Travel medicine : knowledge, attitude, practice and immunisation

Roukens, A.H.E.

Summary and discussion

General introduction – Travel medicine

Global travel has increased dramatically during the past decades, with an estimated growth at approximately 6% per year [1]. Inversely proportional to this increase is a reduction of geographic barriers to pathogens, hence increased potential for spread of infectious diseases. The traveller nowadays comprises a wide variety of individuals with accompanying ways of travelling, requiring a diverse approach to protection during his or her journey, of which several aspects are addressed in this thesis.

According to the Swiss cheese model proposed by James Reason [2], cumulative protective medical measures (barriers) prevent hazards from causing human losses or illnesses. As noted in the introduction of this thesis, this model can be applied to travel medicine, to improve protection against travel-related diseases through knowledge on the following topics; 1. Epidemiology and prevention of travel-related diseases, 2. Morbidity and mortality of these illnesses in specific groups of travellers, 3. Adherence to travel health precautions, 4. Immunological responsivity upon vaccination, and 5. Availability of preventive measures, such as vaccines. In table 1, a schematic application is given of the model to (travel) health care, corresponding with the topics investigated and discussed in this thesis.

Prevention of travel-related disease with regard to specific populations of travellers

Chapter 1

In chapter 1 we describe how indeed travellers' knowledge and attitude can be influenced with a training programme. Long term travellers are notorious non-compliers to malaria prophylaxis [3], which is confirmed by our study population (47% took malaria chemo-prophylaxis) compared to 84% of short term travellers on vacation in high-risk areas [4]. The study of a malaria prevention programme among 2,350 employees working at an oilfield service company showed that a carefully designed malaria awareness training including self-diagnosis and treatment had a significant positive effect on knowledge and attitude towards malaria prevention and doubled the use of malaria chemoprophylaxis (47% of respondents who followed the programme vs. 19% who did not). The relative success of this malaria prevention programme has led to the implementation of the programme in other oilfield service companies (personal communication).

Table 1 Schematic application of the Swiss cheese model to travel health care [2], corresponding with the topics described and discussed in this thesis

Swiss cheese	Representation in health care	Representation in travel health care addressed in this thesis
Slice of cheese	Health care professional	Professional in travel medicine, general internal medicine, public health • Education of public health care and infectious diseases specialists on possibilities of enhancing vaccine stockpiles (YF, rabies) or enhancement of immunity (hepatitis B) • Address medical specialists to point out responsibility in educating specific groups of travellers (transplant recipients, elderly travellers, long term travellers)
	Barrier that protects patient from harm	Preventive measures (non-medical intervention) • Anti-mosquito bite measures in long term travellers Vaccination • Reduce costs / increase availability of YF and rabies vaccines thereby enhancing vaccine utilisation • Increase hepatitis B response in non-responders by intradermal vaccination Increase of chemoprophylaxis use in malaria exposed long term travellers Evaluation of use of antibiotics in diabetic and transplant recipient travellers
	Procedure that alleviates the consequences of an error	Training of malaria exposed long term travellers • Self-diagnosing, testing and treatment
Error		-
Hole	Opportunity for error	Purpose of travel (VFR, expatriate, tourist, migrant) influencing awareness of hazards • Studying travel-related diseases in transplant recipients, diabetics, long term travellers, acknowledging risks Adverse events following yellow fever vaccination • Investigating the pathophysiology of YEL-AVD in elderly travellers Non-adherence to preventive measures and chemoprophylaxis in long term travellers

	Weakness in defences against error	-
Arrow	Series of events leading to medical error	-
Adding a slice		Identify risk for diseases and subsequent education • kidney transplant recipients • long term travellers to malaria endemic countries
	Improve health care safety	Increasing KAP for specific groups of travellers Increase vaccine utilisation (YF, rabies) and immunity against vaccines (hepatitis B)
Plugging a hole		Contribute to scientific knowledge concerning malaria prophylaxis, vaccinating elderly (YF), intradermal vaccination (YF, rabies, hepatitis B)

YF = yellow fever, VFR = visiting friends and relatives, YEL-AVD = yellow fever associated viscerotropic disease, KAP = knowledge attitude and practices.

A realistic approach of protection against malaria for long term travellers, considering the low usage of chemoprophylaxis even after intensive training, would be to prescribe chemoprophylaxis only for the first 2-3 months of their stay abroad. In that period they will get acquainted with local healthcare, and know what to do in case of illness. The availability of self-testing and standby treatment for malaria may offer these travellers an additional safe guard against the serious consequences of falciparum malaria infection, provided that they are properly instructed, by means of hands on training. In line with the Swiss cheese model, this malaria prevention programme comprises separate components that raise awareness and protection, in which a missed step is pre-empted by the next.

Correct performance of dipstick-based rapid diagnostic tests for falciparum malaria in febrile travellers may vary from 69% to 91% depending on whether prior instructions were given [5-7]. Implementation of the malaria prevention programme not only improved knowledge and attitude on malaria but also allowed us to investigate the contribution and drawbacks of the use of rapid diagnostic test for *P. falciparum* by the target population, although we have no control for the result of the test. When a finger prick for self-testing is performed we strongly recommend storing a few drops of blood on filter paper for PCR analysis for *P. falciparum* after returning home, to enable determination of true positive and true negative rates for self-testing and clinical diagnosis of falciparum malaria abroad. This additional information would overcome the major limitation of the study as described in chapter 1, which is that the diagnosis of malaria remains subject to what the participants report.

Chapter 2

In chapter 2, we describe the study of travelling kidney transplant recipients, in which we found that the majority (80%) travelled outside the Netherlands, 43% travelled outside Western Europe (WE), and 34% outside WE and the northern Americas. At least one in five travellers failed to obtain pre-travel health advice for medically more hazardous destinations, defined as destinations for which at least hepatitis A vaccination is required (VAC+). In addition, one in five travellers seeking information did not receive active or passive immunisation against hepatitis A while they should have, nor was immunoprotection confirmed by hepatitis A serology. Furthermore, one-third of the kidney transplant recipients travelling to VAC+ and one-fifth travelling to VAC- countries acquired a travel-related illness, and almost a quarter of the ill travellers needed to be hospitalised. This is a dramatic disease burden, compared to less than 1% hospitalisation of ill, immunocompetent travellers to the tropics [8].

In respect to self-treatment, only 14% of responders with diarrhea reported to have started self-treatment with antibiotics. In conclusion, it is clear that there certainly is room for optimizing care of this vulnerable group. Travel health specialists should deliberate with the traveller's other specialists to develop an appropriate travel advice [9].
Several limitations of this study have been discussed in chapter 2. Although our findings were very similar to those found by other researchers [10], the major drawback is the retrospective, observational study design. To meet with the need for prospective studies in immunocompromised travellers the study described in chapter 3 was designed by the municipal health centre of Amsterdam and performed in cooperation with the Travel Clinic of the Leiden University Medical Centre. This prospective, controlled study was set up to investigate the burden of travel-related diseases in all immunocompromised travellers seeking advice at the travel clinic. Inclusion of travellers using immunosuppressive medication, HIV-infected subjects, asplenic travellers, diabetics and travellers with inflammatory bowel disease together with their healthy travel partner allowed for adjustment of exposition to pathogens while abroad. Inclusion of diabetic travellers was met in 2008, and the analysis of the development of infectious diseases is described in chapter 3 of this thesis. Inclusion of otherwise (non-diabetic) immunocompromised travellers is ongoing, and results of the travel-disease burden for these groups will be published when numbers needed to include are reached.

Chapter 3

The main result of the study described in chapter 3 is the lack of difference in prevalence of travel-related diarrhea between prospectively monitored medication dependent diabetic travellers and their healthy travel-partner (respectively 44% and 41%). Also the prevalence of vomiting, fever, cough, or rhinitis did not differ. This result was unexpected, as in a retrospective population-based survey including 423 insulin dependent diabetics (IDD) and non-insulin dependent diabetics (NIDD), and more than 8000 controls, Bytzer et al. found a significantly higher prevalence of non-travel-related diarrhea among diabetics (adjusted OR 2.06, 95%CI 1.56 - 2.74) [11]. Travelling has also been associated with metabolic dysregulation in 68% of IDD [12], and in the study by Bytzer et al., the increased prevalence of symptoms was correlated with poorer levels of glycemic control [11]. The prevalence of metabolic dysregulation found in our study was low: 4.3% among IDD and 2.4% among NIDD, possibly due to advances in the quality and use of insulin preparations and treatment schedules. [13-15].

Another prominent result is the fact that in spite of specific instructions, 83% of all diabetics with diarrhea did not use their stand-by antibiotic treatment, not even in the case of metabolic dysregulation (in 2 out of 3 diabetics with diarrhea). Considering that 93% of stand-by antibiotics were not used, makes stand-by treatment cost-ineffective. Frequent blood glucose monitoring, adjustments in medication (insulin dosage) and diet are probably more helpful in minimising the impact of diarrhea or fever on metabolic dysregulation. The conclusion of this study is that the advice to use antibiotics for stand-by treatment of travellers' diarrhea is poorly adhered to, and probably not efficacious, and should therefore not be routinely recommended to diabetics, or prescription should be restricted to those in whom metabolic dysregulation is expected, with strict instructions on when to start antibiotics. With respect to the kidney transplant recipients who similarly failed to use their prescribed antibiotics, it should be stressed that they do benefit from the use of antibiotics in case of diarrhea, reflected by the high morbidity of travel-related diseases reported in chapter 2. These results clearly show the difference in immune compromised state and subsequent susceptibility for serious infectious diseases.

Prevention of travel-related diseases by vaccination – protecting specific populations

Chapter 4

As the baby boomer's generation retires, many will have time and money to travel abroad. These elderly travellers are vulnerable to the effects of travel-related stress, transportation environments, foreign disease, temperature extremes, and acute illnesses [16]. With cardiopulmonary, renal and immunological functions declining with longevity, health care professionals are responsible for counselling these elderly travellers on travel preparation (itinerary, medication and insurance), air travel, safety, sun and heat, insect precautions, food and water precautions, and vaccinations.

The immune response to vaccines in elderly can be impaired [17], and may subsequently increase the susceptibility to acquire (travel-related) infectious diseases. In chapter 4 we investigate the immune response against yellow fever vaccine in elderly with respect to the increased risk to serious adverse events.

We demonstrate that in elderly subjects (≥ 60 years), the initial humoral response against yellow fever vaccine is hampered, compared to 40-year younger vaccinees. Significantly lower anti-YF-17D antibody titres are measured at 10 and 14 days after

vaccination in the elderly, but not at 28 days. To our opinion, this may offer a biological explanation for the higher susceptibility to yellow fever vaccine associated viscerotropic disease (YEL-AVD) with increasing age. The yellow fever vaccine contains live attenuated virus that replicates in order to induce an immune response. In general, the viraemia can be detected in 50% of vaccinees and peaks on the 5th day after vaccination [18]. YF-17D viraemia is about 5 $\log_{10}$ lower than viraemia induced by wild type YF virus (1.7 $\log_{10}$ PFU/ml versus 6-8 $\log_{10}$ PFU/ml) [19], reflecting the attenuation of the vaccine strain. An impaired immune response in the first 10 days after vaccination could give ground to a higher YF-17D viraemia, possibly leading to YEL-AVD. The fact that impaired immunity is a risk factor for YEL-AVD is shown by case reports of this fatal condition in immunocompromised persons; HIV [20] and post-thymectomy [21].

The measured difference in antibody response between the younger en elderly subjects is subtle, and could be the reason why it was not picked up by analyzing the response at 30 days post vaccination [22]. Other mechanisms, such as host genetic susceptibility could also play a role in the development of YEL-AVD, as suggested by a recently found heterozygous CCR5 32 mutation in a patient with YEL-AVD [23]. Although not investigated in regard to YEL-AVD, other genetic factors are associated with humoral and cellular response against yellow fever vaccine, and could also play a role in the development of adverse events [24]. Since the condition of YEL-AVD is extremely rare, the development is likely to be multifactorial [25], e.g. a combination of immunosenescence, CCR5 mutations and other yet to be discovered risk factors.

Taken together our results described in chapter 4 and those of Monath et al. [22], indicate that elderly travellers can be adequately protected against yellow fever by vaccination. All subjects showed seroprotection at day 14 after vaccination. Nonetheless, concern about inducing a serious, possibly fatal event by vaccination remains. Since the first cases of YEL-AVD were published, the WHO has strongly advised to weigh the risk of vaccination against the risk of acquiring yellow fever. In the daily practice, this means that severely immunocompromised individuals (HIV infected with CD4 cell counts <200/ml, rheumatologic, transplant or inflammatory bowel disease patients using immunosuppressive medication and patients with a history of thymectomy) are advised not to visit endemic areas. Mildly immunocompromised, including elderly, are vaccinated if they visit yellow fever endemic or transitional regions, and advised to use anti-mosquito bite protection if travelling to low risk regions [19].

The possible role of the immune response lagging behind in the development of YEL-AVD in elderly calls for new approaches to prevent YEL-AVD in elderly who need protection against yellow fever because of their travel destination. In Chapter 6 we obtained equal protective immune response by intradermal injection of a reduced dose of yellow fever vaccine compared to the conventional vaccine dose injected intramuscularly [26]. However, if intradermally injected antigens indeed elicit higher immune responses because they are directly targeted towards the antigen presenting cells (APC) in the skin, injecting even lower amounts of vaccine virus may induce an antibody response without inducing a detectable viraemia. Unfortunately, in animal experiments, ageing is associated with a lower density of Langerhans cells (LC), a population of antigen presenting cells in the skin [26]. Whether this also applies to dermal dendritic cells in humans is unknown.

Another possibility is the injection of inactivated YF-17D, as suggested by Gaspar et al. [28]. The 17DD virus (10^4 PFU/dose) was inactivated by hydrostatic pressure, and inoculated subcutaneously in mice on days 0, 15 and 30. As expected, neutralising antibody titres measured 2 weeks after each vaccination were significantly lower (respectively 10-fold, 4-fold and 10-fold) in the mice vaccinated with the inactivated vaccine compared to the live vaccine. Forty-five days after the first vaccination the mice were challenged with a intracerebrally injected, lethal dose of YF-17DD, and all vaccinated mice survived (irrespective of the live or inactivated vaccine virus). The authors are currently testing the immunogenicity of priming with pressure-inactivated 17DD virus and boosting with the live virus vaccine, which, if successful, would be appropriate for the elderly travellers, as YF-17D viraemia is undetectable in subjects with pre-existent yellow fever immunity [18,26]. In addition, higher doses of inactivated YF-17D should also be tested in order to induce a better response.

Chapter 5

In chapter 5 we focus on subjects who failed to mount a protective immune response to 6 standard intramuscular hepatitis B vaccinations. The biological mechanism for this impaired response remains unidentified. We investigated whether stimulation of the immune response by application of a TLR7 agonist, imiquimod, on the skin prior to intradermal (ID) hepatitis B vaccination would benefit these non-responders (NR). Unfortunately, we found that imiquimod application did not enhance the humoral response. However, irrespective of imiquimod application, 70% of the NR developed a protective immune response after 3 ID hepatitis B vaccinations with 5 μg HBsAg. This is the first study demonstrating the induction of a protective immune response to additional intradermal hepatitis B vaccinations in individuals who failed to respond to

6 prior vaccinations. The presence of high avidity antibodies after the first ID dose suggests that the previous vaccinations did induce the development of a small number of antigen specific lymphocytes, although not enough for a measurable antibody response.

Since the mechanism of non-response to the hepatitis B vaccine is unknown, a rational approach to overcome this defective response remains challenging. Clearly, our approach to enhance antigen presentation with a TLR7 agonist was not effective.
Several hypotheses have been investigated previously to explain non-responsiveness. Besides the well-known demographic and behavioural factors associated with non-responsiveness, such as smoking, obesity, male gender and old age [29-31], genetic associations with non-responsiveness have been identified. The suggestion that MHC-linked genes may also control the human immune response to HBsAg was first made by Walker et al. who observed a significant excess of HLA-DR7 and a total absence of HLA-DR1 in HBsAg vaccinated low or non-responders [32]. Subsequent studies demonstrated that the HLA class II alleles HLA-DR3, -DR7, -DQ2 and -DP11 are associated with low or non-responsiveness and HLA-DR1, -DR5, -DR2, -DQ5 and -DP4 are associated with strong humoral responses to HBsAg vaccination [33]. Since the involvement of MHC is likely to influence the development of the adaptive immune response against HBsAg, the following steps in the response have been investigated: antigen presentation by APC, T helper cell proliferation and regulatory T cell activation. The different steps towards an adaptive immune response following hepatitis B vaccination are illustrated in figure 1 [34].

A defect in epitope selection and presentation was not found in T cell-APC mixing experiments using responder T cells and non-responder APC [35-37]. However, in these experiments non-responder APC were only examined for their capacity to stimulate a recall response to HBsAg in vitro and therefore the role of these APC to induce a primary anti-HBs response in vivo is not determined. With respect to the results described in chapter 5, stimulating APC with imiquimod would be less likely to enhance the antibody production if the in vivo functionality of APC is comparable to the in vitro findings. Another limitation of these T cell-APC mixing experiments, is that they do not exclude a defect in the migration of APC towards the draining lymph node. Nonetheless, if this migration is antigen specific a positive effect of imiquimod would be expected, and if this is not antigen specific, which is more likely, one would expect a much wider range of immune deficiencies in hepatitis B non-responders.

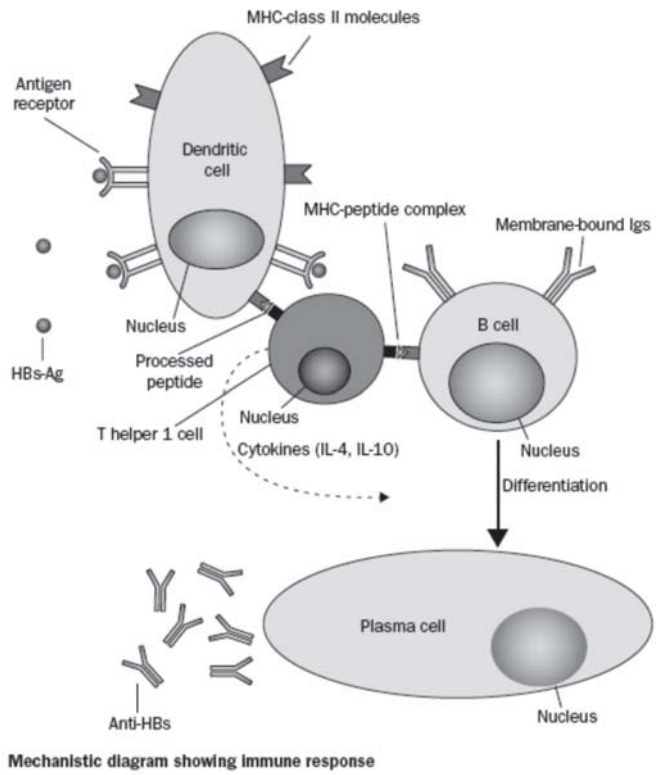

Figure 1 Mechanistic diagram showing the different required steps for the induction of an adaptive immune response against HBsAg. Adapted from ref 34

HBs-Ag = Hepatitis B surface antigen, MHC = major histocompatibility complex, Igs = immunoglobulins. The HBsAg is presented by MHC class II to T helper cells.

A 'hole in the T cell repertoire' as underlying defect in the hepatitis B vaccine response, meaning either the lack of T cells with receptors able to recognize HBsAg peptide-class II complexes or unresponsiveness when T cell receptors do recognize the antigen, was investigated by proliferating T cells of good responders and non-responders after stimulation with HBsAg [38-39]. Desombere et al. demonstrated that the T cell response of good responders to HBsAg vaccine was multispecific and polyclonal (numerous epitopes and restricting elements) whereas the T cell response of poor

and non-responders was paucispecific and oligoclonal (few epitopes and restricting elements). By using a panel of synthetic peptides representing selected sequences of the HBsAg, the specificities of each of these T cell lines were determined, and revealed that the majority of the identified T cell epitopes was located in and around the first hydrophobic transmembranous region of the HBsAg. This was observed in T cell lines from good and poor vaccine responders, without distinction [39]. These data, together with the diminished proliferation capacity and impaired IL-2, IL-10 and IFNg production of non-responder T cells to HBsAg demonstrated by Chedid et al. and Kardar et al. [38,40], suggest that the hyporesponsiveness to HBsAg may be caused by defective T cell recognition of HBsAg which is more likely due to a 'hole in the repertoire' than to inadequate antigen presentation by APC.

Failure of T cells to respond to HBsAg may also be explained by a lack of antigen-specific T cell help or by an excess of antigen-specific suppression mediated by regulatory T cells. Scarce data support this hypothesis, as increased numbers of CD4+CD25+FoxP3+ regulatory T cells were demonstrated in the blood of non-responders after normal hepatitis B vaccination, compared to high-responders [41]. Whether these regulatory T cells are HBsAg specific remains to be investigated.

Although such 'obstinate' non-responders as described in chapter 5 were never studied before, the strategy to overcome nonresponse to HBsAg vaccine is the administration of additional doses demonstrated by Wismans et al. who showed that supplementary vaccination of healthy hypo- and non-responders after standard hepatitis B vaccination induced an anti-HBs titre greater than 10 IU/l in 38% after one and in 75% after three additional doses of 20 micrograms of hepatitis B vaccine given intramuscularly [42]. Others reported seroconversion in 61% of the revaccinated [43]. Our findings found 70% seroconvertion of true non-responders after additional vaccinations. And the T cell proliferation, although decreased, after identical epitope stimulation in non-responders [39] also support this strategy. Non-response is evidently a multifactor mechanism, with a cumulative negative influence of different factors (e.g. age, weight, gender, smoking, HLA-profile) on the adaptive immune response.
In line with our attempt to enhance this response with imiquimod, many other adjuvants are investigated, such as AS04 [44] which elicits a superior response in non-responders compared to the licensed vaccine, although the experimental vaccine contained 40μg HBsAg and the licensed vaccine 20μg HBsAg. Also novel antigenic formulations (PreS/S) are tested in non-responders, and show that the boundaries of the immune response in these subjects can be stretched [45].

Whether the ID route of HBs-antigen delivery is superior to the intramuscular route remains uncertain, as we have not included a control group of participants who received a similar low vaccine dose intramuscularly. On the other hand, the ID vaccination route has shown to be a potential vaccination route for several vaccine antigens [46] and could also have contributed to the high rate of seroprotection with high avidity antibody response in these NR.

Prevention of travel-related diseases by vaccination – increasing vaccine dose availability

Chapter 6, 7 and 8

The intradermal vaccination, described in chapter 5, used to immunise hepatitis B non-responders is a recently rediscovered possibility of vaccine dose reduction through augmented immune stimulation, that received much attention from vaccinologists [46-48]. By reducing the vaccine dose needed for immunisation, costs per vaccine dose decrease and vaccine stockpiles last longer, possibly leading to higher vaccine coverage. In chapters 6, 7 and 8 the intradermal vaccination as an immunity enhancing route of inoculation is studied for yellow fever, a live attenuated vaccine virus, and for rabies vaccine, an inactivated virus.

Intradermal administration of reduced amounts of both vaccines, 1/5th of the yellow fever vaccine and 1/10th of the rabies vaccine, elicited protective immune responses. We have shown that in the case of yellow fever, the reduced dose injected intradermally is non-inferior to the subcutaneous dose, and that the reduced rabies vaccine dose elicits a protective response (in correspondence with WHO definitions of protection), but we have not demonstrated the superiority of the intradermal immunisation *per se* since no comparison was made between the reduced vaccine dose administered intradermally and the reduced dose administered by the conventional immunisation route

In support of the superiority of the intradermal route, Cubas et al. recently showed that intradermal inoculation of virus-like particles (VLPs) of simian-human immunodeficiency (SHIV) in mice induced enhanced immune responses compared to intramuscular, intraperitoneal and subcutaneous inoculation. By optical imaging, the trafficking of the VLPs after immunisation was directly visualized, thereby showing that intradermal immunisation led to the largest level of lymph node involvement for the longest period of time, which correlated with the strongest humoral and cellular

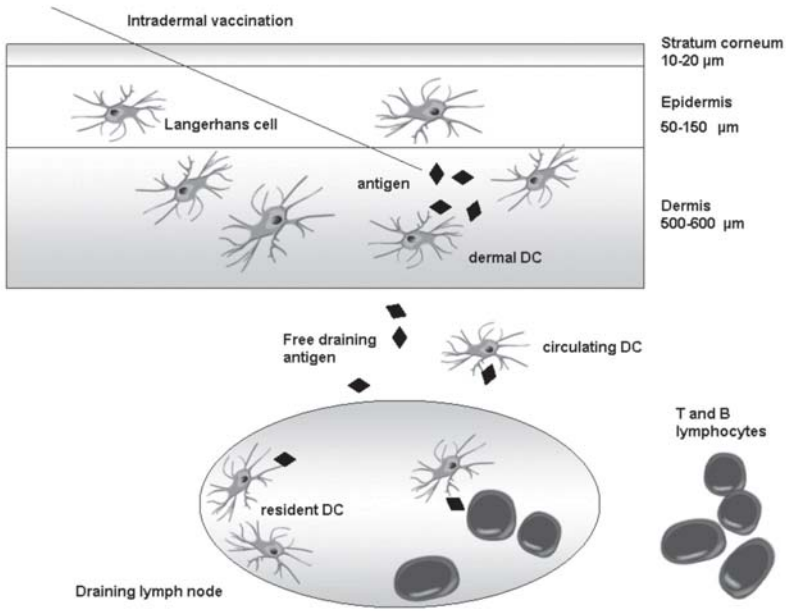

Figure 2 Schematic representation of intradermal immunisation and subsequent initiation of adaptive immune response [after ref 46]

DC = dendritic cell.

immune responses [49]. These findings should now be investigated with respect to other antigenic formulations.

The immune response following intradermal immunisation is depicted in Figure 2. In response to the injected antigen, with or without adjuvant, immature DC's residing at the site of vaccination (LC or dermal DC) undergo a maturation process that is characterised by expression of costimulatory molecules and inflammatory cytokines [50]. With respect to yellow fever, the in vivo sites of replication of YF-17D have been determined in cynomolgus macaques [28]. After subcutaneous inoculation, small amounts of 17D virus were found in the skin at the site of inoculation, in the draining lymph nodes and mesenteric lymph nodes at the peak of viraemia (day 3 for these primates). By day 7, liver, spleen, bone marrow, thymus and adrenal glands were found to harbour YF-17D. The spleen and lymph nodes remained positive for the virus

up to 14 days after inoculation, and by day 46 the virus was undetectable. These data indicate that the attenuated vaccine virus has a tissue tropism similar to that of wild-type YF, and that the initial process of immune activation occurs between the site of inoculation and the draining lymph nodes, similar to inactivated vaccine antigens. Unlike inactivated antigens which are internalised and presented on MHC class II to $CD4^+$ T cells, live virus particles such as YF-17D are classically presented by DC's on MHC class I to $CD8^+$ T cells. Nonetheless, the robust humoral response to YF-17D, besides the elaborate cellular response recently elicited [51,52] cannot be based on MHC class I presentation to CTL's. The vaccine virus activates multiple subsets of DC's by signalling through multiple TLRs, including TLR2, -7, -8, and -9, resulting in diverse types of adaptive immune responses [53]. This was confirmed by Gaucher et al. who showed an increase in proliferating ($Ki67^+$) YF-specific $CD4^+$ T cells (2.3 fold), $CD8^+$ T cells (4.7 fold), non-T cell PBMCs containing monocytes and B cells (1.9 fold) and NK cells (1.6 fold) within the first 14 days after vaccination, returning to insignificant levels thereafter [52]. The higher magnitude of the $CD8^+$ T cell proliferation could reflect the occurrence of the classical MHC class I presentation of live viral pathogens by DC's. The cellular response following YF-17D inoculation has probably been underestimated, since the antibody response has always been regarded the principal mediator of protection, based on protection by passive immunisation [54]. To support the hypothesis of the response being initiated at the site of inoculation, recent data show that YF-17D replicates in DC's and is then rapidly processed [55]. The predilection of YF-17D for DC's of the skin would not be unexpected, given the natural route of infection via mosquito bites. Thus, YF-17D initiates a response via multiple TLR's on cells of the innate immunity, thereby inducing a broad cellular and humoral response, beside the response initiated via replication in DC's which prime naïve CTL's.

Until today, the precise role of distinct DC subsets such as Langerhans cells, dermal DC's, and plasmacytoid DC's in the process of intradermal immunisation remains largely unknown. Besides the involvement of skin resident DC's in the initial antigen-APC contact, circulating DC precursors can be recruited into the dermis upon intradermal vaccination with a soluble protein, via enhanced expression of chemokine receptor/ligand CCR6/CCL20 [56]. After the uptake and processing of antigens, the maturing DC's migrate to the T cell rich areas of the draining lymph nodes, where they express a mature phenotype characterized by $CD11c^{intermediate\ to\ high}$ and $MHCII^{high}$ [50].

The role of migratory DC's upon viral inoculation in the skin with different viruses is not uniform in the induction of $CD8^+$ T cell responses, as shown by the following mice

experiments. For example, in response to Herpes Simplex virus (HSV) the migratory DC's merely ferry viral antigens to the lymph node and immediately transfer the HSV antigens to CD8α^+ DC's residing in the lymph node for cross-presentation [57]. In contrast, He et al. showed that migratory skin DC's did directly present lentivirus derived OVA to lymph node CD8$^+$ T cells, without cross-presentation to lymph node resident DC's [58]. Nonetheless, Allan et al. demonstrated that inhibition of migration of skin DC's, impaired the CTL response in the induction of HSV antiviral immunity [57], thereby implicating the importance of migratory skin DC's.

Besides the trafficking of antigens through migrating DC's, recent research has highlighted the additional role of direct lymphatic drainage of free soluble antigen within hours after inoculation. This free antigen flows through afferent lymphatics into the subcapsular sinuses of the draining lymph node and is taken up and processed by lymph node resident DC's. After 24 hours, a 2nd antigen wave is delivered to the lymph node by influx of dermal DCs (not LCs) [59]. Even though the resident DCs were responsible for the initial T cell activation, the DCs that acquired antigen at the injection site and migrated to the lymph node were needed to sustain the expression of the IL-2 receptor on the T cells.

For the induction of the humoral response, generally the marker of success of vaccination, T cell-dependent B cell responses begin in the T cell-rich areas of the lymphoid organs, where DC's present antigen to antigen-specific T cells in the context of MHC and costimulatory molecules. The antigen-specific B cells then receive signals from the helper T cells, proliferate, and undergo isotype switching. Some of the activated B cells become extrafollicular antibody-secreting plasma cells while others enter germinal centres, where they undergo somatic mutation to generate high-affinity memory B cells and long-lived plasma cells [60,61]. The precise role played by TLRs and DC's in the germinal-centre reaction and in the generation of memory B cells and long-lived plasma cells is poorly understood. Recently, activation of B cells independently of T cells was shown by free protein antigen (green fluorescent protein) flow to lymph nodes upon epidermal inoculation [62]. Regarding vaccine antigens, T cell independent activation of B cells has only been described for polysaccharide antigens.

Several hypotheses have been postulated to explain the relative success of ID vaccination. Firstly, a more direct antigen – APC contact could lead to a smaller 'loss' of antigen in subcutaneous tissue or the blood circulation where possibly less APC's

are present. This hypothesis is particularly attractive in the case of live attenuated viruses, which need to replicate intracellularly in order to induce a potent immune response. For soluble protein antigens, direct flow via the afferent lymphatic vessels could also contribute to the response [59,62]. Interestingly, it has been shown recently that locally activated mast cells can, via enhanced DC migration, augment the immune response to several vaccine antigens such as protein antigens and vaccinia, a live viral antigen [63]. In chapter 7 we describe the protective antibody response to YF-17D in chicken egg allergic individuals. Unfortunately, whether their antibody response was enhanced compared to non-allergic individuals could not be verified, as their response had not been measured at set time points.

Secondly, ID immunisation can trigger the activation and migration of dermal DCs, thereby amplifying the immune response [59]. In contrast, intramuscular immunisation enhances, via the bloodstream, the activation of plasmacytoid DCs (pDCs) which enter the lymph node via high endothelial venules, similar to B en T cells [64]. pDCs are activated through TLR7 and TLR9 signalling, leading to type 1 IFN secretion. Their functional capacity in terms of vaccination (i.e. antigen presentation and T cell priming) remains to be investigated.

Finally, suggested by the findings of Cubas et al., a greater number of lymph nodes engaged upon ID immunisation might be attributed to the lymphatic structure in the intradermal zone. In the skin, lymphatic vessels form two plexuses [65]. The superficial plexus contains branches that drain vertically into larger lymphatic vessels located in the lower dermis and the superficial zone of the subcutaneous tissue. These deep lymphatic vessels contain numerous valves through which antigen can be taken up. In addition, the limited space in the dermis and relatively large volume inoculated, could affect the permeability of the lymph vessels and thereby increasing antigen uptake [46]. This argument of the volume of inoculation influencing the immune response has been suggested by Fox et al. [66], and should be considered when designing new trials studying the immunisation routes.

The growing interest in ID immunisation by vaccinologists has led to the development of many different technologies to accurately administer vaccine doses intradermally. These techniques include fine-gauge needles and microneedle arrays, as well as various types of needle free devices such as jet injectors, and patches. Novel technologies for ID delivery may simplify the logistics of vaccine administration, avoid needle dangers and overcome other drawbacks facilitating vaccination mass campaigns [46-48].

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Samenvatting

Reizigersgeneeskunde; Reizigers beter beschermen tegen infectieziekten

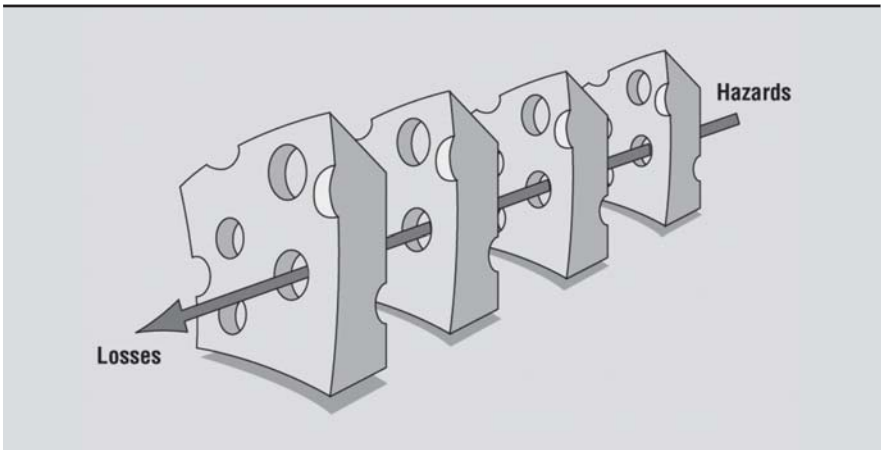
Inleiding

In een tijd waarin steeds meer wordt gereisd naar exotische bestemmingen, worden ook artsen in toenemende mate geconfronteerd met geïmporteerde infectieziekten die niet in hun dagelijkse praktijk voorkomen. Zo werd bijvoorbeeld in 2008 een Nederlandse vrouw in Uganda besmet met het Marburg virus, een zeer besmettelijk virus dat massale bloedingen, falende orgaanfuncties en uiteindelijk de dood tot gevolg kan hebben. De diagnose werd pas een paar dagen na opname in het ziekenhuis vermoed. Afgezien van deze zeer ernstige geïmporteerde infectieziekten, die over het algemeen zeldzaam zijn, krijgt 10% van de reizigers tijdens of kort na hun reis koorts, vaak ten gevolge van infecties van het maagdarmstelsel of de luchtwegen.

Naast het opvangen van reizigers die ziek terugkeren, beoogt de reizigersgeneeskunde ook de reiziger zo goed mogelijk te beschermen tegen mogelijke (infectie) ziekten die voorkomen in het land van bestemming. Deze bescherming kan op verschillende niveaus plaatsvinden, waarvan enkele aspecten worden beschreven in dit proefschrift. Als model voor de bescherming van reizigers in bredere zin, kan het 'Zwitserse kaas model' worden gehanteerd, dat in 2000 beschreven is door James Reason (figuur 1). Reason beschreef dit model als een systeem om menselijke fouten (ten gevolge van een falend systeem) te minimaliseren.

Dit model kan ook toegepast worden op de reizigersgeneeskunde. Voorbeelden van gevaren (hazards) zijn de omstandigheden waarin de reiziger verkeert en die hem of haar kwetsbaar maakt voor besmetting met micro-organismen (bacteriën, virussen, enz) die ziekte kunnen veroorzaken, en de gezondheidstoestand van de reiziger die kunnen maken dat ziekten ernstiger verlopen. Dit laatste is bijvoorbeeld het geval voor reizigers met een verminderde afweer. De verschillende kaasplakken staan model voor de barrières die men kan inbouwen om de kans op besmetting of op een ernstiger verloop van de ziekte te verkleinen. Waar deze bescherming faalt, bijvoorbeeld als een vaccin geen 100% bescherming biedt, vallen er gaten in de kaas. Uiteindelijk leidt de som van barrières en het falen van bescherming tot de kans op ziekte.

De taak van de reizigersgeneeskunde, zoals in andere takken van preventieve geneeskunde, bestaat uit het optimaliseren van deze barrières en het identificeren van de gaten. In dit proefschrift worden een aantal onderzoeken beschreven die dit doel nastreven.



Figuur 1 Zwitserse kaas model van Reason. Hazards = gevaren, losses = schade of ziekte. De verschillende kaasplakken staan voor de verschillende barrières (beschermende maatregelen). Deze zijn schematisch getekend, waarbij in de werkelijkheid de gaten in de verschillende plakken niet overlappen.

Hoofdstuk 1. Langdurig verblijf in malaria gebied: voorkomen en zelf behandelen van malaria

In hoofdstuk 1 werd een malaria preventie programma geëvalueerd. Het programma bestond uit een educatieve training over malaria, een quiz bij aankomst in het land van bestemming, het verstrekken van antimuggenmaatregelen en malariaprofylaxe, testen om malaria bij zichzelf vast te stellen en een geneesmiddel om malaria te behandelen. Het programma werd uitgevoerd in een groep werknemers van oliebedrijven, die gedurende langere perioden in gebieden verbleven waar malaria voorkomt. Hoewel het programma verplicht was, hadden om logistieke redenen niet alle werknemers hieraan deelgenomen, wat de gelegenheid gaf het effect van het programma te onderzoeken. Het is bekend dat deze 'langverblijvers' de beschermende maatregelen tegen malaria niet goed opvolgen. Deze maatregelen omvatten het voorkomen van muggenbeten (malaria wordt door een mug overgebracht), en het innemen van medicatie die de vermenigvuldiging van de malariaparasiet in het lichaam remt. Zoals verwacht was de onderzoeksgroep therapieontrouw (20% nam antimalariamiddelen ter voorkoming van malaria, tegenover 85% van de toeristen naar een malariagebied). Opvallend was echter dat deelname aan het programma dit gebruik verdubbelde (van 20% naar 55%). Voor het gebruik van antimuggenmaat-

regelen werd eenzelfde trend gezien, evenals een gunstige invloed van het programma op de kennis van de deelnemers over malaria. De mogelijkheid om zelf een test uit te voeren om malaria vast te stellen werd eveneens onderzocht. Er werd een daling van het aantal ziekenhuisopnames ten gevolge van malaria gezien bij deelnemers die het programma hadden gevolgd, wat duidt op een eerdere diagnose en behandeling van de ziekte en daardoor minder ernstig beloop.
Een beperking van dit onderzoek was dat de diagnose malaria niet werd gecontroleerd, waardoor de feitelijke juistheid van de zelf-test niet gemeten kon worden. Deze test, indien correct uitgevoerd, zou een uitkomst bieden aan reizigers die symptomen van een malaria infectie hebben en niet direct medische hulp kunnen zoeken.

Hoofdstuk 2. Reizigers met verlaagde afweer: niertransplantatie patiënten

Deze niertransplantatiepatiënten krijgen afweeronderdrukkende medicatie om afstoting van hun getransplanteerde nier te voorkomen. In Hoofdstuk 2 werd onderzocht of deze patiënten met verlaagde afweer op reis gaan en hoe zij zich hierop voorbereidden. Ook werd onderzocht of zij gedurende de reis ziek werden. Een derde van de patiënten was in de voorgaande 5 jaar op reis geweest buiten West-Europa of Noord-Amerika. De helft van de patiënten die reisadvies hadden ingewonnen, deden dit bij hun transplantatie arts; hetgeen wijst op de belangrijke rol van deze arts bij de reisvoorlichting. Opvallend was, dat van de patiënten die ziek werden gedurende hun reis, 25% opgenomen werd in een ziekenhuis. Vergeleken met reizigers zonder afweerproblemen, waarvan 1% van de zieken wordt opgenomen, duidt dit op een verhoogde kans op een ernstiger beloop van ziekte ten gevolge van de transplantatie. Hierbij speelt mogelijk een rol dat deze niertransplantatiepatiënten eerder dan gezonden een ziekenhuis bezoeken bij symptomen van ziekte. Dit onderzoek toont aan dat de voorlichting van deze specifieke groep reizigers kan worden verbeterd door meer aandacht te schenken aan de voorbereiding van de reis, bijvoorbeeld door professioneel advies in te winnen. Hierbij is een belangrijke rol weggelegd voor de behandelende specialist. Door het retrospectieve karakter van het onderzoek zijn de resultaten onderhevig aan de herinnering van de patiënten wat leidt tot een grotere onnauwkeurigheid (afgezien van de resultaten betreffende de ziekenhuisopname). Om deze onnauwkeurigheid uit te sluiten is prospectief onderzoek noodzakelijk.

Hoofdstuk 3. Reizigers met co-morbiditeit: diabetes mellitus

In dit hoofdstuk werd onderzocht of reizigers met suikerziekte (diabetes mellitus) een verhoogde kans hebben op het krijgen van reizigersdiarree. Dit werd namelijk in eerder retrospectief onderzoek beschreven, met als gevolg dat suikerpatiënten op

reis altijd antibiotica meekrijgen ter voorkoming van ernstige ontregeling van hun bloedsuikers ten gevolge van de diarree. Het onderzoek beschreven in hoofdstuk 3 werd prospectief uitgevoerd, wat betekent dat de deelnemers tijdens hun reis dagelijks notitie maakten van het optreden van eventuele symptomen. Omdat het voorkomen van infectieziekten sterk afhangt van de reisbestemming en blootstelling aan micro-organismen, werden naast de diabetische deelnemers ook hun (gezonde) reispartner geïncludeerd. Het belangrijkste resultaat van dit onderzoek was dat suikerpatiënten geen hogere kans hebben op het krijgen van reizigersdiarree dan hun gezonde reispartner. Slechts 2.4 en 4.3% (respectievelijk niet insuline afhankelijke en insuline afhankelijke diabeten) had een ontregeling van de bloedsuiker. Van deze deelnemers had de helft klachten van diarree. Ook bleek uit het onderzoek dat slechts 17% van de diabeten die diarree kregen de voorgeschreven antibiotica innam, wat het nut van het voorschrijven van deze antibiotica nog verder ter discussie stelt.

Hoofdstuk 4. Reizen op oudere leeftijd: implicaties voor vaccinatie met verzwakt levend vaccin

Met een toenemend aantal reizigers in de afgelopen jaren, neemt ook de groep oudere reizigers significant toe. Nu babyboomers de pensioengerechtigde leeftijd bereiken, relatief gezond en vaak niet onbemiddeld zijn, zullen meer en meer ouderen ook verre reizen gaan maken. Deze groep vormt een extra uitdaging om ze weer gezond terug te laten keren naar huis. Afgezien van logistieke maatregelen met betrekking tot geneesmiddelen, verzekeringen enz., neemt het vermogen om beschermende afweer op te bouwen na vaccinatie af met het ouder worden. Om deze afweer te verhogen zou bijvoorbeeld de dosis van het vaccin verhoogd kunnen worden.

Voor het gele koorts vaccin geldt nog een ander probleem. Het gele koorts vaccin bevat levend gelekoortsvirus, dat verzwakt is en dus in principe geen ziekte kan veroorzaken. Na vaccinatie vermenigvuldigt het virus zich in het lichaam. De afweerreactie die daarop volgt en het virus opruimt, zorgt voor een levenslange bescherming tegen de ziekte gele koorts. In de afgelopen jaren werden er wereldwijd tientallen gevallen beschreven waarbij personen na toediening van het gele koorts vaccin de ziekte gele koorts ontwikkelden. Waarschijnlijk zag het vaccinvirus een kans zich veel meer te vermenigvuldigen dan normaal. Uit eerder onderzoek bleek een hogere leeftijd een onafhankelijke risicofactor te zijn voor het ontwikkelen van deze bijwerking. In hoofdstuk 4 werd onderzocht of dit het gevolg was van een tragere afweerreactie bij ouderen waardoor het vaccin virus zich dus langer en meer kan vermenigvuldigen. Een groep jongeren (gemiddelde leeftijd 22) en een groep ouderen (gemiddelde leeftijd

65) werden gevaccineerd en op dezelfde tijdstippen werd de afweer tegen gele koorts gemeten. Uit dit onderzoek bleek dat de beschermende afweer (uitgedrukt in antistoffen) in ouderen inderdaad later opkomt.

Hoofdstuk 5. Verlaagde respons op vaccinatie: hepatitis B vaccinatie

Hepatitis B virus kan een chronische ontsteking van de lever veroorzaken wat uiteindelijk tot leverkanker kan leiden. Omdat dit virus via seksuele weg of bloed wordt overdragen en personen op reis een verhoogd risicogedrag vertonen waardoor ze geïnfecteerd kunnen worden met het virus, worden reizigers in veel landen laagdrempelig gevaccineerd tegen het hepatitis B virus. In Nederland worden reizigers die veelvuldig reizen of lange reizen gaan maken gevaccineerd.

Afgezien van reizigers worden in Nederland verschillende beroepsgroepen, waaronder medisch en paramedisch personeel, gevaccineerd tegen hepatitis B, omdat zij in contact kunnen komen met gecontamineerd patiëntenmateriaal. Helaas maakt 5 tot 10% van de gezonde personen geen afweerstoffen (anti-hepatitis B antistoffen) na vaccinatie. Er is nog geen duidelijke verklaring gevonden voor het uitblijven van deze reactie op het vaccin. Wel zijn mannelijk geslacht, roken, hogere leeftijd en overgewicht geassocieerd met een slechtere afweerrespons. In hoofdstuk 5 werd onderzocht of met een afweerstimulerende crème (imiquimod) deze reactie alsnog op te wekken viel. De crème wordt gebruikt voor de behandeling van (genitale) wratten, waarbij het virus dat deze wratten veroorzaakt (humaan papilloma virus) door gespecialiseerde witte bloedcellen (dendritische cellen) in de huid naar de lymfeklier wordt getransporteerd en er zo een volledige afweerreactie ontstaat. De crème activeert deze dendritische cellen waardoor de afweerreactie wordt gestimuleerd. In het onderzoek werd het hepatitis B vaccin in de huid (intradermaal) geïnjecteerd, onmiddellijk nadat de crème op de huid was aangebracht. Om het effect van de crème te onderzoeken werd één groep met crème gevaccineerd en een groep zonder. Er werd geen verschil gemeten tussen beiden groepen. Wat wel opviel was dat 70% van de proefpersonen, die na 6 eerdere hepatitis B vaccinaties (in de spier) geen beschermende afweerreactie hadden ontwikkeld, dat na intradermale vaccinatie wel deden.

De reden voor het uitblijven van een betere respons na stimulatie met de crème kan zijn dat de afweercellen die gestimuleerd worden niet de cellen zijn die verantwoordelijk zijn voor de slechte afweerrespons. Verder is het mogelijk dat het effect van de crème virus-specifiek is, en dus wel geobserveerd wordt bij infecties met humaan papilloma virus, maar niet bij andere virussen of virusdeeltjes. Het feit dat 70% van de deelnemers (ongeacht de onderzoeksgroep) een beschermende respons ontwikkelde leidt tot de hypothese dat uiteindelijk iedereen afweer tegen dit vaccin kan opbouwen, mits er

voldoende (qua dosis of frequentie) gevaccineerd wordt. Dit is aannemelijk, aangezien de kwaliteit van de antistoffen, uitgedrukt in de sterkte van de binding aan het ingeënte hepatitis B eiwit, al na de eerste vaccinatie hoog was.
De superioriteit van het eenmalig aanbrengen van de afweerstimulerende crème kon in deze groep niet worden aangetoond omdat de bescherming in de controlegroep veel hoger bleek dan verwacht. De grootte van de onderzoekspopulatie was dus te klein om een verschil tussen de controlegroep (zonder crème) en de interventiegroep (met crème) aan te tonen. De superioriteit van de vaccinatie in de huid ten opzichte van de conventionele vaccinatie werd niet aangetoond, aangezien geen controle groep werd geïncludeerd waarbij dezelfde dosis in de spier werd geïnjecteerd.

Hoofdstuk 6 en 7. Gele koorts vaccin dosisverlaging: vaccinatie in de huid

Het gele koorts vaccin dat in hoofdstuk 5 reeds ter sprake kwam, is een levend verzwakt vaccin dat toegediend wordt aan reizigers naar tropisch Afrika en Zuid-Amerika. In de natuur wordt het virus overgebracht door muggen, en infectie bij de mens kan lever-ontsteking, multi-orgaanfalen en uiteindelijk de dood tot gevolg hebben. De enige bescherming tegen gele koorts is vaccinatie, aangezien er geen geneesmiddel tegen de ziekte bestaat. Het vaccin is een levend verzwakt virus dat op kippeneieren wordt gekweekt. Het is een bewerkelijk productieproces waardoor ten tijde van epidemieën gemakkelijk vaccinschaarste kan ontstaan. De wereldgezondheidsorganisatie verhoogt ieder jaar de voorraad gele koortsvaccin om tijdig te kunnen reageren op jaarlijkse epidemieën door middel van vaccinatiecampagnes.
In Hoofdstuk 6 wordt in een vergelijkend gerandomiseerd onderzoek aangetoond dat de intradermale vaccinatie met 1/5de van de normale vaccindosis (0.1ml) een evenwaardige afweerreactie tot gevolg had als vaccinatie van 0.5ml via de conventionele subcutane (onder de huid) weg. Door in tijden van schaarte intradermaal in plaats van subcutaan te vaccineren, kan de voorraad gele koorts vaccin theoretisch vervijfvoudigd worden.
Het onderzoek beschreven in hoofdstuk 6 is het eerste onderzoek waarin aangetoond werd dat intradermale vaccinatie met een kleine dosis gele koorts virus een evenwaardige beschermende afweerrepons tot gevolg heeft. Het succes van deze spaarzame vaccinatiemethode wordt toegeschreven aan de aanwezigheid van specifieke witte bloedcellen (dendritische cellen) in de bovenste lagen van de huid, die de vaccindeeltjes efficiënt kunnen afbreken en presenteren aan de afweercellen in de lymfeklieren, waar uiteindelijk de afweerreactie tegen het virus ontstaat. De subcutane (onder de huid) en intramusculaire (in de spier) vaccinaties maken mogelijk minder aanspraak op deze gespecialiseerde dendritische cellen, waardoor meer

vaccin 'verloren' gaat. Onlangs is in muizen aangetoond, dat als fluorescerende deeltjes in de huid werden ingespoten er meer en langer deeltjes werden teruggevonden in de lymfeklieren, dan wanneer de fluorescerende deeltjes onder de huid, in de spier of in de buikholte werden ingespoten. Dit onderzoek ondersteunt onze bevindingen.

Hoofdstuk 8. Rabiës vaccin dosisverlaging: vaccinatie in de huid

Zoals in hoofdstuk 8 beschreven voor de gele koorts vaccinatie, werd ook de intradermale vaccinatie van rabiës (hondsdolheid) vaccin onderzocht. Evenals gele koorts, wekt dit vaccin (een dood vaccin) na toediening in de huid een goede afweerrespons op. Daarnaast bleek ook een intradermale rabiës revaccinatie (booster) effectief. Een booster is een vaccinatie die het afweergeheugen aanspreekt, na een eerdere (primaire) vaccinatie, waarbij snel een zeer hoge afweerrespons tot stand komt. De intradermale rabiësvaccinatie biedt een uitkomst voor het dure vaccin dat geïndiceerd is voor reizigers die langdurige en avontuurlijke reizen maken. Hierbij is de kans om door een dolle hond, of ander zoogdier besmet met hondsdolheidvirus, gebeten te worden zodanig dat vaccinatie vóór de reis sterk aangeraden wordt. Vanwege de kostenreductie door dosisverlaging kiezen meer reizigers ervoor om zich via intradermale weg te laten vaccineren. De intradermale rabiësvaccinatie wordt om financiële redenen op ruime schaal toegepast in ontwikkelingslanden.

De intradermale vaccinatie als methode om een effectieve afweerrespons op te bouwen wordt de laatste jaren uitvoerig onderzocht met betrekking tot de verschillende vaccins, zoals gele koorts en rabiës, maar ook met betrekking tot de methode van intradermaal vaccineren. Voorbeelden hiervan zijn bijvoorbeeld vaccinatie met zeer kleine naaldjes, of vaccinatie met een pleister, waarbij het vaccin door de huid wordt opgenomen. Deze nieuwe methoden kunnen de toediening en daarmee de implementatie van de intradermale vaccinatie stimuleren.

Beschouwing en conclusie

Bescherming van reizigers tegen infectieziekten kan op verschillende niveaus worden nagestreefd. Het Zwitserse kaasmodel, zoals beschreven in de inleiding van deze samenvatting, geeft weer dat op elk niveau fouten kunnen optreden die kunnen leiden tot ziekte. In dit proefschrift staan verschillende onderzoeken beschreven die kunnen bijdragen aan de bescherming van reizigers tegen infectieziekten.

List of abbreviations

APC	Antigen Presenting Cell
BCG	Bacille Calmette Guérin
CI	Confidence Interval / Cumulative Incidence
CMK	Curative Malaria Kit
CTL	Cytotoxic T Lymphocyte
DC	Dendritic cell
GMT	Geometrical Mean Titre
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSV	Herpes Simplex Virus
ID	Intradermal
IDD	Insulin-dependent Diabetic
IM	Intramuscular
IU	International Units
KAP	Knowledge, Attitude and Practice
KTX	Kidney Transplant Recipient
LC	Langerhans cell
LUMC	Leiden University Medical Centre
MHC	Major Histocompatibility Complex
NA	Neutralising Antibodies / Northern America
NIDD	Non-insulin-dependent Diabetic
NR	Non-responder
OR	Odds Ratio
PBMC	Peripheral Blood Mononuclear Cell
PCECV	Purified Chick Embryo Cell Vaccine
PCR	Polymerase Chain Reaction
PFU	Plaque Forming Unit
RFFIT	Rapid Fluorescent Focus Inhibition Test
RR	Relative Risk
SC	Subcutaneous
RT-PCR	Reverse Transcriptase-PCR
SAE	Serious Adverse Event
SHIV	Simian-Human Immunodeficiency Virus

SOT	Solid Organ Transplant
TLR	Toll like Receptor
VFR	Visiting Friends and Relatives
VLP	Virus-like Particle
VN	Virus Neutralisation
WE	Western Europe
WHO	World Health Organisation
YEL-AND	Yellow Fever Vaccination associated Neurotropic Disease
YEL-AVD	Yellow Fever Vaccination associated Viscerotropic Disease
YFV	Yellow Fever Virus
YF-17D	Yellow Fever 17D (vaccine virus)

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Curriculum vitae

Anna Helena Elvire Roukens werd op 1 juli 1979 geboren in Arnhem. Zij behaalde in 1996 haar eindexamen aan de Europese School Brussel, België. In 1996 startte zij met de studie Biomedische wetenschappen aan de Vrije Universiteit Brussel, waarvan zij in 1997 de propedeuse behaalde. In 1997 startte zij met de studie Biomedische wetenschappen en in 1999 met de studie Geneeskunde aan de Universiteit Leiden. Biomedische wetenschappen rondde zij af met een onderzoeksstage aan de Rockefeller Universiteit te New York, Verenigde Staten van Amerika, waarna zij in 2002 haar doctoraal behaalde. Het artsexamen werd in 2004 afgelegd. Aansluitend startte zij met promotieonderzoek bij de afdeling Infectieziekten van het Leids Universitair Medisch Centrum, onder leiding van dr. L.G. Visser en prof. dr. J.T. van Dissel. De resultaten van het promotieonderzoek staan beschreven in dit proefschrift. Sinds 1 januari 2009 is zij werkzaam als assistent in opleiding Interne Geneeskunde in het Bronovo Ziekenhuis in Den Haag (opleider dr. J.W. van 't Wout). Op 1 mei 2011 zal zij haar opleiding vervolgen in het Leids Universitair Medisch Centrum (opleider prof. dr. J.A. Romijn).

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