subject selection so that a wrong association is found.[11] Table 2 summarizes some methodological aspects of the non-experimental studies that are described in chapters 1, 2, 3, 4, 5, 10 and 11.

TRAVEL AND INFECTION

The first chapter of this thesis describes a study that was designed to improve the quality of medical electives.[12] It describes the health risks and the quality and comprehensiveness of pre- and post-travel care fore a group of Dutch medical students after an elective abroad. Most students engaged in procedures that constitute a risk of blood-borne viral infection, often in countries with high HIV prevalence rates. None of the participants took action following mucosal or percutaneous exposure to potentially infectious body fluids. This was also the case in a survey among British medical students.[13] Furthermore, the allocation of post exposure prophylaxis kits for HIV (PEP) was inadequate. Regarding other health risks: 20% stopped using mefloquine due to adverse effects, which left a sizeable proportion unprotected in countries that are hyperendemic for malaria. Post-travel screening for schistosomiasis, tuberculosis and methicillin-resistant *Staphylococcus aureus* (MRSA) was conducted for approximately half of all students who should have been screened. Based on the results of this study we adopted an integral set of measures to reduce the health risks associated with an elective abroad. Pre- and post-travel consultations as also the distribution of PEP has been centralized and standardized. Furthermore, student and supervisor in Leiden are required to fill out a checklist to assess whether the student is sufficiently experienced to participate in certain procedures, such as

STUDY	STUDY DESIGN	OBJECTIVE	RESPONSE RATE	STUDY SIZE	FOLLOW-UP RATE	STATISTICAL METHODS	POTENTIAL LIMITATIONS REGARDING STUDY DESIGN OR ANALYSIS	ASPECTS RELATING TO GENERALIZABILITY
HEALTH RISKS DURING MEDICAL ELECTIVES ABROAD	COHORT STUDY	QUANTIFY THE COMPREHENSIVENESS OF PRE- AND POST-TRAVEL CARE AND OF TRAVEL RELATED MORBIDITY.	74%	$n = 180$	-	INCIDENCE PROPORTIONS	INFORMATION BIAS (RECALL BIAS, RESPONDENT BIAS)	SINGLE CENTRE, STUDY
INCONVENIENCE DUE TO TRAVELLERS' DIARRHOEA	COHORT STUDY	QUANTIFY THE DEGREE OF INCONVENIENCE CAUSED BY TRAVELLERS' DIARRHOEA.	52%	$n = 406$	96%	INCIDENCE PROPORTIONS AND INCIDENCE RATES	INFORMATION BIAS (RESPONDENT BIAS)	SINGLE CENTRE STUDY
PRE-TRAVEL PREPARATION AND MORBIDITY IN PEOPLE WITH IBD	COHORT STUDY AND CASE-CROSSOVER STUDY	QUANTIFY THE COMPREHENSIVENESS OF PRE- AND POST-TRAVEL CARE AND OF TRAVEL RELATED MORBIDITY.	70%	$n = 277$	-	INCIDENCE PROPORTIONS AND MANTEL-HAENSZEL ODDS RATIO	INFORMATION BIAS (RECALL BIAS, RESPONDENT BIAS)	SELECTION BIAS (SELF-SELECTION BIAS)
POST-TRAVEL SCREENING FOR INTESTINAL PARASITES	COHORT STUDY	QUANTIFY THE INCIDENCE OF ASYMPTOMATIC PARASITIC INFECTION IN TRAVELLERS.	UNKNOWN	$n = 679$	82%	INCIDENCE PROPORTIONS	-	RECRUITMENT AT TWO TRAVEL CLINICS, HALF OF THE PARTICIPANTS WERE STUDENTS
IMMUNE RESPONSE TO SCHISTOSOME ANTIGENS	CASE-CONTROL STUDY	QUANTIFY THE TYPE AND STRENGTH OF THE MEMORY IMMUNE RESPONSE TO SCHISTOSOME ANTIGENS.	-	$n = 21$	-	MANN–WHITNEY TEST	-	-
IMMUNE RESPONSE TO YELLOW FEVER VACCINE	CONTROLLED COHORT STUDY	COMPARE THE IMMUNE RESPONSE TO YELLOW FEVER VACCINE BETWEEN YOUNG AND ELDERLY PEOPLE.	-	$n = 58$	100%	MIXED LINEAR MODEL, CHI-SQUARE TEST, WILCOXON'S RANK TEST	-	SINGLE CENTRE STUDY
IMMUNE RESPONSE TO INFLUENZA A VACCINE	CONTROLLED COHORT STUDY	COMPARE THE IMMUNE RESPONSE TO INFLUENZA VACCINE BETWEEN HEALTHY CONTROLS AND PEOPLE WITH HIV INFECTION.	-	$n = 112$	99%	MIXED LINEAR MODEL, CHI-SQUARED TEST	CONFOUNDING, INFORMATION BIAS	SINGLE CENTRE STUDY

IBD: INFLAMMATORY BOWEL DISEASE.

suturing or assisting in the operating theatre or delivery room. If new skills are to be acquired abroad, it should be specified beforehand whether the medical staff abroad has the time and facilities to supervise and teach new skills. Students also receive a brochure that describes how to act in case of exposure to potentially infectious body fluids. Upon return, all students fill out a standard short web-based checklist which assesses certain health risks, such as exposure to potentially infected body fluids and the risk of schistosomiasis and tuberculosis. The checklist results in a computer generated recommendation stating whether the student needs to contact the occupational health department or another care provider for a post-travel consult. Finally, the department of student affairs is creating a list of so called preferred partners. These are long standing partnerships with hospitals abroad, where medical staff are familiar with supervising foreign students and where student responsibilities and access to care are well-defined. A more comprehensive pre- and post travel survey will assess the effectiveness of the new policy. In addition this study will address other aspects, such as the incidence of culture shock, (traffic) accidents, violence and post-travel irritable bowel syndrome.

For most travellers to the tropics, diarrhoea is the most common health hazard. It can be a major nuisance but it is very seldom fatal. In **the second chapter** we assess the burden of illness due to travellers' diarrhoea in adults who travelled to the (sub)tropics for a median of 23 days.[14] We conclude that conventional definitions of travellers' diarrhoea encompass many mild cases (in our study at least a third of all cases) for which treatment is unlikely to provide a significant health benefit. We recommend that the degree of inconvenience should be incorporated as an endpoint in clinical studies on travellers' diarrhoea. This will enable scientists and policy makers to better distinguish 'significant' travellers' diarrhoea from mild travellers' diarrhoea, thus allowing for a more precise estimate of the size of the target population for vaccination or stand-by antibiotic prescription and of the benefit of such measures.

Chapter three describes a questionnaire study on travel experiences in which we investigated pre-travel preparation of Dutch patients with inflammatory bowel disease (IBD).[15] We also surveyed health problems encountered during travel and investigated whether travel increased the risk of an exacerbation of IBD. Faecal urgency and incontinence were the main IBD-related inconveniences. Onset of a new episode of diarrhoea was reported by 32%, which surprisingly is not higher than the incidence of travellers' diarrhoea in the general population.[16] Probably, people with chronic bowel disease are less inclined to regard gastro-intestinal complaints as new episodes of diarrhoea. We did not find that travel increased the risk of an exacerbation of IBD within a 2-month period after travel. However, the individual's self-reported number of exacerbations over the past 5 years may not be a valid marker for the expected incidence of an exacerbation after travel.

Lastly, pre-travel advice for IBD patients was often deficient. We recommend that physicians caring for patients with IBD raise awareness of the benefit of pre-travel counselling and that they refer patients to travel medicine clinics in a timely fashion. Sufficient time is required to check serology after hepatitis A vaccination in those who use systemic immunosuppressants. Even if seroprotection is not attained after one dose, a second dose is often effective, as has been shown in organ transplant recipients.[17,18]

Chapter four describes a study in which we aimed to determine the utility of routine post-travel screening of asymptomatic long-term travellers to the (sub)tropics for intestinal parasites using molecular diagnostics and for schistosomiasis using serology.[19] Only one infection with *Strongyloides stercoralis* was found in over 400 travelers and no infection with *Entamoeba histolytica* in over 500 travelers. The incidence of infection with Schistosoma spp. was higher. However, each case was associated with exposure to highly endemic lakes in Malawi and Tanzania. We conclude that routine screening of stool samples for parasitic infection is not indicated for asymptomatic people, who travel to the (sub)tropics for up to 3 months. Screening for Schistosoma spp. should be offered to travellers with fresh-water contact in endemic regions. Post-travel screening of specific groups of asymptomatic travellers, such as migrants, expatriates, or aid workers may yield higher infection rates.

Chapter six describes a randomized trial on the efficacy of a live attenuated oral cholera vaccine, CVD 103-HgR, to prevent all-cause travellers' diarrhea.[20] The vaccine failed to provide protection. The power of the study was limited by the unexpected low incidence of LT–ETEC-associated diarrhoea. Other studies that evaluated the protective efficacy of ETEC-specific vaccines also failed to demonstrate clinically important benefits.[21,22] Future studies attempting to prevent travellers' diarrhoea through vaccination should target a broader range of enteropathogenic *Escherichia coli* and other enteropathogens.[23-26] Newer vaccines have therefore included more colonization factor antigens that are expressed by *Escherichia coli*.[27-31] Furthermore, future trials should include large numbers of travellers, or limit the investigation to countries for which detailed data concerning aetiology of travellers' diarrhoea is available. Lastly we recommend that trials should incorporate the degree of inconvenience as a clinical endpoint.

IMMUNITY

Immunology from an evolutionary perspective

From the beginning of their existence, metazoan recruited a basic diversity of molecular categories able to interact with proteins, sugars or lipids, i.e. an innate immune system that was able to recognize pathogens. The interactions with pathogens were articulated to

signalling cascades that were sometimes shared with other functions, such as fertilization control, development, metamorphosis and regeneration pathways. These signals were coupled to a diversified set of effector mechanisms.[32-34] Later in evolution, jawed vertebrates developed a so-called adaptive immune system.[35] This system consists of a set of gene segments that are assembled during the ontogeny of lymphocytes. After selection, it provides each individual with an unparalleled diversity of recognition capacity.[32] According to Du Pasquier, the reason why most life-forms did not develop an adaptive immune system, may be related to the relative value of individuals for the survival of a species. In species with large progenies and in which individuals reproduce only once and relatively early, and in which older individuals are less important for the survival of the species, innate immunity may suffice. This avoids the complexity of an adaptive immune system.[32]

Immunity and vaccines against tuberculosis

In the realm of immunology, a leap of faith may be required to imagine it possible to apply vaccines to prevent infectious diseases, such as malaria and tuberculosis, to which no sterile immunity occurs in people who are infected with the wild type micro-organism. From an epidemiological viewpoint, it is not necessary to achieve complete or ''sterile'' eradication of bacteria to effectively reduce the incidence of active tuberculosis. The natural state of most humans is protective immunity, since only a minority (~5%) develop clinically active tuberculosis after infection.[36] Various host-derived factors increase the risk: malnutrition, aging, stress, type-2 diabetes, vitamin D deficiency and genetic factors that affect innate and adaptive immunity.[37-52] Furthermore, temporary or permanent skewing of the immune system due to co-infections influences cellular immunity and may increase the risk of developing active tuberculosis.[53-58] On the other hand, infection with *Mycobacterium bovis* may mitigate the risk of developing active tuberculosis. This has been demonstrated in the 1940s in Denmark, where the incidence of tuberculosis was compared between the island Zealand and South Jutland.[59,60] On Zealand, bovine tuberculosis had been eradicated by 1930, whereas in South Jutland it was still prevalent at the time. In Zealand the incidence of morbidity due to MTb was higher than in South Jutland.

Morbidity occurs after primary infection, or after reactivation of latent tuberculosis. In areas with a high incidence of MTb infection, re-infection is also an important cause of active tuberculosis. This has been shown by Dutch pathologists, who analyzed lungs of people who died of causes, other than tuberculosis, in the '30s, '50s and '60s.[61-63] Based on histology, they selected cases with a primary calcified complex in the apex of the lung, indicating past or latent MTb infection. They then looked how many of these cases also had morphologically different active complexes, which were categorized as reinfections

FIGURE 1

Tuberculosis in The Netherlands: incidence, mortality, number of patients that have been treated, total number of patients and estimated chance of contracting tuberculosis.
(Reproduced by permission of KNCV Tuberculosefonds. Source: CBS, CIb and NTR).

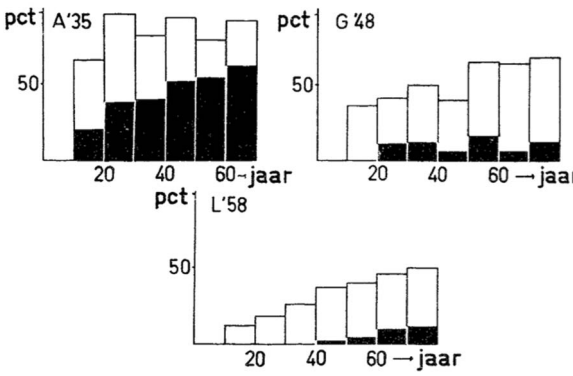

FIGURE 2

Prevalence of tuberculosis infection (total bar charts) and reinfections (filled sections of barcharts) by age and by year in which the studies were conducted.[63] (Reproduced by permission from Nederlands Tijdschrift voor Geneeskunde 1962. Copyright Bohn Stafleu Van Loghum).

with MTb. If these more active lesions were caused by reinfection and not by reactivation of latent MTb, such lesions should be less prevalent in the '60s than in the '30s and '50s, owing to the dramatic decrease in the incidence and prevalence of infection with MTb though time (FIGURE 1). This is exactly what they found (FIGURE 2). This observation has been corroborated by DNA fingerprinting of MTb isolates.[64,65]

Public health programs have had a large impact on the incidence of infection with MTb. This has been achieved by adequate treatment of people with active tuberculosis and by screening and treating the contacts that surrounds such a case. In a similar manner, preventing active tuberculosis with effective vaccines will impact the incidence of MTb infection, by reducing the reservoir of people who can transmit MTb. Vaccination strategies focus on preventing infection and/or active disease by inducing immunity to antigens that are expressed early in the course of infection, such as Early Secretory Antigenic Target (ESAT-6) and Antigen 85 (Ag85B). The subunit vaccine H1 consists of the fusion protein Ag85B-ESAT6.[66-68] To prevent active disease it also seems important to induce immunity to late stage antigens, that are expressed during bacterial dormancy.[52] The vaccine, H56 is based on this concept and combines Ag85B, ESAT-6 and Rv2660c. Rv2660 is expressed in late stage infection.[69]

Correlates of protection against MTb, vaccines and adjuvants

Despite increasing knowledge on the crucial role of individual cell types, genes and molecules in the protective host defence against MTb, we lack a true understanding of what exactly constitutes protection and protective immunity. This creates a roadblock for tuberculosis vaccine development and the identification of surrogate endpoints of protection, that can be used in clinical research.[70] Nevertheless, there is general consensus that a Th1 cell response is essential for bacterial containment during infection.[71] Inducing such a response with subunit vaccines, requires new types of adjuvants. Aluminum salts (i.e. alum), which were the only approved adjuvants until the end of the 20th century, promote the 'wrong' type of immune response, a Th2 antibody mediated response.[72] Initially, alum was added to vaccines, because it caused a precipitate (i.e. solid form), and because of the observation that precipitates improved vaccines' antigenic properties.[73] The mechanism governing the enhanced immunogenicity was thought to be the formation of a depot at the injection site, and subsequent slow release of antigen. This assumption has been disproven.[74] It seems that alum induces cytotoxicity and the release of host DNA, which acts as a damage associated molecular pattern (DAMP); an immunostimulatory signal.[75] Furthermore, alum allows host DNA to access the cytoplasm of dendritic cells (DCs), which activates pathways that promote MHC class II presentation and DC-T-cell interactions.[76] Activation of the inflammasome, directly by alum, or indirectly by local accumulation of uric acid, may also contribute to the adjuvant effect.[74]

In **chapter eight and nine** of this thesis, two clinical trials are described in which two new adjuvants were combined with a MTb subunit vaccine.[77,78] The first adjuvant, IC31® was developed by Intercell AG (Vienna, Austria) and consist of the artificial antimicrobial peptide KLK and the oligodeoxynucleotide ODN1a. KLK acts as a vehicle, enhancing uptake into antigen presenting cells (APC). ODN1a stimulates Toll-like receptor 9 (TLR9) signalling and activates APC. This causes a mixed Th1 and Th2 type response.[79-82] The second novel adjuvant, CAF01, was developed by Statens Serum Institute (Copenhagen, Denmark). It consists of liposomes formed by N,N'-dimethyl-N,N'-dioctadecylammonium (DDA) and of the synthetic immunomodulator α,α'-trehalose 6,6'-dibeheneate (TDB), which is inserted into the lipid bilayers.[83-85] DDA liposomes target cell membranes of APC, which subsequently leads to enhanced uptake and presentation of antigen and a weak Th1 cell response.[86] TDB is a synthetic analogue of the mycobacterial cell wall component trehalose 6,6'-dimycolate (TDM) often referred to as cord factor. TDB stabilizes DDA liposomes and enhances the Th1 and Th17 cell response.[84,85,87-89] This is mediated by recognition of TDB by the C-type lectin Mincle, which induces IL-1 production which in turn induces MyD88-dependent Th1/Th17 cell responses.[90-92]

H1-IC31® induced a long-lasting Th1 cell response in naïve subjects, characterized by IFN-γ producing lymphocytes.[93] The immune response was faster and generally stronger in subjects who had been vaccinated with BCG in the past and in subjects with past or latent MTb infection.[77] H1-CAF01 also induced a robust and long lasting Th1 cell response.[78] Despite these encouraging results, these surrogate immunological endpoints are not true correlates of protection. This is exemplified by a recent large phase 2b trial in which a vaccine consisting of a recombinant strain of modified Vaccinia Ankara virus that expresses Ag85A, induced excellent immune responses, but failed to protect South African infants against active tuberculosis.[94] Commenting on this result, Dr. Dye and Dr. Fine write: "The stakes are high. The venture is costly and risky, but has a huge potential payoff. We need to go on playing the high-stakes game."[95]

Poliovirus eradication, fractional doses and adjuvants

The Global Polio Eradication Initiative is another high-stakes venture.[96] Through thoughtful work, dedication and concerted effort, polio cases have decreased by over 99% since 1988, from an estimated 350 000 cases then, to 416 reported cases in 2013.[97] Furthermore, the last case of infection with poliovirus type 2 occurred in 1999 and of poliovirus type 3 in 2012.[97] In 2015, only 3 countries (Afghanistan, Nigeria and Pakistan) remain polio-endemic, down from more than 125 in 1988. War and displacement of people are currently the main obstacles to achieving complete interruption of the transmission of poliovirus. After eradication, cessation of oral poliovirus vaccine (OPV) is needed to prevent outbreaks due to circulating vaccine derived poliovirus.[98-100] IPV is a factor 20 more expensive than OPV.

Therefore, one of the prerequisites for cessation of the use of OPV is to make IPV affordable and suitable for use in developing countries.[101] Using fractional (reduced) doses may impact affordability and optimize the utilization of the production capacity for IPV. Intradermal administration has the potential to lower the dose without reducing immunogenicity. A needle-free jet injector may be a reliable way to administer vaccines intradermally. In **chapter seven** of this thesis, a study is described that found that fractional-dose intradermal IPV booster vaccination using a jet injection system was well tolerated and immunogenic.[102] Antibody titres in the fractional-dose intradermal group were slightly lower than after standard full-dose intramuscular vaccination. A way to further increase immunogenicity of fractional-dose IPV, may be to add an adjuvant. In mice, an IPV-CAF01 formulation has been tested.[103] IPV-CAF01, containing 2 D-Units (DU) of poliovirus type 1, 2 and 3 was compared to unadjuvanted IPV with either 2 or 20 DU of poliovirus type 1, 2 and 3. Intramuscular (IM) delivery of fractional-dose adjuvanted vaccine induced stronger antibody responses than IM fractional-dose unadjuvanted vaccine. The response to the fractional-dose adjuvanted vaccine was as strong as the response to the full-dose unadjuvanted vaccine. Furthermore, the adjuvant also induced an increased cellular response, as measured by multiplex cytokine analysis. In another experiment, IPV-CAF01 was injected simultaneously at an intradermal and an intramuscular site. Interestingly, this elicited an intestinal immune responses against poliovirus, measured as faecal IgA. This is important, because intestinal immunity shortens the time during which an infected person sheds poliovirus.[104-106] In the IPV vaccination trial described in this thesis, mucosal immunity was a secondary endpoint, which remains to be analyzed.

Digging up memory

In our study on IPV vaccination there was a fast and strong antibody response; i.e. a ≥ 40 fold increase in antibody titre within 7 days after a booster vaccination. This is typical of a memory immune response, which is characterized by a logarithmic increase in antibody titre within days after re-exposure to an antigen, combined with avidity maturation. Such a memory response depends on long lived-memory B cells.[107,108]

In a primary humoral immune response to a novel antigen, antigen-specific helper T cells that have been activated by antigen-bearing dendritic cells trigger some antigen-specific B cells to migrate towards follicular dendritic cells (FDCs), initiating the germinal centre reaction. In GCs, B cells receive additional signals from follicular T cells (Tfh) and undergo massive clonal proliferation, switch from IgM towards IgG, IgA or IgE, undergo affinity maturation and differentiate into plasma cells secreting large amounts of antigen-specific antibodies.[109] At the end of the GC reaction, a few plasma cells exit nodes/spleen and migrate to survival niches, where they survive through signals provided by supporting stromal cells.[110]

The duration of antibody responses is proportional to the number of long-lived plasma cells generated by immunization. In absence of subsequent antigen exposure, antibody persistence may be reliably predicted by the antibody titres that are reached 6–12 months after immunization, i.e. after the end of the short-term plasma cell response. This is illustrated by the accuracy of mathematical models predicting the kinetics of anti-HBsAg and anti-hepatitis A antibodies.[109,111,112] Long-lived plasma cells preferentially reside in niches in the bone marrow,[113] in the spleen[114] and in the tonsils.[115-117]

In parallel to plasma cells, memory B cells are generated in response to T-dependent antigens, during the GC reaction. When memory B cells exit the GC, they migrate to extrafollicular areas of the spleen and lymph nodes.[118] This migration occurs through the blood, in which post-immunization memory B cells are transiently present on their way towards lymphoid organs. The spleen harbours most memory B cells, followed by tonsils, bone marrow and peripheral blood.[119] Their phenotype does not differ in the different compartments. Memory B cells do not produce antibodies and do not protect, unless re-exposure to antigen or cross-reacting antigens drives their differentiation into antibody producing plasma cells. Since the affinity of surface Ig from memory B cells is increased, their requirements for reactivation are lower than for naïve B cells. Memory B cells may thus be recalled by lower amounts of antigen and without CD4+ T cell help. Therefore this reactivation is characterized by a rapid increase of the antibody titer.[109]

As we observed in a study on influenza vaccination in **chapter eleven** of this thesis, rechallenge with influenza subunit vaccines often fails to induce a typical booster humoral response.[120] This is peculiar, since influenza vaccines do induce a memory response with memory B cells and long-lived plasmablasts that can produce IgG antibodies with high levels of somatic hypermutation.[121,122] However, in individuals who have been primed by past vaccination or influenza infection, the recall response may be negatively influenced by residual cross-reactive anti-influenza antibodies. Upon vaccination, antigen-antibody complexes may reduce the load of antigen available for B cell binding. Alternatively, antibodies may have a negative feedback on B cells. Consequently, individuals with residual antibodies to a given antigen may only show a limited increase of their antibody response; such that vaccine responses are better described by the proportion of individuals above a given threshold than by those showing a 2- or 4-fold increase of antibody titers.[109] In chapter eleven geometric mean titers and seroprotection rates (defined as HI titers $\geq$1:40) were the main outcome measures.

Don't just do something, stand there.
Much in medicine remains uncertain. When faced with an incomplete pathophysiological model of a mechanism of disease and with an incomplete understanding of the effects

of treatment modalities, clinicians must rely on controlled studies to determine what is best. When such studies are lacking or when the results cannot be generalized to an individual patient, individual and collective experience must be combined with an understanding of pathophysiology to decide what is good. However, even impeccable logic doesn't always suffice and may have grave consequences.[123] To decide what is wisest, a doctor requires conscious knowledge of his inclination for cognitive error and of the fundament of intuition and reason. "*I call that man awake who, with conscious knowledge and understanding, can perceive the deep unreasoning powers in his soul, his whole innermost strength, desire and weakness, and knows how to reckon with himself.*"[124,1]

[1] *Wach nenne ich den, der mit dem Verstand und Bewusstsein sich selbst, seine innersten unvernünftigen Kräfte, Triebe und Schwächen kennt und mit ihnen zu rechnen weiß.*

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