Travel medicine : knowledge, attitude, practice and immunisation

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Chapter outline

The general introduction provides background information to the field of travel medicine from a historical, public and medical point of view.

Chapter one describes the effect of a malaria prevention programme and performance of self-diagnosis and standby treatment of malaria in long-term travellers to malaria endemic regions.

Chapter two describes the travel-health preparations and travel-related morbidity of kidney transplant recipients travelling to developing countries.

Chapter three reports on infectious complications in travellers with diabetes (insulin and non-insulin dependent), and their use of antibiotics in case of disease ocurrence.

Chapter four reports on the immune response in healthy elderly elicited by the live attenuated yellow fever vaccine. The response in elderly is compared to the response in younger vaccinees.

Chapter five addresses the intradermal Hepatitis B vaccination after topical application of an immunostimulant ointment, as a method to augment the immune response in previously non-responders to the vaccine.

Chapter six describes the non-inferiority of intradermally administered yellow fever vaccine at a reduced dose (0.1ml) compared the the conventional subcutaneous dose (0.5ml), in order to reduce the dose needed to elicit protective immunity.

Chapter seven focuses on the intradermal test dose of yellow fever vaccine in individuals with egg allergy who develop a local skin reaction to the vaccination.

Chapter eight reports on the immunity and safety of the intradermal inoculation route for pre-exposure primary and booster rabies vaccination with a purified chick embryo cell vaccine (PCECV).

The general discussion elaborates on the clinical perspectives of the studies concerning different types of travellers, on the immunology underlying the different routes of vaccination and different types of vaccines, and contemplates on future perspectives in research concerning Travel Medicine.

Introduction

General introduction – Travel medicine

In an epoch where every generation travels more frequently and at longer distances than the previous generation, with a mean increase of 30 million travellers per year from 1995 until today [1], physicians throughout the world are confronted with new diseases. From the perspective of Western medicine, the import of highly contagious exotic infections remains an ominous but realistic threat, as shown by a Dutch patient who returned from Uganda carrying Marburg virus [2]. More than just a threat is the fact that approxmately 10% of travellers to developing countries experience a febrile illness, during or immediately after travel [3]. In absolute numbers, this implies that each year, roughly 4 million travellers appeal to specialised health care, either abroad or at home, because of systemic febrile illness, diarrhea or dermatologic disorders [4].

During the last decades, travel medicine has evolved into a distinct discipline of Infectious Diseases, eventhough transmission of infectious agents into vulnerable populations through travel has been well know for centuries. For example when the Spanish conquistadors invaded the Central and South American continents and annihilated (also by murdering) 95% of indigenous populations [5] . In fact, all major epidemics that have afflicted the human race have been spread internationally by travellers. Examples are the plague, which killed one third of the affected population, [6] throughout Europe between the fourteenth and eighteenth centuries, and syphilis, which is believed to have originally been imported into Europe from the New World by Spanish sailors [7]. Scientific medical publications in the field of travel medicine start to appear in the 1950's with mainly topics on the impact of air and space travel on physical conditions and pre-existing illnesses, and individual reports of observed diseases during journeys (PubMed Database, MeSH terms "Travel Medicine", approximately 6300 hits). By the late 1960's the first randomised controlled trial to investigate antimicrobial prevention of traveller's diarrhea was reported [8], as well as case reports on imported infectious diseases by travellers, such as malaria [9]. In 1970, a novel perspective of travel medicine was introduced, in which travellers were defined as short-term travellers (vacational tourists), long-term travellers (e.g. expatriates) and immigrants and travellers visiting friends and relatives (VFRs) (those originating form tropical countries), among whom different risks of acquiring travel-related diseases could be distinguished [10]. Following closely on new travelling trends, specific norovirus outbreaks among cruise ship passengers were reported [11,12]. Since the 1990's, the

number of scientific articles on Travel Medicine has increased almost threefold compared to the preceding decades (figure 1), implicating the increase of importance to and attention by the medical profession of this discipline of Infectious Diseases.

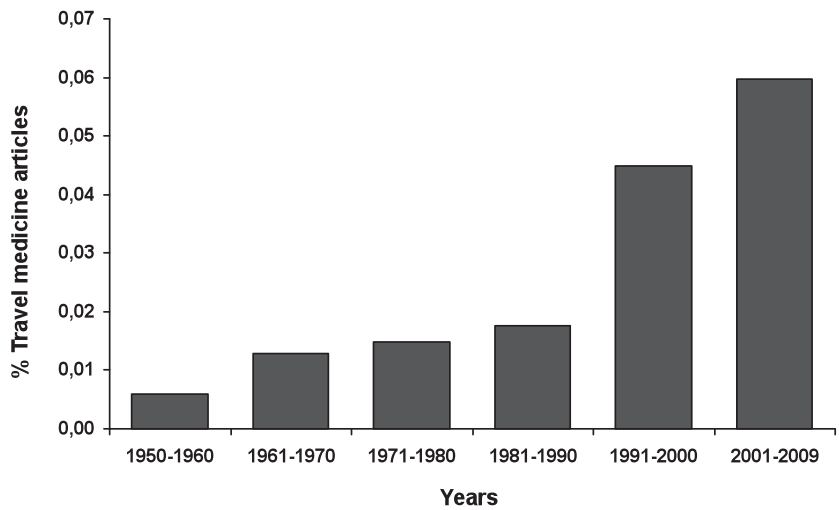

Figure 1 Percentage of articles on travel medicine published (PubMed Database, MeSH Term Travel Medicine, per decade), according to the total number of scientific medical articles published (PubMed Database, total number of articles per decade).

Hand in hand with travelling comes protection against travel-related diseases, which can be achieved on an individual and a population level. As preventive travel medicine covers multiple fields, from training to vaccination, individual and population-wide protection can be achieved on these different levels. A model to explain cumulative protective medical measures, and the occurrence of its failures, was proposed by James Reason as the "Swiss cheese" model [13]. According to this metaphor, in a complex system, hazards are prevented from causing human losses or illnesses by a series of barriers. Each barrier has unintended weaknesses or holes, giving the similarity with Swiss cheese (figure 2).

Defences, barriers, and safeguards occupy a key position in this system approach. By defining the barriers, and the (potential) holes, the system can be improved and the

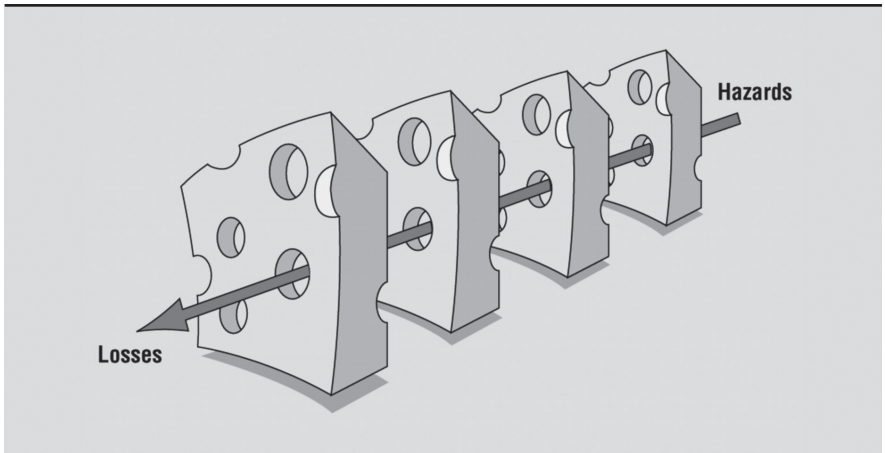

Figure 2 Swiss cheese model of how defences, barriers, and safeguards may be penetrated by an accident trajectory [adapted from 13]. The slices of cheese are schematic and should either be positioned differently, or have different position of the holes, leading to non-overlapping holes.

hazards minimised, which can also be applied to travel medicine. This Swiss cheese model can be applied to the field of travel medicine, in which the slices and holes of the cheese are related to different aspects of protection against travel-related diseases in Table 1.

By applying the model on travel medicine, improvement of the system of protection against travel-related diseases can be achieved through knowledge on the following topics; 1. Epidemiology 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 against vaccination, and 5. Availability of preventive measures, such as vaccines. The research described in this thesis addresses these various topics.

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

Several approaches to inventory the exact burden of these travel-related diseases have shown that the determination of the denominator (i.e. the number of persons exposed to a threat or disease) remains a challenge. A clinically relevant approach to investigate this

Table 1 Application of the Swiss cheese model to travel medicine. Different aspects of the model are related to health care in general and to health care in relation to travel medicine specifically

Swiss cheese model	Representation in health care	Representation in travel health care
Slice of cheese	Health care professional	Health care professional • Travel medicine specialist • General physician • Travel consultant (nurse) • Specialist – Transplant, Rheumatic Diseases, etc.
	Barrier that protects patient	Preventive measures, e.g. • Cook it, Peel it, Boil it or Forget it • Anti-mosquito bite measures • Keep away from stray animals Vaccination Chemoprophylaxis Antibiotics • Preventive • Therapeutic
	Procedure that alleviates the consequences of an error	Information / Training • Travel insurance • What to do when bitten • What to do in case of symptoms • Self-testing • Self-treatment
Error		Lacking scientific data / knowledge Misjudgement of risk by health specialist

Hole	Opportunity for error	Purpose of travel (VFR, expatriate, tourist, migrant) • Altered tendency of seeking health travel advice • Altered conception of risk • Altered motivation for adherence to measures • Itinerary – activities during travelling Adverse events of vaccines or prophylactic medication Self administration of preventive measures • Preventive measures • Chemoprophylaxis
	Weakness in defences against error	Vaccines with <100% protection rate Chemoprophylaxis with <100% protection rate
Arrow	Series of events leading to medical error	Series of events leading to travel-related disease
Adding a slice	Improve health care safety	Identify category of travellers at risk for diseases Extra training for specific groups of travellers Post-travel screening
Plugging a hole		Update scientific data / knowledge Train travel health care specialists / consultants

VFR = Visiting friends or relatives.

burden is to monitor self-reported health problems after travelling to developing countries. However, with this approach, mild or self-limiting illnesses such as diarrhea, mild respiratory infections and skin disorders are either not picked up, or picked up less frequently. In addition, this approach is highly subject to population bias.
Freedman et al. estimated the proportionate morbidity by diagnosis of self reported travel-related disease and geographic region among travellers returning from six developing regions of the world, by using the number of patients with a given diagnosis as the numerator and all ill travellers to a destination as a denominator [4]. Data of 30 GeoSentinel sites, which are specialised travel or tropical-medicine clinics on six continents, contributed to clinician-based sentinel surveillance data on 17.353 ill returned travellers. Besides the limitations of this study, such as probable under-representation of travel-related sexually transmitted diseases and infections with a short incubation period (e.g. dengue), it showed that the proportionate morbidity of diarrhea among returning travellers is highest in all developing regions visited (Southeast Asia, Central Asia, South America, Central America, Caribbean), except for Sub-Saharan Africa, where falciparum malaria accounts for the highest proportionate morbidity [figure 2 from ref 4]. Dengue occurs mostly in visitors to the Caribbean and Southeast Asia, cutaneous leishmaniasis in those who visit Central America and South America, and typhoid fever in travellers to south central Asia. TropNetEurop, a surveillance network of experts in Infectious disease and Tropical medicine throughout Europe, has reported similar trends [14]. Unfortunately, Freedman and colleagues have not analysed in more depth the contribution of the purpose of travel, a well-known risk factor for contracting infectious diseases during travelling.

Bottieau and colleagues, alike the GeoSentinel group [4], investigated self-reported febrile episodes among returning travellers (N=1743), but additionally categorised these travellers into: Western travellers (natives of Western countries visiting the tropics for less than 6 months); expatriates (Western individuals residing for more than 6 months in the tropics); natives of the tropics who have lived for more than 1 year in Europe and returning to their home country to visit friends and relatives (VFR travellers); and foreign visitors or migrants (natives of the tropics arriving for the first time in Europe) [3]. *Falciparum* malaria was more frequently diagnosed in expatriates, VFR travellers, and foreign visitors or migrants, whereas rickettsial infections, dengue, and acute schistosomiasis occurred almost exclusively in Western travellers and expatriates. Prevalence of HIV infection and tuberculosis was much higher in VFR travellers and foreign visitors or migrants. The epidemiology of travel-related diseases generated by these data is important for guiding post-travel diagnosis and empiric therapy as well as

for prioritizing pre-travel intervention strategies. In this thesis, the aim of reducing the risk of malaria in expatriate travellers is discussed in more detail (chapter 4).

Besides distinguishing travellers on the basis of the purpose of travel, they can be categorised according to their immune status. Immunocompromised travellers are more likely to experience severe effects of illness, and less likely to mount a significant response to vaccinations than those without immune disorders [15-21]. The divergent group of travellers with a compromised immunity comprises; 1. Patients on immune suppressive therapy such as solid organ or hematopoietic transplant recipients, patients with Crohn's disease, colitis ulcerosa and rheumatic diseases, 2. Patients with human immunodeficiency virus (HIV) infection, 3. Asplenic travellers, 4. Patients with defective barriers such as skin or mucosal disorders or a reduced gastro-intestinal acid barrier [22]. Although the magnitude of the immune disorder is difficult to quantify, except for the use of the $CD4^+$ T cell count in HIV patients, the overall health of immunocompromised patients improves, and so does their motivation for travel along with the need for specific protective measures. In chapter 2 and chapter 3 of this thesis, the susceptibility of travelling solid organ transplant recipients and diabetics to travel-related diseases and their precautions taken, are discussed in more detail.

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

The paradigm in vaccinology, which has existed since the development of vaccines, is that every population will mount comparable (protective) immune responses to similar vaccine doses and number of dose administrations. This approach has led to population-wide immunisations and hence the control of many infectious diseases, and should therefore always be pursued. However, with current advances in knowledge on individual variability in risk and morbidity of infectious diseases and in vaccine response, a more personalised approach could be strived for [23]. For the development of a personalised vaccination approach, the immune response in specific vulnerable groups must be inventoried and new vaccination methods, adjuvants and schedules should be investigated.

Evident groups targeted by this approach would be the previously mentioned immunocompromised travellers, but also apparently healthy individuals can show a diminished response to vaccines. In these healthy persons, genetics, gender and age are well-known factors that can influence the response to specific vaccines [24].
The success of population-wide vaccination programs, suggests that interhuman genetic differences are negligible in the process of vaccine antigen processing,

presentation and lymphocytic response. However, complex interaction of the Human Leukocyte Antigens (HLA) and peptides derived from pathogens or vaccines are believed to play a role in the magnitude and breadth of the immune response [25,26]. HLA class II alleles influence the humoral response after vaccination, since antibody production is mediated by HLA class II-restricted $CD4^+$ T-cell responses, except for polysaccharide antigen vaccines (e.g. pneumococcal vaccine) in which the response is T cell independent [27]. Indeed, for hepatitis B and measles vaccines, genetic profiles were found to be associated with persistent seronegativity or a low antibody response after vaccination [28-30]. The heritability of the immune response against hepatitis B vaccine is caused for 40% by genes within the MHC (Major Histocompatibility Complex), shown by higher intraclass correlations of MHC identical than MHC different dizygotic twins, and 60% by non-MHC genes [28]. Nevertheless, these genetic profiles do not exclusively account for the magnitude of the response. In the development of antibodies against hepatitis B vaccine, higher age, male gender and smoking also predispose for a lower antibody response [31,32].

In this thesis, two allegedly immunocompetent populations are investigated. The first group are individuals who failed to mount a protective immune response to the hepatitis B vaccine (chapter 5), expressed in antibody level. In these non-responders, the intradermal delivery of the vaccine antigen, along with an immune response modifier, was investigated in an attempt to induce a protective response. The second group are travellers of sixty years or older who received the live attenuated yellow fever vaccine (chapter 4). In the case of yellow fever vaccine, older age is associated with an increased susceptibility to serious adverse events which could hypothetically result from a diminished virus neutralising antibody response.

As the global population in Western countries is ageing, so is the travelling population. The elderly suffer from more frequent and severe infections than younger people [33], and this should increase the awareness in the elderly traveller and in those who give travel health advice. One of the main reasons for the increase in infections observed in the elderly is believed to be 'immunosenescence' [33], which refers to the immune system's diminished function with age. Logically, if the elderly show an increased susceptibility to infections, their response to vaccines could be diminished, and this has indeed been found, e.g. in the case of influenza vaccination. In a review, the clinical vaccine efficacy in young adults was 70-90%, compared to an efficacy of 17-53% in the elderly vaccinated [34], depending on the circulating influenza strains. The phenomenon of immunosenescense is not yet well understood, but the following theories have been proposed: 1. Impaired antigen presentation, 2. Thymic involution leading to decreased naïve T cell production and a

decreased ability to respond to new antigens, 3. Reduced B cell production or isotype switching, resulting in low affinity antibody production, 4. Increased memory T cell numbers which restrict the diversity of the immune cell repertoire and 5. Ageing of the bone marrow stroma leading to decreased survival of plasma cells [35,36]. With more detailed knowledge on the development of the immune response to travel-related vaccines in the elderly, travel medicine could meet with the needs of this growing population.

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

In the scope of a population-wide protection through vaccination, the aim is to create herd immunity in order to significantly reduce pathogen transmission and infection. Of all the goals formulated by the World Health Organisation (WHO) with respect to eradication of vaccine preventable diseases, only smallpox eradication has been achieved sofar [37]. Failure of eradication of infectious diseases through vaccination can be attributed to many factors. Evidently, political and financial reasons are the main hurdles to be taken, but from a scientific perspective other reasons can underly this failure. First, if the infectious agent has a non-human host, i.e. a zoonosis such as yellow fever, vaccination of all susceptible humans would still not eradicate the pathogen. Second, not all vaccines provide 100% protection against infection (e.g. vaccination with the capsular polysaccharide of *Salmonella typhi* (Vi) has a protection rate of 75% against typhoid fever in endemic populations) [38]. Third, immunisation is a human interference with nature, and people who believe this interference is wrong on religious or other grounds will refuse to be vaccinated, hampering eradication of the infectious agent. In the Netherlands, small outbreaks of poliomyelitis and measles occur on these grounds [39]. However, these reasons are probably secondary to the lack of resources to obtain the vaccine coverage that is needed for eradication. By reducing the vaccine dose needed for immunisation, vaccine stockpiles will last longer and costs will decrease, possibly leading to higher vaccine coverage.

A recently rediscovered possibility of vaccine dose reduction that receives much attention from vaccinologists, is vaccination in the skin [40-42]. The skin represents the outermost line of defense against mechanical impacts, temperature, UV-radiation, dehydration and pathogenic microorganisms. It is composed of three primary layers: the epidermis, the dermis and the subcutis (figure 3).

The outer part of the epidermis consists of dead cells (stratum corneum), the inner part of live cells such as keratinocytes, melanocytes and, of special interest for immunisation purposes, dendritic cells which are named Langerhans cells (LC) after their discoverer

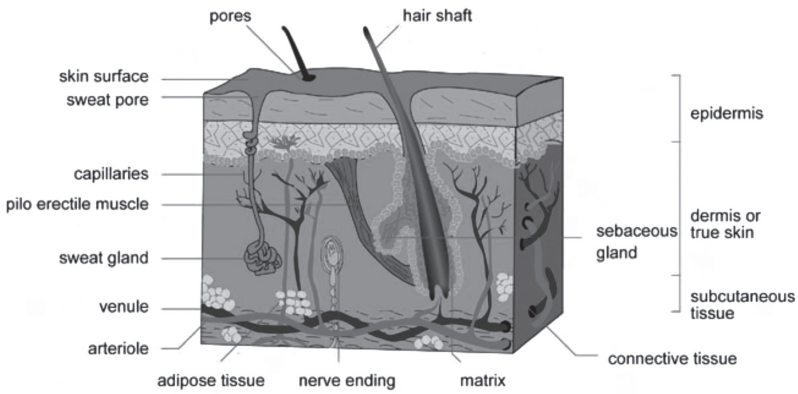

Figure 3 Cross section of the skin. Adapted from the visual dictionary [43].

[44]. These professional antigen presenting cells (APC) account for only 1% of cells, but cover nearly 20% of the surface area due to their horizontal orientation and long protrusions [45]. The dermis is primarily composed of extracellular matrix, and like the epidermis contains dendritic cells (dermal dedritic cells – dermal DC), besides fibroblasts, macrophages and granulocytes. In the dermal layer reside the most superficial glands and lymphatic and blood vessels of the body. LC and dermal DC constantly monitor the (epi)dermal microenvironment by taking up antigen and processing it into fragments that can be recognised by effector cells of the adaptive immune system. LC have classically been thought to be essential for initiating T cell responses to cutaneous antigens, accounting for the success of intradermal vaccination [46]. However, recent data have also highlighted the importance of dermal DC in cutaneous immunity [47,48]. Zhao et al. investigated the contribution of vaginal APCs in immune induction to HSV-2 (Herper Simplex Virus), and revealed that only the $CD11b^+$ dermal DCs, but not Langerhans cells, presented viral antigens to $CD4^+$ T cells and induced Interferon γ (IFN)secretion. Following on these results, Allan et al. provided in vivo evidence that priming of HSV-specific CTLs (Cytotoxic T cells) after skin infection does not require antigen presentation by LCs. Although these results are confined to HSV and may not apply to other pathogens, they do undermine the hypothesis of overall dominance of LCs in an (epi)dermally initiated immune responses.

For immunisation purposes, both could be relevant, as both LC and dermal DC process and present the injected antigen to naïve T cells in the draining lymph nodes [49]. Itano and colleagues demonstrated that after subcutaneous inoculation of antigen, unprocessed antigen drains to lymph nodes within several hours and does not require cell-mediated transport [50]. DC that reside in the lymph node take up and process this antigen and then activate naïve T lymphocytes. A second wave of antigen is delivered to lymph nodes approximately 24 hours later by an influx of dermal DC that express high levels of the antigen. Although extensive T cell proliferation is induced by the first wave of antigen, complete $CD4^+$ T cell differentiation requires the presence of dermal DC. [50].

LC and DC represent the principal APC under steady state condition, which is disrupted during cutaneous vaccination. The inflammatory state initiated by immunisation might induce influx of plasmacytoid DC into the site of injection, contributing to the induction of an adaptive immune response [51]. Based on these data, the success of intradermal vaccination is attributed to efficient vaccine antigen presentation to APC and hence T and B cells, whereas with subcutaneous or intramuscular vaccine administration, the probability of antigen – APC contact is lower. This hypothesis has recently been studied in mice, in which Virus-like particles (VLPs) of simian-human immunodeficiency virus (SHIV) were inoculated intramuscularly, intraperitoneally, subcutaneously and intradermally. With an optical imaging approach to directly visualize the trafficking of the VLPs after immunisation, Cubas et al. showed convincingly that the 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 immune responses [52].

Historically, the route of vaccine administration by needle, i.e. intradermal, subcutaneous or intramuscular, has been reached on arbitrary grounds. The first scientific evidence of vaccination was provided by Edward Jenner, an English doctor who in 1796 successfully inoculated the content of a cowpox bulla -containing vaccinia virus- into the skin of a young boy, rendering him protected against a challenge with the human pox virus (variola) [53]. Almost 100 years later another vaccinology pioneer, Louis Pasteur, developed a post-exposure rabies vaccine, which was administered under a fold of the skin (i.e. subcutaneously) [54]. Apparently, intramuscular injection was initially not the standard immunisation route, and is still not the immunisation route for vaccines as Bacille Calmette Geurin (BCG) and vaccinia. Increased knowledge on vaccine-induced immunity, and enhanced laboratory techniques have contributed to a more ‘educated’ monitoring of immune response, although these measured responses often remain surrogates for protection against infection [55].

In this thesis, the intradermal delivery of Hepatitis B vaccine (chapter 5), yellow fever vaccine (chapters 6 and 7) and rabies vaccine (chapter 8) is discussed, as a method to reduce vaccine dose or enhance vaccine-induced immunity.

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Performance of self-diagnosis and standby treatment of malaria in international oilfield service employees in the field

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Abstract

Background

Falciparum malaria remains a major occupational illness that accounts for several deaths per year and numerous lost working days among the expatriate population, working or living in high-risk malarious areas. Compliance to preventive strategies is poor in travellers, especially business travellers, expatriates and long-term travellers.

Methods

In this cross-sectional, web-based study the adherence to and outcome of a preventive malaria programme on knowledge, attitude and practices, including the practice of self-diagnosis and standby treatment (curative malaria kit, CMK) was evaluated in 2,350 non-immune expatriates, who had been working in highly malaria endemic areas.

Results

One-third (N=648) of these expatriates visited a doctor for malaria symptoms and almost half (29 of 68) of all hospitalisations were due to malaria. The mandatory malaria training for non-immunes was completed by 92% of those who visited or worked in a high risk malaria country; 70% of the respondents at risk also received the CMK. The malaria awareness training and CMK significantly increased malaria knowledge [relative risk (RR) of 1.5, 95%CI 1.2-2.1], attitudes and practices, including compliance to chemoprophylaxis [RR=2.2, 95%CI 1.6-3.2]. Hospitalisation for malaria tended to be reduced by the programme [RR=0.4, 95%CI 0.1-1.1], albeit not significantly. Respondents who did not receive instructions on the rapid diagnostic test were two times [RR=2.3, 95%CI 1.6-3.3] more likely to have difficulties. Those who did receive instructions adhered poorly to the timing of repeating the test. Moreover, 6% (31 of 513) of those with a negative test result were diagnosed with malaria by a local doctor. 77% (N=393) of the respondents with a negative test result did not take curative medication. 57% (252 of 441) of the respondents who took the curative medication that was included in the kit did not have a positive self-test or clinical malaria diagnosis made by a doctor.

Conclusions

This survey demonstrated that a comprehensive programme targeting malaria prevention in expatriates can be effectively implemented and that it significantly increased malaria awareness.

Introduction

Every year, *Plasmodium falciparum* infects 300 to 500 million persons, and kills between one and two million. Particularly sub-Saharan Africa, parts of South America and South-East Asia are affected. Falciparum malaria is also a major occupational illness that accounts for several deaths per year and numerous lost working days among the expatriate population, working or living in high-risk malarious areas. Approximately 1% of all non-immune travellers who acquire *P. falciparum* infection die [1].

Increasing awareness, personal protection measures against mosquito bites, chemoprophylaxis, and early diagnosis and treatment are the mainstay of prevention against falciparum malaria. Compliance to these preventive strategies is poor in travellers, especially business travellers, expatriates and long-term travellers [2]. Moreover, the diagnosis of malaria is often not immediately considered in returning travellers, resulting in treatment delay and subsequent higher morbidity [3].

In 2003, a preventive programme for international employees and contractors working in malaria endemic areas was set up by an oilfield service company to enhance awareness on the dangers of malaria, and to reduce its morbidity and mortality. The cornerstones of this preventive programme were a malaria awareness training programme and provision of a curative malaria kit, which contained dipstick-based strips for self-diagnosis and emergency standby medication for self-treatment of falciparum malaria. In an initial survey, this programme was rated very good to excellent by more than 60% of the respondents [4].

In this cross-sectional study by web-based questionnaire, the adherence to this preventive malaria programme, and the practice of self-diagnosis and standby treatment of presumptive falciparum malaria in the field was evaluated.

Methods

Malaria prevention programme

The malaria prevention programme consists of the following components:

1. Malaria training for non-immunes. This training was mandatory for all non-immune international oilfield service company employees. Any person who had left a malaria endemic country for more than six months was considered non-immune to malaria.
2. Arrival packages were assigned to employees with high-malaria-risk destinations, according to the WHO malaria country definition [5]. A quiz was designed to enhance the awareness of expatriate workers on the risks of malaria and the possible preventive measures.
3. At all malarious locations appropriate preventive measures were provided, including insecticide treated bed nets, routine malaria prophylaxis, insect repellents and insecticide treatments to kill mosquito larvae in company facilities and residences.
4. Malaria hot line. A toll-free telephone line, staffed by multilingual doctors who were specialized in tropical diseases, was available 24 hours a day, seven days a week.
5. A curative malaria kit (CMK) with hands-on training. This kit was developed to address emergency cases of suspected malaria in which an individual was more than 24 hours away from a medical centre. The kit consisted of forehead temperature strips, three dipstick-based, immunological antigen-capture self-tests for falciparum malaria (Paracheck Pf® or Core Malaria Pf®, depending on availability), and curative medication (Coartem®: artemether/lumefantrine). If the self-test was positive the infected person was instructed to start taking the curative medication (four tablets every morning and four tablets every evening for three days), and seek medical assistance as soon as possible. In case of a negative test result, the blood test was to be repeated 12 hours later.

Web-based questionnaire

To evaluate the malaria prevention programme, an e-mail invitation to answer a web-based questionnaire (NetQuestionnaires version NETQ 6.0, the Netherlands) was sent in July 2007 to 8,380 oilfield service company employees, who were registered as non-immune to malaria, and who might have travelled to, lived or worked in a malarious area in the last two years. The survey covered use of the programme in these preceding 24 months.

The web-based questionnaire was accessible from July to September 2007 by a unique link per addressed employee, and could be opened only once. During this period, several reminders were sent to the employees who had not yet accessed the questionnaire. The answers to the questionnaire were analysed anonymously. Gender, age and country of birth was the only personal information requested.

Definitions

Malaria was reported as

1. 'Doctor's diagnosis of malaria'; diagnosed by a local doctor (not necessarily laboratory confirmed)
2. 'Laboratory confirmed malaria'; diagnosed by a local doctor and confirmed by laboratory
3. 'Presumptive malaria'; a positive self-test, or a clinically diagnosed or laboratory confirmed malaria.

The following subgroups were defined:

- to analyse the effect of the malaria prevention programme on several aspects concerning knowledge, attitude and practices (KAP) of malaria:
  1. 'Malaria Prevention Programme' as receiving the training for non-immunes with or without CMK
  2. 'No Malaria Prevention Programme' as receiving neither training nor CMK.
- to analyse the effect of the CMK on malaria KAP:
  1. 'CMK' as receiving the training and the kit
  2. 'No CMK' as receiving the training without the kit.

Statistical analysis

Continuous data were analyzed with Students t-test, categorical data with Chi-square test or Fisher's exact test where appropriate. Corrected relative risk (RR) was calculated from the corrected odds ratio (OR) obtained by logistic regression. Corrected OR was recalculated into RR according to the following formula: RR=OR/((1-P)+(P*OR)), provided by Zhang and Yu [6], as the OR overestimates the RR when prevalence (P) exceeds 10%. Possible confounders for which was corrected by logistic regression are specified for all reported results. *P* values were provided for categorical data with more than two categories. Statistical analysis was performed using a computer-assisted software package (SPSS version 12.0, SPSS Inc., Chicago, IL).

Results

The web-based questionnaire was opened by 3,575 employees, giving a total response rate of 43%. Of these respondents, 2,552 reported to have travelled to malaria endemic countries in the past 24 months, of whom 2350 (92%) completed the questionnaire entirely. Analysis of the answers of all the respondents at risk and of those at risk who completed the questionnaire did not yield different results. Therefore, only the results of the completed questionnaires are reported. The mean time to complete the questionnaire was 12 minutes and 22 seconds.

Study population

The demographic characteristics of the studied population are listed in Table 1.
The malaria countries visited are amongst those with the highest incidence of *P. falciparum* [6]; in descending order of frequency, the most visited countries were: Angola, Cameroon, Nigeria, India, Gabon, Sudan, Equatorial Guinea, Democratic Republic of Congo and Chad. Most respondents visited more than one endemic country; the median of endemic countries visited per respondent was 2 (range 1-105).

Risk of malaria

A comparison was made between the cumulative incidences (CI) of malaria according to work status (Table 2). The CI of acquiring malaria increased according to work status and thus according to time spent in malaria endemic countries. In addition, chemoprophylaxis use by long term travellers was significantly lower (29%) compared to that of rotators and visitors (both 62%) ($p<0.001$). In contrast to the increasing CI of malaria with a longer duration of stay, the CI of being hospitalised for malaria was similar in all groups.

Ninety percent of the respondents who reported to have had laboratory confirmed malaria acquired the disease in sub-Saharan Africa. Malaria was acquired in descending order of frequency in Sudan, Nigeria, Equatorial Guinea, Angola, Chad, Cameroon, Republic of Congo, Gabon, Ivory Coast, India, Benin, Somalia, Uganda and Peru. The considerable burden of malaria in this population was demonstrated by the fact that one-third (N=648) of all respondents visited a doctor for malaria symptoms and almost half (29 of 68) of all hospitalisations were due to laboratory confirmed malaria.

Table 1 Demographic characteristics of study population

Demographic characteristics		N (N total 2350)	%
Gender	Male	2065	88
	Female	285	12
Age (yrs)	mean	36	-
	(range)	(19-63)	
Continent of birth	African	733	31
	European	631	27
	South American	328	14
	Asian	301	13
	North American	174	8
	Arabic	102	4
	Oceanian	64	3
Country of birth	Malaria endemic$	1392	60
	Malaria non endemic	941	40
Working conditions	Outdoor*	1278	54
	Indoor	1072	46
Work status	Long term (>6 months)	1122	48
	Rotator	795	34
	Visitor	342	15
	Other (e.g. spouse)	91	4

Percentages may not add up to exactly one hundred due to rounding off. $Malaria endemic country according to the WHO (5). *Outdoor working conditions include working on a land rig or with seismic crew, off shore, on another field location or on a marine vessel.

Malaria prevention programme

The mandatory malaria training for non-immunes was completed by 92% of those who visited or worked in a high risk malaria country. Overall, 70% of the respondents at risk also received the CMK (Figure 1). Seventy-five percent (N=1229) of respondents who received the CMK were instructed in how to use it, and all (98%) considered the instructions to be clear.

Multivariate analysis showed that respondents who were born in a malaria endemic country were two times less likely to receive the malaria prevention programme [RR 2.0, 95% CI 1.4-2.7]. In addition, women were less likely to receive the CMK [RR 1.4, 95% CI 1.1-1.7]. The effect of the malaria prevention programme and the CMK on

Table 2 Cumulative incidence of malaria per 100 persons according to work status in 24 months. *p-value for malaria diagnosis was obtained by χ^2-test and for hospitalisation with Fisher's exact test. Those who responded to belong to the 'other' group (N=91), instead of the solicited groups, were excluded as their global time of possible exposure to malaria was unclear.

		Cumulative incidence (%) of malaria in 24 months [95%CI]			p-value*
		Visitor (N=342)	**Rotator** (N=795)	**Long term** (N=1122)	
Malaria	Presumptive	2.3 [0.7-3.9]	6.2 [4.5-7.9]	13.7 [11.7-15.7]	<0.001
	Doctor's diagnosis	2.0 [0.5-3.5]	5.7 [4.1-7.3]	12.8 [10.8-14.8]	<0.001
	Laboratory confirmed	1.8 [0.4-3.2]	4.3 [2.9-5.7]	9.7 [8.0-11.4]	<0.001
Hospitalisation for malaria	Doctor's diagnosis	0.6 [0.0-1.4]	1.6 [0.7-2.5]	1.5 [0.8-2.2]	0.6
	Laboratory confirmed	0.6 [0.0-1.4]	1.6 [0.7-2.5]	1.2 [0.6-1.8]	0.5

malaria KAP was therefore corrected for these variables. The distribution of the programme was not influenced by work status, i.e. whether employees were long-term workers, rotators or visitors, neither by working indoors or outdoors.

Respondents receiving the malaria prevention programme reported a twofold higher use of malaria chemoprophylaxis (47% vs. 19%) and had significantly more knowledge about malaria. A similar effect was observed for those who only received the CMK. Those who did not receive the programme were twice as likely not to consider malaria as a threat, nor to take additional anti-mosquito measures (Table 3).

Despite the increased use of chemoprophylaxis by the total group receiving the CMK, a small group (14%, N=226) thought that having the CMK made regular malaria chemoprophylaxis unnecessary. The use of chemoprophylaxis in this group was 49%

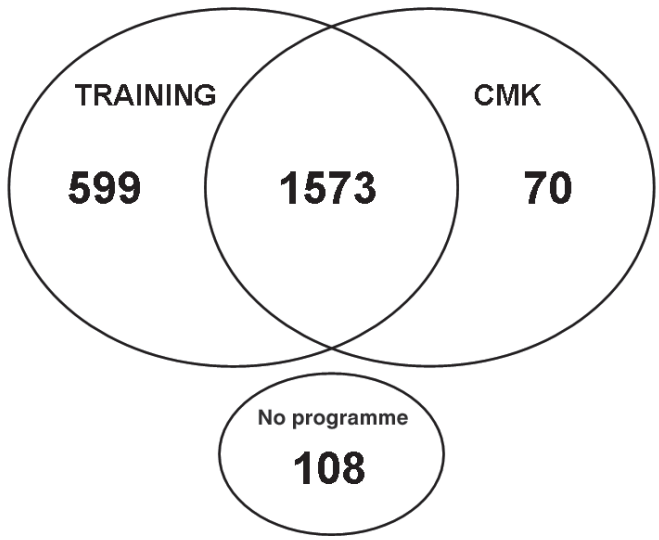

Figure 1 Distribution of the Malaria Prevention Programme in population at risk (N=2350). Numbers represent number of respondents receiving this part of the programme. Training = Training for non-immunes, CMK = Curative Malaria Kit.

in comparison to 60% of those who felt prophylaxis remained necessary with CMK use ($p = 0.001$).

Use of self-test

One-third (N=575) of the respondents who had received the CMK performed the malaria self-test contained in the CMK for presumptive malaria. Forty-nine test results were positive (defined as a positive test result at first or repeated testing), 508 negative and 18 invalid. Two-thirds (N=378) repeated the test, giving a similar result in 79% (19 of 24), 99% (338 of 344) and 40% (4 of 10), respectively. Although it was instructed to repeat the test after 12 hours if the result was negative, only 55% (N=189) adhered to this instruction.

Table 3 Effect of Malaria Prevention Programme on malaria KAP.

	Malaria Prevention Programme				Curative Malaria Kit			
Malaria Knowlegde, Attitudes and Practices	Training and/or CMK	No training and no CMK	RR [95%CI]	Corrected RR# [95%CI]	Training with CMK	Training w /o CMK	RR [95%CI]	Corrected RR# [95%CI]
Chemical prophylaxis use n/N (%)	1049/2242 (47)	21/108 (19)	2.4 [1.8-3.0]	2.5[$] [1.9-3.2]	861/1573 (55)	171/599 (29)	1.9 [1.7-2.1]	1.9[$] [1.6-2.0]
Not considering malaria as a threat n/N (%)	126/1873 (7)	13/97 (13)	0.5 [0.3-0.9]	0.4 [0.2-0.8]	69/1337 (5)	53/480 (11)	0.5 [0.3-0.7]	0.5 [0.3-0.7]
Not inclined to take anti mosquito measures n/N (%)	103/1746 (6)	16/78 (21)	0.3 [0.2-0.5]	0.3 [0.2-0.5]	58/1236 (5)	39/453 (9)	0.6 [0.4-0.8]	0.5 [0.3-0.8]
Correct malaria knowlegde* n/N (%)	977/2242 (40)	28/108 (26)	1.7 [1.3-2.1]	1.6 [1.2-2.1]	745/1573 (47)	202/599 (34)	1.4 [1.3-1.6]	1.4 [1.2-1.5]

RR=Relative Risk.

[#] Corrected for malaria endemic country of birth.

[$] Additionally corrected for work status (long term traveller, rotator, etc.).

* Knowlegde was examined by multiple choice question on the maximum incubation time of Plasmodium falciparum. The denominators for questions on considering malaria as a threat and inclination to take anti-mosquito measures vary, as answers reporting 'neutral' were not used for analysis.

Fifteen percent of the respondents reported having difficulties in using the self-test. Among those, the most frequently reported difficulties were pricking the finger and placing the blood drop on the test strip (Table 4).

Table 4 Difficulties with self-test contained in the CMK reported by respondents who used the test. More than one difficulty could be reported per respondent.

Difficulties with self-test		**N (%)**
Respondents reporting difficulties N performing self-test = 575	85 (15)	
Difficulties N total =85	Finger prick	50 (59)
	Placing blood drop	24 (28)
	Result interpretation	15 (18)
	Identifying lines	13 (15)
	Technical problem kit	12 (14)
	Instructions	10 (13)
	Adherence to waiting time	2 (2)
	Too ill to perform test	1 (1)

Respondents who did not receive instructions with the self-test were two-times more likely to have difficulties [RR=2.3, 95%CI 1.6-3.3] and three-times more likely to have an invalid test [RR=2.9, 95%CI 1.0-8.5]. Respondents with difficulties were 30 times more likely to have an invalid test result [RR=29.6, 95%CI 8.2-106.4], after correction for possible confounding of receiving instructions.

Use of medical care

Almost twice as many respondents with a positive result visited a doctor for malaria symptoms, and a positive test indicated a tenfold higher risk of being diagnosed with malaria. On the other hand, 6% (31 of 513) of those with a negative test result were still diagnosed with malaria by a local doctor, although this diagnosis was 1.5 times less likely to be confirmed by a laboratory (Table 5).

When hospitalisation was employed as an indicator for severity of malaria, performing a test before visiting a doctor for malaria symptoms did not result in more severe malaria in comparison to immediately visiting a doctor (respectively 12%, and 14% hospitalisation in

Table 5 Influence of test performance and result (positive if first or repeated test result was positive) on doctor visit and malaria diagnosis and hospitalisation.

	CMK received				
	Self-test result				
	Positive N=49	**Negative** N=508	**RR** [95%CI]	**Corrected RR#** [95%CI]	**Self-test not performed** N=1068
Visited doctor for malaria symptoms N Yes (%)	40 (82)	233 (46)	1.8 [1.5-2.0]	1.8 [1.4-2.0]	177 (17)
Doctor's diagnosis malaria N Yes (%)	33 (67)	31 (6)	11.0 [8.3-13.2]	10.3 [7.4-12.8]	59 (6)
Laboratory confirmed malaria N Yes (%)	28* (85)	18* (58)	1.5 [1.1-1.7]	1.5 [1.2-1.7]	47* (80)
Hospitalisation for malaria N Yes (%)	4* (12)	4* (13)	0.9 [0.2-2.9]	1.0 [0.2-3.0]	8* (14)

RR=Relative Risk.

RR is corrected for malaria endemic country of birth.

* Denominator is respondents with a doctor's diagnosis of malaria.

those with doctor's diagnosis of malaria, p = 1.0) (table 5). In addition, respondents in whom malaria was diagnosed despite a negative test result, had a similar hospitalisation rate (13%).

Standby emergency treatment

One fifth (N=441) of the respondents took curative medication for malaria. The origin of the curative medication was mostly the CMK (39%) or a local hospital (35%). Ninety percent (N=44) of the respondent with a positive test result and 22% (N=115) of the respondents with a negative test result took curative medication.

Fifty seven percent (N=252) of respondents who took curative medication did not have presumptive malaria. The source of this inappropriately used curative medication was two times more likely to be the CMK than the medication used by those with presumptive malaria (50% vs. 25% respectively).

Effect of the malaria prevention programme on the outcome of malaria

1.1% (N=25) of the respondents who had received the malaria prevention programme was hospitalised for laboratory confirmed malaria in comparison to 3.7% (N=4) of those who did not receive the programme [RR=0.3, 95%CI 0.1-0.9]. However, when corrected for birth in a malaria endemic country the risk of hospitalisation was not significantly reduced [RR=0.4, 95%CI 0.1-1.1]. There was no significant reduction in hospitalisation for those who had received the CMK without training.

Discussion

Falciparum malaria is a severe disease and international employees and contractors working in highly endemic malarious areas are particularly at risk. In this study, it was found that one per 200 employees per year was hospitalised because of laboratory confirmed malaria, and 90% of malaria was acquired in sub-Saharan Africa. The self-test was positive in 8% of the respondents. Malaria was also diagnosed by a medical doctor in 6% of the repondents with a negative test. The malaria awareness training and self-diagnosis and treatment had a significant positive effect on knowledge and attitude towards malaria prevention and doubled the use of malaria chemoprophylaxis. This study also suggests a reduction in hospitalisation for malaria, thus reducing malaria associated morbidity.

Several limitations of this study require attention. First, not all employees responded to the invitation (response rate was 43%), possibly inducing a responder bias. This may have led to an overestimation of the uptake of the programme. On the other hand, some of the respondents did not or partly receive the programme, which allowed to draw seperate conclusions on the contribution of awareness training and CMK. Secondly, neither the result of the self-test nor the diagnosis of malaria by doctor or laboratory was confirmed by an independent test. Therefore, the accuracy of the interpretation of the self-test result by these febrile expatriates remains unknown. However, the endpoint of malaria was considered to be equally (in)accurate in all respondents, meaning that no diagnosis bias was introduced.

This survey showed that sub-Saharan Africa continued to pose the highest risk for the acquisition of malaria, and that long term residents are at the highest risk to contract malaria, although they were not more likely to be hospitalized than rotators or visitors. This could reflect the experience long term travellers have with malaria, being more aware of its symptoms.

The present study confirmed that the compliance of expatriate workers to malaria prophylaxis was poor [7] and decreased with duration of stay [8]. Fifty-five percent of the respondents did not take malaria chemoprophylaxis; for comparison in travellers on vacation in high-risk areas this was 16% [2]. The availability of self-testing and standby treatment with CMK may offer non-compliant employees an additional safe guard against the serious consequences of falciparum malaria if proper medical care is not available. In addition, the introduction of the malaria awareness training and CMK significantly increased compliance to malaria prophylaxis. Despite this increased compliance, 14% of those who received the CMK thought that having the kit made regular chemoprophylaxis unnecessary. Although many of the respondents who felt this way actually did use prophylaxis. The importance of continuing prophylaxis use despite the availability of standby treatment warrants special emphasis in any educational programme.

In experienced hands, the immunological antigen-capture self-test for *P. falciparum* histidine-rich protein-2 or lactate dehydrogenase has shown to be accurate and reliable diagnostic tests for *P. falciparum* infection [9]. However, the correct performance of these dipstick-based rapid tests in febrile travelers may vary from 69% to 91% depending on whether prior instructions were given [10-12]. In the present study, 15% reported difficulties with performing the self-test, and the fact that not receiving CMK instructions was significantly associated with difficulties and invalid test results clearly underscores the need for proper instructions. Only 67% adhered to the instruction to repeat the self-test in case of a negative test result, and 55% adhered to the instructed time interval. The reason for non-adherence to these instructions is unknown. One possibility is that the self-test was not repeated because malaria symptoms had spontaneously resolved. It should be emphasized during the training that repeating the self-test within six hours after a first negative test result is unlikely to be useful as parasitaemia may still be too low to detect.

The introduction of a self-test for malaria aims at decreasing treatment delay in case of a positive test result in the absence of medical care, and at reducing the empirical use of standby treatment medication in case of fever and a negative test result. On the contrary, introduction of a self-test for malaria may increase patients' delay and lead to more severe malaria in case of false negative test result. However, the hospitalisation rate of respondents with a negative test result who were subsequently diagnosed with malaria by a doctor was not significantly increased. This suggests that these patients did not have severe malaria more frequently.

Six percent of respondents who tested negative were still diagnosed with malaria. However, this diagnosis was less likely to be confirmed by a laboratory. It may reflect the possibility of overdiagnosis of malaria by a doctor, since there is anecdotal evidence that in Africa it is common practice to assume malaria, often irrespective of actual complaints [13]. The use of molecular diagnostics has the potential to overcome these limitations. When a finger prick for self-testing is performed we would recommend storing a few drops of blood on filter paper as well for PCR analysis for *P. falciparum* after returning home. This would enable future determination of true positive and true negative rates for self-testing and clinical diagnosis of falciparum malaria abroad.

The use of a self-test had a clear effect on restrictive use of standby medication: 77% of the respondents who had a negative test result did not take standby medication. Standby treatment was used not only by respondents with a positive self-test or medical diagnosis of malaria, but also in 57% who did not have a diagnosis of malaria, a number which has also been reported by others [14, 15]. The CMK may have facilitated this inappropriate use, as the curative medication used by respondents without presumptive malaria originated in 50% from the CMK. This aspect will require future scrutiny; the improper use of self-treatment may result in unnecessary exposure to side-effects and in a delay of diagnosis and treatment of other potentially life threatening diseases.

Conclusions

This survey demonstrates that, with proper instruction and training, a preventive malaria programme can contribute to the awareness of the risks of this disease. The components of this programme that deserve attention are the instructions on the performance of the self-test, the correct use of the curative medication and the need to seek medical care regardless of use of CMK. As it is impossible to make all travellers, irrespective of their purpose or duration of travel, adhere one hundred percent to every preventive measure, the contribution of the separate components which raise awareness and protection is cumulative. For those travellers considered to be exposed to higher risks of infection, such as expatriates, this malaria prevention programme certainly is such a component. Its strength lies in the multi-step design, in which a missed step is pre-empted by the next.

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Health preparations and travel-related morbidity of kidney transplant recipients travelling to developing countries

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Abstract

Background

Improved health of solid organ transplant recipients has possibly led to increased travelling by this group. Since they are thought to be more susceptible to (travel-related) infectious diseases, as a result of their immunosuppressive medication, a survey was performed to investigate their travel profile and occurrence of travel-related diseases.

Methods

A cross-sectional, descriptive study was conducted among Dutch kidney transplant recipients visiting the nephrology outpatient clinic. 290 completed a questionnaire (Q1). Of these, 103 travelled to (sub)tropical regions in the past 5 years. A mailed questionnaire (Q2) concerning occurrence and severity of travel-related diseases was returned by 71 individuals.

Results

Thirty four percent of the respondents had travelled outside Western Europe (WE) and Northern America (NA); 22% of these travellers did not seek pre-travel health advice. Transplant physicians were most frequently consulted for pre-travel advice (53%). Of the respondents travelling outside WE and NA 29% were ill during their most recent journey. Four of seventeen ill recipients (24%) were hospitalised, reflecting the high morbidity of travel-related disease in this patient group.

Conclusion

Our data show that there is need for improvement of pre-travel healthcare, and suggest an important role for transplant physicians in providing adequate counselling.

Introduction

Modern surgical techniques and immunosuppressive therapy have greatly improved the health of solid organ transplant (SOT) recipients resulting in an increased mobility to different parts of the world, including tropical or subtropical destinations. Because of the continuous need for immunosuppression, SOT recipients are more susceptible to travel-related diseases and opportunistic pathogens than immunocompetent travellers [1,2]. A Canadian study revealed that SOT recipients are relatively unaware of these travel-related risks [3]. We investigated the travel health knowledge, attitude and practices of Dutch kidney transplant recipients (KTX) regarding preventive measures, adherence to these measures and consequences of illness while staying abroad, in order to assess the travel-related risks and to improve travel care in this patient group.

Patients and methods

This cross-sectional, descriptive study was conducted at the nephrology outpatient clinic of the Leiden University Medical Centre (LUMC) in the Netherlands from December 2004 until January 2005.

A questionnaire (Q_1) was distributed to all kidney and kidney-pancreas transplant recipients while waiting for their routine visit to their transplant physician. Patients participated on a voluntary basis. Q_1 focussed on demographics, on the medical history regarding organ transplantation, on information regarding travel of the past five years (destination, duration, purpose) and on pre-travel preparations.

Patients who had travelled outside Western Europe (WE) received a second, more extensive questionnaire (Q_2) by mail. Q_2 assessed information on the most recent journey in the past five years (after transplantation) (destination, duration, purpose), on pretravel preparations, on medication taken during travel, on medical illnesses during travel and on its consequences.

To evaluate preventive measures and morbidity according to medical hazard, destinations were categorised into countries with (VAC^+) and countries without (VAC^-) recommendations for vaccination according to Dutch national travel guidelines [4]. VAC^- countries are those in Western Europe, the Northern Americas, New Zealand and Australia.

Statistical analysis was performed using a computer-assisted software package (SPSS version 12.0). Student's-*t*-test was used to compare continuous variables, the

Chi-square test for categorical variables. A 2-tailed p-value <0.05 was considered statistically significant.

Results

Study population

A total of 290 of the approximately 400 individuals visiting the nephrology outpatient clinic completed Q_1. Seventy-two recipients had their transplantation within the previous year. Six of the 72 recently transplanted SOT had travelled, of which 3 outside Western Europe (Curacao, Tunisia and Turkey). Since travelling is dissuaded during the first year after transplantation we excluded this patient group from further analysis (Figure 1).

Q_2 was sent to 94 of the remaining 218 patients who had travelled outside WE, and to an additional 9 patients that had responded to Q_1 after the period of active recruitment (February 2005). Seventy-one of these 103 patients returned Q_2 (70% response rate). Twelve questionnaires were excluded (patients were deceased, had moved, or had their last travel within WE) leaving 59 questionnaires eligible for analysis.

Responders ($N=59$) and non-respondents ($N=32$) to Q2 did not differ in terms of gender ($p=0.5$), age ($p=0.3$), transplant organ ($p=0.4$) and post-transplantation period ($p=0.9$). Demographic characteristics of the study population are listed in table 1. Nine of 59 respondents to Q_2 did not fill out their (immunosuppressive) medication. Usage of the following immuunosuppressive medication was reported ($N=50$): prednisone (95%), mycophenolate mofetil (47%), cyclosporine (42%), tacrolimus (27%), azathioprine (18%), mycophenolic acid (4%) and sirolimus (2%). Most KTX were on double (54%) or triple (42%) immunosuppressive therapy.

Travel profile

The majority (80%) of the respondents to Q_1 reported to have travelled outside the Netherlands (NL), 43% travelled outside WE, and 34% outside WE and the northern Americas (NA) in the previous five years. No differences in gender, age, transplant organ or mean time since transplantation were observed between travelling and non-travelling respondents to Q_1.

The mean duration of travel of the patients travelling outside WE (Q_2, $N=59$) was 24.5 $\pm$ 2.9 (s.e.m.) days. Regions visited by the travellers outside WE (Q_1, $N=94$) were North America (23%), Africa (20%); North Africa, mostly Canary Islands (12%), and South Africa (8%), Eastern Europe (13%), Asia (12%) and Central America (11%),

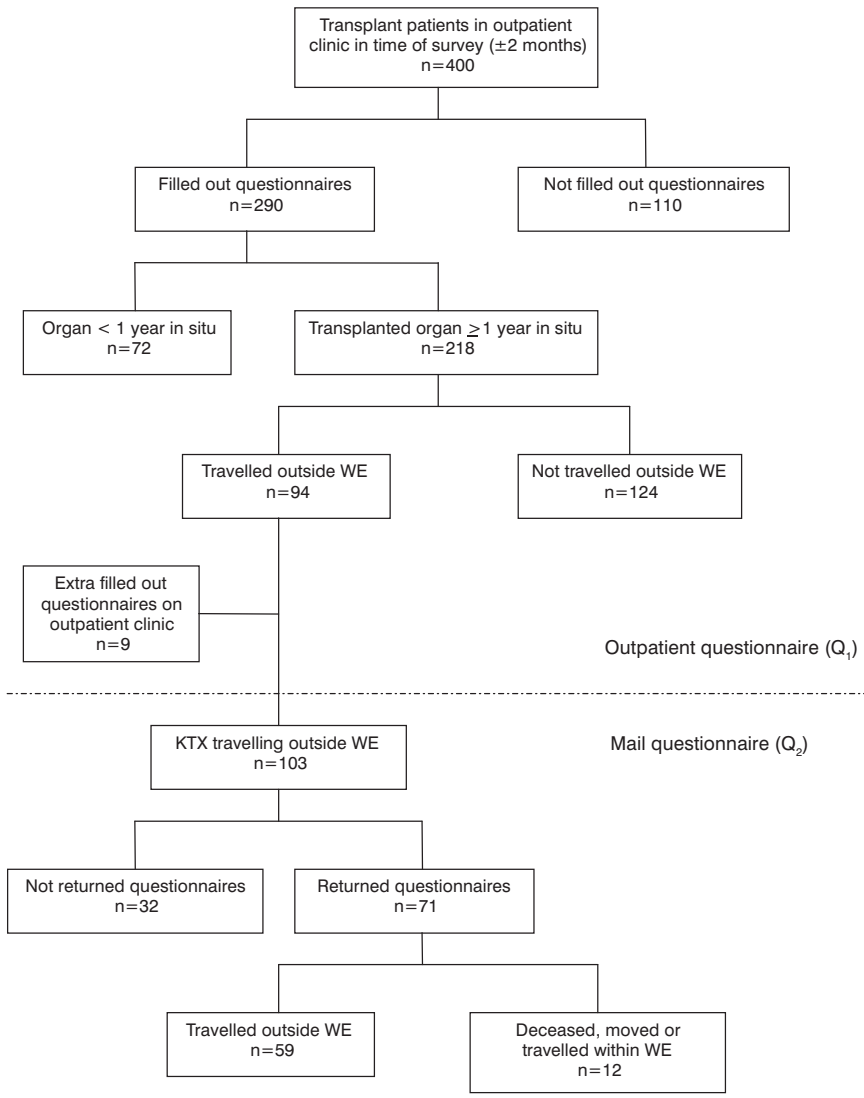

Figure 1 number of subjects enrolled in phase 1 (outpatient questionnaire, Q_1) and phase 2 (mail questionnaire, Q_2) of the survey.

Table 1 Demographics of studied travelling kidney and kidney pancreas transplant recipients

	Q_1 (n=218)		Q_2 (n=59)	
	% or mean (± s.e.m.)	range	% or mean (± s.e.m.)	range
gender (male)	60	-	64	-
age (yrs)	50.5 ± 0.8	23-79	50.5 ± 1.7	24-78
percentage kidney transplants	78	-	83	-
transplant organ in situ (yrs)	9.5 ± 0.5	1-37	9.5 ± 1.0	1-29

Q_1 is the outpatient questionnaire, Q_2 is the mail questionnaire which only the travellers outside Western Europe (N=103) received.

followed by the Caribbean (7%), Oceania (4%), South America (4%) and the Middle East (3%). In the Q2 population the major travel purpose was tourism (73%). Twenty four percent visited friends and relatives (VFR), and 3% travelled for business.

Travel Health Preparations

Pre-travel health information was sought by 5% (Q_1; 4 of 80) of reponders travelling outside NL but within WE, 23% of travellers outside WE but within NA and 78% of travellers outside WE and NA ($p<0.01$). No statistical significant difference regarding gender, age, transplanted organ or time since transplantation was observed between respondents seeking pretravel advice and those not seeking advice (data not shown). Therefore, the data considering kidney and kidney pancreas transplant recipients were analysed as one group (KTX).

Travelling KTX who sought pre-travel advice (Q_2, $N=37$) mostly consulted their transplant physician (53%), followed by a specialised travel clinic (16%) or their general practitioner (14%).

Regarding purpose of travel, travel health advice was sought by 28 of 42 (67%) tourists, and by 7 of 15 (46%) VFR. Responders to Q_2 and travelling to VAC^+ countries ($N=25$) more frequently sought pretravel advice (80%) than Q_2 respondents travelling to VAC^- areas ($N=34$) (50%) ($p=0.03$).

Accuracy of advice on vaccine-preventable diseases

Of the travellers seeking advice and travelling to VAC^+ regions, 15 respondents (79%) were vaccinated according to the national guidelines. The five unvaccinated individuals

travelled to Turkey (*N*=4) or Colombia (*N*=1). Four of these 5 obtained pretravel advice from their transplant physician.

Travel related diseases

Seventeen respondents to Q_2 (29%; 95% CI 17-41) reported being ill during their most recent journey. Diarrhea was most frequently reported (44%; 95% CI 27-61, 14 of 32 reported symptoms), followed by fever (19%; 95% CI 5-33) and symptoms of respiratory tract infection (16%; 95% CI 3-29). Nine of 14 of travellers with diarrhea took additional medication (mainly antidiarrheal medication) of which only 2 reported to have taken antibiotics. No statistical difference was observed in the development of illness according to the number of immunosuppressants taken by respondents (data not shown).

Almost twice as many travellers visiting VAC^+ destinations (40%; 95% CI 21-59) reported symptoms of disease compared to those with VAC^- countries (21%; 95% CI 7-35) however this difference did not reach statistical significance (p=0.1). A significant difference was seen in illness in KTX travellers reporting to have taken oral diabetic medication (80%; 95% CI 45-100 ill) compared to non-diabetic travellers (24%; 95% CI 13-35 ill) (p=0.02).

Consequences of illness for KTX during travel are listed in table 2. Four of the ill recipients (24%; 95% CI 4-48) were admitted to the hospital because of: syncope during a diarrheal episode (*N*=1), *Salmonella* gastro-enteritis with transient renal failure (*N*=1), ulcerative lesions in mouth and throat accompanied by diarrhea and weight loss (*N*=1) and cellulitis of the lower limb (*N*=1).

Table 2 Type of medical care and absence from work due to travel-related disease in transplant recipients during travel outside Western Europe

	percentage of travellers becoming ill (*N*=17)	time (days) (mean ± s.e.m.)	range (days)
Use of additional medication	64	-	-
Contact physician on location	29	-	-
Contact physician in NL	29	-	-
Hospitalisation	24	25±16	1-56
Absence from work due to illness	29	38±16	4-92

NL = the Netherlands.

Discussion

In this survey, 75 of 218 respondents had visited a (sub)tropical destination in the past five years and tourism was the main reason for travelling. At least one in five immunocompromised travellers failed to obtain pre-travel health advice for these medically more hazardous destinations. In addition, there is room for improvement of the accuracy of advice on vaccine-preventable diseases. For example, 21% of 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 (data not shown). Furthermore, one third of the KTX travelling to VAC^+ and one fifth travelling to VAC^- countries acquired a travel-related illness. Diabetic KTX travellers were most at risk. Finally, we found that almost a quarter of the ill travellers were hospitalised compared to less than 1% hospitalisation of ill, short term healthy travellers to the tropics [5].

Some potential limitations of this survey require comment: 1) we analysed travel behaviour of the last five years to reduce recall bias; 2) the number of kidney transplant recipients visiting friends and relatives (VFR) may be underestimated due to a language barrier resulting in failure to return the mailed questionnaire (Q_2). In general, VFR are reported to be at greater risk of acquiring travel-related diseases [6].

Although very few studies have investigated practices, travel-related risks and complications experienced by solid organ transplant recipients travelling, the findings are surprisingly similar [3,7]. In the retrospective, descriptive study of mainly kidney and liver transplants recipients by Boggild et al. tropical destinations accounted for 48% of all travel; 34% travelling outside NA and WE failed to seek pre-travel advice, and in 78% the transplant physician was the source of information [3]. Of the travellers who became ill 56% had travelled to the tropics. In the retrospective, descriptive study of heart transplant recipients by Kofidis et al. travelling overseas was associated with a 45% complication rate in comparison to 22% for European destinations [7].

To conclude, we would like to make the following suggestions for improvement of travel care in this patient group. 1) transplant physicians have a central role in raising awareness of the risks and precautions for foreign travel after solid organ transplantation. A number of web links provide general information on risks and recommended preventive health measures [8]. For more specific advice referral to a specialised travel medicine centre is recommended. 2) As in non-compromised travellers, gastrointestinal and respiratory

tract infections were most frequently reported [9]. Diarrhea can lead to dehydration and may compromise renal function and increase toxicity of immunosuppressive medication [8]. Only 14% of respondents with diarrhea started self-treatment with antibiotics. Therefore, emphasis should be put on the importance of prompt self-treatment with antibiotics to reduce duration and severity of the diarrhea. 3) Finally, the need for prospective studies in this patient group remains.

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2

Symptoms of Infectious Diseases in Travellers with Diabetes: a Prospective Study with Matched Controls

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Abstract

Background

Diabetic travellers to the (sub)tropics are thought to have symptomatic infectious diseases more often and longer than non-diabetics. Evidence for this is needed. This study evaluates whether diabetic travellers are at increased risk of symptomatic infectious diseases.

Methods

A prospective study was performed between October 2003 and February 2008 among adult medication-dependent diabetic travellers, with their non-diabetic, non-immune-suppressed travel companions serving as matched controls. Thus, diabetics and controls were assumed to have comparable exposure to infection. Data on symptoms of infectious diseases were recorded by using a structured diary.

Results

Among 70 insulin-dependent diabetics, the incidence of travel-related diarrhea was 0.99 per person-month, and the median number of symptomatic days 1.54 per month. For their 70 controls, figures were 0.74, and 1.57, respectively ($p>0.05$). Among 82 non-insulin-dependent diabetics, incidence was 0.75, and the median number of symptomatic days was 1.68. For their 82 controls, figures were 0.70, and 1.68, respectively ($p>0.05$). As for other symptoms, no significant travel-related differences were found between diabetics and controls.

Only 17% of diabetic travellers with diarrhea used their standby-antibiotics.

Conclusions

Medication-dependent diabetic travellers to (sub)tropical destinations do not have symptomatic infectious diseases more often or longer than non-diabetics. Although the incidence of metabolic dysregulation among diabetic travellers should be assessed in more detail, routine prescription of stand-by antibiotics against uncomplicated travellers' diarrhea is probably not useful, in particular not for NIDD. Self-treatment should be reserved for more complicated diarrhea.

Introduction

In recent years, the number of travellers to (sub)tropical countries has increased dramatically [1], including those with pre-existing medical conditions such as diabetes. Due to improved awareness and support for diabetic travellers, their number probably will continue to rise [2,3].

Travelling to the (sub)tropics may complicate an underlying medical condition and may require special considerations and advice. For example, it has been suggested that diabetic travellers have a higher risk of metabolic dysregulation and symptomatic infectious diseases [4-6]. Dutch travel guidelines thus recommend that diabetics taking insulin or oral anti-diabetic medication should be prescribed stand-by antibiotics for treatment of diarrhea while in the (sub)tropics [7]. British guidelines likewise advise to consider prescribing a course of antibiotics for diabetic travellers [8]. However, data on the association of diabetes mellitus with tropical infections, and on the benefits of preventive and therapeutic measures are lacking. Even evidence for a causal relation between diabetes and domestic infections is limited and inconsistent [9].

The exact number of diabetics who visit the (sub)tropics is not known. In a study published in 1991, 0.4% of 2445 travellers to a developing country who visited a travel clinic had insulin-dependent diabetes mellitus [10]. Since then, the prevalence of diabetes, both insulin-dependent and non-insulin-dependent, has increased. Annually, about ninety million persons travel to the (sub)tropics from North America and Europe [11], where diabetes prevalence is about 2.8% [12]. Assuming that diabetics travel as frequently as non-diabetics, an estimated 2.5 million diabetics travel annually from North America and Europe to (sub)tropical destinations.

In order to improve travel advice for this substantial group, we conducted a prospective study with matched controls to see if diabetics are more susceptible to symptomatic infectious diseases than non-diabetics. We also studied the usage of antibiotics for stand-by treatment of diarrhea among diabetics.

Methods

Study population

A prospective study with matched controls was performed among travellers who attended the travel clinics of the Public Health Service Amsterdam or the Leiden University Medical Centre between October 2003 and February 2008. All medication-dependent diabetics 18 years or older were eligible if planning to travel to one or more (sub)tropical countries together with a non-diabetic, non-immune-suppressed travel

companion, who was within 10 years of their own age. Thus, the control group was comparable for travel destination, travel duration, and exposure. Tropical or subtropical destinations were defined as those with moderate to high risk on hepatitis A according to the World Health Organization [13].

Insulin-dependent diabetes (IDD) was defined as diabetes mellitus requiring daily insulin treatment, with or without additional oral anti-diabetics. Non-insulin-dependent diabetes (NIDD) was defined as diabetes mellitus requiring only oral anti-diabetics.

Survey methods and definition of symptoms

A standard questionnaire was used to collect data on socio-demographics and medical history. Diabetics and controls were asked to fill out a structured diary about symptoms of infectious diseases, from the day they visited the travel clinic (up to 4 weeks before departure), until 2 weeks after return from travel. Data were collected before departure to gain information about baseline symptoms, and for 2 weeks after return to encompass incubation periods of the most (acute) travel-related infectious diseases. In the results section, the term 'travel-related' refers to the period of travel itself and the two weeks thereafter.

Recorded in the diary were any episodes of fever, diarrhea, vomiting, rhinitis, cough, and signs of skin infection; consultation with a doctor; and whether the diabetics used the stand-by antibiotics or other medication. Fever was defined as a self-measured body temperature of 38.5 degrees Celsius or more. Diarrhea was defined as loose or watery stools. Rhinitis was defined as nasal discharge or congestion. Cough could be dry or productive. Signs of skin infection included redness or (itching) rash, swelling, tenderness, and/or pus-like drainage. The diary also provided for recording non-infectious symptoms and signs, such as dysregulation of blood glucose level. Diabetics monitored blood glucose levels at their own discretion.

All diabetics were prescribed ciprofloxacin (500 mg 2 times a day for 3 days), to be used as immediate self-treatment in case of traveller's diarrhea, according to the Dutch national guidelines on travel advice [7].

Power-analysis showed that 70 pairs were needed to prove a diarrhea outcome ratio of 2 or more, with $\alpha = 0.05$ and power = 80%.

This study was approved by a medical ethics committee. All participants gave their informed consent.

Statistical Analysis

For non-independent, non-matched characteristics, McNemar's statistic testing was performed (SPSS for Windows release 15.0, SPSS Inc., Chicago, USA). A p-value < 0.05 was considered to be statistically significant.

A random effects Poisson regression model was used to calculate incidence rates and accompanying incidence rate ratios (IRR). Incidence rate was defined as the number of symptom onsets divided by the sum of symptom-free days for all individuals during a specific time period. A random effects logistic regression model was used to calculate median number of symptomatic days and accompanying odds ratios. Median number of symptomatic days equals an individual's probability to have a symptom per day. It was calculated to compare the disease burden between the diabetics and non-diabetic controls. In order to express results in units per month, numbers per day were multiplied by 30.

The random effects model takes into account two levels of correlation: 1) diabetics and their travel companions had more or less the same exposure, and thus are not independent; 2) for incidences, there may be repeated episodes of a symptom within an individual; for numbers of symptomatic days, presence of symptoms over the days within an individual are correlated. IDD and NIDD were analyzed separately.

For estimation of the parameters, a Bayesian approach was used, starting with non-informative priors. Posterior distributions were obtained by Markov Chain Monte Carlo methods, using the WinBUGS program [14,15]. Three chains were generated, based on different sets of baseline values. Parameter estimates are the medians of the posterior distributions. The range from the 2.5% to the 97.5% quantile is used to quantify the uncertainty in the parameter estimates. This range can be interpreted as a 95% confidence interval and will be referred to as such. If 1 is not included in the 95% confidence interval of a ratio, the ratio can be considered statistically significant ($p<0.05$).

Results

During the study period, 210 diabetics planning to travel with a non-diabetic, non-immune-suppressed companion were eligible for inclusion: 93 IDD, and 117 NIDD. Of these 210 eligible pairs, 58 (28%) did not participate, citing lack of time (34%), lack of interest (57%) or reasons unspecified (9%). The remaining participants all provided a completed diary.

Characteristics of the study sample

The study sample comprised 70 IDD and their 70 controls, plus 82 NIDD and their 82 controls. Of these 152 pairs, 137 (90%) were included at the Public Health Service Amsterdam, and 15 (10%) at the University Medical Centre Leiden. Table 1 shows the characteristics per type of diabetes.

Sixty-four IDD (91%) and 70 NIDD pairs (85%) matched for country of birth; only 8 IDD (11%), and 12 NIDD pairs (15%) matched for gender (data not shown). The IDD more often had cardiovascular disease and dyslipidemia than their controls ($p<0.05$). There was no difference in the use of gastric acid inhibitors. The NIDD more often had non-ischemic cardiovascular disease and dyslipidemia than their controls ($p<0.05$). Their use of gastric acid inhibitors seemed more frequent, but not significantly.

Incidence rates and number of symptomatic days between diabetics and their controls

Table 2 shows the travel-related symptoms by prevalence, incidence rate, mean duration among symptomatics, and median number of symptomatic days per symptom for IDD and their travel companions. The figure in Table 2 shows the accompanying incidence rate ratios (IRR) and odds ratios (OR) on a logarithmic scale. Likewise, table 3 shows the results for NIDD and their controls.

IDD and controls

The prevalence of travel-related diarrhea was 44% among IDD and 41% among controls. The incidence rate of travel-related diarrhea was 0.99 per person-month versus 0.74; the IRR showed no significant difference. The median number of days with diarrhea was 1.54 per month among IDD, comparable to controls.

Diarrhea outcome measures before travel showed no significant differences between IDD and controls ($p>0.05$) (data not shown).

Diarrhea incidence rate and median number of symptomatic days were higher during travel than before travel, for both IDD and their controls ($p<0.05$) (data not shown).

The IDD and controls did not significantly differ in travel-related incidence rates and median number of symptomatic days for vomiting, fever, cough, rhinitis, and signs of skin infection. Nor did they differ pre-travel, except that the median number of days with cough was lower among IDD ($p<0.05$) (data not shown).

Travel-related and pre-travel outcome measures did not differ significantly, except that cough among IDD increased after departure in incidence rate and median number of symptomatic days ($p<0.05$), although confidence intervals approximated 1 (data not shown).

NIDD and controls

The prevalence of travel-related diarrhea was 39% among NIDD and 43% among controls. The incidence rate was 0.75 per person-month versus 0.70; the IRR showed no significant difference. The median number of days with diarrhea was 1.57 per month among NIDD, comparable to controls.

Pre-travel diarrhea incidence rate and median number of symptomatic days were higher for NIDD than controls ($p<0.05$) (data not shown).
Diarrhea incidence rate and median number of symptomatic days were higher during travel than before travel for both NIDD and controls ($p<0.05$) (data not shown).
Travel-related incidence rates and median number of symptomatic days for vomiting, fever, cough, and rhinitis were comparable between both groups. The travel-related incidence rate and median number of days for signs of skin infection were higher among NIDD than among controls. However, these measures also differed before travel (data not shown) and showed no significant increase after departure (data not shown).
Before travel, incidence rate and median number of symptomatic days for vomiting were higher for NIDD than controls ($p<0.05$) (data not shown).
Travel-related and pre-travel outcome measures did not differ significantly, except that rhinitis and vomiting among controls increased after departure in both incidence rate and median number of symptomatic days ($p<0.05$) (data not shown).

Treatment and doctor consultation

Only 6 out of 31 IDD with diarrhea (19%) used the stand-by antibiotics. Effect on the duration of diarrhea was unclear due to small numbers. Seven (23%) used loperamide or activated carbon, and 3 (10%) used oral rehydration solution. Of 29 controls with diarrhea, 10 (34%) used loperamide or activated carbon, and 1 (3%) used oral rehydration solution (not statistically different from IDD).
Only 5 out of 32 NIDD with diarrhea (16%) used the standby antibiotics. Effect on the duration of diarrhea was unclear due to small numbers. Nine diabetics (28%) used loperamide or activated carbon, and 1 (3%) used oral rehydration solution. Of the 35 controls with diarrhea, 12 (34%) used loperamide or activated carbon, and 1 (3%) used oral rehydration solution (not statistically different from NIDDs).
As to the use of other medication (antibiotics, antipyretics, and anti-inflammatory drugs) and doctor consultations, both IDD and NIDD were comparable to their controls.

Dysregulation of blood glucose

Of 70 IDD, 3 (4.3%) reported dysregulation of blood glucose levels during travel. A 69-year old woman had two hypoglycemic episodes, of which one coincided with non-febrile diarrhea, for which she took stand-by antibiotics. A 47-year old man had a 2-day episode of hyperglycemia without fever or diarrhea. A 25-year old woman had 2 alternating hypo- and hyperglycemic episodes, of which one coincided with non-febrile diarrhea, for which she took no stand-by antibiotics.

Table 1 Characteristics of the insulin-dependent and non-insulin-dependent diabetics, and their controls

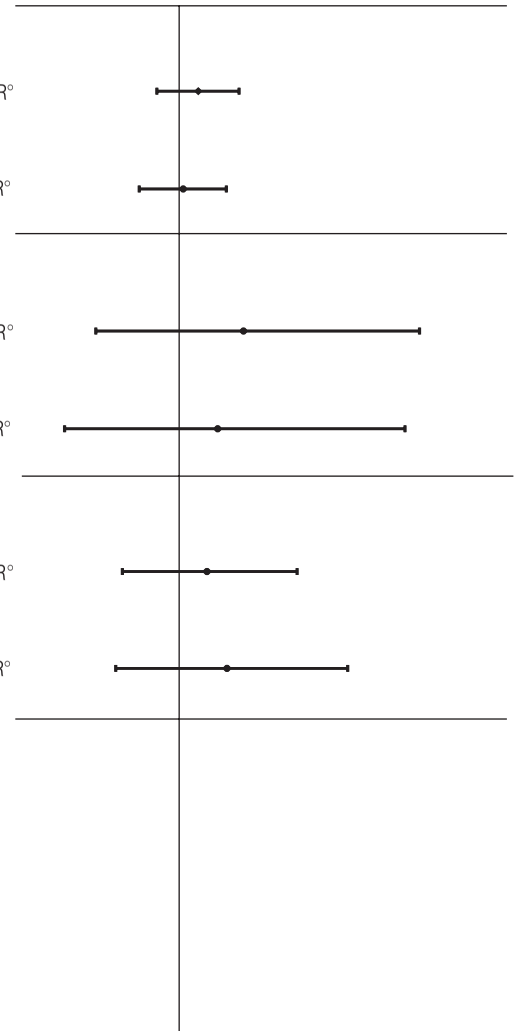
	IDD		Controls		Odds ratio (p-value)	IDD		Controls		Odds ratio (p-value)
Number of participants	70		70			82		82		
Sex										
Male	41	59%	25	36%		41	50%	31	38%	
Female	29	41%	45	64%		41	50%	51	62%	
Median age in years °	48	(34-59)	46	(33-59)		60	(53-67)	60	(51-65)	
Country of birth										
Western country	57	81%	59	84%		51	62%	54	66%	
Non-western country	13	19%	11	16%		31	38%	28	34%	
Travel destination										
Middle East and North Africa	19	27%	Idem			12	15%	Idem		
Sub-Saharan Africa	15	21%	Idem			18	22%	Idem		
Asia	19	27%	Idem			31	38%	Idem		
Latin America	17	24%	Idem			21	26%	Idem		
Median travel duration in days °	21	(13-27)	Idem			20	(15-23)	Idem		
Median duration data collection in days										
Before departure °	16	(10-21)	Idem			15	(11-28)	Idem		
After departure °	34	(27-39)	Idem			33	(28-38)	Idem		

Anti-diabetic treatment												
Metformin	18	26%	0				67	82%	0			
Sulfonylurea / thiazolidinedione drug	9	13%	0				50	61%	0			
Only short-acting insulin	5	7%	0									
Only intermediate-acting insulin	12	17%	0									
Only long-acting insulin	4	6%	0									
Intermediate- and short-acting insulin	11	16%	0									Long- and
short-acting insulin	30	43%	0									Continuous
insulin infusion	8	11%	0									
Comorbidity												
Ischemic cardiovascular disease	9	13%	2	3%	4.5	(0.028)*	11	13%	8	10%	1.4	(0.63)
Other cardiovascular disease	17	24%	8	11%	2.8	(0.047)*	33	40%	19	23%	2.2	(0.035)*
Dyslipidemia	19	27%	1	1%	∞	(0.0001)*	39	48%	9	11%	11.0	(0.0001)*
Asthma/ COPD	2	3%	4	6%	0.5	(0.69)	4	5%	4	5%	1.0	(1.0)
Hypothyroidism	5	7%	1	1%	5.0	(0.22)	2	2%	2	2%	1.0	(1.0)
Gastric acid inhibitor medication	4	6%	6	9%	0.6	(0.73)	10	12%	4	5%	3.0	(0.15)

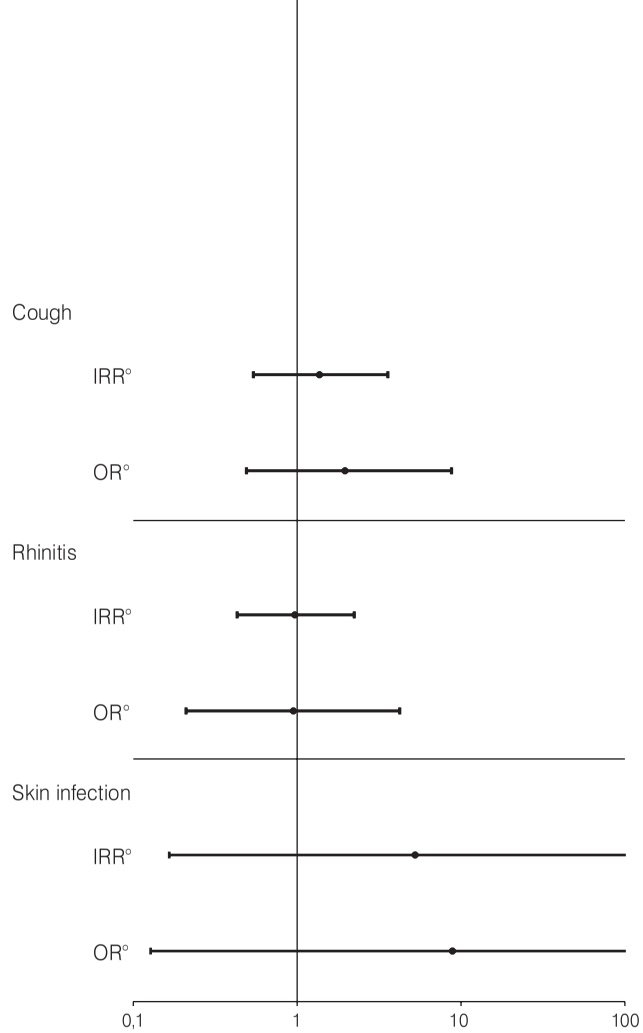
IDD = insulin dependent diabetics.
NIDD = non-insulin dependent diabetics.
° Interquartile range between brackets.
* p-value < 0.05

Table 2 Travel-related symptoms of infectious diseases among insulin-dependent diabetics and their non-diabetic controls

	IDD		Controls		Ratios with 95% confidence intervals, for IDD versus controls
Number of participants	70		70		
Diarrhea					Diarrhea
Prevalence	31	44%	29	41%	
Incidence rate per person-month	0.99	(0.75-1.28)	0.74	(0.54-1.00)	IRR°
Mean duration among symptomatics in days	1.9		2.5		
Number of symptomatic days per month	1.54	(1.30-1.82)	1.57	(1.32-1.85)	OR°
Vomiting					Vomiting
Prevalence	4	6%	2	3%	
Incidence rate per person-month	0.049	(0.014-0.12)	0.022	(0.003-0.074)	IRR°
Mean duration among symptomatics in days	1.0		2.0		
Number of symptomatic days per month	0.049	(0.015-0.12)	0.051	(0.015-0.12)	OR°
Fever					Fever
Prevalence	9	13%	7	10%	
Incidence rate per person-month	0.16	(0.084-0.27)	0.11	(0.053-0.20)	IRR°
Mean duration among symptomatics in days	2.4		1.7		
Number of symptomatic days per month	0.37	(0.25-0.52)	0.18	(0.10-0.28)	OR°



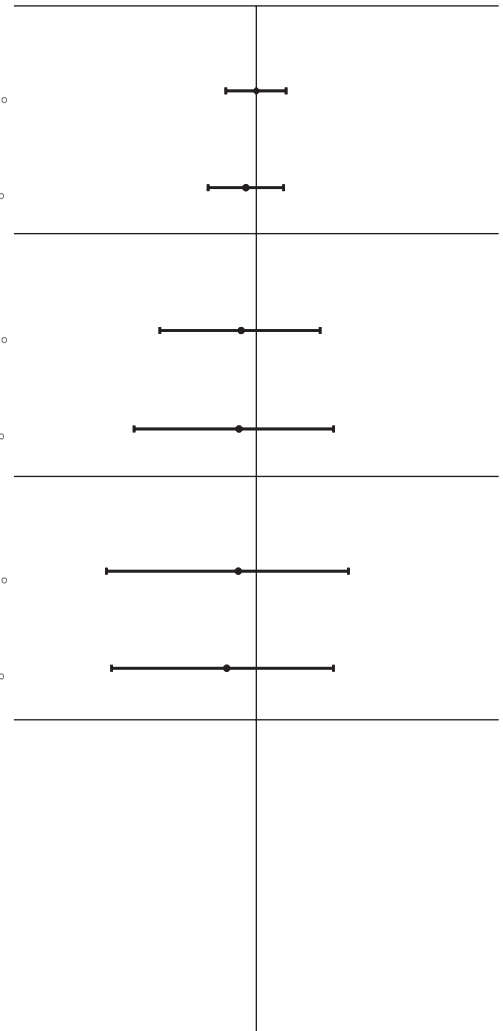
Cough					
Prevalence	16	23%	12	17%	
Incidence rate per person-month	0.32	(0.20-0.48)	0.26	(0.15-0.47)	
Mean duration among symptomatics in days	7.8		7.6		
Number of symptomatic days per month	1.67	(1.45-1.91)	1.39	(1.22-1.58)	
Rhinitis					
Prevalence	19	27%	18	26%	
Incidence rate per person-month	0.37	(0.24-0.58)	0.40	(0.26-0.59)	
Mean duration among symptomatics in days	5.1		6.6		
Number of symptomatic days per month	1.57	(1.35-1.81)	1.89	(1.66-2.12)	
Signs of skin infection					
Prevalence	3	4%	1	1%	
Incidence rate per person-month	0.048	(0.014-0.12)	0.070	(0.025-0.15)	
Mean duration among symptomatics in days	2.0		1.8		
Number of symptomatic days per month	0.11	(0.055-0.18)	0.10	(0.052-0.17)	



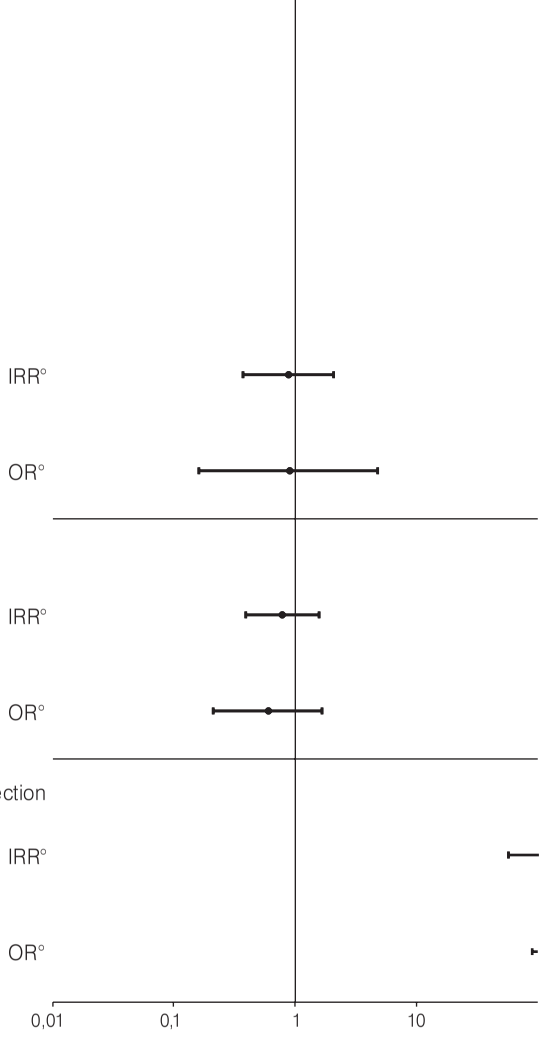
IDD = insulin-dependent diabetics, Numbers between brackets are 95% confidence intervals, IRR: Incidence rate ratio, IDD versus controls, with 95% confidence interval, OR: Odds ratio for the number of symptomatic days, NIDD versus controls, with 95% confidence interval, ° p>0.05, * p<0.05

Table 3 Travel-related symptoms of infectious diseases among non-insulin-dependent diabetics and their non-diabetic controls

	NIDD		Controls		Ratios with 95% confidence intervals, for NIDD versus controls
Number of participants	82		82		
Diarrhea					Diarrhea
Prevalence	32	39%	35	43%	
Incidence rate per person-month	0.75	(0.56-1.00)	0.70	(0.52-0.91)	IRR°
Mean duration among symptomatics in days	2.4		3.0		
Number of symptomatic days per month	1.57	(1.35-1.82)	1.69	(1.45-1.94)	OR°
Vomiting					Vomiting
Prevalence	3	4%	5	6%	
Incidence rate per person-month	0.049	(0.017-0.11)	0.051	(0.018-0.11)	IRR°
Mean duration among symptomatics in days	2.0		1.2		
Number of symptomatic days per month	0.097	(0.044-0.18)	0.060	(0.023-0.12)	OR°
Fever					Fever
Prevalence	5	6%	6	7%	
Incidence rate per person-month	0.051	(0.018-0.11)	0.073	(0.031-0.14)	IRR°
Mean duration among symptomatics in days	2.2		2.7		
Number of symptomatic days per month	0.12	(0.060-0.20)	0.20	(0.13-0.30)	OR°



Cough					Cough	
Prevalence	16	20%	19	23%		
Incidence rate per person-month	0.28	(0.17-0.49)	0.30	(0.19-0.46)		IRR°
Mean duration among symptomatics in days	11.8		8.7			
Number of symptomatic days per month	2.05	(1.88-2.22)	1.79	(1.60-1.99)		OR°
Rhinitis					Rhinitis	
Prevalence	21	26%	26	32%		
Incidence rate per person-month	0.39	(0.25-0.72)	0.48	(0.32-0.74)		IRR°
Mean duration among symptomatics in days	7.8		7.7			
Number of symptomatic days per month	2.11	(1.91-2.33)	2.46	(2.21-2.71)		OR°
Signs of skin infection					Skin infection	
Prevalence	12	15%	0	0%		
Incidence rate per person-month	0.44	(0.23-2.16)	0	(0.00-0.0060)		IRR°
Mean duration among symptomatics in days	10.7		0			
Number of symptomatic days per month	1.68	(1.51-1.85)	0	(0 - 0.0040)		OR°



NIDD = non-insulin-dependent diabetics, Numbers between brackets are 95% confidence intervals, IRR: Incidence rate ratio, NIDD versus controls, with 95% confidence interval, OR: Odds ratio of the number of symptomatic days, NIDD versus controls, with 95% confidence interval, ° $p>0.05$, * $p<0.05$

Of 82 NIDD, 2 (2.4%) reported dysregulation of blood glucose levels during travel. A 63-year old woman had one hyperglycemic episode, coinciding with non-febrile diarrhea, for which she did not take stand-by antibiotics. A 47-year old woman had a 4-day episode of hyperglycemia without fever or diarrhea.

Discussion

This is the first prospective study evaluating whether medication-dependent diabetic travellers to the (sub)tropics are at increased risk for developing symptomatic infectious diseases. Although we hypothesized that they would have symptoms more often and longer than non-immune-suppressed non-diabetics, no differences in travel-related diarrhea, vomiting, fever, cough, or rhinitis were found. The NIDD had signs of skin infection more often than controls, unrelated to travel. A higher incidence rate and burden of non-travel-related signs of skin infection among type 1 and 2 diabetics has been reported before, irrespective of insulin use [9,16]. Why we found increased risk for skin infection only among NIDD and not IDD, may reflect differences in age, exposure, or unknown co-morbidity, such as pre-existing skin disease, carriage of *Staphylococcus aureus*, peripheral neuropathy, or microvascular disease [9,17].

Before travel, disease burden of cough seemed to be lower among IDD than controls. This coincided with a higher prevalence of asthma or chronic obstructive pulmonary disease among the controls, although the difference was not statistically significant ($p>0.05$).

Before travel, outcome measures for diarrhea and vomiting were higher among NIDD than controls. The increased diarrhea might be explained by medication, as the oral anti-diabetic metformin is known for such gastro-intestinal side effects [18]. Also, diarrhea has been associated with metabolic dysregulation. In a retrospective population-based survey including 423 IDD and NIDD and more than 8000 controls, non-travel-related diarrhea was more prevalent among diabetics than controls, with an OR of 2.06 (95% confidence interval 1.56 - 2.74) after adjusting for age and sex [19]. That study linked poorer levels of self-reported glycemic control with a higher prevalence rate of diarrhea.

Our study design had distinctive strengths. Structurally specified data were obtained prospectively and on a daily basis. Data collection started before departure (median 15 days) to gain insight into pre-existing symptoms. It continued until 2 weeks after return from travel to encompass incubation periods of the most (acute) travel-related infectious diseases. With a travel companion serving as a matched control, situational

specifics for diabetics and non-diabetics were comparable, which minimized any differences in exposure to infectious agents between the two groups. Diabetics and controls also matched in age and country of birth. They did not match for gender or for cardiovascular disease and dyslipidemia. However, prospective studies on travel-related infectious diseases found no association of symptoms of infectious diseases and gender [20,21], and we are not aware of any association with cardiovascular disease or dyslipidemia.

The prevalence of diabetes among visitors of our clinic was 3.1%, comparable with the general population [12]. Also, age and male-female ratio of our diabetic subjects were comparable with the general diabetic population. Participants' travel destinations were equally distributed across the four (sub)tropical regions. Their median travel duration of 20 days corresponded well with the median travel duration of the average traveller [22,23]. Thus, the study sample can be considered representative, and results can reasonably be applied to the average diabetic traveller to a (sub)tropical country.

This study has some limitations. Sample size may not have been large enough to detect small differences. Secondly, although the diary provided information on symptom duration, it did not distinguish mild symptomatology from severe. For example, diabetics could have had more bowel movements or more water loss. Thirdly, diabetics and controls differed in counseling and prescription; some diabetics did use the stand-by antibiotics. Therefore, the data may be skewed toward seeing less differences in outcome measures between both groups.

Metabolic dysregulation was minimal among our diabetics: 4.3% among IDD and 2.4% among NIDD. A retrospective, descriptive cohort study among IDD performed in 1996-1997 reported that 68% of 19 IDD travellers to tropical destinations had metabolic dysregulation [4]. Moreover, it found that 55% of those IDD reported dysregulations more frequently during travel than at home. This suggested that travel to the tropics is a risk factor for metabolic dysregulation. However, data were collected retrospectively, by telephone interviewing, and the study size was small and comprised only IDD. Finally, with improvements in the quality and use of insulin preparations and treatment schedules [5,24,25], diabetics might now be more aware and more compliant with anti-diabetic therapy, including its adjustments to travel-related alterations in eating habits, physical exertion, climate, and circadian rhythm.

Nevertheless, the prevalence of metabolic dysregulation in our subjects, may be underestimated, because regular testing of blood glucose levels during travel was not part of the study protocol. The diabetics monitored blood sugar at their own discretion.

Aside from such limitations, our findings represent diabetics who sought pre-travel health advice. One may assume that they had a more than average health awareness, particularly having received travel advice and knowing the objectives of the study.
As to usage of stand-by antibiotics, it was carefully explained to all diabetics. Its importance was emphasized by an experienced travel health expert, and by means of information leaflets. Nevertheless, 83% of all diabetics with diarrhea did not use this treatment, even in the case of metabolic dysregulation. Of 152 stand-by antibiotic courses provided, 141 (92.8%) were not used. Moreover, NIDD did not experience hypoglycemias, only hyperglycemias. Indeed, hypoglycemia is uncommon when using only oral anti-diabetics [26]. Thus, routine prescription of stand-by antibiotics to prevent hypoglycemia during uncomplicated diarrhea is probably not useful. For IDD, monitoring blood glucose more frequently, and adjusting insulin dosage and diet accordingly, are probably more helpful in minimizing the impact of diarrhea or fever on metabolic dysregulation. Stand-by antibiotics may be useful for diabetic travellers to areas where health facilities are lacking or for complicated cases, for example 3 or more unformed stools per 24 hours with accompanying symptoms such as fever, or blood in stools. The merits of this definition could not be assessed in this study.

In conclusion, this study showed that medication-dependent diabetic travellers to (sub)tropical destinations do not have travel-related symptoms of diarrhea, vomiting, fever, cough, rhinitis, and signs of skin infection more often or longer than non-diabetics.
The incidence of metabolic dysregulation among diabetic travellers should be assessed in more detail, but our findings indicate that routine prescription of stand-by antibiotics against mild, uncomplicated travellers' diarrhea is probably not useful, in particular not for NIDD. Self-treatment could be of value for travellers to remote areas or for cases of complicated diarrhea.

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3

Yellow fever vaccination of the elderly: The humoral immune response lagging behind

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Manuscript in progress

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Abstract

Background

Yellow fever vaccine (YF-17D) can cause, although rarely, serious adverse events (SAEs). The mechanism of these SAEs is poorly understood. Older age has been identified as one of the risk factors for developing such events. We investigated the adaptive immune response against YF-17D in elderly subjects, to elucidate the mechanism of SAEs.

Methods

Young volunteers (age range 18-28 yrs, N=30) and elderly travellers (age range 60-81 yrs, N=28) were vaccinated with YF-17D from the same vaccine batch. Neutralising antibody titres and plasma YF-17D RNA copy numbers were measured at day 5 after vaccination. Following vaccination, adverse events were documented in a diary during 3 weeks.

Results

Ten days after vaccination seroprotection (80% virus neutralisation in plaque assay by minimally diluted serum) was attained by 77% (23/30) of the young participants and by 50% (14/28) of the elderly ($p = 0.03$, Chi-square test). At day 10, the younger participants had a Geometrical Mean Titres (GMT) of 0.18 IU/ml, ten-fold higher than the GMT in the elderly (0.017 IU/ml) ($p = 0.004$). At day 14 the GMT also differed (respectively 4.8 IU/ml and 2.7 IU/ml, $p = 0.035$). Seroprotection was attained by all participants (young and elderly) by day 14. At day 5, viraemia was more common in the elderly (18%) than in the younger participants (3%). Viraemia was associated with fever but not with the time to seroprotection. The elderly participants reported fewer mild adverse events.

Conclusion

We found that elderly subjects (age ≥60 yrs) had a slower antibody response against yellow fever vaccine after primovaccination. We hypothesize that this allows attenuated virus to cause higher viraemia that may result in severe disease.

Introduction

The live attenuated 17D yellow fever vaccine is regarded as one of the safest and most effective vaccines [1]. However, it can cause fatal adverse events in immunocompromised individuals [2]. A hampered immune response allows the vaccine virus to replicate unrestrictedly, leading to vaccine-associated disease that resembles wild type yellow fever (yellow fever vaccine associated viscerotropic disease, YEL-AVD). YEL-AVD is fatal in 50% of cases [3]. In the last decade, a series of these serious and sometimes fatal adverse events following yellow fever vaccination appeared in the literature [4-10]. The risk of YEL-AVD increases with a history of thymectomy [11], male gender [12] and old age. For vaccinees of 60-69 years, this risk is estimated to be 1.1:100.000 doses and for vaccinees of ≥70 years it is 3.2:100.000, a 4.4 and 13.4 fold higher risk than for young adults [13].

The higher risk of YEL-AVD in elderly travellers has led to a more restrictive policy towards vaccinating travellers older than 60 years [14,15]. In this group the risk of serious adverse events following vaccination is weighed against the risk of infection, using disease surveillance data of the WHO and reports of yellow fever outbreaks.

The biological mechanism for the association between adverse events and older age has not yet been elucidated [3]. The innate and adaptive immune response wanes with age [16]. However, yellow fever neutralising antibody (NA) levels are equal in elderly and young vaccinees at 30 days following vaccination [17]. Although the immune response eventually leads to equal NA levels, it may be that the response develops more slowly in elderly subjects. This would allow the vaccine virus more time to replicate and cause adverse events.

Methods

Objectives

This study was conducted to determine whether the adaptive immune response to yellow fever vaccine is slower to develop in persons of 60 years or older compared with younger persons (18-40 years). The humoral response to yellow fever vaccination was measured by Plaque Reduction Neutralisation Test (PRNT). Yellow Fever 17D (YF-17D) viraemia after vaccination was quantified by real time PCR (qRT-PCR). In addition, baseline naïve and memory T cells ($CD4^+$ and $CD8^+$) were quantified.

Study design and Participants

In this prospective controlled observational trial, participants were recruited at the Travel Clinic of the Leiden University Medical Centre, and Municipal Health Centres of Leiden and The Hague. Healthy volunteers aged between 18 and 40 years were eligible for inclusion into the control group. Participants in the control group did not need to have an indication for yellow fever vaccination. Healthy travellers aged 60 years or above, who had an indication for yellow fever vaccination based on their travel destination (National Coordination Centre for Travelers' Health, LCR) [18], were eligible for the study group.

Those who had previously received yellow fever vaccine, those who had a compromised immunity due to underlying illness or immunosuppressive medication and –in the young– those who were pregnant were excluded. The study was carried out between April 2008 and April 2009. Vaccinations were administered at the Travel Clinic of the LUMC.

Yellow fever vaccine

The live, attenuated, 17D vaccine used in this study was manufactured on embryonated chicken eggs according to WHO regulations and stored according to manufacturer's guidelines. All administered vaccines originated from the same vaccine lot (Stamaril, Lot no B5355, Sanofi Pasteur, France). The vaccine was administered subcutaneously in the deltoid region of the right arm.

Data collection

At the time of inclusion, data on demographic characteristics of the participants were obtained. Blood samples for the determination of neutralising antibodies (NA) and YF-17D viraemia were collected before (day 0), and 3, 5, 10, 14 and 28 days after vaccination. Peripheral Blood Mononuclear Cells (PBMC) for determination of cellular immunity were collected at day 0 and 14.

Participants were asked to document local and systemic adverse events after vaccination in a three-week diary. Solicited symptoms were: erythema, pain and swelling at the site of injection, fever and myalgia.

Constant virus – varying serum dilution Plaque Reduction Neutralisation Test (PRNT)

The tests were carried out in 6-well plates (Corning Inc., USA) using a slightly modified technique described originally by De Madrid and Porterfield [19]. Briefly, approximately 6×10^5 Vero cells/mL were seeded per well in 6–well plates and cultured to obtain a

confluent monolayer. Sera were complement inactivated at 56°C for 1 hour. Prevaccination sera were tested in 1:16 dilution, to which 100 plaque forming units (PFU) of 17D-YF were added. Postvaccination sera were tested in two-fold dilutions starting from 1:4 to 1:1024. One hundred PFU of YF–17D virus were added to each serum dilution. All test sera were assayed in duplicate. After 1 hour incubation on ice, the mixtures of virus and serum were added to the Vero cell monolayers and incubated for 1 hour at 37°C. An overlay of 2 x DMEM and 2% agarose was added. After 5 days of incubation at 37°C, the overlay was discarded and cell monolayers were stained with crystal violet. Plaques were counted by eye. Virus neutralisation (VN) was calculated for each serum dilution (i) with the following formula: VN(i) = 100 x (number of PFU in diluted postvaccination serum / number of PFU in pre-vaccination serum (in a 1:16 dilution)). The serum dilution at which $\log_{10}$ neutralisation index 0·7 (80% VN) occurred was taken as endpoint, as this corresponds to the generally accepted definition of protection [20]. A reference serum, obtained from the National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/) was used for quantification of International Units per milliliter (IU/mL). In our hands a 0.7 log10 plaque reduction in 1:10 diluted serum corresponds to a titre of 0.5 IU/mL [95%CI: 0.3–0.8 IU/mL] [21]. Similar values have been found by others [22]. Geometrical Mean Titres (GMT) were compared between the two groups.

Flow cytometry

PBMC samples were thawed and allowed to rest overnight in RPMI supplemented with 10% fetal calf serum (Gibco, Breda, The Netherlands). After washing, cells were incubated with ViVid Live/Dead stain (Invitrogen, Breda, The Netherlands) for 10 minutes at 4°C before staining with the following antibodies: CD3-APC-Cy7, CD4-Alexa 700, CD8-Am Cyan, CD45RA-PE-Cy5, CCR7-PE-Cy7 (all BD Biosciences, Breda, the Netherlands) and CD19-Pacific Blue and CD14-Pacific Blue (Biolegend, ITK Diagnostics, Uithoorn, the Netherlands) for 30 minutes at 4°C. Cells were acquired on a LSRII flowcytometer using FACSDiva Software (BD Biosciences) and analysed using FlowJo Software (TreeStar, Ashland, Oregon, USA). Analysis was performed by gating on lymphocytes, followed by selection of live cells that were not CD14 or CD19 positive. Subsequently, $CD3^+$ T cells were selected and samples were gated for $CD4^+CD8^-$ cells and $CD8^+CD4^-$ cells before analyzing memory subpopulations.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Viral RNA was isolated from 200 μl plasma using a MagNa Pure LC Total Nucleic Acid Isolation Kit (Roche Molecular Diagnostics, Penzberg, Germany). cDNA was

synthesized with 10 μl elute (200μl total) in a Tprofessional ThermoCycler (Biometra, Germany), and quantitative reverse transcription-PCR (qRT-PCR) of YFV RNA was performed in a BioRad i-cycler IQ™ real-time PCR detection system (BioRad, Veenendaal, The Netherlands). The following YFV specific primers and probe were used [23]:

YFV-1 (forward)	AATCGAGTTGCTAGGCAATAAACAC
YFV-2 (reverse)	TCCCTGAGCTTTACGACCAGA
YFV-P (probe)	FAM-ATCGTTGAGCGATTAGCAG-BHQ

FAM (6–carboxyfluorescein) was used as 5'-reporter dye and BHQ (Black Hole Quencher) as 3'-quencher dye. In order to quantify YFV RNA, $\log_{10}$ dilutions of in vitro transcribed RNA standards were included as standard curves. RNA copy numbers were calculated with the standard curves from Cycle threshold (Ct) values to compare viraemia in both groups quantitatively.

Ethics

The protocol and consent forms were approved by the Dutch Central Committee of Human Research (CCMO) and by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC) in the Netherlands. The trial was registered under NTR1040 and ISRCTN42180653, (http://irsctn.org). Written informed consent was obtained from each participant prior to inclusion.

Statistical methods

Power calculations were based on an expected 80% virus neutralisation of 95% in the control group and 66% in the elderly group at day 14, based on previous observations at the Travel Clinic (unpublished data). With an α of 0·05 and a β of 0·2, 26 participants per group were needed to confirm a significant difference under these assumed conditions. To take into account a possible lost to follow up of 15%, 30 participants were included per group.

The Student's t-test was used for comparison of the Geometrical Mean Titres (GMT) at which 80% virus was neutralised between the control group and elderly vaccinees. Where appropriate, Chi-square tests were used, and Wilcoxon's test for non-parametrical distributed numerical data. Statistical analysis was performed using a computer-assisted software package (SPSS version 16.0, SPSS Inc., Chicago, IL).

Results

Population

We enrolled 60 volunteers. None of the participants withdrew prematurely. In 2 elderly participants, virus neutralisation already occurred at day 0, meaning that these persons had been vaccinated against yellow fever previously (Figure 1). These two were excluded from further analysis. Baseline characteristics of the study population are given in table 1. Gender and possible previous exposure to flaviviruses did not differ between the groups (Table 1).

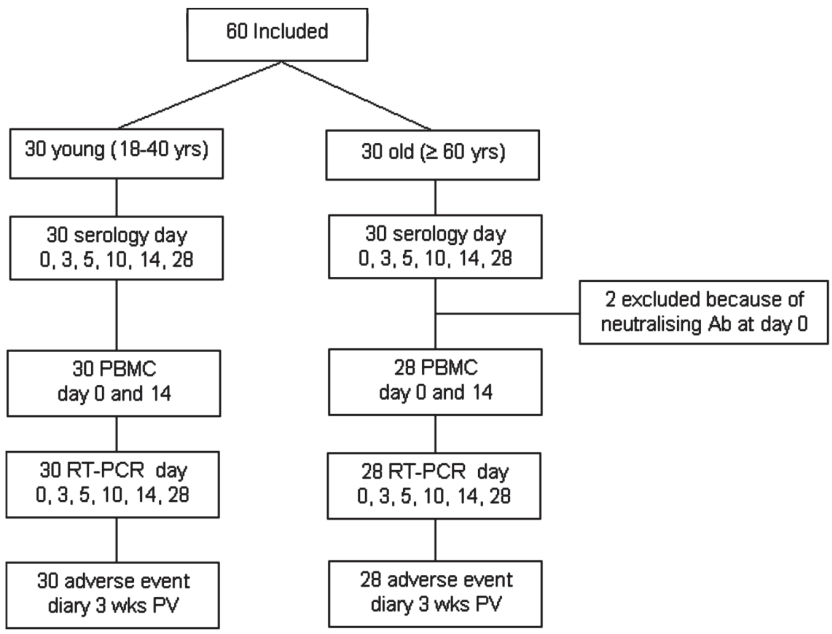

Figure 1 Inclusion of study population. Two participants in the group of ≥ 60 yrs had neutralising antibodies before vaccination. These were excluded from analysis. Ab = Antibodies, PBMC = Peripheral Blood Mononuclear Cells, RT-PCT = Real Time - Polymerase Chain Reaction, PV = Post-vaccination.

Table 1 Demographic characteristics of the study population

Demographic characteristics		Participants		p-value
		Young (18-40 years) N = 30	Elderly (≥ 60 years) N = 28	
Females (%)		22 (73)	20 (71)	0.9
Age (years)	Median	21	66	-
	IQL range	20-22.5	65-69	-
	Range	18-28	60-81	-
Flavivirus[$] N yes (%)		8 (27)	8 (29)	0.9

[$] Flavivirus = possible flavivirus encounter in past five years defined as travelled to flavivirus endemic destination

Neutralising antibody response

At day 3 and 5 after vaccination, no neutralising antibodies were found (data not shown). Ten days after vaccination seroprotection was attained by 77% (23/30) of the young participants and by 50% (14/28) of the elderly group ($p = 0.03$, Chi-square test). This result is also reflected in the height of the antibody concentration (Figure 2). At day 10, the younger participants had a GMT of 0.18 IU/ml, ten-fold higher than the GMT in the elderly participants (0.017 IU/ml) ($p = 0.004$). At day 14, seroprotection was attained by all participants (young and elderly). The GMT at day 14 still differed significantly between the young and the old (respectively 4.8 IU/ml and 2.7 IU/ml, $p = 0.03$). At 28 days after vaccination there was no longer a significant difference in the GMT between the younger and older group (resp. 13.3 IU/ml and 9.0 IU/ml, $p = 0.1$). Female gender or recent travel to flavivirus endemic countries did not result in a significantly different virus neutralisation titre in either group (data not shown).

Vaccine safety

Participants reported the occurrence and duration of adverse events after yellow fever vaccination in a 3–week diary. In younger vaccinated participants vaccination evoked redness and swelling at the site of inoculation more frequently and for a longer period than in the elderly participants. Although not significant (with $\alpha = 0.05$), adverse events occurred more frequently and earlier (respectively 1.3 and 5.3 days after vaccination) in the younger participants compared to the elderly group.

Yellow fever vaccine virus RNA

YF-17D viraemia was measured by qRT-PCR at day 5. The detection limit was 1300

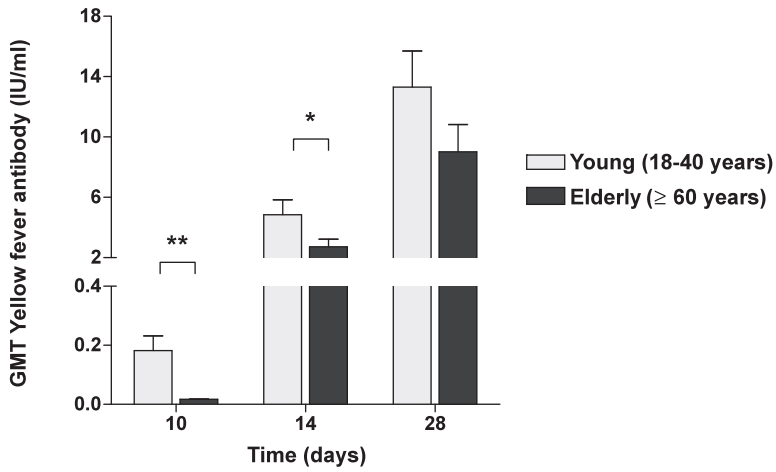

Figure 2 Geometric mean titres (GMT) of yellow fever antibodies in participants with a measurable antibody response at 10, 14 and 28 days after vaccination. No antibodies were measured at day 3 and 5 after vaccination. * p=0.03, ** p=0.004. GMT were analysed with non-parametric test (day 10) and Student's t-test (day 14 and 28). Error bars represent 95%CI.

copies/ml. At day 5 viraemia was detected in more elderly (18%) than young participants (3%) (p = 0.05, Fisher's Exact Test). The one young participant with viraemia did have a protective antibody titre at day 10. Of the six elderly people with viraemia, two had attained seroprotection by day 10 (33%) compared with 12 of 22 (55%) of those without detectable viraemia. In the seven participants with detectable viraemia at day 5, the GMT was 0.3 IU/ml (95% CI 0.1 to 0.8) at day 10, 8.6 IU/ml (95% CI 5.6 to 13.1) at day 14 and 16.5 IU/ml (95% CI 10.9 to 24.8) at day 28. In those without detectable viraemia the GMT was 0.05 IU/ml (95% CI 0.03 to 0.07) at day 10, 3.3 IU/ml (95% CI 2.8 to 3.7) at day 14 and 10.5 IU/ml (95% CI 9.1 to 12.0) at day 28. P-values for the difference in GMT between those with and those without viraemia were 0.14 for the comparison at day 10, 0.06 at day 14 and 0.33 at day 28.

Viraemia was associated with fever. Of the six participants with detectable viraemia, three (50%) had self-reported fever compared with 4 of 47 (9%) who did not have detectable viraemia (p = 0.03, Fisher's Exact Test), The mean number of days between

Table 2 Solicited adverse events after primary and booster YF-17D vaccination. Safety of vaccination expressed in various parameters

Adverse event			Participants		p-value
			Young N=30	Elderly N=28	
Local	Erythema	N yes (%)	8 (27)	2 (7)	0.05
		Mean N days (s.e.m.)	3.4 (±0·8)	2.0 (±1·0)	0.4
	Swelling	N yes (%)	3 (10)	1 (4)	0.3
		Mean N days (s.e.m.)	2·7 (±1·2)	2·0 (-)	0.8
	Pain	N yes (%)	3 (10)	2 (7)	0.7
		Mean N days (s.e.m.)	1·7 (±0·7)	2·0 (±0·0)	0.7
Systemic	Myalgia	N yes (%)	12 (40)	6 (21)	0.1
		Mean N days (s.e.m.)	2·3 (±0·5)	2·3 (±0·2)	0.9
	Fever	N yes (%)	3 (10)	4 (14)	0.6
		Mean N days (s.e.m.)	6·3 (±3.8)	3·0 (±4.0)	0.4
	(N days after vaccination)		1.3 (±1.3)	5.3 (±0.3)	0.09

S.e.m. = standard error of the mean. Fever was defined as self-measured temperature above 38 degrees Celsius.

the vaccination and start of symptoms did not differ between those with and those without viraemia (respectively 3.7 and 3.5 days). Myalgia and injection site reactions were not associated with viraemia (data not shown).

T-cell subsets

We investigated the percentage of naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory (TEMRA) cells in the $CD4^+$ and $CD8^+$ T cell subsets at baseline (day 0). Previous publications have shown that naïve cells are relatively more numerous in younger persons, and that the percentage of more differentiated T cells (EM and TEMRA) is augmented in elderly persons [24]. This was confirmed in our population (Figure 3). The increased percentage of naïve cells in the younger participants, compared with the elderly, was more pronounced in the $CD8^+$ than in the $CD4^+$ T cell subset. In addition, the elderly group had a higher percentage of $CD8^+$ effector T cells. Irrespective of age, no correlation was found between the percentage of naïve T cells and either anti-YF17D GMT or viraemia.

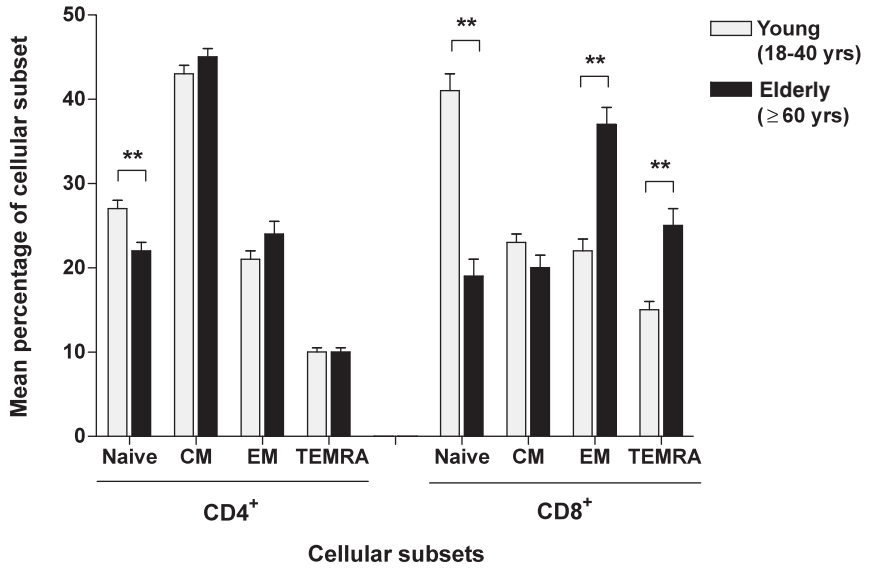

Figure 3 Percentage of T lymphocyte subsets in the study population, divided in $CD4^+$ and $CD8^+$ subsets. Young and elderly participants were compared with respect to their naïve, central memory (CM), effector memory (EM) and terminally differentiated effector memory cells (TEMRA) at day 0 (before vaccination) with Student's t-test. ** $p \leq 0.001$.

Discussion

We demonstrated that in elderly persons (≥ 60 years) the initial humoral response to yellow fever vaccine lags behind that of younger vaccinees. GMT of neutralising antibodies were significantly lower at 10 and 14 days after vaccination but not at 28 days. Five days after vaccination viraemia was more common in the elderly. Viraemia was associated with having a fever but was not associated with the time to sero-protection.

These results may offer a biological explanation for the increased susceptibility to YEL-AVD in old age. Immunological senescence leading to an impaired ability to clear

the vaccine virus has been put forth as a possible reason for increased risk of YEL-AVD in elderly people [17]. In a retrospective study of two large clinical trials of two YF-17D vaccines from different manufacturers, involving a total of 4,532 subjects, neutralising antibody responses at 30 days after vaccination were equivalent in younger and elderly subjects. Due to the retrospective nature, early responses (i.e. < 30 days after vaccination) could not be compared and were assumed to be equal in both groups. Our results show that this assumption needs to be modified.

We observed a striking resemblance with respect to the occurrence of adverse events between the previous analysis of yellow fever vaccination in elderly subjects and our study (AE) [17]. Overall, the incidence injection site adverse events was lower in elderly than in younger subjects. If injection site reactions are a result of immune activation, observing less adverse events in elderly subjects could reflect a weaker or slower immune response in elderly people. Similarly, this line of argumentation is consistent with the later onset of adverse events in the elderly compared with the younger subjects (5.3 versus 1.3 days).

Neutralising antibodies are the gold standard for monitoring the immune response against yellow fever vaccine. This has in part a biological reason. In vitro, the antibodies inhibit viral replication. In vivo, passively immunised primates are protected against challenge with wild-type yellow fever [25]. Therefore, the delayed humoral response in the first two weeks after vaccination in the elderly could enable augmented virus replication. Whether this hampered antibody response coincides with an impaired cellular immune response remains to be studied.

In the elderly, an impaired innate and acquired immunity [26,27] is generally held responsible for the increased susceptibility of the elderly to infections, and reduced ability to respond to vaccines [28]. Clinical examples of this hampered response against vaccines are the influenza vaccine, with an efficacy between 70% and 90% in those under 65 years of age, but of 30% to 40% for those over 65 years of age [29], the pneumococcal polysaccharide vaccine [30] and hepatitis B vaccines [31]. On a cellular level, it has been shown that the subset of naïve T lymphocytes decreases dramatically with age (possibly due to thymic involution), together with an increase of effector T cells, which we also demonstrated in our study population. This altered distribution of lymphocytes at specific differentiation stages may restrict the diversity of the immune cell repertoire, leading to a diminished response to neoantigens, like yellow fever vaccine [27,28].

Beside the immunosenescence in elderly subjects, other hypotheses on the mechanism of YEL-AVD have been postulated. For example, it is possible that the vaccine virus reverts or mutates to a more virulent form. However, extensive genetic analyses of the viral strains extracted from patients with YEL-AVD do not provide evidence to support this hypothesis [3]. The hypothesis of host genetic susceptibility for developing YEL-AVD seems more plausible. Pulendran and colleagues found a heterozygous CCR5Δ32 mutation in a patient who suffered from YEL-AVD [32]. Since the prevalence of heterozygosity of the CCR5Δ32 mutation in the general population is 15% [33] and the occurrence of YEL-AVD among yellow fever vaccinees is significantly less [13], other host factors (e.g. immunosenescense) must also play a role in the development of YEL-AVD [34]. On the other hand, milder forms of YEL-AVD might occur more frequently, but might not be severe enough to be published, thus introducing publication bias. In addition to the hypothesis of genetic susceptibility, recently discovered genetic host factors, including complement protein C1qB and eukaryotic translation initiation factor 2 alpha kinase 4- (an orchestrator of the integrated stress response) predicted YF-17D $CD8^+$ T cell responses with up to 90% accuracy. A B-cell growth factor, TNFRS17, predicted the neutralising antibody response with up to 100% accuracy [35].

Although occurrence of YEL-AVD is very rare, fear of this adverse event could reduce utilisation of yellow fever vaccine. An "International Laboratory Network for Yellow Fever Vaccine-Associated Adverse Events" has been established in 2008, to complement the USA and the European Yellow Fever Vaccine Safety Working Groups [36]. Its goal is to determine the pathogenesis of severe adverse events following yellow fever vaccination through systematic and coordinated laboratory evaluation of reported cases. With this study, we contribute to this goal. A greater understanding of the pathogenesis of YEL-AVD may lead to new approaches to prevent this serious complication. One possibility may be to inject much less vaccine virus in a more immunostimulant manner (eg. intradermally) [37].

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Intradermal Hepatitis B vaccination in non-responders after topical application of imiquimod (Aldara®)

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Abstract

Background

Five to ten percent of immunocompetent persons fail to develop a protective immune response to hepatitis B vaccination, and are defined non-responders (NR). We investigated the immune response to intradermal hepatitis B vaccination after pre-treatment of the skin with the TLR7 agonist imiquimod.

Methods

Twenty-one non-responders (anti-HBs < 10IU/L after at least 6 intramuscular hepatitis B vaccinations) were randomly assigned to the control group (N=11) or the experimental group (N=10). Participants in both groups received 3 intradermal vaccinations with 5μg HBsAg (0.125mL) at 0, 1 and 6 months. In the experimental group, the dermal site of injection was pre-treated with 250 mg imiquimod ointment. Anti-HBs antibodies were determined at 0, 1, 2, 6 and 7 months.

Results

In both study groups, 70% of the participants developed a protective immune response (anti-HBs ≥ 10IU/L), after the 3rd intradermal vaccination.

Conclusion

The application of imiquimod on the skin prior to intradermal vaccination did not enhance the humoral response to hepatitis B vaccine. However, irrespective of imiquimod application, 70% of the NR who had not responded to 6 previous intramuscular vaccinations, developed a protective immune response with high affinity antibodies after 3 ID hepatitis B vaccinations with 5 μg HBsAg.

Introduction

The immune response to the injected hepatitis B surface antigen (HBsAg) can vary greatly in healthy subjects [1]. Whereas most healthy vaccinees develop an adequate antibody response, defined as an anti-hepatitis B surface antigen (anti-HBs) titre of ≥100 IU/L, five to ten percent of immunocompetent persons fail to develop a protective immune response and never reach an anti-HBs titre exceeding 10 IU/L; these are defined as non-responders (NR) [2]. True NR, defined as NR to 2 series of hepatitis B vaccinations are presumed unlikely to develop adequate anti–HBs titres with further vaccine doses, although no thorough research has been performed to confirm this [3]. The protective efficacy of hepatitis B vaccination is directly related to the induction of anti-HBs antibodies [4-6]. An antibody titre of ≥10 IU/L measured 1–3 months after the administration of the last dose of the primary vaccination series is considered to be a reliable marker of immediate and long-term protection against infection, and those who have an anti-HBs titre of ≥10 IU/L are considered to have protective immunity.
Non-responsiveness to the vaccine has major implications for health care workers and sexual partners of HBV carriers in low endemic countries. In terms of biological mechanisms, non-responsiveness to hepatitis B vaccination has been associated with the presence or absence of specific of MHC alleles. The most pronounced associations with non-responsiveness were with excess of HLA-DR3, -DR7, -DQ2 and -DP11 and with absence of HLA-DR1, -DR5, -DR2, -DQ5 and -DP4 [7-8]. Other characteristics correlated with an inadequate anti-HBsAg response are; higher age, obesity, male gender, and cigarette smoking [9-11].

Several strategies to increase the immune response to the hepatitis B vaccine in NR have been investigated, and comprise of an additional series of standard vaccinations, vaccination of HBsAg combined with other antigens or additional adjuvants [12-14], or alternative routes of administration. The most commonly chosen strategy is to give an additional series (1 to 3 vaccinations) of conventional intramuscular vaccinations, leading to seroconversion in 61% of the revaccinated [15].
Another alternative to enhance immunogenicity in NR would be to administer hepatitis B vaccine in the dermal layer of the skin, instead of injecting intramuscularly. Although the intradermal (ID) vaccination route has shown to elicit slightly lower antibody responses in healthy subjects [16-22], in hepatitis B vaccine low responders (anti-HBs of 10-100 IU/L) and NR, the ID vaccination yielded slightly higher antibody titres compared to intramuscular (IM) vaccination during the first 6 months after vaccination [15,23,24].

In this study, we combined the ID vaccination route with local stimulation of dermal antigen presenting cells as a new approach to obtain a protective antibody response in true hepatitis B vaccine NR. Imiquimod (Aldara®) activates antigen presenting cells (APCs) through the toll-like receptor 7 (TLR7) and is registered for the treatment of (genital) warts and basal cell carcinoma.

Methods

Objectives

This study was conducted to determine whether in NR to hepatitis B vaccination, pre-treatment of the injected skin with a TLR stimulant (Aldara®, one sachet (250mg) applied on 20cm^2 skin) before ID hepatitis B vaccination (5μg; 0·125 mL) would elicit a higher antibody response compared to ID vaccination (5μg; 0·125 mL) without pre-treatment of the skin. Efficacy of vaccination was determined by serum anti-HBs antibody measurement.

Study design and Participants

The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC, The Netherlands; protocol P05.187), and registered in the Dutch Trial Register (#NTR1043). Written informed consent was obtained from each participant.

Healthy volunteers of 18 years and older with a history of at least 2 series of hepatitis B vaccination (one series comprises 3 vaccinations of at least 10µg HBsAg per vaccination) and no postvaccination antibody titre of ≥10 IU/L, were eligible for inclusion. Participants were recruited via the University Medical Centres of Leiden and Utrecht (the Netherlands). Records of previous hepatitis B vaccinations and antibody responses were obtained. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, pregnant volunteers and those with (possible) autoimmune disorders. The study was carried out between May 2007 and October 2008. Subjects were randomly assigned to the experimental (with imiquimod pretreatment of the injected skin) or control (without pre-treatment of the injected skin) group. Randomisation was performed with the use of sealed envelopes containing the vaccination code balanced through in permuted blocks of 4.

Hepatitis B vaccine

The hepatitis B vaccine used in this study, HBVAXPRO® 40μg HBsAg/mL, is a recombinant vaccine with alum adjuvant, manufactured by Sanofi Pasteur MSD (Lot

no ND37720) and stored according to manufacturer's guidelines. Multiple dosages (maximally 4) were obtained from one vial for ID vaccination. One ID vaccination of 0·125mL contained 5μg HBsAg.

Vaccination and data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained. Participants received 0·125mL hepatitis B vaccine (equivalent to 5μg HBsAg) intradermally on the dorsal side of the right forearm at 0, 1 and 6 months. This vaccination site enables careful monitoring of possible adverse events. The quality of the ID injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test) [http://www.cdc.gov/tb/pubs/Mantoux/part1.htm (accessed 27th of March 2009)], with 6 mm being the lowest acceptable diameter. In the experimental group, a square surrounding 20 cm^2 (equal of 7·9 square inches) was marked on the dosal side of the forearm. The participant applied the content of one sachet of Aldara® (5g, 50mg/g) to the marked surface on the skin. This is the advised dosage per application for the treatment of skin lesions. After the ointment was taken up by the skin (in approximately 3 minutes), the vaccine was injected in the centre of the marked area. The oinment was removed by the participant by washing after 6 hrs. In the control group, the vaccine was administered without pre-treatment of the skin.

Blood samples were collected before vaccination (time point 0), and at 1, 2, 6 and 7 months. In the first blood sample (at time point 0) HBsAg and anti-HBcore antibodies were measured as control for infection with HBV.

Participants were asked to document clinical symptoms (local and systemic) after vaccination in a four-week diary. Solicited symptoms were; erythema, pain and swelling at the site of injection, fever and myalgia. Severity of adverse events was documented as – (absent), +/- (mild), + (moderate) or ++ (severe).

Anti-HBs detection

Anti-HBs titres were assessed by the ARCHITECT Anti-HBs assay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's instructions, and were expressed in International Units (IU)/L .

Anti-HBs avidity determination

Avidity of anti-HBs antibodies was measured in duplo by adding 0M (PBS), 2M, 4M and 6M urea to the serum of non-responders who mounted an antibody concentration ≥ 30 IU/l after three intradermal vaccinations (with or without imiquimod). The avidity

index was calculated as the ratio of anti-HBs with 6M urea (dilution 1:1) to anti-HBs in PBS (dilution 1:1). As a control, the avidity index of healthy responders (anti-HBs ≥ 50 IU/l after 3 hepatitis B vaccinations) was measured. The antibody concentration had no effect on the avidity index, measured by diluting serum (2-, 5- and 10-fold) in the presence of 6M urea (data not shown). In five study participants who mounted a protective response anti-HBs avidity was determined longitudinally throughout the course of the 3 intradermal vaccinations, to envisage the process of avidity maturation.

HLA allele determination

Study participants were typed for HLA-DRB1, -DQB1 en DPB1 as described previously [25], in the European Foundation of Immunogenetics (EFI)-accredited HLA laboratory of the Department of Immunology and Haematology, LUMC, the Netherlands. Briefly, DNA was isolated using a commercially semi-automated beads based assay (Chemagen, Baesweiler, Germany). The HLA-DRB and DQB typing was performed with a reversed approach of the PCR/SSOP technique and HLA-DPB1 were determined with a commercially available beads-based hybridization assay (Tepnel, Stanford, CT. USA). The interpretation of the raw data was carried out with computer assisted analysis software [26].

Statistical methods

Power calculations were based on an expected seroconversions (anti-HBsAg titre ≥10 IU/L) of 60% in the experimental group [15] and 10% in the control group. With an α of 0·05 and a β of 0·2, the sample size is 10 participants per group. To take into account a possible lost to follow up of 30%, we aimed to include 13 participants per group.

To correct for skewing of the data, antibody concentrations after experimental and control ID vaccination were log-normalised. Differences between vaccination groups were investigated under the hypothesis that a change from baseline of each endpoint was identically distributed within each of the two vaccination groups against the alternative that median change-from-baseline within one vaccination group differed from the other group. The hypothesis was tested using the non-parametric Wilcoxon's test. Moreover, differences in antibody titres before and after first and second intradermal vaccination with or without imiquimod pretreatment were tested using analysis of variance on the log-transformed data. Where appropriate, Chi-square test or Fisher's exact test was used. Statistical analysis was performed using a computer-assisted software package (SPSS version 14.0, SPSS Inc., Chicago, IL).

Results

Study population

Forty of the 70 non-responders from our database responded to the letter of invitation to participate, of whom 19 chose not to participate due to the travel distance to either of the two university hospitals (Leiden or Utrecht). Ten participants were included in the experimental group and eleven in the control group. One participant in the control group withdrew after the second vaccination due to local pigmentation at the site of vaccination. The data of this person were included in analysis per time point. The total inclusion was stopped at 21 instead of 26, as the calculated number of participants (10 per group) was reached and no further withdrawal occurred. Characteristics of the participants are given in table 1. Hepatitis B surface antigen and anti-HBcore was undetectable in all participants.

Table 1 Demographics and vaccine history of study population

Characteristics	Imiquimod N=10	Control N=11	p-value
Sex (female) (%)	6 (60)	5 (45)	0.5
Age (years) (IQR)	39 (26-52)	36 (23-47)	0.6
BMI (kg m^{-2}) (IQR)	25.4 (22-28)	25.2 (22-27)	0.9
Smoking N (%)	3 (30)	3 (27)	1.0
Total HBsAg (μg) before inclusion (mean) (IQR)	78 (60-75)	62 (60-60)	0.3
Time since last HB vaccination (years) (IQR)	1.7 (0-4)	4.1 (1-9)	0.1
Median anti-HBs titre after last IM vaccination (IU/L) (IQR)	2.3 (0.0-6.0)	2.0 (0.0-5.7)	1.0
Median anti-HBs titre at start trial (IU/L) (IQR)	0.1 (0.0-5.6)	0.0 (0.0-1.0)	0.4

P-values were calculated with χ^2 -test, Fisher's exact test, Wilcoxon or Student's t-test where appropriate. HBsAg = Hepatitis B surface antigen. BMI = Body Mass Index. IM = Intramuscular. IQR = Interquartile range. No significant differences were found between the imiquimod and control group (p-values > 0.1).

Vaccine administration

The mean diameter of the cutaneous wheal that appeared after intradermal vaccination (total N=62) was 10.4 mm, with a range of 8-14 mm.

Topical Imiquimod application and anti-HBs response

The anti-HBs titre was measured at each vaccination and one month after each vaccination. Evaluation of the overall anti-HBs antibody responses before and after

the vaccinations revealed the development of antigen-specific humoral responses after vaccination in most of the volunteers, the overall height of which did not depend on pre-treatment with imiquimod (Figure 1). Boosting of the responses by a third vaccination 6 months after the first two was observed, and again, did not differ between the experimental and control group. Responders with high anti-HBs remained good responders throughout the study, and those with low anti-HBs remained low responders.

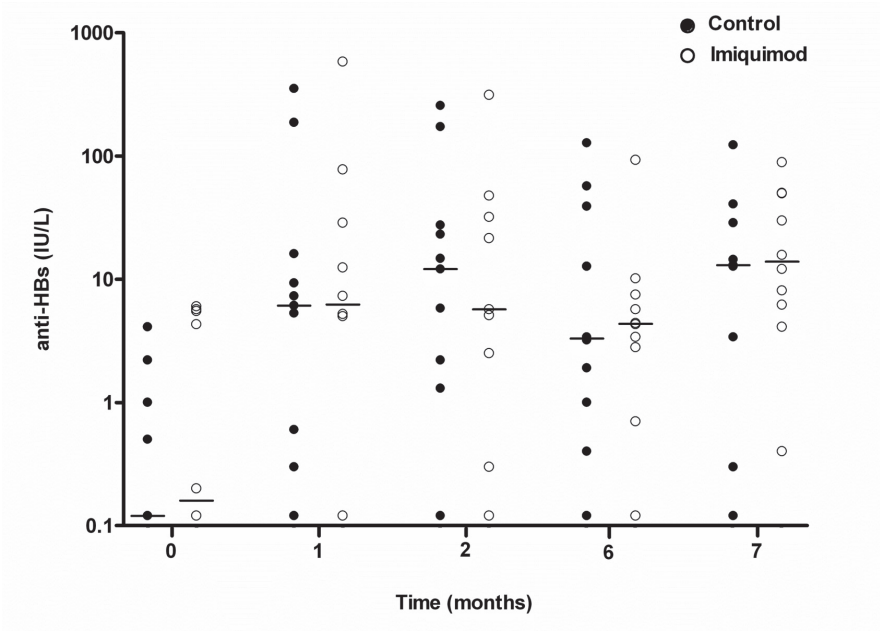

Figure 1 Serologic response (anti-HBs) according to time of vaccination

Intradermal vaccinations were performed at 0, 1 and 6 months. Bars represent medians. Anti-HBs titres of 0.00 were recoded into 0.12 in order to appear in the figure. Each dot represents one participant, dots can overlap.

When a seroconversion of anti-HBs ≥ 10 IU/L was taken as an endpoint, no difference was measured in the time of achievement of this seroconversion in the imiquimod and the control group (figure 2) (Logrank test, $p = 0.8$). One month after the last vaccination, 7 of 10 (70%) of the experimental group and 8 of 11 (73%) of the control group developed an anti-HBs titre ≥ 10 IU/L ($p = 0.9$, Fisher's exact test).

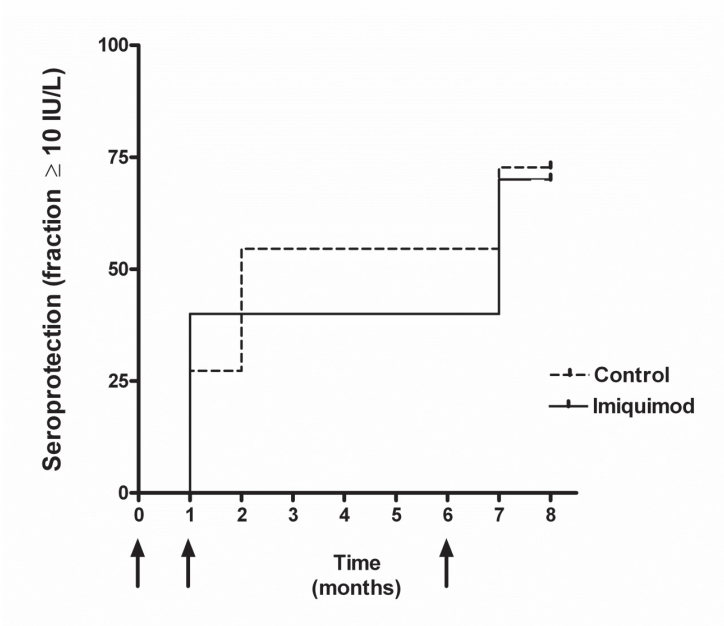

Figure 2 Cumulative percentage of participants achieving an anti-HBs titre of ≥10 IU/L

Arrows indicate intradermal vaccinations. Imiquimod N = 10, control N = 11.

5

Avidity of anti-HBsAg antibodies

In healthy responders to the hepatitis B vaccine, avidity maturation occurs during the vaccination series [27]. Since the participants in our study had at least 6 vaccinations to which they did not mount a protective response, we measured the avidity of anti-HBs antibodies of those in whom the additional ID vaccinations did induce an antibody response of ≥ 30 IU/l (N=7). The avidity index (anti-HBs in 6M urea / anti-HBs in PBS) of these previous non-responders was 0.72 (95%CI 0.61-0.93). The avidity index of healthy controls (N=9) in whom 3 intramuscular hepatitis B vaccinations induced an anti-HBs ≥ 50 IU/l was 0.60 (95%CI 0.35-0.85). Anti-HBs avidity after the first ID vaccination did not differ from the anti-HBs avidity after the last ID vaccination (data not shown). Apparently, in this group of previous non-responders, no avidity maturation occurred but instead, the anti-HBs response immediately showed high avidity, comparable to that in healthy vaccinees after a primary vaccination series.

Responding vs. non-responding participants

To gain more insight in the development of a protective serologic response in the study population, several known associations were investigated. These were: sex, age, weight, smoking, and history of hepatitis B vaccination. After multivariate analysis, increased age was associated with non-response in this trial (Table 2).

Table 2 Demographics and history of hepatitis B vaccination of study population according to serologic response after ID vaccination

Characteristics	Responder (≥10 IU/L) N=15	NR (<10 IU/L) N=6	uni variate p-value	multi variate p-value
Sex (male) (%)	5 (33)	5 (83)	0.06	-
Age (years) (mean) (IQR)	31 (22-45)	52 (49-58)	0.001	0.02
BMI (kg m-2) (mean) (IQR)	24.8 (21-27)	26.5 (24-29)	0.4	-
Smoking N (%)	6 (40)	0 (0)	0.1	-
Total HBsAg (μg) before inclusion (mean) (IQR)	64 (60-60)	84 (60-120)	0.4	-
Time since last HB vaccination (years) (IQR)	2.2 (0-3)	4.8 (1-10)	0.1	-
Anti-HBs titre after last IM vaccination (IU/L) (IQR)	2.4 (0.0-6.3)	0.0 (0.0-5.3)	0.5	-
Anti-HBs titre at start trial (IU/L) (IQR)	0.1 (0.0-4.3)	0.0 (0.0-0.0)	0.007	0.6

P-values were calculated with χ^2 -test, Fisher's exact test or Student's t-test where appropriate.
NR = Non-responder. BMI = Body Mass Index. IM = Intramuscular. IQR = Interquartile range.

Distribution of HLA alleles associated with antibody response after Hepatitis B vaccination

Both study groups were comparable with respect to HLA-DR and HLA-DP distribution. Alleles strongly associated with a non/poor response (DR3, DR7, DP11) [9] were present in 13 of 21 participants (6 in the imquimod group and 7 controls); alleles strongly associated with a high response (DR1, DR5, DR2, DPB1*0401) were present in 4 participants (2 in the experimental group and 2 controls). The comparison between the median anti-HBs antibodies titres of the different HLA-groups is reported in table 3.

Adverse events

Participants reported adverse events in a four-week diary after each vaccination. At any time point, the application of imiquimod did not elicit any additional local erythema or tenderness, nor any systemic symptoms, except myalgia after the 1st vaccination (table 4). However, the ID vaccination of the vaccine did induce local

Table 3 Comparison of the peak antibody titre (within one participant during the study) between groups determined by HLA alleles associated with high of low response to hepatitis B vaccination [9]

HLA-DR and -DP allele association with response	Peak antibody titre median (IU/L)	Interquartile range
Non-response (N=13)	15.7	6.2 – 23.1
Neutral (N=4)	39.7	29.5 – 83.8
High response (N=4)	177.1	1.9 – 409.2

Non-reponse: DR3, DR7 and/or DR11, neutral: None of or both the HLA-DR and –DP alleles mentioned, high response: DR1, DR5, DR2, and/or DPB1*0401.

erythema in approximately 80%, swelling in 75% and pruritus in 30% of participants. In 11 of 21 participants, the first and second vaccination were still visible as a pigmented area at the time of the last blood sampling.
Severity of local adverse events was documented as – (absent), +/- (mild), + (moderate) or ++ (severe), according to participants' experience. There was no difference between the control and experimental group in regard to the severity of the adverse events. The participants who reported redness and swelling experienced these events as mild (66%), moderate (32%) and severe (2%).

Discussion

In this study, application of the TLR7 agonist, imiquimod, on the skin prior to intradermal vaccination did not enhance the humoral response to hepatitis B vaccine in previously hepatitis B vaccine NR. However, irrespective of imiquimod application, 70% of these 'true' 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 did not respond to 6 previous vaccinations. The presence of high avidity antibodies after the first 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.

Although the number of participants in this study is low, the 70% seroprotection rate obtained in this group of NR, who were consecutively enrolled without further selection,

Table 4 Adverse events after ID hepatitis B vaccination with or without pre-treatment with imiquimod of the vaccinated skin

Adverse event			Vaccination preceding adverse events (month)					
			0		1		6	
			IMQ	CON	IMQ	CON	IMQ	CON
Local	Erythema	%	90	73	89	73	90	80
		N days (mean)	19	27	20	23	18	20
	Swelling	%	70	73	89	73	70	70
		N days (mean)	10†	25†	15	22	16	19
	Pain	%	20	0	11	9	10	0
		N days (mean)	5	-	10	1	5	-
	Pruritus	%	30	27	33	18	40	20
Systemic	Myalgia	%	40†	0†	22	9	0	0
		N days (mean)	3	-	4	5	-	-
	Fever	%	0	0	0	0	10	0
		N days (mean)	-	-	-	-	1	-

Number of days are calculated for those who reported this adverse event. IMQ = Imiquimod (experimental) group, CON = Control group. † $p<0.05$ with Fisher's exact test or Student's t-test.

strongly suggests that similar protection rates may be reached in unselected groups of NR. 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. The local adverse events induced by the ID delivery of the vaccine (with aluminiumhydroxide as adjuvant) were perceived as mild to severe, and one participant withdrew because of sustained pigmentation at the site of vaccination. The etiology of this adverse event may be due to local granuloma formation in response to the aluminum adjuvant present in the vaccine. No systemic reactions occurred. If ID hepatitis B vaccination is pursued for research or clinical purposes, we suggest to vaccinate in a less visible site than the forearm, e.g. in the shoulder or back.

The lack of a beneficial effect of imiquimod on the immune reactivity to the vaccine in our study was unexpected. Several publications support the local immune boosting effect of imiquimod. For instance, Aldara®, with imiquimod as its active substance, is registered for the treatment of (genital) warts and basal cell carcinoma, and has recently been shown effective in the treatment of vulvar intraepithelial neoplasia (VIN)

[28]. After treatment with imiquimod, the antigen is processed and presented to cells of the adaptive immune system leading to clearance of the virus and subsequent clearance of the lesions [29]. In addition to functional maturation [30], imiquimod induces migration of dendritic cells from the dermis to draining lymph nodes [31,32]. Subcutaneous administration of imiquimod as vaccine adjuvant simultaneously with the antigen of interest, has shown to induce enhanced responses towards the administered antigen [32,34], However, ID vaccination combined with imiquimod as an adjuvant in mice, failed to increase the response towards the injected antigen [33]. In that study, imiquimod was topically applied for 24 hours before vaccination, possibly decreasing the density of local APCs at the time of vaccination. For this reason, we applied the imiquimod ointment immediately prior to vaccination. We assumed that simultaneous administration of imiquimod and antigen would allow for an enhanced antigen presentation, but a poor penetration of imiquimod through the skin may have disturbed this timing. Perhaps a more frequent application of the ointment would have enhanced its effectivity [35]. Secondly, if the hampered immune response in non-responders is due to a defect on the level of T cells or B cells instead of dysfunctional antigen presentation [36], stimulating APC would be less likely to enhance the antibody production. Finally, although in animal models imiquimod (administered either topically or systemically) has demonstrated adjuvant activity in vaccines using antigenic peptides [37], proteins [38], and DNA [39], the immunostimulating effect of imiquimod in clinical setting was most evident in HPV infection [28,40]. It is possible that HPV infection downregulates a specific aspect of the immune reponse, which is specifically upregulated by imiquimod. This would imply that the immunostimulating effect is virus or antigen specific.

The unexpected high rate of seroprotection observed in this study population places the hypothesis of the 'true' non-responder who will never respond to the hepatitis B vaccine into a new perspective. On the other hand, the ID vaccination route has shown to be a potential vaccination route for several vaccine antigens [41] and could also have contributed to the high rate of seroprotection with high avidity antibody response in these NR. The beneficial effect of the ID route of hepatitis B vaccination in NR should now be confirmed in a larger cohort.

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Intradermally Administered Yellow Fever Vaccine at Reduced Dose Induces a Protective Immune Response: A Randomized Controlled Non-inferiority Trial

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Abstract

Background

Implementation of yellow fever vaccination is currently hampered by limited supply of vaccine. An alternative route of administration with reduced amounts of vaccine but without loss of vaccine efficacy would boost vaccination programmes.

Methods

A randomised, controlled, non-inferiority trial was conducted in a Dutch university centre between August 2005 and February 2007. A total of 155 primary vaccinated and 20 previously vaccinated volunteers participated. Participants were randomly assigned in a 1:1 ratio to receive intradermal (i.d.) vaccination with live attenuated yellow fever 17D vaccine at a reduced dose (1/5th; 0·1 mL) or the conventional subcutaneous (s.c.) vaccination (0·5 mL). Antibody neutralisation titres were determined at 2, 4 and 8 weeks and 1 year after vaccination by counting the reduction in virus-induced plaques in the presence of serial serum dilutions. Adverse events were documented in a 3-week dairy. Viraemia was measured 5 days after vaccination.

Results

From 2 weeks up to one year after vaccination, the maximum serum-dilution at which 80% of the virus plaques were neutralised, which indicates protection against yellow fever, did not differ between those given a reduced i.d. dose or standard s.c. dose of vaccine. In all cases the WHO standard of seroprotection (i.e. 80% virus neutralisation) was reached (in 77/77 and 78/78, respectively). Similar results were found in the previously vaccinated individuals. Viraemia was detected in half of the primary vaccinated participants, which was not predictive of serological response. In revaccinees no viraemia was detected.

Conclusions

Intradermal administration of one fifth of the amount of yellow fever vaccine administered subcutaneously results in protective seroimmunity in all volunteers. Albeit this vaccination route should enable vaccination of five-times as many individuals at risk for disease, these results should now be confirmed in field studies in areas with potential yellow fever virus transmission to change vaccination policy.

Introduction

Yellow fever is a re-emerging viral hemorrhagic febrile illness in tropical and sub-tropical areas of Africa and remains a major health threat in South-America. It is estimated to affect 200.000 individuals annually of whom approximately 30.000 die worldwide [1]. The virus is transmitted by infected *Aedes* mosquitoes, and may cause a wide spectrum of disease, from mild symptoms to severe illness accompanied by fever, hepatic and myocardial injury, renal failure, hemorrhage, and even death. There is no curative treatment for yellow fever, making vector control and vaccination essential ingredients in the prevention of yellow fever morbidity and mortality.
Although this flavivirus has never emerged in Asia, the Asian continent is considered vulnerable to future introduction of the virus, because of the presence of a large susceptible human population, the presence of the urban vector and increasing international travel [2]. Also Western countries may be at risk: for instance, in the Netherlands, the *Aedes albopictus* mosquito was introduced via imported bamboo from China, and its capability of transmission of flaviviruses is currently under investigation.

Thus, there is a potential risk for large epidemics of urban yellow fever now that migration of people from rural areas may introduce the virus into areas of high human population density, such as large African and South-American cities. During yellow fever epidemics in non-immune populations, case-fatality rates may be as high as 50% [3]. In case of simultaneous outbreaks in megacities the current emergency stockpile of yellow fever vaccine of 6 million doses will not be sufficient to protect the large populations from the disease [4].

Yellow fever vaccination is the single most important and effective means to prevent the occurrence of yellow fever, and carries a low risk of serious adverse events. The live-attenuated 17D vaccine provides protective immunity within one to two weeks in 95% of those vaccinated [5]. The World Health Organization (WHO) therefore strongly recommends to include yellow fever vaccination in at-risk countries, as part of the routine childhood immunisation program. However, hampered by a limited vaccine supply, this recommendation has not yet been acted upon as epidemic emergencies have priority. Besides mass immunisation campaigns in response to epidemic outbreaks and planned routine childhood immunisation programmes, yellow fever vaccination is used for preventive immunisation of travellers to endemic regions [6]. Therefore, to circumvent the consequence of current shortage of vaccine supplies,

there is an urgent need to find alternatives for the current standard of yellow fever vaccination, i.e., the subcutaneous administration of 0·5 mL 17D vaccine.

In general, the route of administration of a particular vaccine, e.g., intramuscular, subcutaneous or intradermal, appears to have been reached at arbitrary historical grounds. For the yellow fever vaccine, subcutaneous administration of 0·5 mL followed the initial human trials in which yellow fever 17D (YF–17D) vaccines were first put to extensive use. However, for some vaccines already, for instance rabies, hepatitis B and influenza vaccines, the classical subcutaneous or intramuscular routes have been challenged by the apparent efficacy of intradermal administration using appreciably smaller amounts of vaccine [7-10]. The safety and efficacy of this route of administration has not been addressed for the yellow fever vaccine. Interestingly, already in 1943, at the dawn of yellow fever vaccine development, Fox and colleagues observed an immune response after intradermal administration of the YF–17D vaccine [11]. However, the population investigated was small and the method used to assess antibody responses is irreconcilable with current definitions of seroprotection formulated by the WHO. Furthermore, scarification experiments with yellow fever vaccine conducted in the 1950s revealed a lower response rate when compared to subcutaneous inoculation [12,13].

In this study we investigate the efficacy of intradermal inoculation of yellow fever vaccine at one fifth the amount given subcutaneously, as a potential strategy to reduce costs and increase vaccine coverage in areas with limited amounts of vaccine available for mass vaccination as well as for travellers to these areas. Furthermore, to elucidate requirements for the induction of an effective immune response to yellow fever vaccination we assessed antibody responses in relation to post-vaccination viraemia in both primary and revaccinated individuals.

Methods

Objectives

This study was conducted to determine whether reduced dose i.d. yellow fever vaccination (1/5th; 0·1 mL) would be as efficacious and safe as the conventional s.c. vaccination (0·5 mL). Efficacy of vaccination was measured by virus neutralisation plaque reduction assay.

Study design and Participants

Healthy volunteers of 18 years and older were eligible for inclusion. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, pregnant volunteers and those with chicken egg allergy. The study was carried out between August 2005 and February 2007. Subjects were randomly assigned by the investigator (AHR) to either receive intradermal (i.d.) (experimental vaccination group) or subcutaneous (s.c.) (conventional vaccination group) yellow fever vaccination. Randomization was performed with the use of sealed envelopes containing the vaccination code balanced through in permuted blocks of 10 each. Vaccinations were administered at the travel clinic of the LUMC by the investigators who were trained in both methods of vaccine administration.

In the experimental vaccination group, participants received 0·1 mL YF–17D vaccine intradermally on the dorsal side of the right forearm. The syringe which was used for i.d. administration is identical to the syringe used for administration of tuberculin in the Mantoux test. The quality of the i.d. injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test) [14], with 6 mm being the lowest acceptable diameter. The conventional vaccination group received 0·5 ml YF–17D vaccine subcutaneously in the right upper deltoid region.

17D Yellow Fever Vaccine

The live, attenuated, 17D vaccine used in this study was manufactured on embryonated chicken eggs according to WHO regulations and stored according to manufacturer's guidelines. All administered vaccines originated from the same vaccine lot (Stamaril, Lot no Y5597, Sanofi Pasteur, France). A single vaccination dose of 0·5 ml contained approximately $3{\cdot}5 \times 10^4$ plaque forming units (PFU), measured in two randomly selected vials. Multiple dosages (maximally 4) were obtained from one vial for i.d. vaccination. After reconstitution, vials were stored at 4°C and discarded after maximally 4 hours.

Data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained, including information on possible flavivirus exposure (defined as travel to a flavivirus endemic country) in the 5 years prior to entering the study and previous yellow fever vaccination. Blood samples were collected in all (155) primary vaccinated participants before vaccination, and 4 and 8 weeks after vaccination. An additional blood sample was collected 2 weeks after vaccination in 55 primovaccinees (the last 55 consecutive subjects entering the study) to investigate the kinetics of the neutralising antibody response in more detail.

Extra ethylenediamine tetraacetic acid (EDTA) blood samples were collected 5 days after vaccination in the first 24 consecutive primovaccinees entering the study.
In 20 previously vaccinated participants blood was drawn before vaccination, and 5 days and 2 weeks after booster vaccination (figure 1). Approximately one year after vaccination, one additional blood sample was taken from all participants who could be contacted (96 participants). A financial compensation was given for every blood sample collection at completion of the study. None of the participants withdrew prematurely.
Participants were asked to document clinical symptoms (local and systemic) after vaccination in a three-week diary. Solicited symptoms were; erythema, pain and swelling at the site of injection, fever and myalgia. Severity of adverse events was documented as – (absent), +/- (mild), + (moderate) and ++ (severe).

Constant virus – varying serum dilution Plaque Reduction Neutralisation Test (PRNT)

The tests were carried out in 6-well plates (Corning Inc., USA) using a slightly modified technique described originally by De Madrid and Porterfield [15]. Briefly, approximately 6 x 10^5 Vero cells/mL were seeded per well in 6–well plates and cultured to obtain a confluent monolayer. Sera were complement inactivated at 56°C for 1 hour. Prevaccination sera were tested in 1:16 dilution, to which 100 PFU of 17D-YF were added. Postvaccination sera were tested in two-fold dilutions starting from 1:16 to 1:512. 100 PFU YF–17D virus were added to each serumdilution. All test sera were assayed in duplicate. After 1 hour incubation on ice, the mixtures of virus and serum were added to the Vero cell monolayers and incubated for 1 hour at 37°C. An overlay of 2 x DMEM and 2% agarose was added. After 5 days of incubation at 37°C, the overlay was discarded and cell monolayers were stained with crystal violet. Plaques were counted by eye. Virus neutralisation (VN) was calculated for each serum dilution (i) with the following formula: VN(i) = 100 x (number of PFU in diluted postvaccination serum / number of PFU in pre-vaccination serum (in a 1:16 dilution)). For comparison of i.d. and s.c. vaccination, serum dilution at which $\log_{10}$ neutralisation index 0·7 (80% VN) occurred was taken as endpoint, as this corresponds to the generally accepted definition of protection [16].

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

RT–PCR of YF–17D was performed at the department of Virology of the Erasmus Medical Centre according to Nijhuis and colleagues [17]. Briefly, viral RNA was isolated and reverse transcribed (Taqman Reverse Transcription Reagents, Applied

Biosystems International). cDNA synthesis was performed in a J Mini Gradient Thermal Cycler (BioRad, Netherlands) for real-time PCR, the following YF specific primers and probe were used [18]:

YFV-1 (forward)	AATCGAGTTGCTAGGCAATAAACAC
YFV-2 (reverse)	TCCCTGAGCTTTACCAGA
YFV-P (probe)	FAM-ATCGTTGAGCGATTAGCAG-BHQ

with FAM (6–carboxyfluorescein) as 5'-reporter dye and BHQ (Black Hole Quencher) as the 3'-quencher dye. Real-time PCR was monitored on ABI Prism 7500 Seq. Detection System (Applied Biosystems International). Cycle threshold (Ct) values were used to compare viraemia in i.d. and s.c. groups quantitatively.

Ethics

The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC), the Netherlands (ISRCTN46326316). Written informed consent was obtained from each participant.

Statistical methods

Power calculations for primovaccinees were based on a one-sided non-inferiority according to Armitage P., et al. [19], formula 18.5, with a maximally acceptable difference (δ) of 0·04 between the experimental and conventional vaccination group, α of 0·05, β of 0·2 and a π (overall probability of positive response) of 0·99 [5], which makes $\sigma^2 = 0{\cdot}0099$. The number of participants needed to confirm non-inferiority of low dose i.d. vaccination under these assumed conditions are 77 per group. For the antibody response in previously vaccinated individuals receiving a booster vaccination, basic descriptive statistics are used. It was anticipated that the small number in this subgroup would not allow a definite conclusion concerning non-inferiority and no power calculation was performed. Twenty previously vaccinated persons were included to monitor possible trends in interference of neutralising antibodies in yellow fever vaccination. Paired t-test was performed to calculate their increase in neutralisation after booster vaccination and linear regression was used to calculate influence of circulating antibodies on booster vaccination. Neutralising capacity of sera after i.d. and s.c. vaccination were compared with Student's t-test. Where appropriate, Chi-square tests were used, and Wilcoxon's test for non-parametrical distributed numerical data. Statistical analysis was performed using a computer-assisted software package (SPSS version 12.0, SPSS Inc., Chicago, IL).

Results

Study population

We enrolled 175 volunteers from August 2005 to February 2007 (figure 1). Baseline characteristics of the study population are given in table 1.

Concerning the accuracy of i.d. vaccine delivery, the mean diameter of the cutaneous wheal measured after vaccination was 8 mm (range 6-10 mm), indicating that all (N=87) i.d. vaccination wheals met the minimal requirement for acceptable size.

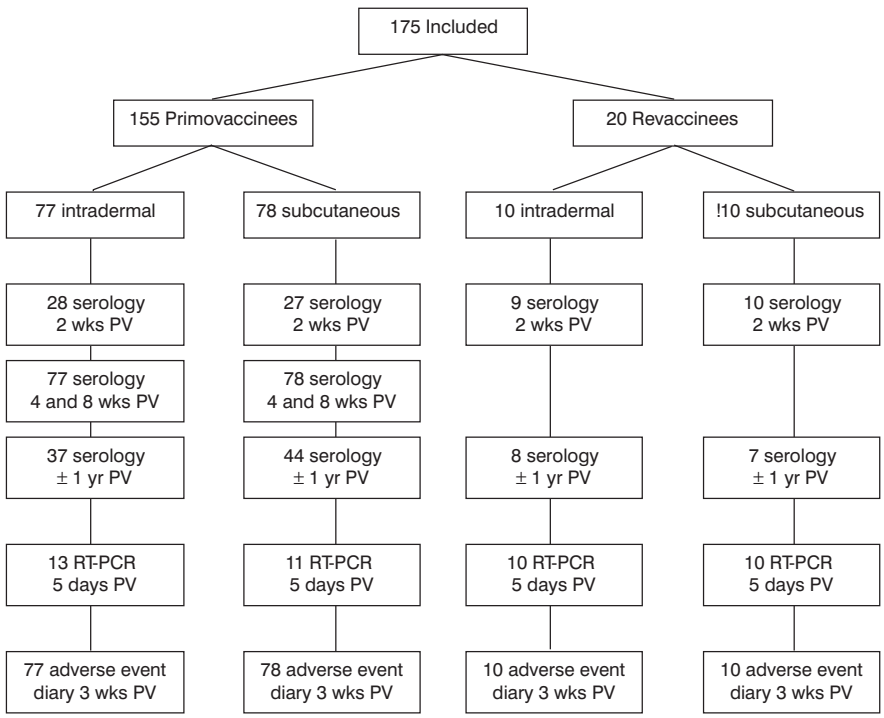

Figure 1 Flow chart of study participants

Included study participants from August 2005 until February 2007. PV = post vaccination. RT−PCR = Reverse transcriptase polymerase chain reaction. Wks = weeks and yr = year.

Table 1 Comparability of intradermally and subcutaneously vaccinated groups

Participants		Vaccine administration		
		Intradermal	Subcutaneous	p-value
Primovaccinees	N Female (%)	56 (73)	65 (83)	0·1
	Mean age (range)	27 (18-61)	25 (19-70)	0·2
	Flavivirus[$] N yes (%)	33 (43)	26 (33)	0·3
Revaccinees	N Female (%)	7 (70)	8 (80)	-
	Mean age (range)	30 (20-50)	34 (21-48)	0·4

Age and gender distribution in primary (77 i.d., 78 s.c.) and booster (10 i.d., 10 s.c.) vaccinated populations. YF-17D = yellow fever vaccine virus. [$] Flavivirus = possible flavivirus encounter in past five years defined as travelled to flavivirus endemic destination.

Vaccine efficacy

Four weeks after vaccination, 80% virus neutralisation (VN) at the least diluted serum (dilution of 1:16) was achieved by 77 of 77 of the intradermally and by 78 of 78 of the subcutaneously vaccinated primovaccinees. The percentage of VN in both study groups was linearly correlated to serum dilution at all time points measured (data not shown). Ninety percent neutralisation was achieved by 70 of 77 (91%) and by 69 of 78 (89%), respectively. Plotting of neutralisation indices against serum dilution showed similar kinetics of i.d. and s.c. vaccination at all measured time points (data not shown). This allowed us to compare the serum dilution at which 80% of yellow fever was neutralised, which is similar in both groups at all time points measured (figure 2).

No difference in percentage of virus neutralisation was measured in either (i.d. or s.c.) group between male and female participants, nor between recent travel to flavivirus endemic countries or not (data not shown).

Neutralising capacity of 1:16 diluted prevaccination serum of previously vaccinated participants ranged from 2% to 97% reflecting the wide range of years since their last YF vaccination (0·5 to 18 years). The mean percentage of VN by the least diluted serum before vaccination in the i.d. group was 77% (range 51%–97%) and in the s.c. group was 74% (range 2% - 97%). All revaccinees reached protective neutralisation immunity 2 weeks (19/19) and 1 year (15/15) after vaccination.

Both the i.d. and the s.c. group of revaccinated participants showed a significant rise in VN after booster vaccination. The mean increase in percentage of neutralisation by serum (dilution 1:16) before and 2 weeks after vaccination in the i.d. vaccinated participants was 18% (95% CI; 8%–28%) and 20% (95% CI; 4%–36%) in the s.c. group

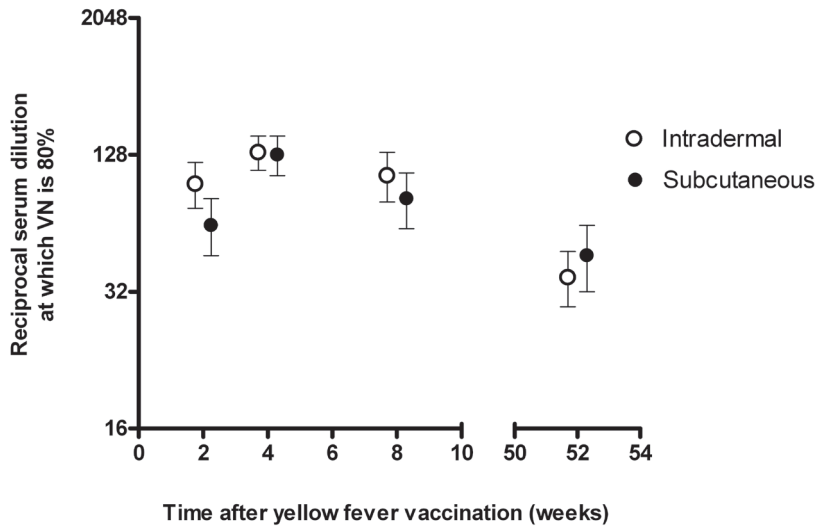

Figure 2 Protective virus neutralisation after intradermal or subcutaneous vaccination against yellow fever

Comparison of reciprocal serum dilutions at which 80% of yellow fever virus is neutralised in constant virus – varying serum dilution test after intradermal and subcutaneous YF vaccination in primary vaccinated participants (n=155). Bars represent 95% Confidence Intervals (CI). Virus neutralising capacity of serum in both groups was performed at similar time points but indicators are juxtaposed for visual enhancement. VN = virus neutralisation.

(data not shown). To investigate the influence of prevaccination neutralising antibody titre on postvaccination VN, pre- and post vaccination serum dilutions at which 80% VN occurred were plotted (figure 3). In linear regression analysis, an increase in post-vaccination VN correlated significantly with a higher prevaccination antibody titre (coefficient 0.54, p=0·02). Thus, the presence of circulating neutralising antibodies in this population did not inhibit a booster response.

Viraemia was measured by RT-PCR 5 days after vaccination in 24 primovaccinees and all revaccinees (N=20). In the latter no YF-17D RNA was detected in the blood. The percentage of primary vaccinated subjects positive for YF virus detection was comparable in the i.d. (7 of 13, 54%) and s.c. (5 of 11, 45%) group, as were the mean Cycle threshold (Ct) values (35·86 cycles and 37·52 cycles, respectively).

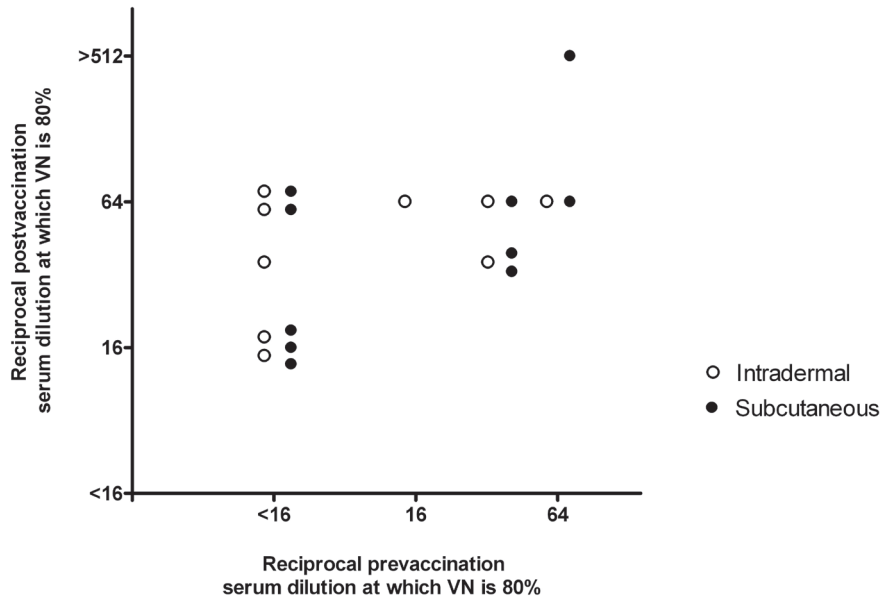

Figure 3 Pre- and post vaccination virus neutralising capacity of serum of previously vaccinated participants

Pre- and postvaccination (2 weeks) serum dilutions at which 80% VN occurred in previously vaccinated participants. When 80% VN was not reached by the least diluted serum (1:16), samples were defined as <16 (reciprocal serum dilution). VN = Virus neutralisation.

No difference was measured in the serum dilution at which 80% VN occurred 4 weeks after vaccination between those with and those without viraemia, irrespective of the route of vaccine administration (figure 4).

Vaccine safety

Participants reported duration and severity of adverse events after yellow fever vaccination in a 3–week diary. In primary vaccinated participants i.d. vaccination evoked redness and swelling at the site of inoculation more frequently and for a significantly longer period than after s.c. vaccination ($p<0{\cdot}001$). Itching at the site of injection was also reported more by i.d. vaccinated primovaccinees ($p=0{\cdot}02$). The s.c. vaccinated primovaccinees reported significantly longer pain at the site of injection

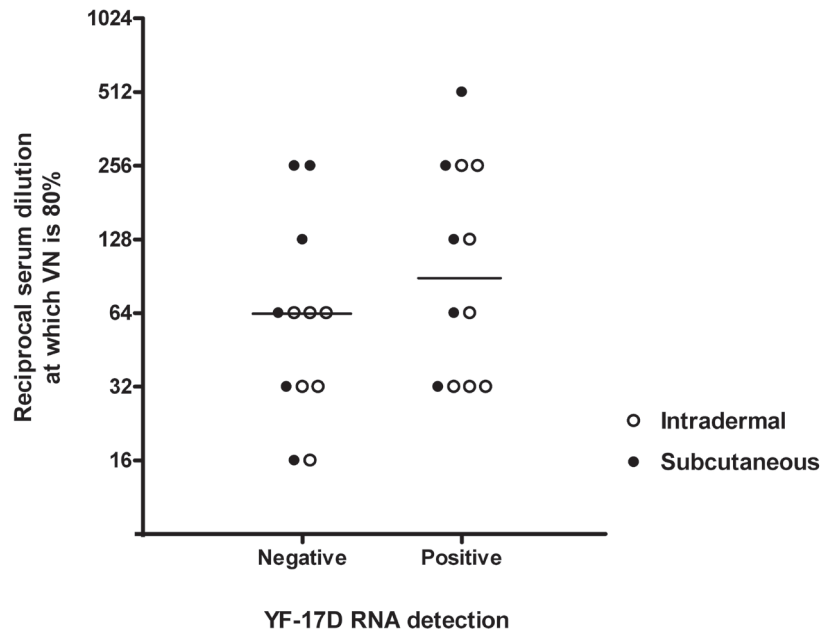

Figure 4 Virus neutralising capacity of YF-RNA negative and positive sera

Comparison of reciprocal serum dilutions, of serum obtained 4 weeks after vaccination, at which 80% VN occurred between positive and negative YF-17D RNA detection by RT-PCR in primary vaccinated participants (N=24). Bars represent the median reciprocal serum dilution. VN = Virus neutralisation.

(p=0·03), and more s.c. primary vaccinated participants reported myalgia (p<0·01) (table 2). In previously vaccinated participants, a similar trend of adverse events was monitored except for myalgia.

The severity of adverse events due to vaccination, which was reported on a 4-level scale (-, +/-, +, ++), did not reveal a difference in experienced discomfort (both local and systemic) between the i.d. and s.c. group. Of the reported adverse events, 2/3rd was experienced as mild (+/-) and 1/3rd as moderate (+). No i.d. vaccinated and 3 s.c. participants rated their events as severe (++).

Table 2 Solicited adverse events after primary and booster YF-17D vaccination

Adverse event			Primary vaccination (N=155)		Booster vaccination (N=20)	
			Intradermal N=30	Subcutaneous N=28	Intradermal	Subcutaneous
Local	Erythema	N yes (%)	63 (82)	25 (32)	6 (60)	1 (10)
		Mean N days (s.e.m.)	4·3 (±0·5)	1·1 (±0·2)	3·2 (±1·0)	1·0 (±0·9)
	Swelling	N yes (%)	52 (68)	9 (12)	6 (60)	0 (0)
		Mean N days (s.e.m.)	2·6 (±0·4)	0·3 (±0·1)	2·6 (±0·9)	-
	Pain	N yes (%)	6 (8)	15 (19)	2 (20)	0 (0)
		Mean N days (s.e.m.)	0·1 (±0·06)	0·6 (±0·2)	0·2 (±0·1)	-
	Severity	N +/- (mild)	39	15	3	-
		N + (moderate)	24	9	2	2
		N ++ (severe)	-	1		-
Systemic	Myalgia	N yes (%)	12 (16)	27 (22)	1 (10)	1 (10)
		Mean N days (s.e.m.)	0·4 (±0·1)	0·7 (±0·1)	0·1 (±0·1)	0·7 (±0·7)
	Fever	N yes (%)	4 (5)	8 (10)	0 (0)	0 (0)
		Mean N days (s.e.m.)	0·1 (±0·03)	0·2 (±0·06)	-	-
	Severity	N +/- (mild)	9	17	-	-
		N + (moderate)	3	8	1	1
		N ++ (severe)	-	2	-	-

Safety of vaccination expressed in various parameters. Severity of adverse events could be graded with - (absent), +/- (mild), + (moderate) and ++ (severe). S.e.m. = standard error of the mean.

Discussion

Intradermal administration of 1/5th of the conventional yellow fever vaccine dose was non-inferior to standard subcutaneous vaccination of the full dose as far as protective immune response and safety is concerned: at 2, 4 and 8 weeks after administration, as well as one year later, the titres of yellow fever-neutralising antibodies were identical in individuals being primary vaccinated intradermally or subcutaneously. Both i.d. and s.c. administration of the vaccine resulted in protective seroimmunity in all subjects.

Finally, the kinetics of the immune response were similar in both groups with neutralising antibody responses peaking at 4 weeks after vaccination.

Several aspects of this study require comment. First, assuming 99% seroprotection after primary vaccination in both groups, the population size in this study does not allow to detect differences less than 4% between the experimental (i.d.) and conventional (s.c.) vaccination groups. However, the numbers are sufficient to reliably measure a log 0·7 virus neutralising capacity in at least 95% of those vaccinated intradermally, which meets the minimal required percentage of seroprotection after vaccination, as defined by the WHO [20]. Second, the viral dose contained in the trial vaccine was $3{\cdot}5 \times 10^4$ PFU/0·5mL, which is equivalent to approximately 5×10^3 Mouse Lethal Dose $(MLD)_{50}$ (21). A fivefold reduction of vaccine dose for i.d. delivery then still contains the minimal potency requirement (1×10^3 MLD_{50}) as defined by the WHO [20], meaning that the results of this study cannot exclude that s.c. vaccination with 0·1 mL dose might be protective. Several lines of evidence however suggest that this may not be the case. More than sixty years ago Fox and colleagues verified the protective efficacy of human serum from vaccinees in a mouse challenge model and observed that at a similar vaccine dose, sera from intradermally injected subjects were more efficacious than sera of those injected subcutaneously [11]. Additionally, 0·1 mL s.c. delivery of a live attenuated chimeric flavivirus vaccine against Japanese encephalitis in non-human primates resulted in a 7–fold lower neutralising antibody response compared to 0·1 mL i.d. delivery by micro needle [22]. Finally, this study has been performed in healthy adult volunteers who represent travellers to and not individuals living in an area of potential yellow fever transmission. This study should be repeated in a population living in a yellow fever endemic area, to account for differences in skin tissue composition, possible interactions by cross-reactive antibodies against other flaviviruses, and possible decreased immune response due to malnutrition or chronic parasitic infections.

In regard to the reproducibility of these results, the significant variation in viral load between YF-17D vaccine batches is of importance. The batches generally contain 5-50 times the minimal required potency dose to account for possible loss during storage and transportation [5]. The YF-17D batch used in this study contained five times the minimal required potency dose, and is therefore at the low side of the batch-variability in viral vaccine load. Intradermal YF-17D vaccination with other batches will thus yield similar results, as no other batch is likely to contain less virus particles.
Correct i.d. vaccination is technically more demanding than subcutaneous or

intramuscular vaccination. By introducing a minimal diameter cutoff of the cutaneous wheal following i.d. vaccination, we allowed to control for proper i.d. delivery of the vaccine. To our opinion, this simple test is a valuable tool to ensure correct i.d. vaccination.

Local adverse events such as erythema and swelling were reported to occur longer in the i.d. vaccinated group. This is consistent with other intradermally administered vaccines [7,8], and might represent the inflammatory reaction due to activation of local immunomodulating cells. To our opinion this increased duration of local adverse events will not be a reason to renounce the new cost-effective method of yellow fever vaccination investigated, as they were not experienced as more severely than the adverse events in the s.c. group. Evidently, adverse events with a frequency beneath 1/77 after low dose i.d. vaccination could not be detected in this study.

The participants who had been previously vaccinated against yellow fever all showed seroprotection after booster vaccination, irrespective of their pre-booster VN capacity, implying that circulating neutralising antibodies did not interfere with the induction of a booster response. Furthermore, this study shows that detectable YF–17D replication as evidenced by viraemia in the week after vaccination, was not required for induction of a booster response, which is consistent with previous findings by Reinhardt et al. [23].

The enhanced efficiency of the i.d. route of vaccination may be explained by direct targeting of antigen presenting cells (APCs) in the dermis and epidermis. Despite the possibility of YF–17D replication in dendritic cells [24], Palmer and colleagues found this replication to be restricted due to rapid processing of the virus [25]. Together with the fact that despite the lower vaccine dose the number of intradermally vaccinated participants in which viraemia was measured was not reduced, it is likely that an even more reduced vaccine dose (than fivefold reduction) administered i.d. could induce a protective immune response.

The findings of this study have the following practical implications: 1) in case of an outbreak of urban yellow fever or vaccine shortage for travellers to endemic areas, i.d. administration of yellow fever vaccine will allow immunisation of at least four times as many individuals as s.c. vaccination with the same limited vaccine supply, 2) provided that these results can be confirmed in field studies in areas with potential yellow fever virus transmission, the i.d. vaccination strategy could be implemented in routine immunisation programmes and support the ‘yellow fever risk reduction initiative’

launched by the WHO and UNICEF to envisage the immunisation of 48 million people in 12 high-risk countries between now and 2010 [26], 3) finally, these results suggest that travellers with a possible history of egg allergy in whom an i.d. test dose of 0·1 ml YF–17D vaccine yielded a strong local urticarial reaction do not need further vaccination, but this should always be checked by virus neutralisation tests.

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Reduced intradermal test dose of yellow fever vaccine induces protective immunity in individuals with egg allergy

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Abstract

Background

Persons with a history of egg allergy are susceptible to developing a strong urticarial or anaphylactic reaction to the yellow fever vaccine. Therefore, in these persons a test dose (1/5th of the coventional dose) is administered intradermally, in order to monitor the local skin reaction.

Methods

The neutralising antibody response after the yellow fever vaccine (YF-17D) skin test was measured in 7 egg allergic persons in whom further vaccination was abandoned because of a strong local urticarial reaction to the YF-17D vaccine test dose.

Results

We found that this test dose of 0.1 mL of YF-17D vaccine was sufficient to induce a protective antibody response in all 7 subjects.

Conclusion

Intradermal injection of 1/5th dose of the yellow fever vaccine appears to be sufficient, in non-allergic as well as allergic persons, and non-inferior to the subcutaneous full dose.

Introduction

The yellow fever vaccine is considered to be one of the most effective and safe vaccines since its development in the 1930's. Mild adverse reactions such as low-grade fever, myalgia, and local redness or tenderness at the site of injection occur in 10-30% of vaccinees, 2 - 6 days after vaccination [1]. More serious adverse events, such as yellow fever vaccine-associated neurotropic disease (YEL-AND) or viscerotropic disease (YEL-AVD), have been reported, but are very rare (0.3-0.4 per 100.000 administered doses) [2]. In addition to these adverse events that are typically related to viral replication, anaphylactic reactions probably triggered by the hydrolysed porcine gelatin or egg proteins present in the vaccine, have been reported with a risk of 0.8 per 100.000 doses [3].

Because the yellow fever 17D vaccine strain (YF-17D) is propagated on embryonated chicken eggs, a history of acute hypersensitivity to eggs or egg products is a contraindication to vaccination. If a subject with a probable history of egg allergy is planning on traveling to an area with a significant risk for contracting yellow fever a test dose of the vaccine can be given under close medical supervision. According to the Dutch guidelines of the National Coordination Centre for Travelers' Health a test dose of 0.1 mL of YF-17D vaccine (1/5th of the normal vaccine dose) is administered intradermally, and a control dose of 0.1 mL physiologic saline (0.9% NaCl) is injected intradermally in the contralateral arm [4]. The test is read after 30 minutes. If the diameter of the cutaneous wheal of the test dose is less than 2 times the diameter of the saline control, the skin test is considered negative and the remaining 0.4 mL of vaccine is administered subcutaneously. In case of a positive skin test, further vaccination is abandoned [4].

In 1943, Fox and colleagues observed a protective immune response after intradermal administration of the YF-17D vaccine [5]. However, the population investigated was small and the methods used to assess antibody responses are irreconcilable with current definitions of seroprotection as formulated by the WHO. We have recently shown that intradermal vaccination with 0.1 mL YF-17D vaccine induced protective neutralising antibody levels in healthy volunteers [6]

To ascertain this protective response also occurs after the YF skin test in egg allergic individuals, we measured the neutralising antibodies in 7 persons in whom further vaccination was abandoned because of a strong local urticarial reaction to the YF-17D vaccine test dose.

Methods

Serum samples of immunocompetent individuals who had received the yellow fever vaccine test dose in our hospital since 2000 (start of registration), and who developed a positive skin reaction were tested. Serum of 7 of 11 registered patients with a positive skin test could be obtained. The live, attenuated, YF-17D vaccine that was used (Arilvax®, Medeva, Belgium, or Stamaril®, Sanofi Pasteur, France) was stored according to manufacturer's guidelines. Administration of the test dose (performed as described previously [6]) and close medical observation of the subjects was performed at the outpatient travel clinic of the Leiden University Medical Centre (LUMC). One individual was hopitalised for observation during the procedure because of the anticipated risk of anaphylaxis.

Neutralising antibodies were measured by constant virus – varying serum dilution Plaque Reduction Neutralisation Test (PRNT), using a slightly modified technique originally described by De Madrid and Porterfield [7]. Briefly, sera were complement inactivated at 56°C for 1 hour. Postvaccination sera were tested in two-fold dilutions starting from 1:16 to 1:512. One hundred Plaque Forming Units (PFU) of YF–17D virus were added to each serum dilution. All test sera were assayed in duplicate in 6-well plates. Virus neutralisation (VN) was calculated for each serum dilution (i) according to the following formula: VN(i) = 100 - (number of PFU in diluted postvaccination serum / number of PFU in medium)*100. The highest serum dilution at which at least 80% virus neutralisation occurred (a $\log_{10}$ neutralisation index of 0·7) was taken as endpoint, as this corresponds to the generally accepted definition of protection [8]. A reference serum, obtained from the National Institute for Biological Standards and Control (http://www.nibsc.ac.uk/) was used for quantification of the antibody response in International Units per milliliter (IU/mL). In our hands a 0.7 log 10 plaque reduction in 1:10 diluted serum corresponds to a titre of 0.5 IU/ml [95%CI 0.3 – 0.8 IU/ml] (unpublished data). Similar values have been found by others [9].

Results

The characteristics of the vaccinated individuals, their skin reaction and antibody response to the vaccine test dose are given in table I. Similar to our findings in healthy volunteers, we found that the test dose of 0.1 mL of YF-17D vaccine was sufficient to induce a protective antibody response in all 7 subjects with egg allergy (Table 1), with a mean concentration of 5.3 IU/ml [99% CI 2.0-8.6 IU/ml]. No adverse reactions

Table 1 Characteristics of egg-allergic persons and outcome of test dose vaccination

N	Age (yrs)	Sex	YF vaccine[a]	Year vacc	Wheal control (mm)	Wheal test dose (mm)	Time vacc serology[b] (wks)	Serum dilution VN80%[c]	Calculated serum dilution VN80%[d]	IU/ml[e]
1	17	F	A4AO55AC	2000	7	16	9	1:16	1:30	1.5
2	20	M	A 510494	2003	5	35	5	1:128	1:245	11.7
3	1	M	A 763219	2003	4	16	3	1:128	1:145	6.9
4	28	F	A 763219	2004	0	15	8	1:32	1:55	2.6
5	26	F	A 763219	2004	7	30	4	1:64	1:110	5.3
6	53	M	A 765135	2005	7	32	5	1:64	1:115	5.5
7	2	M	S A5323	2007	6	13	32	1:64	1:70	3.3
Mean [99%CI]									1:110	5.3 [2.0-8.6]

[a] YF = yellow fever, A = Arilvax, S = Stamaril.
[b] Time vacc serology is the time between test dose administration and serology.
[c] Serum dilution VN80% is the highest serum-dilution at which at least 80% VN occurred. VN = virus neutralisation in plaque reduction neutralisation test.
[d] Calculated serum dilution was the exact graphical serum dilution at which 80% VN occurred.
[e] IU/ml was calculated with the calculated serum dilution VN80%, according to the reference serum (143 IU/ml, VN80% at serum dilution 1:3000).
CI = Confidence Interval.

additional to the local wheal formation were observed in the individuals at the outpatient clinic. The hospitalised patient developed a sensation of swelling of the tongue that responded to treatment with antihistamines.

Discussion

Travellers with egg allergy in whom vaccination was abandoned after the YF-17D test dose are very likely protected by the test dose. Apparently, the wheal-and-flare formation within 30 minutes after vaccine administration did not affect the formation of neutralising antibodies against yellow fever virus. The effect of mast cell degranulation on viral entry and replication remains unknown and could be important for the response to intradermal yellow fever vaccination. It has been shown recently that locally activated mast cells can actually enhance the immune response to a vaccine antigen [10].

Although all 7 egg allergic individuals were protected against yellow fever, the sample size of this study is too small to conclude that documentation of this protection by virus neutralisation test is no longer needed. Post-vaccination testing would no longer be required if 100% success rate of intradermal test dose vaccination would be achieved in 72 egg allergic individuals, corresponding to the lower boundary of the 95% confidence interval of the percentage of individuals who should be protected after YF-17D vaccination according to the WHO. In conclusion, these results show that, similar to healthy (non-allergic) individuals [6], subjects with a history of egg allergy in whom an intradermal test dose of 0.1 mL YF−17D vaccine yielded a strong local urticarial reaction, are able to develop a protective immune response and do not need further vaccination.

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Reduced dose pre-exposure primary and booster intradermal rabies vaccination with a Purified Chicken Embryo Cell Vaccine (PCECV) is immunogenic and safe in adults

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Abstract

Background

Pre-exposure vaccination of persons at risk with intradermally administered reduced dose cell culture rabies vaccines remains controversial in low-enzootic countries.

Methods

In a prospective clinical trial of adult volunteers (N=25), we studied the immune response to purified chick embryo rabies cell vaccine (PCECV) administered intradermally at a reduced dose (0.1mL) in a three-dose schedule (0, 7 and 21 days). In 10 subjects, immunogenicity of intradermally administered one-dose booster vaccination with 0.1 mL PCECV was investigated.

Results

All participants were seroconverted 3 weeks after primary and 1 week after booster vaccination, (antibody titre >0.5 EU/mL, measured by enzyme linked immunosorbent assay). Local adverse events such as erythema and swelling were moderate and transitory.

Conclusion

The intradermal vaccination route offers an efficacious and cost-reducing strategy to increase the accessibility of cell culture rabies vaccines.

Introduction

Rabies virus is transmitted through contact of saliva of a rabid animal with a person's mucosa or a skin lesion. Infection results in an encephalitis for which currently no antiviral treatment is available [1]. Because of the almost invariable fatal outcome after infection, medical care facilities in high-enzootic areas and travel clinics in non- or low-enzootic areas focus on prevention by vaccination either before a potential or shortly after a possible exposure. Individuals eligible for vaccination are the exposed population living in or travelling to enzootic areas, or persons who may be exposed to rabies by nature of their occupation [2].

Pre-exposure vaccination, which consists of a three-dose schedule on day 0, 7 and 21 (or 28), induces long-lasting memory, eliminates the need for rabies immunoglobulins (RIG), and reduces the number of days of post-exposure vaccination in case of possible exposure to the virus from five to two.

In areas where high rabies virus transmission occurs, intradermal (i.d.) pre- and post-exposure vaccination against rabies with a reduced vaccine dose is a widely accepted, safe, efficacious and cost-reducing strategy to increase the accessibility of more expensive cell culture rabies vaccines, and to phase out the use of nerve tissue rabies vaccines [3-6]. In travel clinics in non- or low-enzootic countries, pre-exposure rabies vaccination takes up an important and relatively expensive part in the prevention of travel-related diseases. Low-budget long term travellers such as backpackers at risk are more inclined to opt for pre-exposure rabies vaccination if vaccine costs are low.

However, western travel clinics are hesitant to implement the i.d. administration of cell culture rabies vaccine with a tenfold reduced dose for pre-exposure prophylaxis. Several reasons could underlie this reluctance to vaccinate more economically: 1) intramuscular (i.m.) vaccination results in higher antibody titres when compared to i.d. administration, even though it has been shown with several cell culture derived rabies vaccines that antibody titres induced by i.d. vaccination with 1/10th of the i.m. dose reach adequate levels as defined by the World Health Organization (WHO) [2, 3, 7-10], 2) i.d. vaccination is technically more demanding than the i.m. route, thus requiring a more trained staff, 3) i.d. rabies vaccination can induce more local adverse events than i.m. vaccination [7, 8, 11] and 4) not all official advisory institutions agree on the interchangeability of i.d. administration of the different cell culture rabies vaccines; i.e., human diploid cell vaccine (HDCV), purified chick embryo cell vaccine (PCECV), purified duck embryo vaccine (PDEV) and purified Vero cell rabies vaccine (PVRV). The Centre for Disease Control (CDC) for example recommends using only

HDCV for i.d. administration and the WHO advocates the i.d. application of any cell culture rabies vaccine, provided that the country adopting this i.d. regimen repeats immunogenicity studies with the selected vaccine in their own population [12, 13].

In the setting of pre-exposure prophylaxis, we investigated the efficacy and safety of pre-exposure i.d. primary (three-dose schedule of 0.1 mL) and booster (one dose of 0.1 mL) rabies vaccination with PCECV, in an adult population.

Methods

Study design

Travellers of 18 years and older, with an indication for pre-exposure rabies vaccination according to Dutch medical travel guidelines [14] were eligible for inclusion. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, travellers taking chloroquine or hydroxychloroquine, pregnant travellers and those allergic to chicken eggs. Written informed consent was obtained from each participant. The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Centre (LUMC) (protocol number P05.093), the Netherlands. The study was carried out between August 2005 and July 2007. Vaccinations were administered at the travel clinic of the LUMC by the medical travel consultants who were trained in methods of i.d. vaccine administration.

Subjects received 0.1 mL PCECV i.d. in the dorsal side of the right forearm in a 3-dose schedule (0, 7 and 21 days, one vaccination each time). This site of administration was chosen in order to be able to distinguish between adverse events of i.d. rabies vaccination and other vaccines administered in the deltoid muscle, in case of multiple vaccinations for travel purposes. Additionally, the i.d. vaccination in the dorsal side of the forearm facilitated the monitoring of adverse events by the participants (compared to the deltoid region). The syringe that was used for i.d. administration is identical to the syringe used for administration of tuberculin in the Mantoux test. The quality of the i.d. injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test), with 6 mm being the lowest acceptable diameter [15]. Booster vaccination consisted of one i.d. vaccination with 0.1 mL PCECV, approximately 1.5 years (range 16 – 20 months) after the primary series.

Rabies vaccine

The PCECV used in this study contained ≥2.5 IU/mL of Flury low egg passage (Flury-LEP) rabies strain that was grown in chick embryo fibroblasts, inactivated by β–propionolactone, and purified by density gradient centrifugation (Rabipur®, Novartis Vaccines and Diagnostics GmbH & Co KG, Marburg, Germany). Multiple doses (maximally 8) were obtained from one 1.0 mL vial (0.1 mL per i.d. vaccination). After reconstitution, vials were stored at 4°C and discarded after maximally 8 hours.

Data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained. Blood samples were collected in all primary vaccinated participants before vaccination (day 0), and 3 weeks after their last vaccination (day 42). Rabies vaccination was offered for free and a financial compensation was given for every blood sample collection at completion of the study.

Participants were asked to document local and systemic symptoms after each vaccination in a diary. In case of swelling at the site of injection the maximum diameter was documented by the participant.

Antibody detection against rabies

Antibody titres against rabies were measured using a commercial in vitro diagnostic ELISA (PLATELIA™ RABIES II kit, Bio-Rad, France) according to manufacturer's instructions. Briefly, a 96-well microplate coated with rabies glycoprotein was used. This viral envelope protein is responsible for the induction of neutralising antibodies [16]. The enzymatic conjugate consisted of a protein A from *Staphylococcus aureus* coupled with peroxidase. Positive controls, which are calibrated against WHO standards, allowed the quantitative determination of anti-rabies antibody titre in the serum, which were expressed as Elisa Units (EU) per mL.

The ELISA PLATELIA™II rabies test reaches 98.6% sensitivity and 99.4% specificity in comparison to the virus neutralisation assay, the rapid fluorescent focus inhibition test (RFFIT). There is a strong concordance between the two methods as demonstrated by the linearity of the correspondence between titres obtained by PLATELIA™ RABIES II and those by RFFIT in the range 0–4 IU/mL ($r^2 = 0.94$), and the cut-off level of 0.5 EU/mL corresponds to the internationally recommended 0.5 IU/mL threshold [17].

Statistical analysis

Student's t-test was performed to compare geometrical means of antibody titres and occurrence of adverse events after primary and booster vaccination. Correlation

between antibody titres after primary and titres after booster vaccination, and between the occurrence of adverse events and the height of the antibody response were analyzed by Pearson correlation on logarithmically transformed antibody titres. Calculation of the population size was based on a pilot study we performed preceding this study. In order to show immunogenicity in all participants (with $\alpha = 0.05$ and $1\text{-}\Delta = 80\%$) expressed as an antibody titre above 0.5 EU/mL, 25 participants were to be included, taking into account a withdrawal of 20%. Statistical analysis was performed using a computer-assisted software package (SPSS version 12.0, SPSS, Inc., Chicago, IL).

Results

Demographical characteristics of study cohort

Twenty five participants with a median age of 25.5 years (range 22-59 yrs) were included to receive the primary i.d. vaccination series. Nine of these primary vaccinated participants were male. Ten participants could be contacted after 1.5 years for the revaccination. Their median age was 24.5 years (range 23-59 yrs) at time of inclusion, and two of these participants were male.

Intradermal vaccination

The mean diameter of the arisen cutaneous wheal measured after vaccination was 8 mm (range 7-10 mm), indicating that all i.d. vaccinations (N=85) were performed correctly according to our standard.

Immunogenicity after primary and booster vaccination

Primary i.d. vaccination with PCECV in a three-dose 0.1 mL regimen induced antibody titres ≥0.5 EU/mL in 25/25 participants. Booster vaccination with one dose 0.1 mL PCECV induced protective titres in 10/10 participants (table 1). The geometric mean titre (GMT) after booster vaccination was significantly higher when compared to the GMT following primary vaccination ($p = 0.02$), indicating a good anamnestic response. Half of the boostered participants showed an antibody titre above 30 EU/mL (table 1), which is considered predictive for a longer duration of seroconversion after i.m. vaccination [17].

Correlation between immunogenicity after primary and booster vaccination

The divergent antibody responses to primary and even more to booster vaccination (ranges 2.9 – 52.4 EU/mL and 3.9 – 94.0 EU/mL, respectively), allowed to investigate if a high immunologic response after primary vaccination could predict a high

Table 1 Immunogenicity after (3-dose) i.d. primary and (1-dose) i.d. booster vaccination with PCECV (0.1mL/dose)

Vaccination	Time after vaccination	GMT	Minimum (EU/mL)	Maximum (EU/mL)	95% CI of the GMT	n/N titre>0.5 EU/mL	n/N titre>30 EU/mL
Primary	Day 0	0.0	-	-	-	0/25	-
	Day 42	10.7	2.9	52.4	8.3 – 13.1	25/25	4/25
Booster	Day 550	0.9	0.2	2.5	0.0 – 3.4	8/10	0/10
	Day 7 PB	4.8	0.9	19.0	2.1 – 7.5	10/10	0/10
	Day 14 PB	23.9	3.9	94.0	21.2 – 26.6	10/10	5/10

Booster vaccination was performed approximately 1.5 years after primary vaccination (day 550, or day 0 PB). PB = post booster vaccination

response after booster vaccination. However, no correlation was observed (coefficient = 0.2, p = 0.6) (data not shown) when logarithmically transformed antibody titres after primary vaccination were plotted against the titres after booster vaccination. This lack of intra-individual consistency as far as the height of the antibody response after vaccination is concerned, is demonstrated by the multiple crossing lines (figure 1).

Safety of primary and booster vaccination

Local erythema and swelling at the site of injection occurred in 96% of participants after primary vaccination and in all subjects after booster vaccination. A trend towards more severe local adverse events was documented after booster vaccination, e.g. a mean diameter of swelling twice the diameter after primary vaccination (table 2).
In addition, we investigated if the severity of adverse events corresponded with the height of the vaccine induced antibody response. For primary as well as for booster vaccination, no correlation was found between the severity of adverse events and the height of the antibody titre (correlation coefficients of 0.06, p = 0.8 and 0.2, p = 0.6, respectively) (data not shown).

Discussion

Reduced dose intradermal pre-exposure vaccination with PCECV resulted in protective antibody titres in all primary and revaccinated healthy adult volunteers. This finding is consistent with a study performed in children aged 5 to 8 in Thailand by Kamoltham

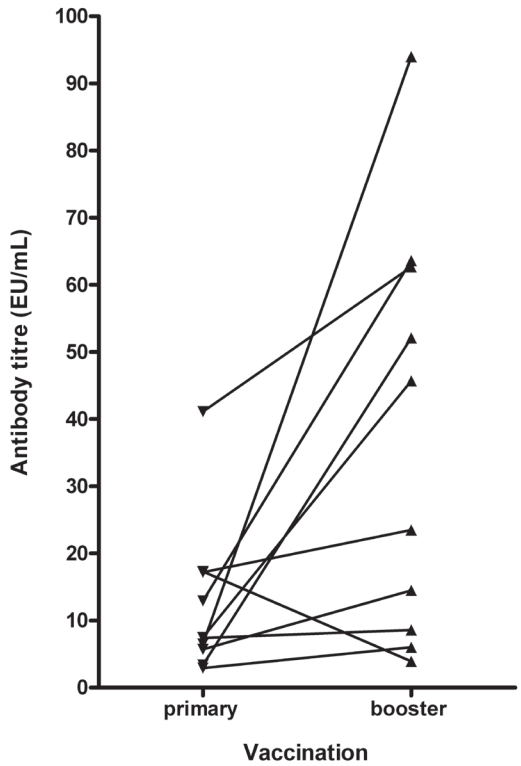

Figure 1 Correlation between antibody response after primary and booster vaccination within participants (N=10)

et al., in which the efficacy of i.d. PCECV vaccination has been shown with a simulated two-dose booster vaccination post-exposure schedule [19].

In addition, the i.d. booster after 1.5 years resulted in an antibody titre above 30 EU/mL in half of the participants, reflecting the possibility of a long-lasting immune response. According to Strady et al. [18] these 'good responders' have an almost 100% probability of staying protected during the following 10 years. This is of importance for those at continuous risk of exposure to rabies virus. Consistent with the population boostered intramuscularly with HDCV or PVRV by Strady et al., i.d. booster with PCECV elicited poor and good responders (figure 1). This dichotomy was

Table 2 Adverse events after i.d. primary and i.d. booster vaccination (day) with PCECV. Lymph node swelling ocurred in the ipsilateral axilla

Adverse event			Vaccination preceding adverse events			
			Primary vaccination (day)		Booster vaccination	
			0	7	21	
Local	Erythema	n/N	24/25	24/25	24/25	10/10
		N days (mean)	4.6	5.5	4.3	6.6
		95% CI of mean	3.8 – 5.4	3.9 – 7.1	3.2 – 5.4	5.8 – 6.4
	Swelling	n/N	22/25	20/25	21/25	10/10
		N days (mean)	4.3	4.9	4.3	5.5
		95% CI of mean	3.3 – 5.3	3.1 – 6.7	2.7 – 5.9	3.9 – 7.1
		Diameter (mm)	17.0	15.1	20.3	40.1
		min – max (mm)	0 - 40	0 - 45	0 - 80	5 - 100
		95% CI of mean	11.7 – 22.3	8.5 – 21.6	9.7 – 30.9	18.7 – 61.5
	Pain	n/N	4/25	4/25	6/25	2/10
		N days (mean)	0.5	0.5	0.7	0.6
		95% CI of mean	0.0 – 1.0	0.0 – 1.0	0.2 – 1.2	0.0 – 0.9
	Pruritus	n/N	12/25	3/10		
Systemic	Myalgia	n/N	2/25	2/25	1/25	1/10
		N days (mean)	0.3	0.3	0.2	0.3
		95% CI of mean	0.0 – 0.7	0.0 – 0.7	0.0 – 0.6	0.0 – 0.9
	Fever	n/N	0/25	1/25	1/25	0/10
		N days (mean)	0.0	0.0	0.1	0.0
		95% CI of mean	-	-	0.0 – 0.3	-
	Lymph node swelling	n/N		2/25		1/10
Overall severity of adverse events*			+/-	+	+	+
Range of severity of adverse events*			− to +	− to ++	− to ++	+/- to ++

Severity of adverse events was documented as – (absent), +/- (mild), + (moderate) or ++ (severe), according to participants experience. *Severity concerned local adverse events.

not observed after primary vaccination and may therefore be attributed to a difference in induction of memory after primary vaccination.

The population size of this study was adequate to demonstrate the immunogenicity of primary and booster i.d. vaccination with 0.1 mL PCECV, but insufficient to determine differences between adverse events after primary and booster vaccinations.

Furthermore, no comparison was made with intramuscularly vaccinated participants since i.d. and i.m. rabies vaccinations have previously been compared with other vaccines than PCECV [4, 7-11].

Local adverse events occur more frequently after i.d. than after i.m. vaccination, as has been demonstrated with various vaccines such as influenza [20, 21], yellow fever (unpublished data), and also with rabies vaccine [7, 8, 11]. Because the local adverse events after i.d. PCECV administration may hamper the acceptance of this vaccination route, recipients should be informed on the occurrence of transitory local erythema and swelling. Systemic symptoms such as myalgia and fever have not been reported more frequently after i.d. vaccination with PCECV [7]. In our experience adverse events are seldom a reason for vaccinees to discontinue the i.d. vaccination.

The severity of adverse events was not correlated with the height of the antibody response after primary as well as booster vaccination. Neither does the antibody level after primary vaccination predict the response to booster vaccination, implying that poor or good responders to rabies vaccine cannot be identified until boostered.

Although widely used for Mantoux testing and BCG vaccination, i.d. vaccination is technically more demanding. To ensure correct i.d. vaccination, we introduced a minimal cut-off diameter of the cutaneous wheal following i.d. vaccination. If the wheal does meet not the required diameter; vaccination should be repeated.

In conclusion, the findings of this study have the following practical implications: 1) up to eight times as many individuals can be vaccinated intradermally with 0.1 mL PCECV compared to i.m. vaccination. By clustering travelers who will be at risk of exposure to rabies virus, travel clinics in low enzootic countries can adopt this method of economic pre-exposure vaccination without changing their vaccination schedule 2) introduction of the diameter cut-off for the wheal after i.d. vaccination allows for control of i.d. vaccine delivery, and 3) the cost-saving strategy should further encourage pre-exposure immunisation in high-enzootic countries, where focus on rabies prevention is still mainly on post-exposure prophylaxis.

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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 CCR5Δ32 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.

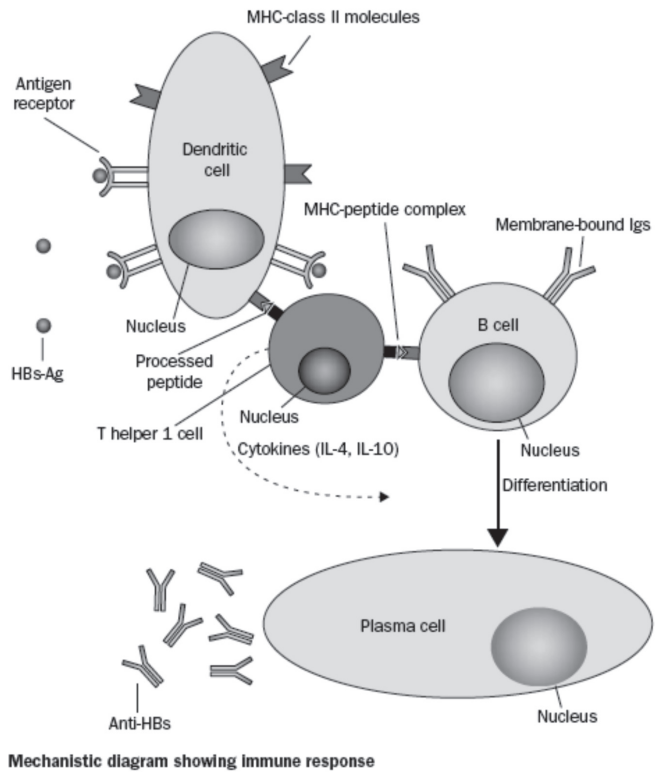

Figure 1 Mechanistic diagram showing the different required steps for the induction of an adaptive immune response against HBsAg. Adapted from ref 34

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

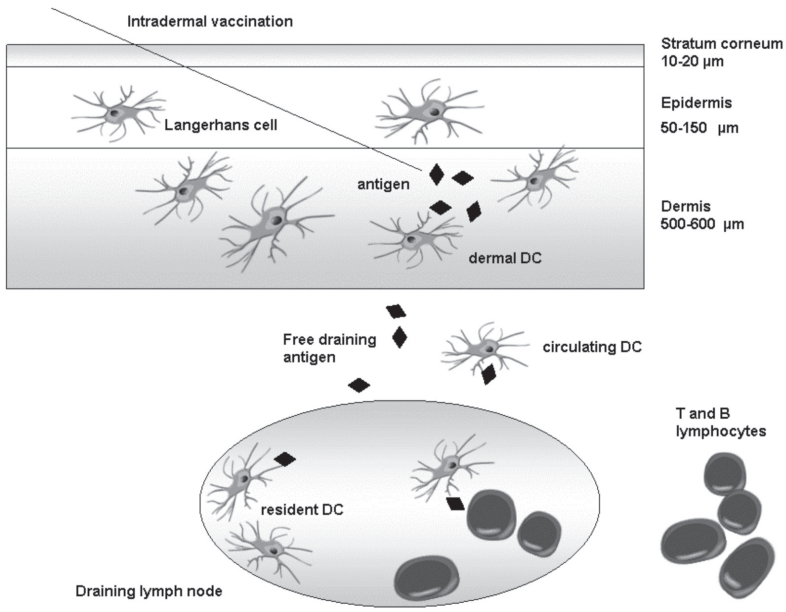

Figure 2 Schematic representation of intradermal immunisation and subsequent initiation of adaptive immune response [after ref 46]

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

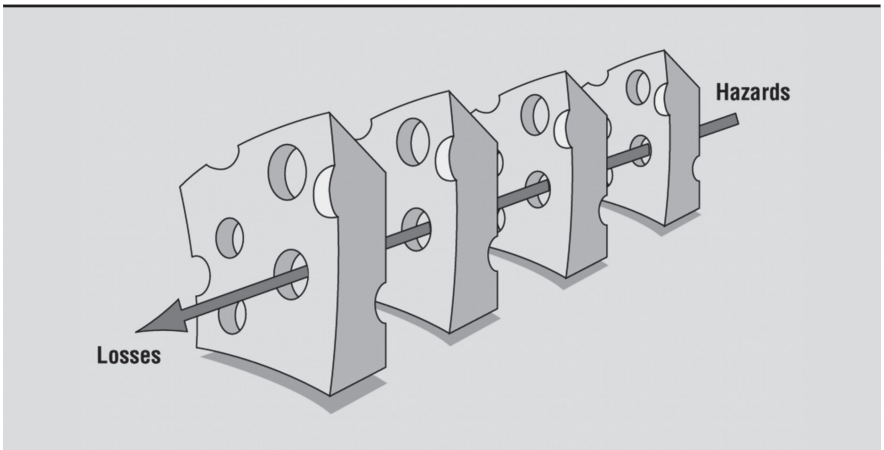
Inleiding

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).